Advances in Dairy Technology

Looking Beyond 2020

Volume 32, 2020

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Advances in Dairy Technology

Volume 32

Proceedings of the 2020
Western Canadian Dairy Seminar

Editors
Divakar Ambrose and Masahito Oba

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Preface

As program co-chairs we take great pleasure in delivering the 38th Annual Western Canadian Dairy Seminar (WCDS) and welcoming you to Red Deer, Alberta. The theme of the 2020 seminar is "Looking Beyond 2020". This year's seminar has the following seven sessions:

- Future Matters
- Secrets of Healthy Feeding
- Human and Cattle Well-Being
- Student Research and Producer Panel
- Nurturing the Next Generation
- Enhancing Herd Fertility
- Herd Health – Protect, Monitor, Investigate

The continued success of the WCDS is attributable to the guidance, support and commitment of the WCDS Advisory Committee. The Advisory Committee includes representatives from academia, the provincial and federal governments, Alberta Milk, Alberta Veterinary Medical Association, Animal Nutrition Association of Canada, dairy equipment manufacturers, financial institutions, milk processors, pharmaceutical industry, as well as representatives from each of the four Western provinces. We thank the Advisory Committee members for their input and advice in shaping this year's program. We specially acknowledge the tremendous support of our conference coordinator, Kate Davies, who always goes the extra mile to ensure that the excellence of the seminar is maintained.

We are fortunate to the have some of the best speakers in the world at this year's WCDS. As teachers, researchers, extension specialists and industry experts, our speakers are not only leaders in their areas of expertise, but they are also passionate about engaging with and learning from the industry. To ensure that effective knowledge-sharing occurs both ways, we encourage attendees to connect with speakers through active participation in question sessions and panel discussions, and at informal settings. Our special thanks to all sponsors for their continued financial support. Without the generous support of our sponsors it would not be possible to bring you the speakers of this caliber while keeping the registration fee quite affordable.

The WCDS was founded in 1982 with a vision to support the growth and success of the Canadian dairy industry. Every year the dairy industry looks to the WCDS for solutions to emerging issues, and for new research results. As in the past years, we are thrilled to see attendees from a broad cross section of the dairy industry, including producers, nutritionists, consultants, veterinarians, services companies, government, university/college personnel and students. We truly hope that the networking and knowledge-sharing that occurs at this year's WCDS is beneficial to all participants and has a positive impact on our dairy industry.

Once again, we extend a warm welcome to you and thank you for your participation. We wish everyone a wonderful time learning and networking over the next three days.

Divakar Ambrose & Masahito Oba
Program Co-Chairs

Advisory Committee

Our thanks to the following individuals and organizations for the time and effort that they have devoted to organizing the 2020 Western Canadian Dairy Seminar

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Lethbridge AB

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Edmonton AB

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Looking Beyond 2020

Program

Tuesday, March 10th

10:00 am – 5:00 pm Pre-Conference Tour
This tour will visit three local dairy farms with the focus on progressive and innovative dairy managers.   **TOUR SPONSOR**: Arm & Hammer Animal & Food Production
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1:30 pm – 4:30 pm Pre-Conference Workshop: *Milk as a Diagnostic Tool for Nutritional Management.* **Facilitator:** Rick Grant, William H. Miner Agricultural Institute
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6:30 pm – 9:00 pm Registration   *Exhibition Hall Entrance*

8:00 pm – 10:00 pm Opening Reception   *Exhibition Hall*
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Wednesday, March 11th

 7:30 *Breakfast – Exhibition Hall*   **SPONSORS:** Agribution Canada & Canadian Nurs-ette

Wednesday AM, Plenary Session   *Mediterranean Ballroom*

 8:30 *Welcome*: **Nelson Dinn, University of British Columbia,** Chair, WCDS
      Advisory Committee

  Introduction and Conference Overview: **Divakar Ambrose, Alberta Agriculture and Forestry**, Co-Chair, WCDS Program Committee

Session I. Future Matters

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8:50 *Can You Use Technology to Uber Proof Your Business?* – **Marty Seymour, Farm Credit Canada**   **DIAMOND SPONSOR**: Farm Credit Canada

9:50 *Nutrition break – Exhibition Hall*

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                                      Phibro Animal Health Corporation

                          EXHIBITOR COFFEE SPONSOR: Gem Silage Products
              MILK FOR NUTRITION BREAKS: DLS Barn Solutions / Jourdain

10:20 *Working Towards a More Socially Sustainable Dairy Industry* – **Nina von Keyserlingk, University of British Columbia**

SPONSOR: Feed Supervisor – Hoof Supervisor - SCiO

11:05 *Sustainability of Animal Agriculture in the Global Food System* – **Robin White, Virginia Tech**   SPONSOR: GEA Farm Technologies Inc.

11:50 **Speaker Panel**

12:10 **Lunch** – *Exhibition Hall*   SPONSORS: Diamond Hoof Care Ltd & TD Agriculture Services Alberta

Wednesday PM, Concurrent Sessions

Session II. Secrets of Healthy Feeding   *Milan Room*

SPONSOR: Dairytech

1:40 *Relationships Between Fiber Digestibility and Particle Size for Lactating Dairy Cows*– **Rick Grant, William H. Miner Agricultural Institute**

SPONSOR: Alberta Animal Nutrition Lectureship

2:20 *Corn Silage: Managing the Manageable*– **Bill Mahanna, Pioneer Hi-Bred**

SPONSOR: Cargill Feed & Nutrition

3:00 ***Nutrition break*** – *Exhibition Hall*   SPONSORS: Grooving Gord Alberta Ltd. & Pro-Line Manufacturing Inc.

3:30 *Managing Dietary Variation to Maintain or Improve Efficiency* – **Bill Weiss, Ohio State University**   SPONSOR: SoyPLUS/SoyChlor

4:10 *Feeding for a Healthy Liver: The Role of Methionine and Choline in Transition Cows* – **Heather White, University of Wisconsin-Madison**   SPONSOR: SoyBest

4:50 **Speaker Panel**

Session III. Human and Cattle Well-Being   *Monaco Room*

SPONSOR: Grober Nutrition

1:40   *Five Habits of Highly Effective Farmers* – **Lisa McCrea, Agwest Veterinary Group**   SPONSOR: DeLaval

2:20 *How to Do More for Mental Health in Agriculture* – **Adelle Stewart, The Do More Agriculture Foundation**   SPONSOR: The Co-operators – Central AB

3:00 ***Nutrition break*** – *Exhibition Hall*   SPONSORS: Grooving Gord Alberta Ltd. & Pro-Line Manufacturing Inc.

3:30 *Transporting Cattle in 2020: Research and Regulation Update* – **Karen Schwartzkopf-Genswein, Agriculture and Agri-Food Canada**

4:10 *Three Ways to Lose Money on the Farm: A View from the Udder* – **Ronald Erskine, Michigan State University**   SPONSOR: Hipra Canada

4:50 **Speaker Panel**

6:00 **Cash Bar**

7:00 **Banquet –** *Tuscany Room* – Comedian **Matt Falk**

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Thursday, March 12<u>th</u>

7:30 *Breakfast – Exhibition Hall*

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Session IV. Student Research and Producer Panel

<div align="right">

Mediterranean Ballroom

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8:30 *Student Research Presentation Competition*

- *Early Identification of Cows at Risk of Metritis Using Calving Factors and Activity Monitors –* **Janet Bauer, University of British Columbia**

- *Effects of Feeding Hay and Calf Starter as a Mixture or as Separate Components to Holstein Calves on Intake and Growth –* **Lauren Engelking, University of Alberta**

- *Dairy Production Performance Replacing Corn and Barley Silages with Whole Crop Faba Bean Silage in Western Canada –* **Victor Guevara, University of Saskatchewan**

- *The Effects of Concentrate Feeding Level and Rate of Increase When Offered Through an Automatic Milking System on Fresh Cow Performance –* **Jennifer Haisan, University of Alberta**

- *Impact of Concentrate Allowance on the Behavior and Production of Dairy Cows Milked in a Free Traffic Automated Milking System –* **Anna Schwanke, University of Guelph**

9:50 ***Nutrition break** – Exhibition Hall*   **SPONSORS**: Elanco Animal Health & Richardson Oilseed Limited

10:20 *Bio-Security Management – Producer Panel Presentations*

- **Jesse Houweling, Houweling Farms, Coaldale, AB**

- **Joel Huizing, Country Charm Farms Ltd, Abbotsford, BC**

- **Jay Olyniuk, Rayner Dairy Research and Teaching Facility, Saskatoon, SK**

<div align="right">

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4:50 **Producer Panel**

12:00 **Lunch** – *Exhibition Hall*   **SPONSORS**: ABS Global (Canada) Inc. & Bricon Safety Grooving Ltd

Thursday PM, Concurrent Sessions

Session V. Nurturing the Next Generation   *Milan Room*
SPONSOR: Alta Genetics Inc.

1:30 *Managing Calf Health and Performance in utero* – **Geoffrey Dahl, University of Florida**

2:10 *New Concepts in Calf Nutrition: The First Week of Life* – **Michael Steele, University of Guelph**   **SPONSORS**: Fortified Nutrition Limited

2:50 ***Nutrition break*** *– Exhibition Hall*   **SPONSORS**: PMT & Westway Feed Products

3:20 *Rethinking Ruminal Acidosis in Dairy Calves* – **Anne Laarman, University of Alberta**   **SPONSOR**: Lallemand Animal Nutrition

4:00 *Economics of Raising Dairy Replacement Heifers* – **Michael Overton, Elanco Animal Health**   **SPONSOR**: MNP

4:40 **Speaker Panel**

Session VI. Enhancing Herd Fertility
SPONSOR: Lactanet Canada   *Monaco Room*

1:30 *Trouble-Shooting Reproduction Issues* – **Jocelyn Dubuc, University of Montreal**

2:10 *Potential Impact of Viral Dseases on Conception Rates in Cattle* – **Claire Wathes, Royal Veterinary College, United Kingdom**   **SPONSOR**: Vetoquinol

2:50 ***Nutrition break*** *- Exhibition Hall*   **SPONSORS**: PMT & Westway Feed Products

3:20 *New Strategies to Maximize Pregnancy Outcomes* – **Jeffrey Stevenson, Kansas State University**   **SPONSOR**: Vandenberg Hay Farms Ltd

4:00 *Genetics and Economics of Using vitro-Produced Embryo Transfer in Dairy Herds* – **Albert De Vries, University of Florida**   **SPONSOR**: WestGen

4:40 **Speaker Panel**

5:00 – 6:30 **Cocktail Reception**   *Exhibition Hall*
RECEPTION SPONSORS: Alberta Milk / BC Dairy Association / Dairy Farmers of Manitoba / SaskMilk
HOT APPETIZERS SPONSOR: Cambridge Red Deer Hotel
CHEESE SPONSOR: DLS Barn Solutions/Jourdain

Friday, March 13<u>th</u>

7:30 **Breakfast** – Exhibition Hall   **SPONSORS**: Holstein Canada & Jaylor

Session VII. Herd Health – Protect, Monitor, Investigate
SPONSOR: Merck Animal Health   **Tuscany Ballroom**

8:30 *When (Before) Disaster Strikes: Preparation For a Disease Outbreak* – **Keith Lehman, Alberta Agriculture and Forestry**

9:10 *Investigating Fetal Calf Loss* – **Cameron Knight, University of Calgary**

Student Research Presentation Competition – Awards Announcement

9:50 **Nutrition break** – Exhibition Hall   **SPONSORS**: Boehringer Ingelheim & Schippers Canada Ltd

10:20 *Use of Big Data to Monitor Herd Health*– **Heather White, University of Wiscon**

11:00 *Antibiotic Therapy: It's Not the Drugs, It's How We Use Them* – **Ronald Erskine, Michigan State University**

11:40 **Speaker Panel**

12:00 **Closing Remarks**

Platinum Seminar Sponsors: BMO Bank of Montreal
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Next Western Canadian Dairy Seminar
March 9 – 12, 2021

Can You Use Technology to Uber Proof Your Business?

Marty Seymour

Director, Industry & Stakeholder Relations
Farm Credit Canada, Regina, Saskatchewan

Farming today is not what it was 50, 25, or even five years ago. The pace of change has never been this fast and it will never be this slow again. Farmers, ranchers and food companies all face the same disruptive question: "If the future is different than the past, how safe is my business?" For most farms today, technology offers solutions to labour, food safety, risk management and efficiency, but many farms struggle with the complexity or return on investment for some of the technologies available.

Seventy-five percent of Canadian dairy farms adopted new technology in the last year according to research completed by the Farm Credit Canada Vision Panel in 2018. This is a number we can all be proud of, but it also means one in four farms did not adopt new technology. For those skeptical of new technology, it's ok not to be an early adopter. My rule of thumb for skeptics who may wait to see if there is value in the latest idea or gadget is: "You don't have to have the latest technology, but you have to compete with those who do."

Agriculture appears to be ripe for a massive data harvest. Companies, including Google, Amazon and IBM, are lining up to help agriculture enthusiasts harvest and make sense of mountains of industry data. The challenge is a general lack of understanding of what problem we are trying to solve with each data set.

Artificial intelligence (AI) offers a huge upside for looking at these massive agricultural data sets. Computing power, as well as algorithm accuracy, has improved data analysis substantially. A challenging obstacle to AI application in biological systems is inconsistent data, which leads to difficulty in writing effective herd health management solutions. In the near term, we have sensors and apps on our phones to identify heat cycles or feed intake of individual animals. These innovations allow farms to expand and grow, and make it easier to train new employees entering the industry without livestock backgrounds.

Robots show the most shine when it comes to attracting interest in the farming community. The advancement of the dairy robot from 1999 to 2019 has been exponential. Robotic milkers have become an economically viable solution in all kinds of farm models. The next generation of robots is going to surface in autonomous agriculture. The farms of the future are likely to be sized and scaled around the use of robots and driverless technology. It also means the farmer of the future may need to have a bias to technology savviness in addition to animal behaviour.

The next frontier of disruption in modern agriculture is anchored in a shift in consumer preferences. The shift in the millennial diet from milk and animal proteins to plant-based alternatives is rooted in social values. These dietary changes are likely to have the biggest change on production agriculture because the current supply chain and food production model in North America is based on animal proteins.

The agri-food industry will continue to evolve. There are a couple fundamental truths the ag community must embrace: 1) consumers will always want choice, and 2) health is one of the key drivers in many food choices people make. The dairy industry has much to offer in both these categories.

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Working Towards a More Socially Sustainable Dairy Industry[1]

Marina A. G. von Keyserlingk* and Daniel M. Weary

Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC
*Email: marina.vonkeyserlingk@ubc.ca

▪ Take Home Messages

- The available evidence suggests that attempts to reduce transparency about farm practices will erode public trust in farming.

- Industry-led efforts to educate the public about farming practices are not an effective method of changing public attitudes about farming.

- A sustained process of respectful engagement between individuals working in agriculture and the general public can help improve dialogue and better identify areas of shared values and where there are important disconnects between public values and current practices.

- Members of the dairy industry need to better identify and articulate a core set of shared values and develop a vision for how practices on farm can better align with these values

▪ Introduction

Animal welfare is an important social concern. To better retail social licence, those not directly involved in farming, including the general public and other supply chain interests, must be accepted as credible stakeholders in the discussions on the way farm animals are cared for. In our presentation we will discuss different ways the industry has responded to increasing societal interest and concern, including attempts to shield practices from public view (e.g., via so called "Ag-gag" laws), attempts by the industry to 'educate' the public, and more rarely, sustained attempts to engage respectful two-way discussions with the public. We end our paper with a call for the dairy industry to take leadership over this process, by clearly articulating our shared values and a long-term vision for the industry that ensures that these values are reflected in our practices.

▪ Closing the Barn Doors

A natural response to criticism is to simply close the door in the hope that reducing the supply in information will help prevent further criticism from taking place. The idea is simply that if people cannot see the practices, then there will be nothing to criticize. This approach may work in the short term but is unlikely to be effective in the longer term. Indeed, efforts to reduce transparency can harm the reputation of agriculture. For example, 'Ag-gag' laws attempt to prevent the filming and distribution of undercover videos. We undertook a study where we experimentally assessed the views of U.S. participants that were told about these laws (Robbins et al. 2016).

---

[1]This summary of some of our work is based on two papers: 1) M.A.G. von Keyserlingk and D.M. Weary 2016. Stakeholder views, including the public, on expectations for dairy cattle welfare presented at the Western Canadian Dairy Seminar in 2016 and a peer reviewed paper by D.M. Weary and M.A.G. von Keyserlingk. 2017. Public concerns about dairy-cow welfare: how should the industry respond? Animal Production Science 57, 1201–1209 2018.

Our findings indicated that when people were made aware of Ag-gag laws they were less likely to see farmers as trustworthy sources of information (Figure 1), were more likely to support the introduction of new animal welfare laws, and were more likely to believe that the welfare of the animals on farms was poor.
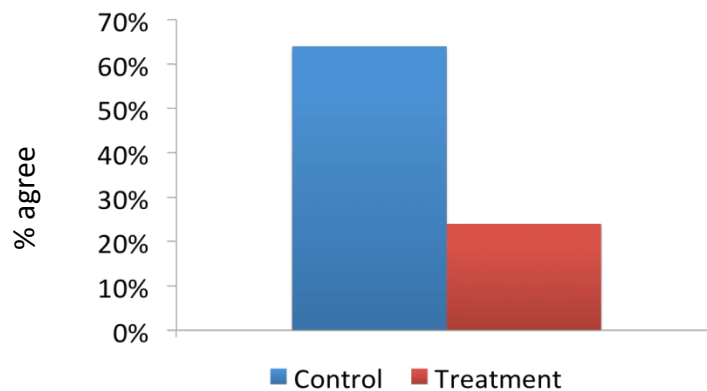
Figure 1. Participants exposed to information about 'Ag-gag' laws were less likely to agree that farmers are trustworthy sources of information (adapted from Robbins et al., 2016.)

▪ Educating the Public

People within the livestock industries sometimes feel that the public is ignorant of farming practices, and greater acceptance of current practices could be achieved if there was a concerted effort to better educate the public about farming. Although it may be true that knowledge about agricultural practices is often low, there are several reasons to believe that efforts to educate the public are unlikely to improve acceptance of current practices.

In 2014, shortly after the release of an undercover video showed footage of disturbing animal handling on a dairy farm in British Columbia, Canada, we undertook a study where we tested the hypothesis that education would result in an increased confidence that dairy cattle indeed have a good life on Canadian farms (Ventura et al., 2016). We examined the views of individuals participating in a 'slow-food' tour; these individuals were interested in where their food came from, but had little previous exposure to dairy. The 50 individuals all agreed to answer five basic questions about dairy farming before the tour and then asked the same questions after they toured a working dairy farm. On average, participants were able to correctly answer three out of five questions before the tour, and this increased to four out of five after the tour, showing that they learned something on the tour. Visiting the dairy farm seemed to address some of the concerns; after the tour most participants were satisfied that cows were provided adequate access to food and water and that they were handled appropriately. However, most participants left the farm with more concerns then when they started (Figure 2), and on average the perception that cows on the farm led a reasonably good life was reduced after the tour. Thus, when visitors learned more about dairy farming, they came away with more concerns, including, for example, lack of pasture access and early separation of cow and calf. This work, along with other research, highlights the lack of relationship between attitudes and knowledge (Hansen et al. 2003). People's views are highly related to their values, and these values are not easily shifted.
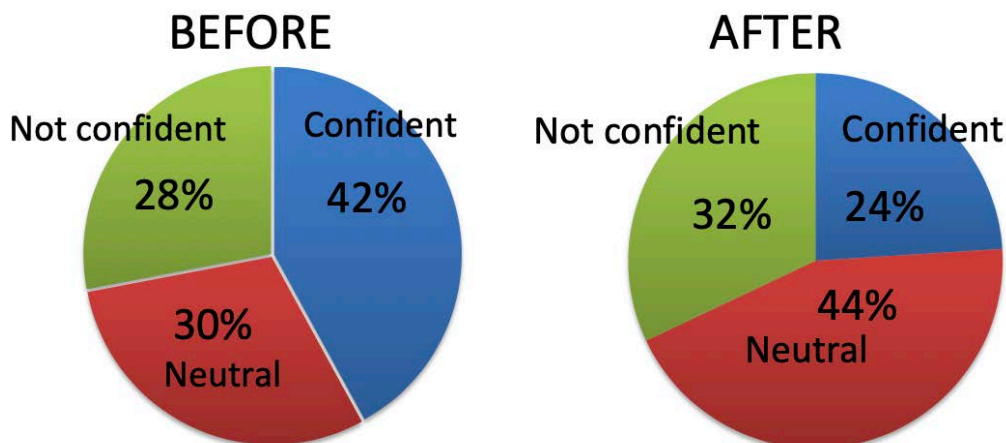
Figure 2. Participants were asked how confident they were that dairy cattle have a reasonably good life before (BEFORE) going on a self-guided tour of the UBC Dairy Education and Research Centre farm. Following the tour of the farm they were asked again (AFTER) how confident they were that dairy cattle have a reasonably good life. (adapted from Ventura et al., 2016)

Education efforts should also consider the ratio of naïve consumers and citizens to decision-makers within the supply chain. As Aerts (2013) argued "it is easier [for citizen advocacy groups] to convince five (or fifteen) buying directors than five (or twenty-five) million consumers." Corporations can be major buyers of dairy and can require that suppliers meet their specific animal welfare standards. Thus, industry engagement efforts must extend beyond direct messaging to the general consumer and include citizens who are most interested in the issues and are likely to influence corporate and government responses.

▪ Engaging the Public

Rather than focusing on one-way efforts to 'educate' the public, the dairy industry might instead consider developing methods of facilitating constructive, informed engagement among the stakeholders. We suggest that this approach will likely be more effective in identifying shared values and potential approaches likely to find general appeal.

At the University of British Columbia (UBC) we have been using web-based surveys to provide opportunities for the public to tell us how they envision the dairy industry of the future. In one such survey (Cardoso et al., 2016), U.S. participants were invited to respond to the following open-ended question: What do you consider to be an ideal dairy farm and why are these characteristics important to you? Respondents focused their responses mainly on animal welfare and quality of milk (Figure 3), but also mentioned social, economic and ecological issues. Providing assurances that animals are well treated, developing methods to incorporate pasture access, and assurance of healthy products without relying on antibiotics or hormones may help provide the dairy industry social license to operate.
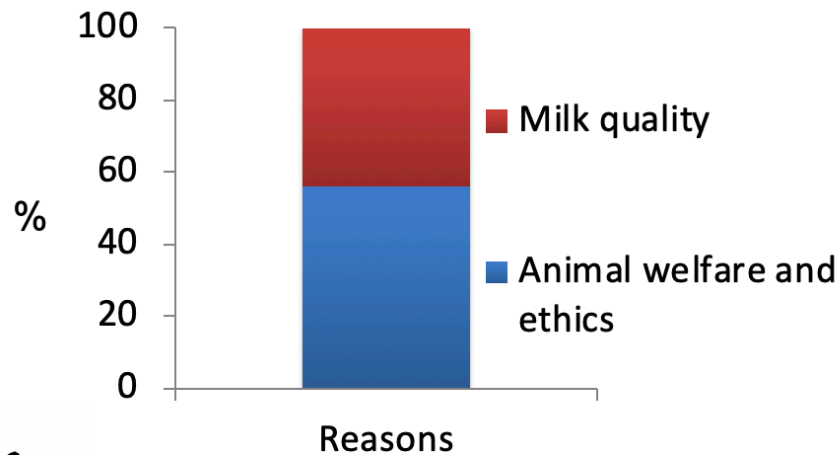
Figure 3. Survey results of 453 U.S. participants who responded to the following open-ended question: What do you consider to be an ideal dairy farm and why are these characteristics important to you? (Cardoso et al., 2016)

Our hope was that by asking the public to tell us how they envision the ideal dairy farm we could begin to identify areas where current practices were out of step and consider methods that better align with public values. This type of approach can provide the industry a basis for predicting which factors are likely to come under increasing criticism and where research efforts should be devoted.

▪ Final Thoughts

Our perspective is that rather than attempting to shield practices from public view or focusing on one-way industry efforts to 'educate' the public, we should develop methods of facilitating constructive, informed engagement among the stakeholders. We suggest that this approach will likely to be more effective in identifying shared concerns and potential solutions likely to find general appeal.

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Sustainability of Animal Agriculture in the Global Food System

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▪ Take Home Messages

- Agriculture contributes approximately ¼ of global greenhouse gas emissions and uses over $^2/_3$ of global freshwater water withdrawals.

- The total environmental impacts of agriculture have increased over time and are proportional to the growing population.

- When scaled per capita, agriculture has increased the food availability per person while reducing resource use.

- Approximately 30% of the global population suffers from micronutrient deficiencies. Animal products provide high-quality sources of micronutrients for human diets.

- Improving efficiency of animal agriculture has and will continue to improve sustainability of animal agriculture in the global food system.

- Advancements and adaptations in our understanding of atmospheric science must be taken into account when considering greenhouse gas emissions mitigation strategies.

- Improved discussion of the socioeconomic benefits of livestock in the global agricultural system is needed in future assessments of agricultural sustainability.

▪ Agriculture, Diets, and Sustainability on the Global Stage

Over the past several decades, a number of government, popular press, and peer-reviewed sources have propagated the idea that reducing consumption of animal source foods is beneficial for human and environmental health. Surveying the peer-reviewed literature, it is understandable that this message has been so readily adopted by such a wide variety of sources. Undoubtedly, there is a correlation between animal product production and increased environmental impact, just as there is a correlation between consumption of livestock products and negative human health outcomes. However, correlation is not causation. In response to the expanding rhetoric surrounding reducing consumption of animal products, several papers have been published highlighting logical flaws and limited causal relationships in the linkages between animal-source food products and negative human or environmental health outcomes. To better understand how the available data support (or fail to support) common discussion points regarding agricultural environmental impact and the role of agriculture in promoting healthy diets, we will assess claims made within the executive summary of the EAT-Lancet report (Willett et al., 2019) relative to the global agricultural database available from the United Nations Food and Agricultural Organization (FAO, 2019).

Is Food Production the Largest Pressure Caused by Humans on Earth?

Several popular press articles and recent peer-reviewed papers claim that agriculture is one of the major factors driving climate change. According to the Contributions of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, electricity and heat production

account for 25% of emissions and other energy sources add another 10%. Industry accounts for 21%, transportation for 14%, and buildings for 6% of total global emissions. Agriculture is combined with forestry and all other land uses and accounts for 24%. From a carbon emissions standpoint, agriculture is globally responsible for just under a quarter of emissions, which is similar to the proportion of emissions assigned to the industry and electricity sectors. For countries with more developed agricultural systems, this proportion is much lower. For example, in the United States and Canada, agriculture accounts for less than 10% of total emissions (U.S. EPA, 2010).

In addition to highlighting agriculture as a major contributor to climate change, agriculture is commonly cited as being a major user of water globally. According to the UNFAO (Aquastat, updated November 2016), 2,769 km^3 of water is used for agriculture per year. This water use accounts for 69% of total global water withdrawal per year. Undoubtedly, this suggests that the claims that agriculture is a water-intensive process are accurate. To better understand where water withdrawals are occurring, we can look at the distribution of water withdrawal by region. Agriculture in Asia accounts for 2,069 km^3 (75%) of agricultural water use. Although the AquaStat database makes it difficult to discern specifically which commodities are contributing to this substantial water use in Asia, it is still an important consideration. Efforts to reduce agricultural water use would be most effective if targeted at these major water-using production systems.

Land is another resource commonly discussed when evaluating the environmental impact of agriculture. According to the UNFAO (FAO, 2019) in 2015, 32% of the global land area was tree-covered areas, 14% was terrestrial barren land, 14% was grassland, 13% was herbaceous crops, 12% was shrub-covered areas, 7% was sparsely natural vegetated areas, and the remaining 7% included artificial surfaces, inland water bodies, mangroves, glaciers, and woody crops. Of all land, only 27% is associated with agricultural activities like cropping and grassland production. Although this is a somewhat imperfect way to characterize land used for agriculture it does suggest that we feed the world's population from a fairly small land base. Continuing to preserve our non-agricultural and non-artificial land areas is a priority for numerous environmental stewardship groups.

Depending on the environmental impact considered, agricultural production systems contribute substantially to environmental pressures globally. In the case of water use, agricultural production is the largest single pressure. In the case of greenhouse gas emissions (GGE), agriculture is just one of many players contributing to atmospheric CO_2 concentrations. Making sure we accurately represent the role of agriculture in contributing to global environmental impact is a critical first step in characterizing strategies to maintain agricultural sustainability long-term. To simply state that agriculture is the greatest single contributor to environmental impacts globally is a gross over-simplification.

What Data are Available Regarding Food Insufficiencies Globally?

Another common discussion point surrounding the sustainability of agricultural systems centres on the challenge of feeding the growing global population. World population was approximately 1.6 billion people in 1900. That population had doubled to 3 billion by the mid 1970s and doubled again to 6 billion by the year 2000. By the year 2100, estimates suggest the population will nearly double again to roughly 11 billion people (U.S. Census Bureau, 2008). This exponential growth of the global population is concerning, namely because of the limited resources available to support the growing population. In 1798, Thomas Malthus wrote the "Essay on the Principle of Population", which put forth the idea that the power of population growth is infinitely greater than the power in the earth to produce sustenance for that growing population. The demise of society predicted by Malthus in the late 1700s did not come about thanks to the advancements in society associated with the Industrial Revolution. A second wave of concern over the growing global population was apparent in the late 1960s and early 1970s and can be attributed to a variety of sources, the most common of which is the 1972 report "Limits to Growth" put out by the Club of Rome. Again, the works of this time period focused on the feedback loop where a growing population impacts its supporting environment in a manner than negatively impacts the environment's ability to provide sustenance for that population. Although the Green Revolution is attributed to starting prior to the Club of Rome report, it was ultimately the advances in agricultural productivity associated with

that movement that thwarted the demise of society projected in the "Limits to Growth" publication. Nearly 50 years later, we are undergoing a similar degree of concern regarding how we will be able to meet the needs of our rapidly growing population given the agricultural resources available.

A useful exercise to better understand exactly where our shortfalls are, in terms of feeding the global population, is to assess how well our agricultural system provides for our current population. In practice, this can be evaluated based on reports of undernourishment. According to the UNFAO (FAO, 2019) between 2015 and 2017, an average of 803 million people (approximately 10.5% of the global population) were categorized as undernourished. The FAO defines undernourishment as the proportion of the population whose habitual food consumption is insufficient to provide the dietary energy levels that are required to maintain a normal active and healthy life. According to the same data source, 685 million people (8.9% of the global population) are severely food insecure. The FAO defines severe food insecurity as a situation where a person has run out of food and gone a day or more without eating. Clearly, these are extreme examples of nutritional inadequacy, and correspondingly reflect a fairly small proportion of the population.

Possibly a greater concern regarding dietary adequacy is micronutrient deficiencies. The World Health Organization tracks micronutrient deficiencies and estimates the proportion of the global population experiencing micronutrient deficiencies to be over 2 billion (WHO/UNICEF, 1995). Micronutrient deficiencies are of greatest concern in vulnerable populations such as pregnant women, children, and the elderly. Globally, roughly 40% of pregnant women and 42% of children suffer from anemia, typically caused by insufficient consumption of Fe and vitamin B_{12}. Aggregated global estimates of the prevalence of vitamin A deficiency are more challenging to find. Vitamin A deficiencies in pregnant women range from less than 5% in the Americas, Europe and Russia to over 20% in Northern Africa, the Middle East, and Central/East Asia. Vitamin A deficiency in children is less than 5% in most countries but exceeds 60% in Sub-Saharan Africa and South Asia. Zinc deficiency is another major micronutrient deficiency globally and follows a similar pattern to vitamin A deficiency.

Comparison of Diets in the Context of Feeding the Growing Global Population

In principle, we can assess global production of nutrients and how they match to a calculated global requirement of nutrients by comparing theoretical diets at the global scale. Given that the EAT-Lancet reference diet was proposed as a diet to support planetary health, it is a logical starting point for this type of comparison. If the ideal diet proposed in table 1 of the EAT-Lancet report was scaled to the current global population, it would require only 21% of current cereal grain, 15% of tuber, 75% of vegetable, 63% of fruit, 85% of dairy, 35% of meat, and 41% of egg production. Legume and nut production would require expansions of 186% and 341%, respectively. If the reference diet was scaled to feed 10 billion people, it would use 28% of the cereal grains, 20% of the tubers, 100% of the vegetables, 84% of the fruits, 47% of the meat, and 55% of the eggs produced today. To feed 10 billion people, current dairy production would need to increase 13%, and legume and nut production would need to be expanded by 280 and 486%, respectively.

Scaling the diet to meet the needs of a 10-billion-person population highlights several challenges with implementation. First, food waste, particularly waste of vegetables, fruits, legumes and nuts, would need to be virtually eliminated. Currently, the FAO estimates that 45% of fruits and vegetables are wasted globally. As such, reducing wastage represents a major undertaking. Another challenge with scaling this diet to meet the needs of 10 billion people is the need to dramatically expand production of legumes and tree nuts.

We can evaluate an alternative diet to showcase the usefulness of this comparison approach. The EAT-Lancet recommendations would limit meat consumption to 90 g/d, increase egg consumption to 23 g/d, decrease legume consumption to 25 g/d, and reduce nut consumption to 4 g/d. These recommendations can scale to 10 billion people within the bounds of the current food production system. Importantly, this diet has a lower energy excess than the EAT-Lancet reference and has a very similar pattern of nutrient

excesses and deficiencies, making it equally as feasible from a human nutrition perspective. With this alternative diet, there is only one major challenge: that of amending the food waste problem.

Importantly, what the comparison of diets highlights is the fact that we can feed a population of 10 billion people with our current agricultural system. This fact in itself highlights some challenges, in principle our food production system is perfectly adequate; however, in practice a large proportion of our global population is exposed to micronutrient deficiencies, nutritional inadequacy, or severe food insecurity. This contrast between the principle and the practice suggests that socioeconomic factors play a major role in the global distribution of food resources.

Do Current Diets and Agricultural Trends Support the UN Sustainable Development Goals?

Most of the global literature on food security focused on how agricultural systems contribute to the UN Sustainable Development Goals. The UN Sustainable development goals seek to:

1. end poverty in all its forms everywhere
2. eliminate hunger
3. ensure healthy lives and promote well-being for all at all ages
4. improve availability and quality of education
5. ensure gender equality
6. ensure availability of clean water and sanitation
7. ensure availability of affordable and clean energy
8. improve accessibility to decent work and enhance economic growth
9. build resilient infrastructure, promote sustainable industrialization and foster innovation
10. reduce inequality within and among countries
11. make cities inclusive, safe, resilient and sustainable
12. ensure sustainable consumption and production patterns
13. take urgent action to combat climate change and its impacts
14. conserve and sustainably use the oceans, seas and marine resources
15. sustainably manage forests, combat desertification, halt and reverse land degradation, and halt biodiversity loss
16. promote just, peaceful and inclusive societies
17. revitalize the global partnership for sustainable development.

Over the 57 years between 1960 and 2017, the UNFAO data (FAOStat data, downloaded 1/16/2019) suggest that availability of plant and animal products have increased by 3 kg/person/year and 0.36 kg/person/year, respectively (Figure 1). Over the same timescale, crop and grassland area has decreased by 0.0071 ha/person/year, agricultural water use has decreased by 2.5 cubic meters/person/year, and agricultural greenhouse gases have increased by 1.2 kg of CO_2 equivalents/person/year. The historical data suggest the agricultural system is providing more food from fewer resources.
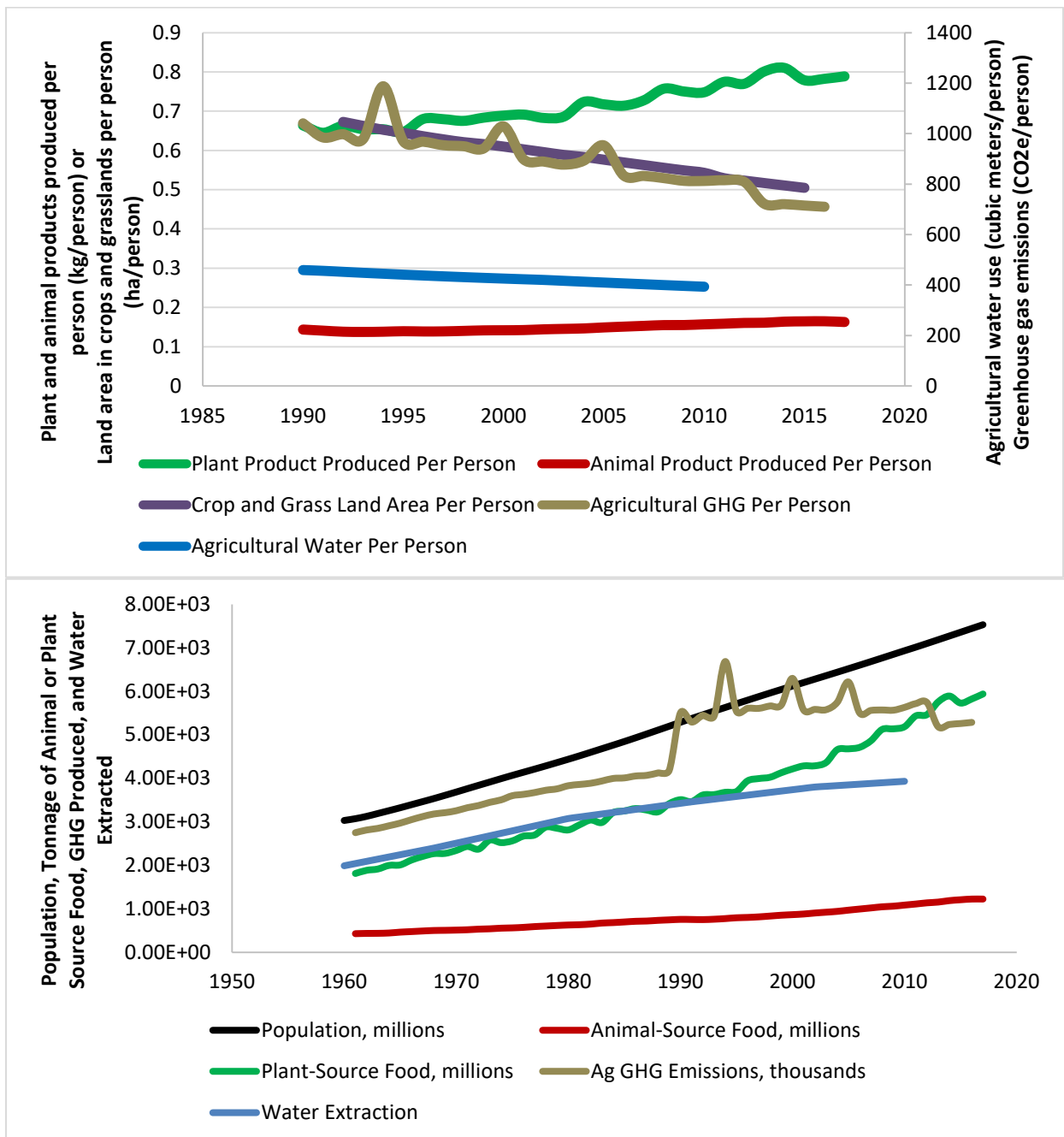
Figure 1. Per capita (upper) and total (lower) production of plant and animal products, greenhouse gas emissions, and usage of water and land for agriculture. Data obtained from UNFAO, 2019.

In addition to the environmental and food availability trends discussed above, the UNFAO data (FAOStat data, downloaded 1/16/2019) suggest that undernourishment has decreased by 11.8 million people per year from 2000 to 2016. Similarly, the proportion of people using safely managed sanitation has increased by 0.75% per year, the dietary energy adequacy of diets has increased by 0.41% per year and the per capita protein supply has increased by 0.30 kg/person/year. The historical data suggest that shifts and improvements in the agricultural system over the past 15 years have supported improved food availability (goal 2), enhanced accessibility of sanitation (goal 6), reduced climate intensity of agriculture per person (goal 13), and limited expansion of cropping and grazing land (goal 15). As such, a major goal of our

agricultural systems moving forward should be to maintain these positive trends to continue to contribute to the UN Sustainable Development Goals.

▪ Considerations for Animal Product Production

It is quite common to discuss the importance of animal-source foods independent of the rest of the agricultural system, even though these foods are implicitly linked to other aspects of the food production system. Animal source foods are commonly cited as major contributors of greenhouse gas emissions and resource use. Animal products are also touted as contributing to negative human health outcomes like cardiovascular disease, colorectal cancers, and all-cause mortality.

What Nutrients are Provided by Animal-Source Foods?

Animal source foods, such as meat, milk and eggs, provide high concentrations of essential micronutrients in a food that also contains relatively low concentrations of energy (Gleason and White, 2019). Meat, milk and eggs are classified as complete proteins, meaning that they contain all ten essential amino acids. Amino acids are the building blocks of protein and are essential for the majority of biological processes. Meat is also a major source of Vitamin B_{12}, a nutrient essential for maintaining brain and nervous system function, and normal energy metabolism. Meat, milk and eggs also provide high concentrations of other important vitamins such as choline (nervous system development) niacin (energy production and metabolism), riboflavin (energy metabolism), thiamin (energy production and nervous system function), and vitamin B_6 (brain and nervous system function). These foods also provide high concentrations of essential minerals like iron (cognitive health and oxygen delivery), phosphorus (bone and tooth health), potassium (blood pressure), selenium (cellular integrity), and zinc (immune system function).

The FAO and WHO data on food insecurity provide some interesting insight into which nutrients are most important for human health globally. Only 10% of the global population classify as being unable to access sufficient energy (undernourished); however, over 28% of the global population experiences micronutrient deficiencies. The 'obese and undernourished' phenomenon is an embodiment of this issue — humans have adequate access to energy resources, but we lack availability of high-quality (and low energy) sources of micronutrients. Animal source foods are exactly that; they provide high concentrations of micronutrients with comparatively low concentrations of energy. This means that humans can consume animal products to meet their micronutrient needs without exceeding their energy requirements. It is particularly important to note that animal-source foods are good sources of several of the micronutrients with particularly high global deficiency prevalence, such as iron, vitamin B_{12}, and zinc. Improved focus on the global importance of micronutrient supplies will be critical to better characterizing the importance of animal-source foods in feeding the growing global population.

How Have the Environmental Impacts of Producing Animal-Source Foods Changed Over Time?

Globally, as the population has increased, so too has the production of animal-source foods and the environmental impact of agriculture (Figure 1). However, adoption of more advanced farming practices over this timescale has also contributed to the reduction in per-capita environmental impact of agriculture. Similar comparisons of historical versus modern environmental impacts of beef and dairy products in the United States also suggest that as livestock operations modernize and improve efficiency, they also reduce environmental footprints (Capper, 2011; Capper and Bauman, 2013).
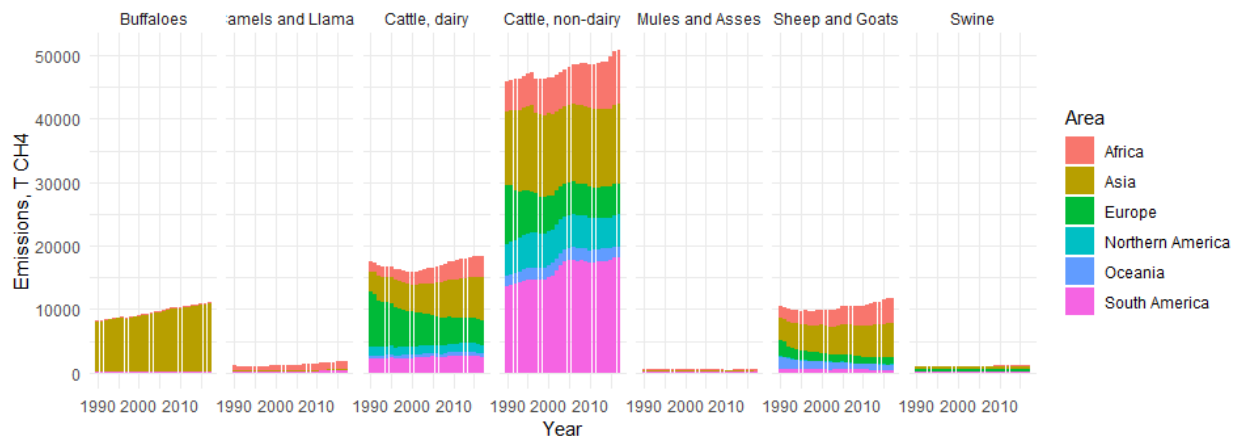
Figure 2. Breakdown of enteric methane emissions globally based on livestock species and emissions area. Data obtained from UNFAO, 2019.

There are important regional differences in environmental impacts of livestock products that should be considered when characterizing agricultural environmental impact and identifying areas for most optimal improvements. If we look at enteric (expelled by burping) and manure methane emissions, for example, these differences become quite apparent. For example, the enteric methane emissions from dairy cattle decreased between 1990 and 2000, largely led by decreases in Europe. However, as the dairy industry in Asia and Africa has expanded, emissions from those locations have replaced the emissions previously produced from the European herd.

It is also useful to compare the relative importance of different species in contributing to global emissions. Beef cattle (categorized above as Cattle, non-dairy) contribute the majority of methane emissions globally. While emissions in North America, Asia, and Oceana have remained fairly constant over the past several years, emissions increases in South America and Africa have contributed to a consistent rise in methane emissions since 1990. Similarly, increasing populations of buffalo in Asia have contributed to a similar rise in methane emission from these animals since 1990. Emissions from camels, llamas, mules, donkeys and swine are fairly minimal compared with emissions from cattle, sheep, goats, and buffaloes.

Enteric emissions, although a major contributor to emissions from animal agriculture, represent a controversial source of environmental impact because they are short-lived in the atmosphere. Currently, when estimating a carbon footprint, methane emissions are multiplied by a global warming potential to convert to carbon dioxide equivalents. Allen et al. (2018) highlighted that this somewhat misrepresents the true importance of methane because it is a short-lived pollutant. If we are not adding any new methane to the atmosphere, we are not adding 'new warming'. Thus, Cain et al. (2019) proposed an alternative, time-based method for accounting the global warming potential of methane emissions. Improved incorporation of this approach into agricultural environmental impact assessments will be crucial in determining where and how we should invest our mitigation efforts.

What is the Impact of Removing Agricultural Animals?

The common methods for comparing environmental impact of livestock products (comparing based on environmental footprints or comparing dietary environmental footprints) is logically flawed. First, it is not logical to compare food products based on their environmental footprints both because humans consume differing quantities of food products and because humans obtain different utility from different food products. Utility here is used as a nebulous term on purpose and can represent the net of nutrient provision, gustatory satisfaction, and all other immediate benefits obtained from consumption of food. Although it is more appropriate to compare foods based on how they are implemented into different diets, comparison of diets in terms of health and environmental outcomes largely ignores the practical

consideration of how the agricultural system will support scaling of such diets to population level.

The above example of the EAT-Lancet diet scaled to a population level highlights why this consideration is important. We have a finite timescale within which we must make alterations to our agricultural system to support the growing global population. If solutions will take longer than that timescale to implement, or are otherwise physically challenging to implement, it is unlikely that they will be truly sustainable solutions. Massive, wholescale changes in the agricultural system take time to implement, and the nature of these changes must be considered when evaluating the true benefits or challenges associated with different dietary patterns.

Dramatic shifts within the agricultural system also may have unintended, collateral effects. The analysis by White and Hall (2017) suggested that when animals were removed from the U.S. agricultural system, a 2.9% reduction in total U.S. greenhouse gas emissions could be anticipated with addition of several nutrient limitations at a population scale. This analysis highlighted the importance of collateral effects like increasing production of synthetic fertilizer, reduced access to byproduct disposal from animal products, and increasing emissions accounted to field crop production in the event that animal agriculture is disbanded. Although a preliminary discussion, the work highlights the need to take a more systems-oriented view on evaluating the impacts of shifts in our agricultural system.

Suffice to say, animal agriculture is an important part of our agricultural system because the system evolved with animals as a part. The practice of agriculture is said to originate in the Neolithic Revolution, where early humans transitioned from hunter-gather societies to agricultural societies. This revolution occurred independently and at different times in developing societies around the globe. In each case, cereal grains were domesticated, followed by livestock (Scanes, 2018). This co-evolution of crop and animal agriculture is evidenced by all societies today. Not a single agricultural system globally contains only one of these types of agriculture because animals and crops co-exist and provide complementary utility to human societies. Improved attention to the historical linkages and progress of global agricultural systems is another useful strategy to assess those factors that influence the sustainability of food production systems. Fundamentally, the fact that the agricultural system has been able to adapt and improve to meet the growing needs of the global population speaks to the sustainability of its basal structure.

▪ Socioeconomic Considerations for Animal Product Production

A final factor important to consider when discussing sustainability of animal products in the global agricultural system is socioeconomic considerations. Sustainability is broadly defined as a balance of social, environmental, and economic considerations. Although it is relatively easy to quantify and make numeric comparisons on economic and environmental bases, social dimensions of livestock production are consistently more challenging to benchmark.

There are several different social dimensions considered by previous analyses. These dimensions include things like the social acceptability of products produced, the welfare of businesses and employees, and the linkages (or lack thereof) between the consumers and producers of a product. More broadly, the social contract within human societies regarding choosing one's own lifestyle also falls under the social dimension. It is perhaps because of the complexity and wide-ranging nature of these social considerations that they are not widely incorporated into assessments of agricultural sustainability.

Accounting for factors like worker welfare and business health can be readily accomplished through surveys and assessment of business finances. It is sensible that these factors must be considered when assessing sustainability of an agricultural production system because economics and the well-being of a business operator are major factors contributing to the longevity of a business. If workers are in danger or do not enjoy their jobs, it is unlikely that they will continue to participate in that job long term. High worker turnover rates can be a challenge for animal health and well-being because it takes time for new employees to be trained. Perhaps more importantly, if sole proprietors or business owners cannot make a

living by their chosen occupation, we cannot expect them to continue that occupation long term. As such, ensuring the security of working conditions on farms and the economic viability of farming operations is an important component of ensuring the sustainability of agricultural systems long term.

Another dimension of the socioeconomic component of sustainability is the linkage, or lack thereof, between consumers and producers. Globally, the fraction of the population associated with agriculture is shrinking rapidly. This shrinking means that fewer people grow up with an understanding and appreciation of where their food comes from. As a result, we have a number of disconnects in understanding regarding agricultural practices and how or why they are implemented. These disconnects are present both within the developed world and within the developing world. Unfortunately, they often focus on technologies employed to enhance efficiency. Use of technologies such as antibiotics, hormones and growth promoting technologies dramatically impacts the efficiency and the economics of livestock operations. As our social license to operate and use these products is impaired either by public opinion, purchasing choices, or policy decisions, it becomes increasingly apparent that improved communication between food producers and food consumers is needed to ensure the long-term sustainability of agricultural operations. This need extends beyond animal agriculture, including all other aspects of the global agricultural system.

The long-term role of socioeconomic pressures in dictating the sustainability of the global agricultural system should not be underestimated. Major objectives in this area should include fostering improved communication and transparency within and outside the agricultural system, working to improve economic viability of businesses and welfare of workers, and the social license to grow animals for food.

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MORE THAN JUST FEED

Relationships Between Fibre Digestibility and Particle Size for Lactating Dairy Cows

Rick Grant, Wyatt Smith, and Michael Miller

William H. Miner Agricultural Research Institute, Chazy, NY
Email: grant@whminer.com

▪ Take Home Messages

- Feed intake and milk production are influenced by dietary neutral detergent fibre (NDF), although it does not explain all of the observed variation.

- Consideration of dietary physical form or particle size (i.e., physically effective NDF; peNDF) and digestibility or indigestibility (i.e., undigested NDF at 240 hours of in vitro fermentation; uNDF240) improves the prediction of dry matter intake (DMI) and milk production.

- A new concept that combines peNDF and uNDF240, called peuNDF, appears to be useful when interpreting and predicting cow DMI and energy-corrected milk responses to diets that are based on corn and haycrop silages.

- When forage fibre indigestibility is greater than desired, a finer chop will boost DMI to levels comparable to lower uNDF240 diets. However, we need to avoid chopping low uNDF240 forages too finely.

- Although the concept of combining physical effectiveness factor (i.e., particle size) with uNDF240 is encouraging, additional investigations with legumes, pastures, and non-forage sources of fibre are needed to test how robust the relationship is between peuNDF and DMI across a wide range of diet types and feeding environments.

▪ Introduction

Portions of this conference proceedings have been previously published in the Proceedings of the Cornell Nutrition Conference for Feed Manufacturers (Grant et al., 2018), the Four-State Dairy Nutrition and Management Conference (Grant et al., 2019), and the Minnesota Nutrition Conference (Grant et al., 2019).

Economic, environmental, and social considerations encourage the use of more forage in dairy cattle rations (Martin et al., 2017). Although regional economics and forage availability determine the balance between dietary forage and non-forage sources of fibre, we appear to be at the threshold of a new era in our ability to effectively feed fibre to lactating dairy cows. Nutritionists have long realized that NDF content alone does not explain all of the observed variation in dry matter intake (DMI) and milk yield as the source of forage and its concentration in the diet vary. Incorporating measures of fibre digestibility and particle size improves our ability to predict DMI and productive responses.

Waldo et al. (1972) recognized that cellulose needed to be fractionated into digestible and indigestible pools for calculation of digestion rates. Together with Van Soest's development of the detergent system of feed analysis (Van Soest, 1994), these two concepts transformed ruminant nutrition. The recognition that there is an indigestible portion of fibre led to research that improved our understanding of the digestibility of fibre in ruminant diets and the development of dynamic models of fibre digestion. Recent research has focused on a three-pool model of ruminal NDF digestion: indigestible NDF measured as undigested NDF at 240 hours of in vitro fermentation, (uNDF240), a fast-fermenting pool of NDF and a slow-fermenting

pool of NDF (Mertens, 1977; Raffrenato and Van Amburgh, 2010; Cotanch et al., 2014). To date more research has focused on defining biologically relevant digestion pools than particle size pools within the rumen, although both digestion and particle size characteristics of a fibre particle are important for explaining ruminal fibre turnover (Mertens, 2011). In a classic paper, Mertens (1997) laid out a comprehensive system for integrating NDF content and particle size, based on the 1.18-mm dry sieved fraction of particles, known as peNDF. Although the peNDF system is based solely on particle size as a measure of physical form, it explains a substantial amount of the variation in chewing activity, ruminal pH, and milk fat elicited when feeding different sources of forage.

Recently at Miner Institute, we have focused on the relationship between undigested and physically effective NDF (peNDF) and have conducted studies designed to assess the relationship between dietary uNDF240 and particle size measured as peNDF. The potential relationships between peNDF and uNDF240 are a hot topic among nutritionists with several practical feeding questions being asked in the field:

‣ What are the separate and combined effects of peNDF and uNDF240 in diets fed to lactating cows?

‣ Can we adjust for a lack of dietary peNDF by adding more uNDF240 in the diet?

‣ If forage uNDF240 is higher than desired, can we at least partially compensate by chopping the forage finer to maintain feed intake?

The bottom line question becomes: are there optimal peNDF concentrations as uNDF240 content varies in the diet and vice versa? The answer to this question will likely be affected by the source of fibre—forage or non-forage—since they differ dramatically in fibre digestion pools and particle size. Some nutritionists have even questioned how important particle size actually is as we better understand fibre fractions (i.e., fast, slow, and uNDF240) and their rates of digestion. This is a complicated question, but the short answer appears to be, yes, particle size is important, although for reasons we haven't always appreciated, such as its effect on eating behaviour, even more so than on rumination.

▪ Miner Institute Study: Undigested and Physically Effective Fibre

Dietary Treatments: peNDF and uNDF240

To begin addressing the questions above, we conducted a study in 2018 to assess the effect of feeding lower (8.9% of ration DM) and higher (11.5% of ration DM) uNDF240 in diets with either lower or higher peNDF (19 to 20 vs. approximately 22% of ration DM). The diets contained approximately 35% corn silage, 1.6% chopped wheat straw, and chopped timothy hay with either a lower physical effectiveness factor (pef; fraction of particles retained on ≥ 1.18 mm screen; 0.24) or a higher pef (0.58).

We used a Haybuster (DuraTech Industries International, Inc., Jamestown, ND) with its hammer mill chopping action to achieve the two particle sizes of dry hay. In addition, for the lower forage diets we partially replaced the timothy hay with nearly 13% pelleted beet pulp to help adjust the fibre fractions. The lower uNDF240 diets contained about 47% forage and the higher uNDF240 diets contained about 60% forage on a DM basis (Table 1).

In studies of this nature, dietary uNDF content can be varied by using the same forages harvested at differing stages of maturity which results in diets that differ in uNDF at similar forage percentages. These studies need to be conducted as they will provide a clean comparison of the effects of dietary forage content and fibre digestibility characteristics. In the present study, as a first effort at examining the relationships between uNDF and particle size, we simply adjusted forage content of the diet as a practical means of manipulating dietary uNDF, while understanding that this approach inevitably confounds forage content with dietary uNDF fractions.

Table 1. Ingredient and chemical composition of experimental diets (% of DM).

	Low uNDF240[1]		High uNDF240	
	Low peNDF[2]	High peNDF	Low peNDF	High peNDF
Ingredients				
Corn silage	34.7	34.7	34.7	34.7
Wheat straw, chopped	1.6	1.6	1.6	1.6
Timothy hay, short chop	10.5	---	24.2	---
Timothy hay, long chop	---	10.5	---	24.2
Beet pulp, pelleted	12.9	12.9	0.4	0.4
Grain mix	40.3	40.3	39.1	39.1
Composition				
Forage	46.8	46.8	60.5	60.5
aNDFom[3]	33.1	33.3	35.7	36.1
uNDF240om	8.9	8.9	11.5	11.5
peNDFom	20.1	21.8	18.6	21.9
peuNDF240[4]	5.4	5.9	5.9	7.1

[1]Undigested NDF at 240 hours of in vitro fermentation.
[2]Physically effective NDF.
[3]Amylase-modified NDF on an organic matter (OM) basis.
[4]Physically effective uNDF240 (physical effectiveness factor x uNDF240).

A New Concept: Physically Effective uNDF240

To explore the relationship between physical effectiveness and uNDF240 among these four diets, we calculated a "physically effective uNDF240" (peuNDF = pef x uNDF240). This value ranged from 5.4% of DM for the low uNDF240 + low peNDF diet to 7.1% of DM for the high uNDF240 + high peNDF diet (Table 1). By design, the two intermediate diets contained 5.9% of ration DM as peuNDF240. An important assumption underpinning our focus on a peuNDF value is that uNDF240 is uniformly distributed across the particle size fractions, particularly above and below the 1.18 mm screen when a sample has been dry sieved. Current research at the Miner Institute Forage Laboratory indicates that uNDF240 is relatively evenly distributed above and below the 1.18 mm screen for the diets fed in this study, with the average difference between the larger and smaller particle fractions being about 9% across the four diets.

When feeding these four diets, we expected the 'bookend' diets to elicit predictable responses in DMI based on their substantial differences in uNDF240 and particle size (Harper and McNeill, 2015). We considered them as bookends because these diets represented a range in particle size and indigestibility that would reasonably be observed in the field for these types of diets. Most importantly, we focused on the two intermediate diets to determine if they would elicit similar responses in DMI given their similar calculated peuNDF content.

The high uNDF240 + high peNDF diet did limit DMI compared with the lower uNDF240 diets (Table 2). When lower uNDF240 diets were fed, the peNDF did not affect DMI. But, a shorter chop length for the higher uNDF240 diet boosted DMI by 2.5 kg/day. As a result, NDF and uNDF240 intakes were highest for cows fed the high uNDF240 diet with smaller particle size. Overall, and as expected, uNDF240 intake was greater for the higher vs. lower uNDF240 diets. The important take-home result is the 0.45% of body weight DMI of uNDF240 for cows fed the high uNDF240 diet with hay that had been more finely chopped (Table 2).

The intake of peuNDF (calculated as the product of pef and uNDF240) was stretched by the bookend diets: 1.47 versus 1.74 kg/d for the low-low versus high-high uNDF240 and peNDF diets, respectively. Of greatest interest, we observed that the two intermediate diets resulted in similar peuNDF intake; we were able to elicit the same intake response by the cow whether we fed lower uNDF240 in the diet chopped

more coarsely, or whether we fed higher dietary uNDF240 but with a finer particle size.

Table 2. Dry matter and fibre intake for cows fed diets differing in uNDF240 and peNDF.

Measure	Low uNDF240[1]		High uNDF240		SE	*P*-value
	Low peNDF[2]	High peNDF	Low peNDF	High peNDF		
DMI, kg/d	27.5[a]	27.3[a]	27.4[a]	24.9[b]	0.6	<0.01
DMI, % of BW	4.02[a]	4.04[a]	3.99[a]	3.73[b]	0.10	0.03
NDF intake, kg/d	9.12[b]	9.06[b]	9.74[a]	8.96[b]	0.19	0.008
uNDF240om[3] intake, kg/d	2.41[c]	2.43[c]	3.11[a]	2.87[b]	0.05	<0.001
uNDF240om intake, % of BW	0.35[c]	0.36[c]	0.45[a]	0.43[b]	0.01	<0.001
peNDFom intake, kg/d	5.56[b]	5.94[a]	5.07[c]	5.44[b]	0.11	<0.001
peuNDF240[4] intake, kg/d	1.47[c]	1.59[b]	1.61[b]	1.74[a]	0.03	<0.001

[abc]Means within a row with unlike superscripts differ ($P \leq 0.05$).
[1]Undigested NDF at 240 hours of in vitro fermentation.
[2]Physically effective NDF.
[3]Organic matter.
[4]Physically effective uNDF240 (physical effectiveness factor x uNDF240).

Lactational Responses to peNDF and uNDF240

An important question becomes: does lactational performance track with these observed responses in feed intake? Generally, unadjusted milk and energy-corrected milk (ECM) production responded similarly to peuNDF intake (Table 3). In particular, production of ECM was lowest for cows fed the high-high uNDF240 and peNDF diet and greatest for the low-low diet (Table 3). Tracking with DMI, the ECM yield was similar and intermediate for the low-high and high-low uNDF240 and peNDF diets. Interestingly, milk fat percentage appeared to be more related to dietary uNDF240 than peNDF content. More research is needed to understand the relative responsiveness of milk fat to uNDF240 and peNDF.

Table 3. Milk yield, composition, and efficiency of solids-corrected milk production.

Measure	Low uNDF240[1]		High uNDF240		SE	*P*-value
	Low peNDF[2]	High peNDF	Low peNDF	High peNDF		
Milk, kg/d	46.1[a]	44.9[ab]	44.0[bc]	42.6[c]	0.9	<0.01
Milk fat, %	3.68[b]	3.66[b]	3.93[a]	3.92[a]	0.10	0.03
Milk true protein, %	2.93[a]	2.88[ab]	2.96[a]	2.84[b]	0.06	0.04
Milk urea N, mg/dl	8.5[c]	9.4[bc]	10.1[ab]	11.0[a]	0.6	<0.01
Energy-corrected milk, kg/d	47.0[a]	45.7[ab]	46.4[ab]	44.6[b]	0.9	0.03
ECM/DMI, kg/kg	1.71[ab]	1.68[b]	1.70[ab]	1.79[a]	0.04	0.02

[abc]Means within a row with unlike superscripts differ ($P \leq 0.05$).
[1]Undigested NDF at 240 h of in vitro fermentation.
[2]Physically effective NDF.

Milk true protein appeared to be boosted by lower peNDF and cows fed the high-high uNDF240 and peNDF diet had the lowest milk protein percentage, with cows fed the low-high uNDF240 and peNDF diet being intermediate (Table 3). The milk urea nitrogen (MUN) concentration was reduced first as dietary uNDF240 decreased and then as peNDF decreased within a level of uNDF240.

Chewing Response to peNDF and uNDF240

Dietary uNDF240 and peNDF had a greater impact on eating than on ruminating time (Table 4). The substantial effect of dietary fibre characteristics on chewing during eating and total time spent eating has

been observed in multiple studies. A recent review found that higher forage content, greater NDF or peNDF content, and lower NDF digestibility may all increase time spent eating for a wide range of forages (Grant and Ferraretto, 2018). In our study, cows fed the high-high uNDF240 and peNDF diet spent 45 minutes/day longer eating and yet consumed nearly 3 kg/day less DM than cows fed the low-low uNDF240 and peNDF diet (Table 4). We need to bear in mind that dietary uNDF240 content was varied by adjusting the forage percentage in the diet. In future studies, we need to assess whether similar results would be obtained if uNDF240 content were adjusted by varying harvest date and forage maturity.

A practical management question is whether or not cows would have sufficient time to spend at the bunk eating a diet with greater dietary uNDF240 that is too coarsely chopped? And if we consider an overcrowded or otherwise competitive feed-bunk environment, the constraint on feeding time could be even more deleterious.

Cows fed the high-high peNDF and uNDF240 diet had the greatest eating time compared with cows fed the low uNDF240 diets (Table 4). Finely chopping the hay in the high uNDF240 diet reduced eating time by about 20 minutes/day and brought it more in-line with the lower uNDF240 diets.

Table 4. Chewing behavior as influenced by dietary uNDF240 and peNDF.

	Low uNDF240[1]		High uNDF240		SE	*P*-value
Measure	**Low peNDF[2]**	**High peNDF**	**Low peNDF**	**High peNDF**		
Eating time, min/d	255[b]	263[b]	279[ab]	300[a]	12	<0.01
Ruminating time, min/d	523	527	532	545	16	0.36

[abc]Means within a row with unlike superscripts differ (*P* ≤ 0.05).
[1]Undigested NDF at 240 h of in vitro fermentation.
[2]Physically effective NDF.

Part of the reason why eating time was more affected than was rumination time is related to the observation that cows tend to chew a bolus of feed to a relatively uniform particle size before swallowing. Grant and Ferraretto (2018) summarized research that showed that particle length over a wide range of feeds was reduced during ingestive chewing to approximately 10 to 11 mm (Schadt et al., 2012). Similarly, in our current study, we confirmed that cows consuming all four diets swallowed boli of total mixed ration with a mean particle size of approximately 7 to 8 mm (Table 5) regardless of uNDF240 or peNDF content of the diet.

Table 5. Particle size of swallowed total mixed ration bolus vs. diet offered (% retained on sieve; DM basis).

Diet	Sieve size, mm						Mean particle size, mm
	19.0	**13.2**	**9.50**	**6.70**	**4.75**	**3.35**	
Low peNDF[1], low uNDF240[2]	3	27	33	20	10	7	9.36
High peNDF, low uNDF240	12	27	29	16	9	6	10.42
Low peNDF, high uNDF240	9	21	23	22	14	11	9.19
High peNDF, low uNDF240	32	13	17	20	11	7	11.55
Bolus							
Low peNDF, low uNDF240	1	11	38	26	14	10	7.96
High peNDF, low uNDF240	3	11	22	29	20	16	7.46
Low peNDF, high uNDF240	2	11	26	29	19	13	7.51
High peNDF, low uNDF240	5	12	19	28	21	14	7.78

[1]Physically effective NDF. [2]Undigested NDF at 240 hours of in vitro fermentation.

Ruminal Fermentation: peNDF and uNDF240

Mean ruminal pH followed the same pattern of response as DMI and ECM yield (Table 6). Although not significant, time and area below pH 5.8 numerically appeared to be more related with dietary uNDF240 content than peNDF. Total volatile fatty acid (VFA) concentration followed the same pattern as DMI, ECM yield, and mean ruminal pH, with cows that consumed similar peuNDF240 having similar total ruminal VFA concentrations (Table 6). Tracking with milk fat percentage, the ruminal acetate + butyrate:propionate ratio was more influenced by uNDF240 than by peNDF.

When we assessed ruminal pool size and turnover, we found that the pool size of NDF tended to be greater for cows fed higher uNDF240 diets and that the pool size of uNDF240 was greater for cows fed these same diets (Table 6). Ruminal turnover rate of NDF tended to be slower for cows fed the higher uNDF240 diets with the high-high uNDF240 and peNDF diet having the slowest ruminal turnover of fibre. Overall, the differences among diets in ruminal pool size and turnover were small but it appeared that higher uNDF240 diets increased the amount of uNDF240 in the rumen and slowed the turnover of NDF. The higher ruminal NDF turnover for cows fed the finely chopped high uNDF240 diet helps to explain the observed increase in DMI.

If future research confirms the results of this initial study, it suggests that when forage fibre digestibility is lower than desired, a finer forage chop length may well boost feed intake and lactational response. The enhanced lactational performance was associated with less eating time as well as more desirable ruminal fermentation and fibre turnover for cows fed the higher uNDF240 diet with lower peNDF.

An important topic remains how rumen fermentable starch may interact with various dietary concentrations of uNDF240 or peuNDF240. On-going studies at the Institute aim to answer this question.

Table 6. Ruminal fermentation and dynamics of fibre turnover.

Measure	Low uNDF240[1]		High uNDF240		SE	*P*-value
	Low peNDF[2]	High peNDF	Low peNDF	High peNDF		
24-h mean pH	6.11[b]	6.17[ab]	6.22[ab]	6.24[a]	0.05	0.03
Time pH < 5.8, min/d	253	208	166	164	61	0.24
AUC, pH < 5.8[3]	52.0	49.6	33.5	30.0	15.0	0.29
Total VFA, mM	122.8[a]	120.6[ab]	118.3[ab]	112.3[b]	4.1	0.05
Acetate+butyrate:propionate	3.33[c]	3.39[bc]	3.58[a]	3.54[ab]	0.16	<0.01
Ruminal pool size, kg						
OM	12.7	12.3	12.9	12.4	0.5	0.44
aNDFom	8.2	7.9	8.7	8.4	0.4	0.06
uNDF240om	3.8[b]	3.7[b]	4.5[a]	4.4[a]	0.2	<0.01
Ruminal turnover rate, %/h						
OM	8.7	8.8	8.4	8.0	0.4	0.15
aNDFom	4.4[x]	4.4[x]	4.2[xy]	3.9[y]	0.2	0.04
uNDF240om	2.7	2.8	3.0	2.7	0.1	0.29

[abc]Means within a row with unlike superscripts differ ($P \le 0.05$).
[xy]Means within a row with unlike superscripts differ ($P \le 0.10$).
[1]Undigested NDF at 240 h of in vitro fermentation.
[2]Physically effective NDF.
[3]Area under curve pH < 5.8; ruminal pH units below 5.8 by hour.

▪ Preliminary Synthesis: Physically Effective, Undigested NDF vs. Dry Matter Intake and Milk Responses

We have combined data from four experiments conducted at the Institute to further explore the relationship between dietary uNDF240 and DMI and ECM yield as well as the relationship between dietary peuNDF240 and DMI and ECM yield. The dietary formulations for these three studies were:

Study 1: the study just described (see Table 1; Smith et al. 2018a; 2018b).
Study 2: approximately 50 or 65% forage in the ration DM, with 13% haycrop silage (mixed mostly grass), and between 36 and 55% corn silage (either brown midrib 3 or conventional) in the ration DM (Cotanch et al., 2014).
Study 3: approximately 42 to 60% corn silage (brown midrib 3 or conventional) and 2 to 7% wheat straw (finely or coarsely chopped) in the ration DM (Miller et al., 2017).
Study 4: approximately 55% conventional or brown midrib 3 corn silage and 2.3% chopped wheat straw (Miner Institute, unpublished, 2019).

Details of ration formulation may be found in the references for each study. All of the diets fed in these four experiments were based heavily on corn silage, contained some combination of haycrop silage and chopped straw, and in Study 1 (the current study) two of the diets also contained substantial pelleted beet pulp to formulate the lower uNDF240, lower forage diet.

Figures 1 and 2 illustrate the relationships that we observed when we combined the data from these studies. For these types of diets, both uNDF240 and especially peuNDF240 appear to be usefully related with DMI and ECM production.

At the moment, it is important to restrict these inferences to similar diets (i.e., corn silage with hay and fibrous byproducts) because more research is required with varying forage types and sources of uNDF (forage vs. non-forage) to determine the robustness of the relationships shown in Figures 1 and 2. In particular, legumes such as alfalfa contain more lignin and uNDF240 but have faster NDF digestion rates than grasses, and we might expect different relationships between dietary uNDF240 and DMI for legume- vs. grass-based rations. In fact, research has shown that high levels of uNDF240 intake may be achieved when lactating cows are fed finely chopped alfalfa hay (Fustini et al., 2017) in part because alfalfa contains more uNDF240 than do grasses (Palmonari et al., 2014; Cotanch et al., 2014).

Interestingly, a 2018 field study using 59 commercial dairy herds assessed the influence of corn silage uNDF measured at 30 and 240 hours with near infrared reflectance spectroscopy on herd DMI and performance (Geiser and Goeser, 2019). Negative relationships between uNDF240 and DMI and and between uNDF240 and ECM were noted. In the future, we hope that potential relationships between uNDF, peuNDF, and DMI and milk yield will be explored for a wide range of diets and management scenarios on commercial dairy farms.
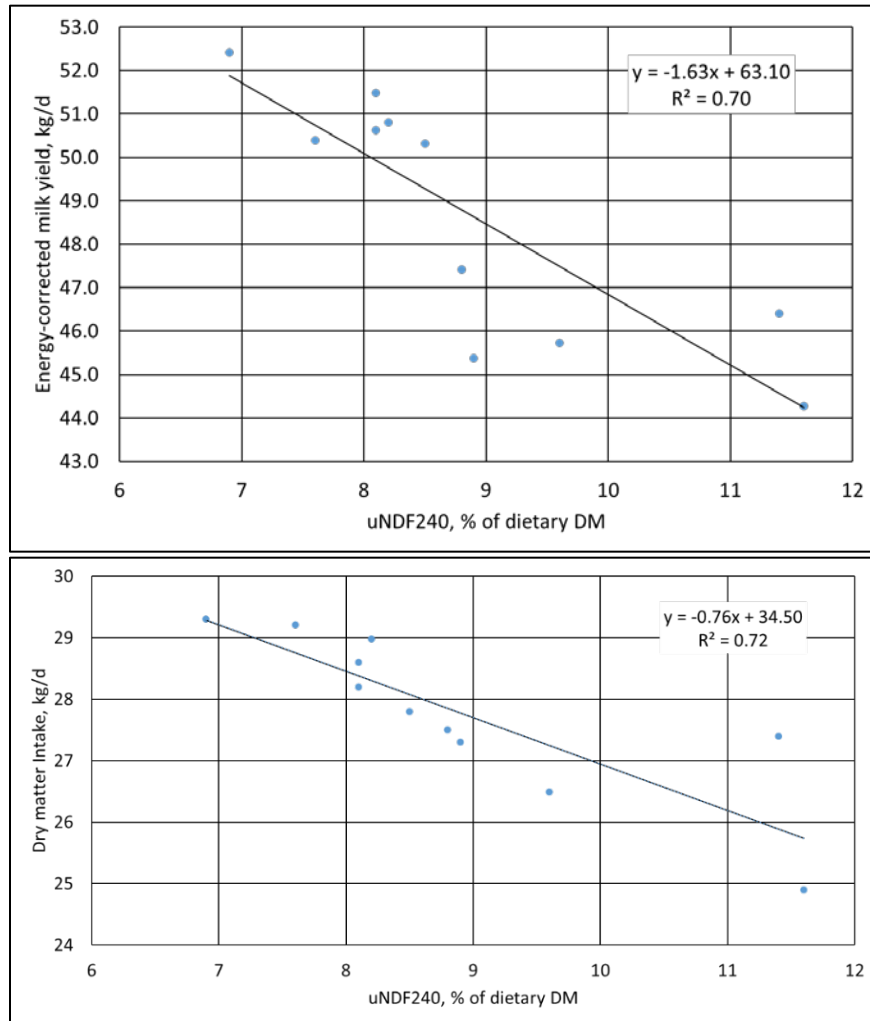
Figure 1. Relationship from four studies between dietary uNDF240 and DMI and ECM yield for cows fed diets based on corn silage, haycrop silage, and chopped wheat straw.
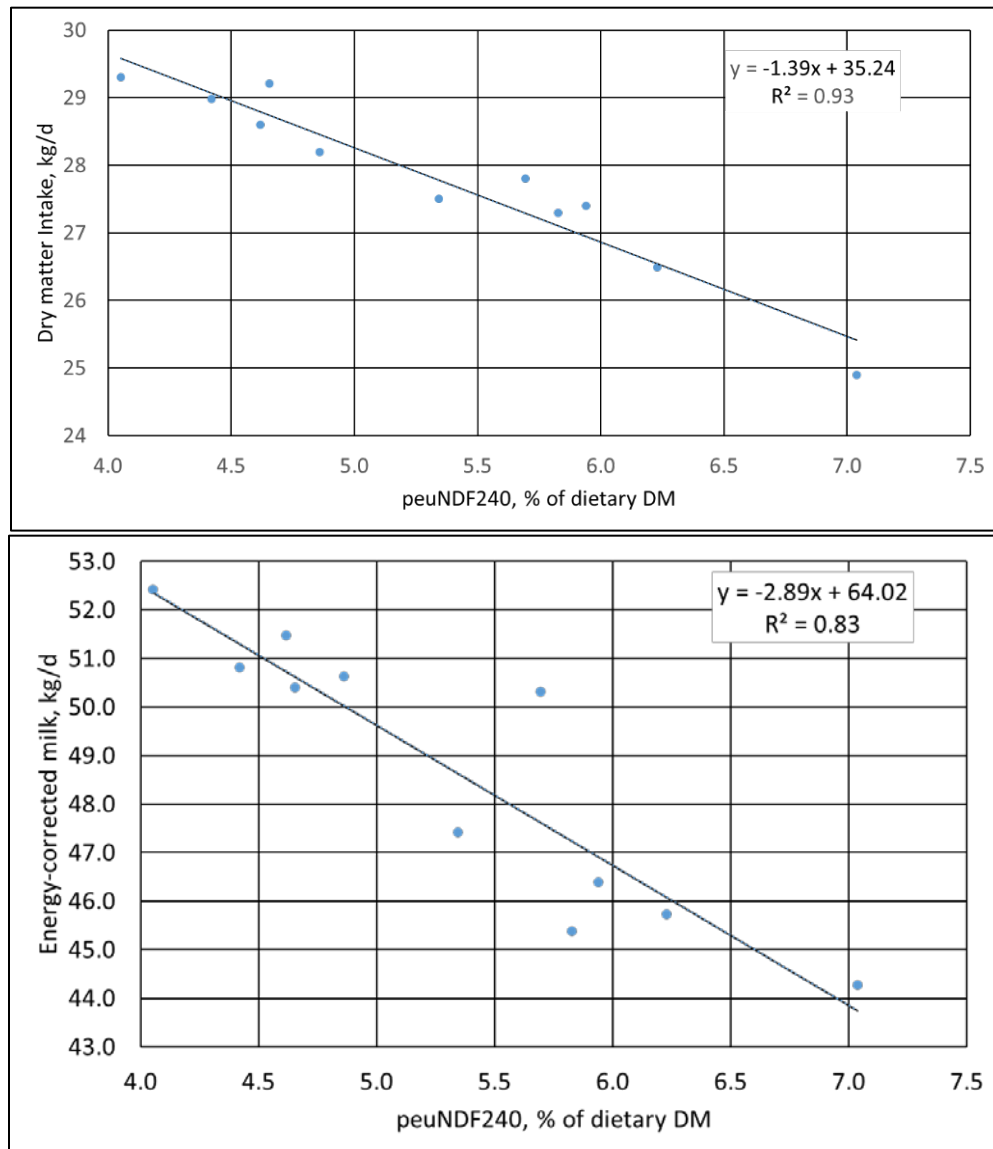
Figure 2. Relationship from four studies between dietary peuNDF240 and DMI and ECM yield for cows fed diets based on corn silage, haycrop silage, and chopped wheat straw (peuNDF240 = physically effective undigested NDF measured at 240 hours of in vitro fermentation).

▪ Summary and Perspectives: A Tale of Two Fibres

The calculated 'physically effective uNDF240' (pef x uNDF240) appears to be a useful concept when interpreting cow response to the diets fed in this study and studies with similar types of diets. Our goal is not to invent yet another nutritional acronym but to focus on a potentially useful concept. We were able to elicit the same response by the cow whether we fed lower uNDF240 in the diet with greater peNDF, or whether we fed higher uNDF240 but chopped the dry hay more finely. In other words, the peuNDF240, or integration of pef and uNDF240, was highly related to DMI and ECM yield.

If future research confirms this relationship between dietary uNDF240 and DMI, it suggests that when forage fibre digestibility is lower than desired, a finer forage chop length will boost feed intake and

lactational response. In addition to investigating potential and probable differences between legumes and grasses, we also must understand the potential responses to forage and non-forage sources of fibre.

As Charles Dickens wrote in his classic novel *Tale of Two Cities* "It was the best of times, it was the worst of times." When it comes to fibre, it looks like we can have the best of times when we are able to integrate two measures of fibre—uNDF240 and peNDF—when formulating rations (Grant, 2018). Research is needed to test this relationship in alfalfa-based diets, pasture systems, and other feeding scenarios that differ markedly from a typical Northeastern and upper Midwestern U.S. diet based primarily on corn silage.

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Corn Silage: Managing the Manageable

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▪ Take Home Messages

- Corn hybrid genetics set the potential for silage yield and nutritional value, but crop management, growing environment, ensiling efficiency and feed-out management determine the outcome.

- Agronomic strengths and weaknesses, dry matter yield and starch content are the primary traits to consider when selecting hybrid genetics for silage production. The best non-brown midrib (non-BMR) silage hybrid is typically a tall, high-yielding grain hybrid.

- Dry matter yield is influenced by hybrid genetics, hybrid maturity, planting population, growing season, plant height at the ear (biomass yield), and kernel maturity at harvest.

- While both fibre and starch digestibility are important when balancing diets, they should not be a focus when choosing non-BMR silage hybrid genetics because of limited genetic variation among hybrids harvested at typical silage maturities.

- High fibre digestibility can be achieved by planting BMR hybrids, harvesting healthy, disease-free plants and potentially by high-chopping plants.

- Starch digestibility can be optimized by aggressive kernel processing and allowing four to six months in fermented storage before feeding.

- Silage growers have little control over growing environment (other than fertility and irrigation) but once genetics are selected, they can exert influence over silage yield and dietary impact by harvest maturity, chop length, degree of kernel processing, and ensiling and feed-out management.

- Producers must pay attention to potential for conjugated linoleic acid production induced by low ruminal pH from changing corn silage starch digestion in high corn silage dairy diets to avoid butterfat depression problems.

▪ Introduction

There has been a trend in recent years for dairy producers to feed higher inclusion rates of corn silage in their lactating cow diets. Martin et al. (2017) reported that between 1982 and 2017, alfalfa hay, hay crop silage and green chop acreage in the U.S. decreased by 32%. At the same time, corn silage acres remained unchanged (with declining cow numbers) but because of improved genetics and crop management, corn silage production increased by 33%. The primary reasons why corn silage is becoming the preferred fermented forage for many producers include: 1) high yields compared with other forage options, 2) easily fermented and palatable feed, 3) high starch content, 4) desirable and more consistent fibre digestibility than multiple-harvest forages,5) lower labour-intensity because of one harvest period, and 6) efficient utilization of manure.

Once hybrid genetics are selected and planted, there are six main areas over which silage producers have some control in optimizing silage yield and quality: 1) harvest maturity and moisture, 2) chop length, 3) degree of kernel processing, 4) inoculation, 5) bunker and pile management, and 6) feed-out rate and management.

Hybrid Genetic Selection

Before the late 1980s, many nutritionists used book values for the nutritional value of corn silage because little research had been conducted to understand how hybrids differed in terms of nutrient content. Early work by Pioneer, in conjunction with the University of Idaho (Hunt et al., 1992) was the first published research showing significant differences between six hybrids grown at two locations in Idaho and California and harvested at three maturity points. Further research (Hunt et al., 1993) from the University of Idaho showed significant differences in animal growth performance between two commercially available hybrids commonly used in the Northwest at that time.

Agronomics

Important genetic agronomic traits that deserve selection consideration for a silage hybrid include 1) heat units (crop heat units [CDU] or growing degree units [GDU]) to both silk and physiological maturity (kernel black layer), 2) plant height, 3) stress emergence, 4) drought tolerance, 5) fungicide response, 6) suitability to high populations, and 7) resistance ratings to important local diseases (e.g. foliar diseases, ear moulds). Some of these traits are delivered via genetic modifications and some by natural genetic adaptability or resistance. Other traits commonly reported by seed companies such as stalk/root strength, grain dry down and test weight are of little importance to silage growers. Silage producers should never rely solely on seed company catalog ratings; they should seek the advice and knowledge of local seed sales professionals who have local experience and the opportunity to observe hybrids over multiple years in experimental plots before commercialization.

Producers should select silage hybrids that are five to ten relative maturity days longer than would be typically be grown for grain in their geography because the heat units are not needed to mature the crop to dry grain (combining) maturity. This approach will help maximize silage yield and starch content. If maturity is too long for the growing zone, starch levels and total yield may be compromised by planting a hybrid whose heat unit requirements exceeds the average killing frost date.

Silage growers will often reduce risk by spreading the pollination period between hybrids. However, planting hybrids with differing kernel physiological maturity ratings may not always provide the desired effect because the hybrids could have similar CHU or GDU to silk. Growers should consult CHU or GDU to silk ratings to see the relative difference in timing of pollen shed and silk emergence. It is difficult to compare CHU or GDU to silk across companies because industry standards are lacking for how heat unit and maturity ratings are determined.

Earlier silking hybrids generally move north of their adapted zone and more readily adapt to higher elevations. If moved too far north or in elevation, late silking hybrids may not reach physiological maturity before the first killing frost or may have reduced grain yield potential if abnormally late silking exposes the crop to cooler temperatures during starch fill.

Dry Matter Yield

Once proper maturity and agronomic traits are decided, the next trait that should be considered is dry matter (DM) yield. In silage, this is primarily determined by the amount of starch and height of the plant at the point of ear attachment (biomass). Longer-season hybrids generally have more yield potential than do shorter season hybrids. However, it is important to select hybrid maturities that allow healthy plants to mature to at least ½ milk line before silage harvest given that starch content is highly correlated with DM yield. Corn grain typically contributes 45–50% of silage DM yield. The corn kernels in silage, because of their starch and oil content, are responsible for 60–70% of the plant's energy content, with the remaining 25% of the energy coming from cell walls (neutral detergent fibre [NDF]) and 10% from cell contents of the vegetative parts of the plant.

Concurrent with the continual 1–2 bushels/acre/year increase in North American corn yield is a parallel

tonnage increase in corn silage yields. This is not surprising given the relationship between starch content and silage yield. Much of the increase in grain and silage yield in the last 15 years can be attributed to plant breeding efforts that produced hybrids which tolerate the stress of high plant populations. In the early 1980s plant populations were about 24,000 plants per acre. Today, we see most silage growers successfully planting and harvesting high quality corn silage at plant populations between 32,000–40,000 plants per acre depending upon geography and growing conditions. A summary of University of Wisconsin silage hybrid plot results from 1995 to 2007 (Lauer, 2014) showed that the top three drivers of silage DM yield were: 1) kernel maturity at harvest, 2) hybrid genetics, and 3) planting date (longer season hybrids typically yield more total DM).

Starch Content

Kernel maturity at the time corn silage is harvested is a significant driver of silage DM yields. A healthy corn crop can deposit as much as 1.0 percentage unit additional starch every two days from 1/3 milk line to physiological maturity (black layer). Harvesting when kernels are immature (e.g., ⅓ milk line) will result in lower DM yield compared with harvesting at later kernel maturities (e.g., ¾ milk line). Research conducted by Pioneer in conjunction with the University of Illinois reported kernels could increase in starch content by over 25% from ½ milk line to black layer maturity (Walker et al., 2010). Kezar (1989) reported starch increase of 22% between ⅓ and ⅔ milk line. Delaying harvest to allow kernels to more fully mature requires a plant that maintains good late-season plant health. This is a constant goal of all corn breeders and is aided by fungicide use in those geographies prone to foliar diseases (Mahanna and Thomas, 2012).

Corn silage DM content of 30–32% is often referenced as being the goal for silage stored in bunkers or drive-over piles. This traditional DM recommendation comes from two perspectives: 1) ensuring enough moisture for adequate silage compaction, and 2) fearing that delaying harvest will result in significant reductions in fibre digestibility. However, technologies have advanced in bunker and pile management (improved compaction capacity, oxygen-barrier file, inoculation), and plant genetics now allows for targeting ¾ milk line (approximately 36–38% whole plant DM) in healthy plants to capture more starch. A review by Pioneer of all published corn silage literature in the Journal of Animal Science and the Journal of Dairy Science (Owens, 2018) found that in healthy plants, fibre digestibility declined only minimally (2–3 percentage points) from ⅓ milk line (~30–32% DM) to ¾ milk line (36–38% DM). Corn is a "modified grass", but generations of corn breeding efforts for improved late-season plant health has allowed corn plants to retain high fibre digestibility, even in later maturities, while the kernel is still depositing valuable starch.

When selecting silage maturities, it is often advantageous to select a slightly shorter-season hybrid and let it mature to ¾ milk line rather than selecting a longer-season hybrid thinking it will produce more biomass. However, if that longer-season hybrid encounters an early killing frost, starch deposition will be terminated, which will negatively impact both DM yield and energy density.

Fibre Digestibility

A trait of minimal importance during silage hybrid selection is fibre digestibility. The growing environment (e.g., amount of moisture the plant receives during vegetative growth stage) is three times more influential on fibre digestibility than is hybrid genetics (Owens, 2012). While fibre digestibility is highly heritable, variation among elite silage genetics is minimal. Despite years of academic research to improve fibre digestibility, limited success has been achieved.

Lauer (1997) summarized the annual corn grain and silage trials at the University of Wisconsin and published the order of items considered important for selection of a corn hybrid. First, the hybrid must be adapted to the area of production in terms of maturity, standability, disease and insect resistance, and drought tolerance. The hybrids that meet those criteria should then be ranked in order of yield potential. Hybrids with high grain content typically have high silage yield. Last, quality (e.g., NDF digestibility for

silages) should be considered. Except for hybrids with the BMR trait that consistently have higher NDF digestibility, the environmental conditions for plant growth and harvest timing have a greater impact on yield and nutritional value (NDF and starch content and digestibility) of corn silage than does genetics (Owens, 2014).

University or company plot data show that only a few standard hybrids are statistically different when grown in the same plot. Only the BMR hybrid entries consistently show a statistical advantage in fibre digestibility. A 3–4 percentage point difference in NDF digestibility is needed to be biologically significant given the inherent error of measuring NDF in laboratory fibre digestion methodologies (Hall and Mertens, 2012; Mertens, 2019).

Corn silage plots of several hybrids grown in Michigan in a drought year followed by a normal precipitation year resulted in lower starch levels in the drought silage but a 20% improvement in NDF digestibility (NDFD) along with lower lignin levels in the silage. By tassel stage, plant stover growth has terminated. Under wetter than normal growing conditions during vegetative growth stages, plants have longer internodes and grow taller. Differences in lignin content are difficult to measure but increased lignin cross-linkage to hemicellulose is likely the cause for lowered fibre digestibility in these plants. This may be why corn silage grown under irrigation appears to be lower in fibre digestibility than the same hybrids grown in dryland conditions (Mahanna, 2010).

In drier than normal vegetative growth environments, internode length is shorter and fibre digestibility of the plants tends to be higher than in plants grown in normal conditions (Van Soest, 1996). While total biomass yield may be lower due to a shorter plant, fibre digestibility is typically higher. Also, with a shorter plant, starch is further concentrated. Research at Cornell University suggests the moisture the plant receives is seven times more influential on fibre digestibility than are the heat units the plant receives (VanAmburgh, 2015). The growing environment post-tassel appears to have minimal effect on fibre digestibility but does exert a significant influence on ear development and silage starch content. Unlike starch digestibility, fibre digestibility does not change during fermented storage so the fibre digestibility at harvest will be the fibre digestibility for the entire feed-out period.

While there is an abundance of knowledge about how to irrigate corn for grain yield, there is a lack of information about how to irrigate the corn plant for silage production. Granted, starch will drive yield and overall energy density, but what is of interest are vegetative stage irrigation regimes that might manipulate fibre digestibility. Agronomists are wary of reducing irrigation schedules with pivot irrigation given concerns about not being able to keep up with plant evapotranspiration needs. Producers using flood irrigation may be in a better situation to experiment with reducing irrigation during vegetative stages to increase fibre digestibility without reducing plant growth. These growers should then fully irrigate as the plant enters the reproductive stage to ensure high starch content. This is an area in need of further research.

Another issue related to growing environment is within-field variability. Corn silage fields do not possess the same soil profile, water-holding capacity or fertility. Unpublished Pioneer data suggest within-field variability in fibre digestibility and starch content may be greater than the differences between hybrids (Bolinger, 2019). One way for silage feeders to manage this variability is to "face" the entire bunker or pile and in this way, average out the variation that might exist in any one area of the bunker or pile.

While growers have limited control over the growing environment, they do have control over chop height as a method to manipulate fibre digestibility. A review of 11 published studies on high chopping corn silage by researchers at Pennsylvania State University (Wu and Roth, 2003) reported that increasing chop height from seven inches to 20 inches increased fibre digestibility by 6.7% and concentrated starch by 6%. Research by Pioneer and the University of Idaho demonstrated all hybrids do not respond to high chopping in the same manner. There appears to be a strong genetic by environment interaction with high chopping. To predict what effect high chopping might have on increasing fibre digestibility, plants must be chopped at different heights and analyzed to see if increasing chop height is worth the loss in stover and

effective fibre. Unpublished research by Pioneer indicates that for every 4–6 inches of increased chop height, the average hybrid will be reduced in yield about 1 ton (30% DM) per acre.

One of the newest laboratory analytical measurements relating to forage fibre is undigested NDF (uNDF). The research is clear that NDF does not degrade in the rumen at a constant rate, but rather as three pools: fast, slow, and undigested NDF. Large slow and uNDF pools in the forage and diet cause greater rumination and slower eating speeds but problematically, lower intake potential due to increased rumen fill. One of the advantages of corn silage as the primary forage ingredient is that it typically has the lowest uNDF of all forages. The uNDF is further diluted in a diet that incorporate corn silages possessing high starch content. Nutritionists are starting to observe depressed DM intake and lower milk production when total uNDF240 (NDF that is still undigested after a 240-hour incubation) intake/cow/day for forages (over 4 mm in length) in the entire diet exceeds about 5.0–5.5 lbs (or about 0.35–0.40% of body weight). Undigested NDF is only appropriate for cows where DM intake is limited by rumen fill, which is typical of intakes during peak milk production. Exceeding these amounts may lower peak production, especially if cow persistency is high.

An exception to ignoring fibre digestibility has been the plant breeding efforts around commercializing BMR hybrids with reduced lignin content in the stalk and leaves resulting in improved fibre digestibility. Rate and extent of ruminal NDF digestion are greater for corn hybrids whose parents possess the BMR mutant, but compared with non-BMRBMR (standard) hybrids, most BMR hybrids have lower silage yields and greater susceptibility to nutrient and water stress (Owens, 2014).

The main nutritional advantage of BMR silage is higher fibre digestibility due to less lignin, which interferes with rumen bacteria degradation of cell walls. Higher fibre digestibility impacts: 1) the amount of forage in the diet (typically more forage equates to a cheaper ration), 2) energy obtained from the corn silage, and 3) amount of forage cows can consume per day. Fibre in BMR hybrids appears to be more fragile and exits the rumen faster than does fibre from standard hybrids. While DM yields of BMR hybrids are behind standard silage hybrids by 5–15% depending on geographical yield potentials, some silage growers and their nutritionists are adopting agronomically improved BMR hybrids and are willing to sacrifice yield to obtain higher fibre digestibility. This is not that different from alfalfa growers harvesting at late-bud stage rather than full-flower, sacrificing alfalfa yield to obtain forage with higher fibre digestibility and intake potential.

Silage producers who are considering BMR hybrids need to have realistic expectations including: 1) potential for more agronomic risk, i.e., reduced standability, 2) reduced yields, 3) additional land base because of reduced yields and extra inventory because of higher feed intake of BMR silage, and 4) possible need to segregate this silage given the biggest benefit will be in diets fed to transition and early-lactation cows. High chopping, while increasing fibre digestibility, will not drive DM intakes as much as the fragile fibre found in BMR hybrids.

Current estimates are that BMR hybrids constitute less than 10% of all North American silage acres. However, as their agronomics and yield improve, it is conceivable that 20 years from now, almost all silage hybrids will have the BMR trait. Another limiting factor to BMR adoption has been the difficulty of price discovery between silage growers and large dairies buying that silage. While the BMR trait should drive intakes and offer feeding advantages, especially to transition and high-production groups, it is difficult for dairies to know how much to pay for BMR silage to compensate growers for increased agronomic risk and lower yields. Commercialization of new BMR genetics with more parity yield to standard silage hybrids should help resolve these impediments to acreage growth.

Starch Digestibility

The starch in corn silage is from relatively immature kernels (pre-black layer) and the desired starch level is a moving target. The fact that the starch is from a relatively immature kernel is important because

kernels at this stage do not contain very much vitreous starch, which is the starch that is responsible for the high test weight in fully mature kernels at combining maturity.

The 7-hour ruminal starch digestibility of new-crop corn silage is about 70% and drifts upwards (about 2 percentage units/month) for about six months before plateauing (Mahanna, 2007). Junges et al. (2017) recently published research suggesting that the bacterial activity, not just acid load, appears to cause the solubilization of the proteinaceous matrix surrounding corn starch granules that results in increased ruminal starch digestion over time in fermented storage.

The greatest mechanical tool to improve corn silage in recent times has been the development and adaption of on-chopper kernel processors that significantly improve both ruminal and total tract starch digestibility. One of the first studies involving kernel processors (Andrae et al., 2001) showed kernel processing increased in situ 24-hour starch digestion from 73.4% to 85.8%. More recent studies show significant increases in improved kernel processing scores with silage harvested with a Shredlage® processor (Ferraretto et al., 2018). Today, very little corn silage is harvested in the U.S. that has not been kernel processed at the time of harvest. The main factors influencing kernel damage at the chopper are: 1) chop length (shorter chop length typically results in better kernel processing if effective fibre from corn silage is not an issue), 2) synchronized timing between header and feed rolls, 3) roller mill wear, 4) roller mill gap setting (typically 1 to 3 mm), and 5) roller mill differential speed (many at 50% or greater).

Many laboratories offer kernel-processing scores (Ferreira and Mertens, 2005) that are helpful to nutritionists balancing diets. There is, however, a need for protocols to ensure corn silage is being evaluated for processing at the time it is being harvested. Pioneer developed a field test employing a 1-liter cup where the goal is to have less than two whole or half kernels in that volume of silage. Fecal starch analysis can be a good post-harvest indicator of degree of kernel damage. In a Pioneer field study of the high-production strings in 32 Wisconsin dairies, only two of the dairies showed more than the goal of < 3% fecal starch and those two dairies had poor corn silage processing scores (Powel-Smith et al., 2015).

There have been recent discussions about the value of soft-floury (low vitreous, low prolamin) endosperm in corn silage kernels. There does not seem to be significant variation in the amount of hard, vitreous starch or starch digestibility in corn silage given the immature kernel maturity (pre-black layer) at normal corn silage harvest. Pioneer field studies from side by side trials (hybrids grown in the same field receiving the same environment) showed no significant difference in 7-hour ruminal starch digestibility between advertised "floury-kernel" and normal hybrids at silage (or high-moisture corn) kernel maturities (Wiersma et al., 2015). Ohio State University researchers concluded that the amount of vitreous starch in corn silage kernels was of relatively little value whereas the amount of vitreousness in dry corn grain should be considered, particularly to help growers know when to grind corn more finely (Firkins, 2006). This makes sense because the level of vitreousness increases as kernels mature past black layer as reflected in differing test weights (more vitreous starch) among hybrids at combining maturity. The fine grinding of dry corn, commonly practiced in the dairy industry, is supported by research in France (Ramos et al., 2009) showing that the negative effects of flint corn (very high vitreous levels) on total tract starch digestion could be eliminated by grinding dry corn to 550 microns.

- **Plant Population**

It is important to target plant population based on individual hybrid recommendations. Typical seed corn germination is about 95%. Overplanting by at least 5% can help reduce the effects of germination-induced skips and for expected reductions due to insects and soil conditions.

Summarizing corn population research is difficult because varying maturities across diverse growing environments make it difficult to draw sweeping conclusions. However, over the last 25 years the average U.S. corn planting population has risen from 23,000 plants per acre (PPA) to about 30,000 PPA. High-yielding environments allow for increasing populations to 36,000–38,000 PPA depending upon individual

hybrid genetics. Higher population increases competition among plants for water, sunlight and soil nutrients. Pioneer has conducted studies comparing hybrids sold during previous decades. There is modest improvement in grain yield production because of higher leaf area index, efficiency of leaf photosynthesis, number of kernels per ear and weight of each kernel. However, the genetic selection of corn hybrids for stress tolerance has accounted for the majority of the 1.0–1.5 bushels/year grain yield increase over the past 80 years. This is a result of higher population increasing the number of ears per acre. More precise soil fertility practices and technology traits that improve resistance to insect and weed pressure have also significantly improved average yields.

Further driving yield is that the average grower is planting earlier than in the past, somewhat the result of improvements in seed treatment options. Silage growers should be cautioned not to rely on ear flex scores when considering planting populations. Ear flex refers to the ability of a plant to extend ear size as plant density is reduced or as growing conditions improve. Many seed companies have abandoned evaluating ear flex and advise growers to rely on actual population recommendations from research trials planted at upwards of 70,000 PPA. Ear flex scores have their primary utility in deciding if a hybrid can deliver higher yields under possible replant situations such as emergence problems or hail, which reduces populations to less than 12,000 PPA (Thomas and Mahanna, 2011).

It is important to differentiate between grain production and silage production when discussing plant populations. The effect of lower plant populations on increasing grain yield is greater in low grain-yielding environments (< 130 bushels/acre) and makes variable rate seeding more beneficial in lower yield environments. Grain yields tend to drop off gradually with higher populations, although much less than hybrids of decades ago that were prone to barrenness under high plant densities. Due to improved hybrid stress tolerance, many seed companies routinely evaluate hybrids at plant populations as high as 50,000 PPA.

There also appears to be slight differences in ideal plant populations by hybrid maturity. Shorter-season hybrids (< 100-day hybrids) tend to show the greatest grain response to higher populations, followed by 101–113-day hybrids and finally longer-season hybrids (> 113 days). Researchers theorize that higher populations overcome some of the disadvantages of smaller stature and lower leaf area index exhibited by shorter-season hybrids. A few seed companies provide a planting rate calculator to determine economic grain planting rates based on hybrid genetics, yield environment, seed cost and grain price.

Silage is a more complex situation. Traditional recommendations have been to increase plant populations in hybrids destined for silage by 10–20% per acre. However, with the increasing value of starch, newer recommendations suggest planting silage at no more than 2,000–3,000 PPA above the recommended planting population for that hybrid if planted for grain. Higher populations might provide more yield of stover but reduce yields of starch (grain; Lauer, 2008). Higher plant populations tend to decrease stalk diameter and increase potential for lodging. This is much less of a concern for silage than for grain corn harvested at a much later maturity. Research has consistently demonstrated that higher populations (upwards of 40,000–42,000 PPA) increase silage yield while decreasing quality only slightly. The decrease in quality is caused by increased stover yield diluting the grain (starch) portion of the plant causing slightly higher fibre levels.

Some earlier research suggests the smaller diameter stalk found in higher populations altered the rind: pith ratio causing slightly lower fibre digestibility. Research conducted in 2008 and 2009 with conventional, leafy and BMR hybrids planted at populations ranging from 25,000–40,000 PPA showed no significant effect of increasing population on fibre digestibility (Thomas and Mahanna, 2011). There are some silage growers who prefer to plant at lower populations, more optimal to grain yield, in an attempt to increase the starch content of silage in response to increasing supplemental grain prices.

- ## Frosted Corn

Corn plants that have been frosted prior to harvest can experience premature leaf or whole-plant death. The plant may remobilize stored carbohydrates from the leaves or stalk tissue (leading to standability issues and potentially reduced fibre digestibility) to the developing ears but yield and nutritional potential will still be lost mostly from the cessation of starch deposition.

Approximate yield losses due to premature death of leaves (but not stalks) are 36, 31, and 7% when the leaf death occurs at R4 (dough), R5 (early dent), and ½ milk line (R5.5) stages of kernel development. Loss of nutrient value from leaf loss or undesirable microbial or fungal growth can be minimized if the crop is harvested as soon as possible after the frost. Post-frosted corn is predisposed to spoilage organisms with the onset of warm days and cool nights, coupled with high humidity from rainy or drizzly conditions. Fortunately, husks tend to open and dry down rapidly following a frost, which mitigates the ear condensation, although stalks will retain considerable moisture. Fungi growth often attributed to conditions set up by a frost is commonly active in the field prior to the frost event.

Corn that has experienced a killing frost at ⅓ to ½ milk line maturity will typically be below 72% moisture and can be harvested soon after the event. Corn that is pre-dough stage will be too wet (> 75% moisture) to harvest and may require several days in the field to dry to acceptable harvest moistures (to prevent excess effluent). If the frost event did not freeze kernels and only damaged the top of the plant leaving leaves around the ear still healthy, the plant will continue to mature and lay down starch in the kernel.

Leaves of immature frosted plants make the crop appear very dry but most of the moisture is in the stalk; starch, which serves to dry down the plant, is lacking in these plants, further adding to the moisture problem. If harvest must proceed, it is possible (but laborious and inconvenient) to add dry materials (e.g., dry corn, beet pulp) to the silage to increase the DM. For example, one bushel of dry corn per ton of immature silage will increase the silage DM by only 1.5% units. Immature corn that has experienced a killing frost will have high sugar content in the stalk from sugars that will not be translocated to the kernel. This helps to improve the crop's nutritive value to offset reduced starch levels. However, these excess sugars will also provide nutrients for spoilage organisms to grow during feed out. These high sugar corn plants will also have a natural population of fermenting bacteria (epiphytes) that will be greatly reduced by the frost event. For these reasons, a combination *L. buchneri* inoculant is highly recommended. A "combination" product means that the inoculant contains both homofermentative strains to quickly reduce pH along with a *L. buchneri* strain to inhibit yeast at feed out.

Cotanch (2015) investigated the impact of frost and subsequent mould and fungal growth on NDF digestibility, using corn that experienced a hard frost that killed much of the top third of the plant. The crop remained in the field for another week until it dried down enough to harvest, and during that time, experienced significant mould and fungal growth on the damaged portion. Frost and resulting fungal deterioration of corn leaves resulted in a 6% unit drop in NDF digestibility (30-hour) and a 5% unit increase in uNDF30 (undigested NDF after 30 hours of incubation) compared to that of the lower, healthy green leaves. The frost and subsequent mould and fungal growth not only reduced the energetic value of the crop but also decreased intake potential because of the increased uNDF. The researcher concluded that NDFD and uNDF are influenced not only by hybrid selection or crop maturity at harvest, but also by any anti-nutritional factors such as of the quality of growing season, presence of weeds, and pest or fungal damage.

- ## Chop Length

It is difficult to offer generalized chop length recommendations because proper length depends on several factors including: 1) the need for physically effective NDF (peNDF) levels in the ration, 2) particle size of the other dietary ingredients, 3) the type of storage structure, and 4) silage compaction capabilities and unloading methods (e.g., silo unloaders, bunker facers). Other factors affecting chop length include the need to chop finer to damage corn kernels if on-chopper processing is not available or to chop longer to

compensate for particle reduction from bagging or feed mixing.

In general, shorter chop tends to improve compaction in the storage structure and increases surface area of fibre (or kernels) to improve rate of digestion by rumen bacteria or intestinal enzymes. Longer chop increases the peNDF of the feed; however, excessive length can contribute to sorting by cattle in the feed bunk. Producers should work with the harvesting crew and nutritionist to decide on the proper compromise, recognizing that particle length in the final ration is what is most important. Start at the feed bunk and work backwards to the amount of each feedstuff in the ration and how much peNDF each one of those feeds needs to contribute to the entire diet.

▪ Ensiling Issues

Corn silage fermentation can be simplified into three phases. Silage experiences aerobic (with oxygen) conditions during harvest and filling, followed relatively quickly by anaerobic conditions that initiate lactic acid bacterial (LAB) growth and pH decline, and finally, back to aerobic conditions during feed out.

The natural microbial (epiphytic) populations that exist on the fresh corn crop at harvest exert a tremendous influence on the stability and feeding value of the resulting ensiled feed. Factors such as temperature, humidity, solar radiation, plant maturity and moisture influence the type and quantity (colony forming units (cfu) per gram of forage) of epiphytes populating the crop. The goal of ensiling is to stabilize the crop via the action of LAB. This reduces pH through the efficient conversion of sugars to lactic acid. As livestock operations transitioned to larger bunkers and drive-over piles, it created a greater need to reduce aerobic deterioration on the face of the silage during feed out.

The ensiling advantage to corn silage is that the crop is high in sugars and low in buffering capacity and is not wilted on the ground for exposure to soil-borne spoilage organisms. The ensiling negatives of corn silage, especially if the crop is stressed by drought or early frost, is high yeast counts. The proliferation of yeast in silage re-exposed to oxygen at feed out can have a detrimental impact on DM loss, heating and palatability. In the presence of oxygen, certain yeast species can metabolize lactic acid, causing an elevation in silage pH which reduces the inhibitory effect on other heat-generating spoilage organisms such as mould, bacilli and acetobacter species. Yeast and acetobacter can also produce aromatic compounds such as esters, aldehydes and ethyl acetate (smells like fingernail polish) which can significantly reduce feed palatability (Mahanna et al., 2018).

The impact of yeast and other spoilage organisms can be minimized by proper harvest moisture (to reduce silage porosity), silage compaction, the use of an oxygen-barrier film and plastic cover, silage facing equipment and the use of silage inoculants containing viable strains of *Lactobacillus buchneri* (Kleinschmit and Kung, 2006).

▪ Feeding Issues

Corn silage inclusion rates in dairy diets are on the rise because of high yields, energy density, consistency and palatability. When formulating diets around corn silage, nutritionists should focus on starch content and digestibility, NDF content and digestibility and physical attributes such as peNDF, kernel damage and feed storage and delivery management.

The starch in high corn silage-based diets (e.g., > 8 kg corn silage DM/cow/day) is often considered a "villain" when herds experience erratic intake, butterfat depression or inconsistency in manure scores. However, the villain image has lessened as 7-hour ruminal starch digestibility laboratory values have become readily available allowing nutritionists to adjust for fermentation-induced increases in ruminal starch availability by reducing both quantity and ruminal fermentability of supplemental starch sources.

Linoleic acid, found in corn germ, can also play a role in butterfat depression (Baldin et al., 2018). Ruminal starch overload and lowered rumen pH can result in reduced intakes and facilitate the

conversion of linoleic acid to conjugated linoleic acid, that can have a huge impact on fat yield (Mahanna, 2009). Butterfat depression can be avoided given current understanding of the trans-fatty acid theory of butterfat depression along with the ability of ration software to track estimates of unsaturated (especially linoleic acid) intakes (Perfield and Bauman, 2005) and the recent development of fatty acid milk analysis (Dann, 2017),

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Managing Dietary Variation to Maintain or Improve Efficiency

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▪ Take Home Messages

- Feed composition data can vary for several reasons: lab analytical variation (usually quite small), sampling variation (ranges from small to very substantial), farm to farm variation (very large) and true within farm variation (degree of variation depends on many factors).

- Nutritionists often confuse sampling variation with true variation and reformulate a diet when it is unnecessary. This can be detrimental to production.

- Because of the size of sampling variation, especially for many forages, duplicate independent samples should be taken (at least occasionally). Results from the samples should be compared and averaged. Using an average of duplicate samples reduces the probability of unnecessary reformulation.

- Composition of total mixed rations is usually less variable than composition of feeds. Highly variable feeds are often cheap and when used in a TMR at low inclusion rates do not greatly add to diet variation.

- Research evaluating the effects of diet variation on lactating cows is limited but the available data show that cows can handle some variation without adverse effects provided, on average over a period of a few days, a good diet is fed.

- Systematic (i.e., planned) variation in diet dry matter may increase production.

- Oscillating concentration of dietary crude protein from deficient to adequate every other day shows promise in improving nitrogen use efficiency without affecting milk production.

▪ Introduction

With respect to nutrition, we are concerned about variation in ingredient composition (variation caused by the feed), batch to batch variation in total mixed ration (TMR) composition (variation caused by the feeder), and variation in nutrient requirements and feed intake of cows within a pen. Some sources of variation do create problems and we should adopt protocols and procedures that reduce that variation. Other variation is largely uncontrollable so we need to adopt procedures that reduce the effects of that variation. Lastly, some forms of variation may actually enhance production and efficiency and that variation should be exploited. This paper will discuss how we can manage variation in feed and diet nutrient composition and how we can use systematic variation to increase milk yields and perhaps nutrient efficiency.

▪ Variation in Feed and Diet Nutrient Composition

What Causes Variation in Feed Composition Data?

Before we can manage variation in diet composition, we must understand the cause of the variation. Feed composition variation can be divided into farm to farm variation, variation over time, sampling variation and analytical variation (Table 1). Farm to farm variation is by far the largest source of variation and analytical variation is usually the smallest. Sampling and day to day variation can be substantial depending on the type of forage and the skill of the sampler. For this discussion, we are using corn silage as an example, but this is true for any feedstuff. The nutrient composition of multiple corn silages could vary (or differ) because they are from different hybrids, they grew on different soils under different weather conditions, they were harvested at different maturities, there were differences in harvest and storage methods, etc. This variation is called 'true variation' and if not managed could affect the cow. On a given farm, many of these sources of variation are small or nonexistent. For example, all silage may have been harvested the same day and stored in the same structure. Furthermore, soil and weather is usually fairly similar within a farm. However, across farms, these factors may differ substantially so that the nutrient composition of corn silage across farms is much more variable than within a specific farm. Although farm to farm variation is huge, it is managed by sampling the corn silage on each farm and using farm specific composition data in diet formulation. The high degree of farm to farm variation also means that you should not use average values obtained from the literature. The silage on your farm may be close to the mean (average) but it also may be very different. Within a farm, if the corn silage is sampled multiple times (for example each month), the nutrient composition will probably vary. Month to month variation could be true variation (depending on how good the sampler is) and affect the cow (for example, the sample taken in January was grown on a drought-stressed field but the sample in February was from a field that was irrigated) but the January and February samples could also differ because of lab error and sampling variation. Sampling and analytical variation is not 'true' variation and will not necessarily affect the cow. If a corn silage sample was sent to a lab and for some reason you asked them to analyze it twice for protein, the values they get should be very close but probably are not identical; that difference is analytical variation or error. If you had a 500 kg pile of corn silage that was going to be put into a mixer to feed a group of cows and you grabbed a handful of silage and put it into a bag and then took another handful and put that into a different bag, and sent both bags to the lab the difference between those two samples is sampling variation. In some situations, results from the two samples from the same pile will differ by a very large amount. The reason this variation should not affect the cow is because all 500 kg is going to be put in the mixer and mixed up. It will be almost impossible for a cow to only eat silage from one of the sampling sites. However, if sampling variation is large, a nutritionist should not have much confidence in the data from a single sample because the formulated diet may be wrong (and cows will respond negatively). Taking multiple samples and averaging the values is the best option. If sampling error is large, the sampler should follow established protocols (discussed below). Formulating a diet based on bad nutrient composition data can be a very costly mistake.

Table 1. Contribution of different sources of variation to overall variation in nutrient composition of corn silage and hay crop silage. Data are from 11 commercial dairy farms; silages were sampled for 14 days in duplicate (St-Pierre and Weiss, 2015). Farm represents farm to farm variation, Day is day to day variation, Sampling is variation caused by sampling and Analytical is lab variation.

Nutrient	Farm	Day	Sampling	Analytical
Corn silage		% of total variation		
DM*	88.9	5.2	3.3	2.6
NDF*	72.6	9.3	13.9	4.3
Starch	72.5	6.8	18.0	2.7
Hay crop silage				
DM	82.6	11.2	5.4	0.8
NDF	89.1	5.1	4.8	1.0
CP*	82.1	4.7	10.7	2.6

*DM is dry matter, NDF is neutral detergent fibre, and CP is crude protein

Control and Interpreting Variation in Composition Data

Analytical variation from good labs is usually quite small and is usually not an issue and will not be discussed. Sampling variation, however, can be substantial depending on the expertise of the sampler and the type of feed being sampled. You can estimate sampling variation by taking at least two independent samples of a feed at about the same time and sending the samples to a lab. Independent means that you take multiple handfuls or scoopfuls of feed, put them into a bucket, mix and then place a subsample into a bag to send to the lab. That entire process is repeated. If the composition values of the two samples are very similar, sampling error is low, otherwise, sampling procedures should be improved. We conducted an experiment to determine sampling variation for corn silage and haycrop (mostly alfalfa) silage. The silages were sampled on 50 farms in the U.S. each month (with duplicate independent samples). Within a farm, over the 12-month period, the average range in dry matter (DM) concentration and neutral detergent fibre (NDF) concentration for corn silage were each about 4 percentage units (Figure 1). For DM, sampling variation accounted for about 30% of the variation and true variation was 70% of total variation. For NDF, sampling and true variation each contributed about 50% of the total variation. The average range in variation for DM within a farm for haycrop silage was 8.5 percentage units (25% of that was from sampling variation) and for NDF the average range was about 5 units and 30% of the total was from sampling. The bottom line is for corn silage much of what we think is true variation is actually sampling variation. To manage this variation you need to follow good sample taking procedures and you should take replicate samples and average the values. Good sampling techniques include mixing what you are going to sample as much as possible before sampling. If you take a grab sample from the face of a bag of corn silage, the sample represents that specific site in the silo. Rather than taking grab samples from the face of the silage, collect samples from the loader bucket when the TMR is being made. When you do that your sample represents the totality of what is being fed We sample physical components of a feed (e.g., a piece of corn cob) we do not sample specific nutrients. Therefore, sampling procedures that allow for segregation of different particles will increase sampling variation if the different particles have different nutrient composition. Corn silage is arguably the most difficult feed to sample properly. It comprises particles that differ greatly in shape, size, density and nutrient composition. Pulling a handful of silage from the face of a bag or bunker silo can result in an enrichment of specific types of particles. Not only should the face of a bunker silo never be sampled because of the real risk of getting killed by a silage avalanche it also can result in a biased sample. Longer pieces (usually leaves and stalks) can be stuck in the silage mass and the handful of silage you pull away will be enriched with smaller particles (likely higher starch particles). Removing a sample with your palm facing down allow smaller particles to drop away, which could reduce the starch concentration of the sample and enrich its NDF concentration. Because of size and density, with movement, larger particles tend to rise to the top of a pile and small particles migrate to the bottom. Not sampling all the vertical strata of a pile could result in a biased sample. Using good sampling techniques and taking duplicate independent samples and

averaging the lab results from the two samples will reduce the chance of causing variation in diet composition by reformulating the diet when in reality the diet really did not change.

Managing True Variation in Feed Composition

If a TMR is made correctly (i.e., the recipe is followed exactly), its nutrient composition should be less variable batch to batch or day to day than the nutrient composition of the individual ingredients that go into the mixer because the nutrient composition of the ingredients varies independently. On any given day the NDF concentration of the corn silage may be higher than average but the NDF in the alfalfa may be lower so the two deviations partially cancel out. Using simple probabilities with only two ingredients, 25% of the time both ingredients will have greater than average concentrations of a nutrient, 25% of the time they both will have concentrations less than average and 50% of the time one feed will be higher than average and the other feed lower than average, which will partially cancel out the variation. If the recipe is not followed, then TMR variation will be increased. This can be managed by proper training of the feeder and using TMR monitoring software.

Because the composition of a mix of ingredients is usually less variable than that of individual ingredients, feeds that are highly variable can be successfully used in a TMR if inclusion rates are kept low. Highly variable feeds are often very cheap and if they provide less than about 10% of the total nutrient supply, including them in a mixed diet will have negligible effects on diet variation. Highly variable, cheap feeds can be fed at higher inclusion rates, but diets may have to be formulated differently, which may reduce the cost saving. Using a feed that is highly variable increases the risk that you may feed a diet that is deficient in a nutrient, which can reduce milk production, or is in excess of a nutrient or substance that is detrimental to the cow, reducing milk yield or maybe causing health issues. The only way to reduce risk of an excess is to limit inclusion rate but the risk of a deficiency can be reduced by over formulation. For example, with more consistent ingredients you may formulate a diet to have 16% crude protein (CP) but with highly variable ingredients, you may need to formulate for 16.5 or 17% CP. The saving in feed cost from using a highly variable cheap feed must be more than the cost of increased protein supplementation.
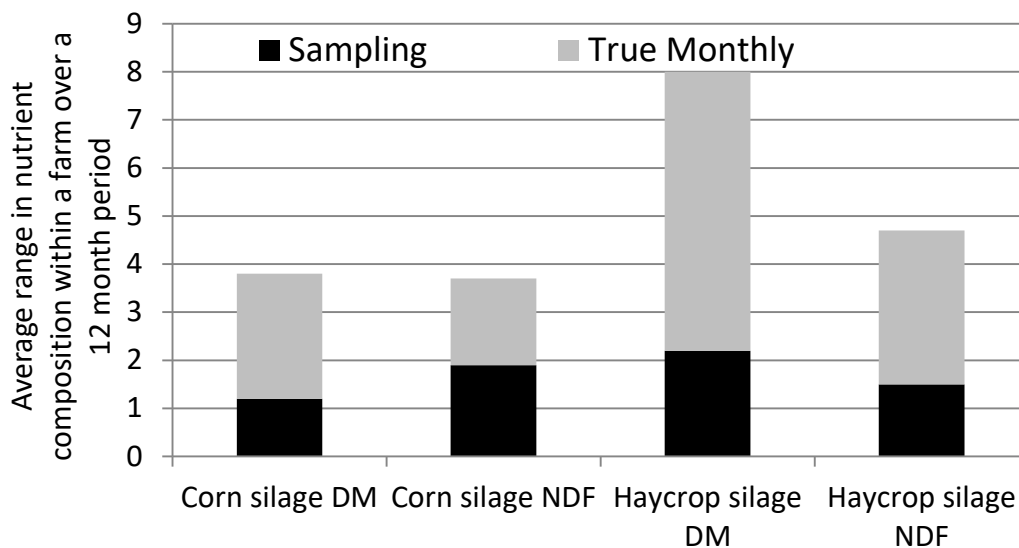
Figure 1. The average range in dry matter (DM) and neutral detergent fibre (NDF) in corn silage and haycrop silage. Variation caused by sampling was determined by duplicate sampling of each silage and true month to month variation was determined by subtracting sampling variation from total variation within each farm. Ranges were determined on 50 commercial dairy farms in the U.S. and silages were sampled each month for 12 months (St-Pierre and Weiss, 2015).

▪ How Much Variation in Nutrient Composition Can Cows Handle?

For years (probably decades) the dogma was that cows need consistent diets and that day to day variation in nutrient composition will have negative effects on cows. However, until very recently, the effects of diet variation on cows were never researched. We conducted a series of experiments evaluating how variation in forage NDF, CP, fat, forage dry matter and forage to concentrate ratio effects short term (usually 3 weeks) production by cows. Across all the studies the results were quite surprising. Feeding a diet with 4.8% fat for four days, then switching to a diet with 7.0% fat for four days and repeating that cycle reduced milk production by 1 kg/d compared with feeding a diet with 5.8% fat continuously (Weiss et al., 2013). On average, both treatments contained the same concentration of fat and the supplemental fat was highly unsaturated (corn oil). Variation also reduced daily dry matter intake (DMI) by about 1 kg. The negative effect of variation appeared to be cumulative. The longer the cows were exposed to the variation, the worse was the negative effect on DMI and milk production.

Because silage is often exposed to rain and snow, its DM concentration can change abruptly. We conducted an experiment to determine whether an abrupt change in silage DM affected midlactation cows. In this experiment (McBeth et al., 2013) we had a control treatment (no change in silage DM content) a treatment in which silage DM content was decreased by 10 percentage units by the addition of water with no other changes in the diet other than increasing feed delivery rate (unbalanced diet), and a treatment that included the wetted silage but we adjusted the forage to concentrate ratio so that nutrient composition (except for DM) was the same as the control (balanced diet). The wetted silage treatments were fed for three consecutive days twice during the 21-day experiment. During the six days the wetted silage was fed, the unbalanced diet had 1.5 percentage units less NDF, 2.6 percentage units less forage NDF, 2 percentage units more starch and 0.1 percentage units more CP. The treatment with the highest milk yield was actually the treatment we hypothesized would be the worst. When cows were abruptly changed to the unbalanced diet, they produced an average of about 1 kg more milk than cows fed the control. This occurred even though the wetted silage was only fed for six days out of the 21-day experimental period (during 15 days of the experiment all cows were fed the exact same diet).Over the 21-day period, milk fat yield did not differ between cows on the unbalanced treatment and control cows but cows on the unbalanced treatment produced about 20 g more protein per day (P < 0.05). Cows initially reduced DMI when fed the wetted silage whether diets were adjusted or not and it took cows one to two days to return to normal intake (Figure 2). The surprising finding was that for a day or two after cows were switched back to the normal silage, they ate more than control cows. Incorporating "controlled variation" into diets may be a way to increase production. Once or twice a week cows could be fed a wetter diet with about 5 percentage units less forage and 5 percentage units more concentrate for 3 days and then switched back to normal diet. For this to work, however, excess feed has to be delivered so that when cows want to increase intake, feed is available.
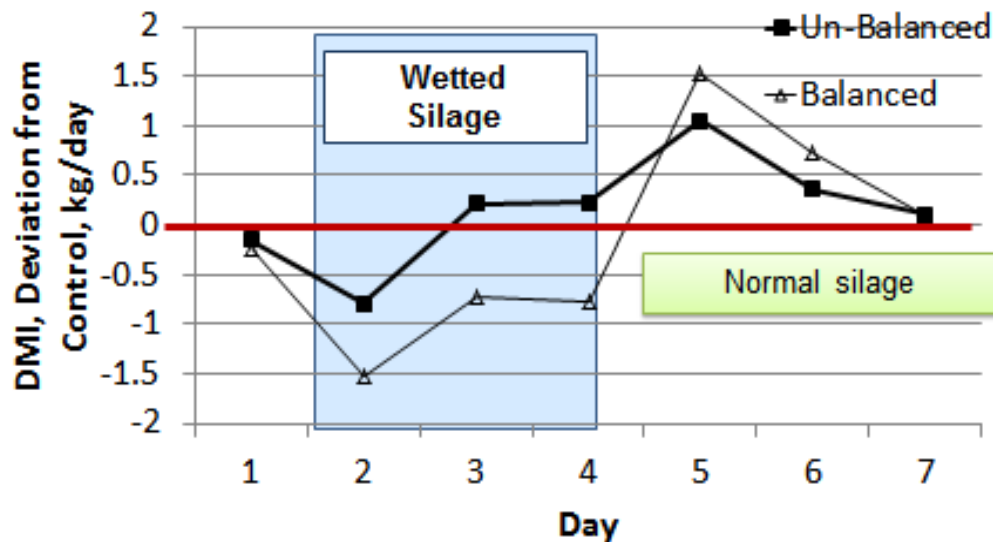
Figure 2. Effect of abruptly changing the DM of silage on DMI of dairy cows. On all days except for 3 days (shaded box) all cows were fed the same diet. During the experimental period, one group of cows was fed the control diet, another set of cows was fed wetted silage (10 percentage unit decrease in DM) with no change in diet (Un-Balanced) and a third set of cows was fed wetted silage, but forage to concentrate ratio was adjusted so on a DM basis, the diet was the same as the control. On all days excess feed was delivered so that the feed bunk was never empty. When cows were first switched to the wetted silage, DMI decreased and it took 2 or 3 days for cows to adjust and return to normal intakes. When cows were switched back to the control diet, cows continued to eat more feed for 1 or 2 days.

In a third experiment we changed the concentration of forage NDF day to day in a random pattern by using forages that differed greatly in quality (Yoder et al., 2013). The proportion of concentrate in the diet and the composition of the concentrate did not change over days. The day to day variation was much greater than what would be typical on a commercial farm. In general, DMI and milk yield followed the expected pattern. On days when cows were fed high forage NDF, DMI and milk yield decreased (usually with a 1-day lag) and on days when cows were fed diets with low forage NDF, intake and milk yield increased. Over the 21-day experiment, the variable treatment had the same average forage NDF as the control diet, which was very consistent day to day. Average intake and milk production (24.5 kg/day DMI and 43 kg/day milk) were the same for both treatments although day to day variation in intake and production was much greater for the variable treatment. We used a random pattern for the variable treatment but generally a diet with high or low NDF was never fed for more than two or three days in a row, which may be why we did not see any overall negative effects. Feeding a bad diet (e.g., excess forage NDF) for more than two or three days in a row will likely reduce DMI and milk yield, and cows may not recover. This experiment does not show that the diet fed to cows does not matter because over a period of a few days, the diet was on average well-balanced. Furthermore, we did not evaluate inconsistencies in making the TMR; we evaluated changes in forage composition. What this study does show is that cows can handle some variation in forage quality without negative effects. If you obtain composition data from a new sample of forage and it differs from the previous sample you do not have to rush to change the diet. You should evaluate the data and try to determine whether it is a real change and then reformulate if necessary. Cows do not need to be fed perfectly every day but they need to be fed a good diet when averaged over a few days.

We and others have also evaluated if oscillating dietary CP concentrations over time affects production and nitrogen (N) utilization efficiency (i.e., g of N for growth or milk N/g of N intake). Oscillating involves feeding a lower CP diet (below requirement) for one or two days followed by feeding a diet that meets requirements for one or two days and that pattern is repeated for weeks. In some experiments, oscillation

reduced manure N excretion and increased protein efficiency (we could feed on average a lower protein diet and maintain production). In growing sheep and steers (Cole, 1999; Archibeque et al., 2007; Doranalli et al., 2011), oscillating dietary CP from deficient to adequate levels (average of 10% to 16% CP) every 48 hours reduced the CP required to maintain a similar average daily gain as feeding a higher, adequate level continuously. This also reduced manure N excretion and ultimately improved N balance at the same average CP intake. For growing animals oscillating CP generally improves efficiency but inadequate data are available with lactating cows to reach a conclusion (Figure 3).
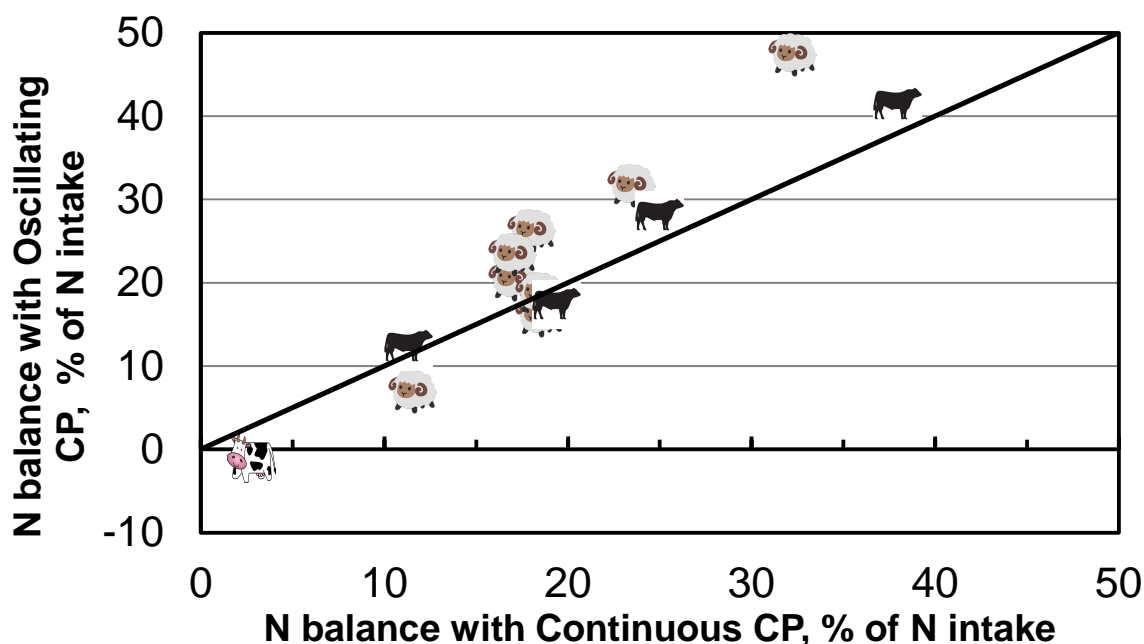
Figure 3. Comparison of nitrogen (N) balance as a % of N intake when oscillating the day-to-day CP concentration versus feeding a similar average CP level continuously to ruminants [adapted from (Reynolds and Kristensen, 2008) with added data]. The different symbols represent the type of animal used in the experiment [sheep (n = 8), beef cattle (n = 4) or lactating dairy cows (n = 1)] and the line represents equal N balance among treatments. Symbols above the line mean that N balance was improved when oscillating CP compared to feeding a similar average CP level continuously.

Fewer data are available for lactating dairy cows. In our first experiment at OARDC (Brown, 2014), oscillating CP every 48 hours from 10.3 to 16.4% CP did not affect N utilization efficiency (g of milk N/g of N intake) compared with continuously feeding a diet with 13.4% CP (i.e., average for the oscillation treatment). However, the oscillation treatment numerically decreased milk production compared with continuously feeding the 13.4% CP diet (33.8 vs. 34.7 kg/day, $P < 0.15$). Less milk production negates any improvement in income over feed cost or reducing the environment impact of dairy farms. However, feeding a low protein diet (10.3%) for 48 hours may have been too long. When we compared production within days, milk yield was reduced about 2 kg/day on the second day of feeding the 10.3% CP diet compared with the first day and remained low on the first day of feeding 16.4% CP diet. The change in production always lagged 1 day after the diet change.

The results above led to our last experiment. We hypothesized and tested whether oscillating the CP concentrations of a marginally deficient diet every 24 hours would improve milk production and reduce manure N excretion in dairy cows. In a 50-day feeding trial, 30 mid-lactation Holsteins were fed one of three treatments: 1) adequate protein fed continuously (16.2%), 2) marginally deficient protein fed continuously (14.1%), or 3) 24-hour oscillations from adequate (16.2%) to deficient protein (11.9%) to be

on average equal to the marginally deficient protein diet fed continuously. Total output of urine and feces was used to measure protein digestibility and N balance. Compared with the marginally deficient protein diet fed continuously, oscillating the same average protein level numerically reduced DMI; however, milk yield was similar whether oscillating or feeding a static concentration (Table 2). Milk protein yield was also similar among diets. Milk urea-N tended to be higher with the oscillating CP treatment vs. continuous feeding of the marginally deficient CP diet; this may have occurred because these cows had a higher protein digestibility than cows fed a constant protein diet. However, more of the CP digested was excreted as N in urine rather than being used for milk protein synthesis or increasing body protein stores (i.e., N balance; Table 3). Based on N balance, body composition measurements, and plasma markers of catabolism (i.e., 3-methyl-His), we had no evidence oscillating CP resulted in mobilizing body reserves to support higher milk yield at a lower intake. Overall, this work indicates large, daily changes in dietary CP or oscillating dietary CP concentrations every 24 hours to dairy cows is not detrimental for milk production, but further research is needed before using this feeding method to enhance production efficiency and reduce manure N excretion.

Table 2. Effects of static or oscillating dietary CP concentrations for 50 days on intake and production.

| | Treatment[1] | | | | P-value | |
	16.2%CP	14.1%CP	Oscillating CP	SEM[2]	16.2 vs. 14.1%CP	14.1%CP vs. oscillating
DMI, kg/day	22.9	23.2	22.2	1.08	0.59	0.11
Milk, kg/day	36.6	35.1	35.3	1.81	0.02	0.78
ECM[2], kg/day	36.3	34.9	33.8	1.89	0.19	0.35
ECM/DMI	1.59	1.52	1.52	0.04	0.13	0.98
Milk fat, %	3.22	3.36	3.10	0.17	0.45	0.17
Milk protein, %	2.94	3.03	2.90	0.05	0.04	0.01
Milk lactose, %	4.83	4.81	4.80	0.07	0.57	0.99
Milk fat, kg/day	1.21	1.19	1.10	0.09	0.77	0.27
Milk protein, kg/day	1.10	1.06	1.05	0.06	0.14	0.59
Milk lactose, kg/day	1.82	1.69	1.73	0.05	0.01	0.24
MUN, mg/dL	12.8	10.2	10.9	0.74	0.01	0.10

[1] Treatments were adequate CP fed continuously (16.2% CP), marginally deficient CP fed continuously (14.1% CP); or 24-h oscillations from adequate (16.2% CP) to deficient CP (11.9% CP)
[2] Energy corrected milk, kg/day = 0.327 × milk, kg/day + 12.95 × milk fat, kg/day + 7.65 × milk protein, kg/day

Table 3. Effects of static or oscillating CP concentrations on CP digestion and N intake and excretion.

| | Treatment[1] | | | | P-value | |
	16.2%CP	14.1%CP	Oscillating CP	SEM[2]	16.2 vs. 14.1%CP	14.1%CP vs. oscillating
CP digestibility, %	65.2	61.7	65.3	1.44	0.09	0.07
N intake, g/day	561	512	474	16.9	0.01	0.03
N digested, g/day	366	317	310	14.2	0.01	0.66
Milk N, g/day	174	179	164	5.63	0.50	0.06
Urine N, g/day	185	124	151	12.6	0.01	0.13
Retained N, g/day	5.5	14.2	-5.4	10.0	0.49	0.11
Milk N, % of digested N[2]	48.1	56.7	53.8	2.37	0.02	0.35
Urine N, % of digested N[2]	50.5	39.5	49.6	3.10	0.02	0.04

[1] Treatments were adequate CP fed continuously (16.2% CP), marginally deficient CP fed continuously (14.1% CP); or 24-h oscillations from adequate (16.2% CP) to deficient CP (11.9% CP)
[2] Digested N was corrected for negative N retention and then used for milk and urine-N calculations.

▪ Conclusions

The nutrient composition of feeds and diets varies sample to sample, whether those samples are taken the same day or months apart. Some of this variation is real (a new cutting of hay, a new batch of distillers grains, or today's silage came from a weedy part of a field) but much of the sample to sample variation we observe is simply caused by sampling variation. The feed or diet may not have changed at all, but the sample we took was not a good representation of the feed. If we assume all changes in nutrient composition are real and reformulate a diet based on that change, the resulting diet may be wrong. Diets should be formulated based on average composition from at least two samples, and nutritionists should evaluate their sampling skills by comparing results from duplicate independent samples. We are currently investigating whether we can use 'controlled' variation to enhance production, and contrary to standard dogma, intentionally varying certain diet components systematicaly may improve production and efficiency. Feeding cows diets that oscillate between adequate and deficient concentrations of CP every other day may improve productive efficiency. Likewise, reducing the forage to concentrate ratio of a diet by adding water to silage for a period of 3 days once per week may also enhance production. The use of controlled variation in diet composition in dairy diets deserves additional research.

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Feeding for a Healthy Liver: The Role of Methionine and Choline in Transition Cows

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▪ Take Home Messages

- Choline is a quasi-vitamin that is degraded in the rumen, becoming unavailable to the cow unless supplemented in a rumen-protected form.

- Choline plays a key role in liver lipid metabolism and consistently results in increased milk yield and fat-corrected milk yield when supplemented during the transition to lactation period.

- Methionine is an essential amino acid that is available to the animal within microbial crude protein; however, that supply may not be sufficient for high producing animals.

- Methionine supplementation consistently results in an increase in milk protein yield and plays a role in mediating inflammatory response.

- Limited work has examined choline and methionine simultaneously. Data in cows and cell culture lacks evidence of a significant interaction on production outcomes or indicators of metabolic function or health, which suggests that there are unique biological roles of each nutrient.

▪ The Transition to Lactation Period

The transition for the dairy cow from being dry to lactating is a period of metabolic challenge (Grummer, 1993; Drackley, 1999); however, this period also holds great opportunity for improvements in animal efficiency and health. Many of the challenges associated with the transition to lactation are rooted within energy balance. The voluntary feed intake reduction around the time of calving, coupled with increases in energy requirements to meet the needs of lactation, result in cows entering a state of negative energy balance (NEB) around calving. During periods of NEB, triglycerides (TG) are mobilized from fat stores and the resulting fatty acids and glycerol backbone are transported to the liver to help alleviate NEB. Glycerol can serve as a glucose precursor in the liver, and fatty acids provide milk fat precursors in the mammary gland or are oxidized for energy in the liver. Oxidation of nutrients, including fatty acids, is essential for liver cell functions, including fuelling the energetically expensive pathway of gluconeogenesis (glucose synthesis), by which most of the glucose supply in ruminant animals is generated. The onset of NEB also creates a deficiency in glucose, amino acids, and other nutrients because of low dry matter intake (DMI) during a time of elevated nutrient requirements.

▪ Choline

Choline is a quasi-vitamin that is essential in many species and serves as a precursor for phospholipid synthesis for cell membranes and lipid (fat) transport, as a component in acetylcholine (a predominant neurotransmitter), and as a methyl donor. In nonruminant animals, choline deficiency results in fatty liver, exemplifying its importance in liver function and how it could be applicable to ruminant liver health. Understanding the benefit of choline for ruminants has largely focused on reductions of liver lipid concentration (discussed below). If not fed in a rumen-protected (RP) form, most dietary choline is degraded during rumen fermentation.

Supplementation of rumen-protected choline (RPC) to peripartum dairy cows has been of interest because of the consistent increase in yield of milk or fat-corrected milk. Meta-analyses are a useful tool that allow us to determine if a treatment has an effect across a range of environments and animals by re-analyzing data from multiple studies with similar objectives. A meta-analysis by Arshad et al. (2019), which focused on RPC supplementation and included 23 experiments (74 treatment means; 1,938 cows), demonstrated a significant increase in pre- and postpartum DMI (0.28 and 0.47 kg/d, respectively), increased energy-corrected milk (ECM; 1.61 kg/day weighted mean average), and increased fat and protein yield (0.08 and 0.06 kg/day, respectively). Benefits of RPC supplementation do not appear to be dependent on prepartum dietary energy (Zenobi et al., 2018c) or body condition score (Bollatti et al., 2019).

It is challenging to determine effects of nutritional interventions on health incidences within a single study because of limited animal numbers; therefore, another benefit of meta-analyses is to examine health events over a large pool of animals. Within the meta-analysis, incidence of retained placenta and mastitis, but not displaced abomasum, ketosis, or metritis, were reduced by RPC supplementation. Interestingly, supplementation of RPC may have benefits on production that persist beyond the supplementation period, as demonstrated by tendencies for improved milk yield at 15 and 40 weeks postpartum and improved milk components at 15 weeks postpartum after supplementation during the 3 weeks before and 3 weeks after parturition (Zenobi et al., 2018c). When supplementing prepartum, we also have the potential to influence the calf developing in utero, and improved average daily gain from calving or weaning to 50 weeks of age, and improved immune status and response to a bacterial challenge have been observed (Zenobi et al., 2018a; Zenobi et al., 2018b).

Choline can be used by many tissues within the body but metabolism is primarily within the liver (Pelech and Vance, 1984). The classically described benefit of RPC supplementation is a reduction in liver fat accumulation across the transition to lactation. A decrease in liver fat has been observed with peripartum RPC supplementation (14.4 to 15 g/day choline) in several (Cooke et al., 2007; Elek et al., 2008; Lima et al., 2012; Zom et al., 2011; Goselink et al., 2013) but not all (14.4 to 19 g/day choline) (Piepenenbrink and Overton, 2003; Zahra et al., 2006; Zhou et al., 2016c; Zenobi et al., 2018c) transition cow studies. When supplemented to dry, pregnant cows that were feed restricted to mimic the NEB aspect of the transition period, RPC supplementation lessened how much fat accumulated within the liver (Cooke et al., 2007; Zenobi et al., 2018d). When RPC was supplemented after fatty liver induction using the same model, supplementation reduced liver fat (Cooke et al., 2007), suggesting an ability of RPC to aid in recovery from fatty liver.

Choline is a key component of very low density lipoprotein (VLDL), particles that aid in transport of fat from the liver to other tissues. The mechanism of RPC action to reduce liver fat is thought to be through increased phosphatidylcholine (a component of cell membranes and VLDL) synthesis and thus increased VLDL package and export from the liver, as in nonruminants. It is very challenging to measure blood VLDL in ruminants because of the differences in lipid profile and because the mammary gland in dairy cows takes up more fat from the blood compared with the mammary gland of other species because of the greater extent of milk fat synthesis. Markers of VLDL secretion were increased in transition cows supplemented with RPC that showed reduced liver TG accumulation (Goselink et al., 2013). To narrow in on the effect of choline supplementation on VLDL export, bovine liver cells can be isolated and cultured in vitro, allowing for specific examination of many treatments and outcomes. Quantification of VLDL export from liver cells in culture by ELISA assay indicated an increase in VLDL export with increased choline supplementation (Chandler and White, 2017). In addition, recent advanced laboratory techniques have confirmed the ability of RPC supplementation to increase phosphatidylcholine concentrations in lipid-rich lipoproteins isolated from plasma of non-lactating cows (Myers et al., 2019).

Increases in milk fat yield may be reflective of increased VLDL export from the liver because the VLDL can subsequently be taken up and used by the mammary gland. Despite this, improvements in lipid metabolism may not fully explain production advantages observed with RPC supplementation because production responses have been seen without a decrease in liver fat (Zenobi et al., 2018c). Previously, it

was noted that decreased liver fat may allow for increased liver gluconeogenesis, the pathway by which the liver makes glucose (Drackley, 1999; Goselink et al., 2013). Gluconeogenesis produces glucose for release into circulation for immediate use, or as glycogen that is stored in the liver for quick release when needed. Increased liver glycogen has been observed with RPC supplementation in cows (Piepenbrink and Overton, 2003; Zenobi et al., 2018d) and liver cell culture (Chandler and White, 2019) and may reflect greater rates of gluconeogenesis. Just as supply of glucose to the mammary gland via increased hepatic glucose production can support increased milk yield, increased mammary gland lipid uptake may support improved milk or milk fat yield.

▪ Methionine

Methionine (Met) is an essential amino acid and often one of the first two limiting amino acids in dairy cow diets. It is critical to many pathways in the body and is involved in DNA methylation, creatine synthesis, and glutathione synthesis. During lactation, methionine demands increase because it is an essential amino acid for milk protein synthesis. Although dietary methionine is degraded during rumen fermentation, it is still supplied to the cow because it is a key component of microbial crude protein (protein produced by the rumen bacteria); however, high producing dairy cows likely need more methionine than microbial protein can provide.

Supplementation of Met has been through RP Met, a Met analogue 2-hydroxy-4-(methylthio)-butanoic acid (HMB), or the isopropyl ester of HMB (HMBi). Supplementing RP Met consistently results in increased milk protein yield and increased DMI (Zanton et al., 2014). This response is logical given the potentially limited supply and important role of essential amino acids for milk protein synthesis during the early postpartum period and throughout the entire lactation. Response of RP Met supplementation on milk yield is less consistent, especially during the transition period. For example, supplementation of HMBi or RP Met during the peripartum period did not affect milk yield but did increase milk protein percent in one study (Ordway et al., 2009); in another study, similar supplementation resulted in a 2.4 and 4.3 kg/day increase in milk yield for HMBi and RP Met, respectively (Osorio et al., 2013).

A key role of Met outside of amino acid function is as a methyl donor (a methyl group contains one carbon atom bonded to three hydrogen atoms; it is usually part of a larger molecule). Methionine is part of the transmethylation pathway that generates S-adenosylmethionine (SAM), the universal methyl donor of the body (Figure 1). The complexity of the figure indicates the intricate balance of methyl donation to SAM and the potential depletion of the methionine supply for other roles. After methyl group donation, Met becomes other intermediates unless it is regenerated by adding a methyl group back to the molecule via the transmethylation pathway. This regeneration requires a methyl group to be donated from another nutrient: folate, betaine, or choline (via betaine as an intermediate). Interestingly, when choline is provided in a cell culture model, expression of genes involved in transmethylation is increased, regardless of how much Met the cells are provided (Chandler and White, 2017). This highlights the priority of the cell for regenerating Met and the potential for other methyl donors to support the regeneration.
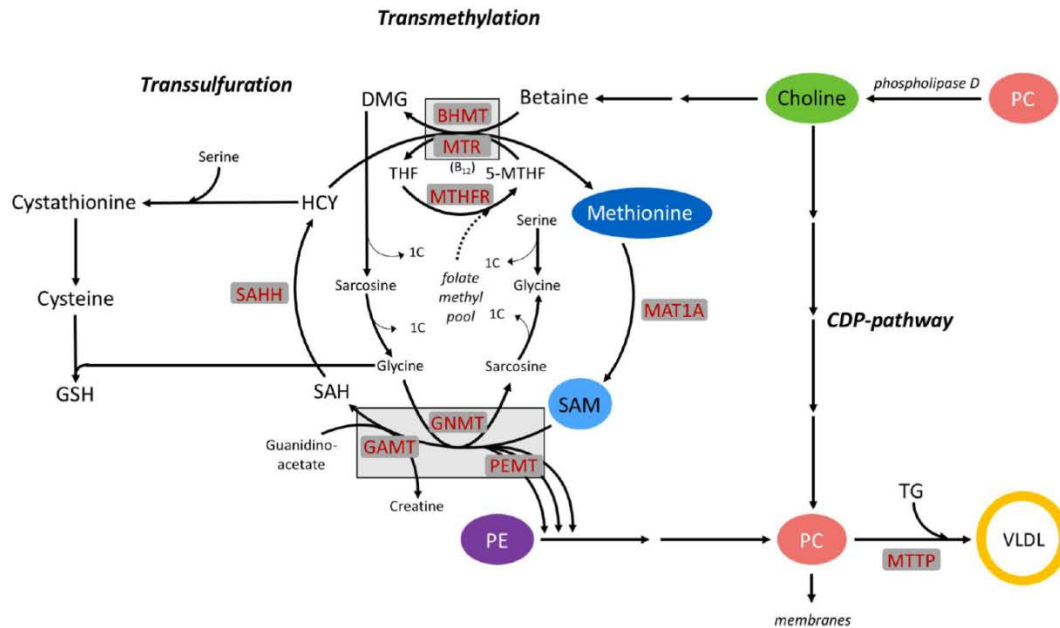
Figure 1. Intersection between pathways of choline and methionine metabolism in the transmethylation pathway. The abbreviations shown in red represent key enzymes that control methyl group transfer. Other abbreviations are: dimethylglycine (DMG), glutathione (GSH), homocysteine (HCY), S-adenosylmethionine (SAM), S-adenocylhomocysteine (SAH), tetrahydrofolate (THF), phosphatidylethanolamine (PE), phosphatidylcholine (PC), and very low-density lipoprotein (VLDL). (Chandler and White, 2017).

The methyl cycle also highlights two other potential roles for Met. In addition to generation of phosphatidylcholine from dietary choline to support VLDL synthesis, phosphatidylcholine can also be synthesized through three sequential methylations of phosphatidylethanolamine to phosphatidylcholine, known as the PEMT pathway. While both pathways are biochemically possible, the PEMT pathway is energetically expensive and relies heavily on SAM methyl-donation. Cell culture experiments measuring the key gene (*PEMT*) involved in the PEMT pathway demonstrated decreased gene expression with both Met and choline supplementation, despite increased VLDL export with choline supplementation (Chandler and White, 2017). Conversely, increased gene expression was observed with Met supplementation in one transition cow study; however, VLDL were not quantified and liver lipids did not change (Zhou et al., 2016c). Across four transition cow studies and one study involving feed restricted, non-lactating cows, Met supplementation either did not change (Bertics et al., 1999; Osorio et al., 2013; Piepenbrink et al., 2014; Zhou et al., 2016c) or increased liver lipid accumulation (Preynat et al., 2010).

Perhaps the most interesting role of Met is related to the generation of glutathione (Figure 1) and the role in inflammatory response. As a sulfur-containing amino acid, Met is the principal precursor for the synthesis of glutathione (Brosnan and Brosnan, 2006), which serves as an antioxidant. Liver concentrations of glutathione decrease postpartum and take nearly 3 weeks after calving to return to postpartum concentrations, but supplementation with RP Met increased liver glutathione concentrations (Osorio et al., 2014). Addition of Met in liver cell cultures experiments resulted in increased glutathione and decreased lipopolysaccharide-induced inflammatory response (Zhang and White, 2017). Supplementation of RP Met in transition cows also reduced markers of oxidative stress (Zhou et al., 2016a,b). These studies indicate an exciting role for Met in improving inflammatory status in transition dairy cows.

▪ Potential Interactions between Choline and Methionine

The potential interactions between choline and Met are highlighted through the roles of each nutrient in VLDL synthesis and methyl donation. Limited transition cow studies have examined the effects of Met, choline, and the interaction of the two. In two transition cow studies where cows received either no treatment, choline, Met, or choline and Met (Sun et al., 2016; Zhou et al., 2016c), there were no significant interactions on production or markers of health. Consistently, cell culture models exposed to Met and choline across a range of treatment doses demonstrated no significant interactions on expression of key genes (either methyl donation pathways or gluconeogenesis) or metabolites (glycogen, cellular lipid, VLDL, etc.) (Chandler and White, 2017; Chandler and White, 2019). These data support separate biological priorities for choline and Met. Ultimately choline and Met both play a key role in transition cow liver health.

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Five Habits of Highly Effective Farmers

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▪ Take Home Messages

- **Be proactive:** Being proactive allows you (farmers) to rapidly anticipate and identify problems as they appear and make appropriate adjustments to minimize the negative effects.

- **Realize a purpose for your business:** Successful farmers have taken the time to think about not only what they do but why they do it. Build a clear mission, vision, and core values for your farm. Make sure the entire team know your purpose.

- **Begin with the end in mind:** Goal oriented farmers always have the end in mind, with a clear vision of where they are going and how they are going to get there. Knowing where your farm will be in 5, 10, and 15 years allows you to have clearly defined goals.

- **Prioritize and manage:** Once you have an end goal, priorities can be set for what needs to happen to move in a straight line toward your goal, managing all areas of your business to get there.

- **Benchmark and analyze your performance:** Progressive farms set performance goals and review them often. Don't compare yourself to the average, but rather to the top 25%.

- **Sharpen the saw:** Renewing yourself physically, spiritually, mentally and socially will help you achieve the other five habits.

▪ Introduction

Dairy farms all over the world continue to progress and be passed down from one generation to the next. Meanwhile, market sensitivities, consumer and regulatory requirements, labour availability, farm demographics, and technological advancements all place demands on our dairy community. Despite these pressures, successful farms have found a way to adapt and thrive. Top performing dairy farms have the distinct ability to step back and objectively assess their businesses and themselves. They are constantly doing SWOT analysis (strengths, weaknesses, opportunities and threats) of the business and conditions, and fine-tuning strategies to adapt or adjust to the circumstances. These farms are not distracted by or influenced by things that they can do nothing about.

American author Stephen Covey (1989) wrote a bestselling book over 25 years ago titled *7 Habits of Highly Effective People* that has become a timeless influential handbook for successful people and is still relevant today. This article highlights key habits that the most effective farmers practice every day.

▪ Habit 1: Be Proactive

Being proactive is a state of mind and at the core of highly effective farmers. By concentrating on their circle of influence (Figure 1) and choosing how they respond to circumstances they have no control over, proactive farmers can change the nature of the results. Traditionally, our fathers and grandfathers ran their dairy farm in a reactive manner, being affected by the physical and social environment around them. Reactive farmers are driven by feelings, circumstances and conditions, waiting until there is a problem and then dealing with it.

Today, farmers are learning to be proactive, making decisions based on their core values. They are still influenced by the outside environment; however, they are self-aware enough to recognize that they can choose how they will respond to a given situation. They rapidly anticipate and identify problems as they rise, and in some cases, before they arise, and make the appropriate adjustments to minimize the negative effects. A farm may not be able to control whether its forage is too high in potassium or too low in energy due to weather conditions when making it, however a proactive farm will recognize its forages limitations and make the necessary adjustments without waiting for the cows to get milk fever or ketosis.
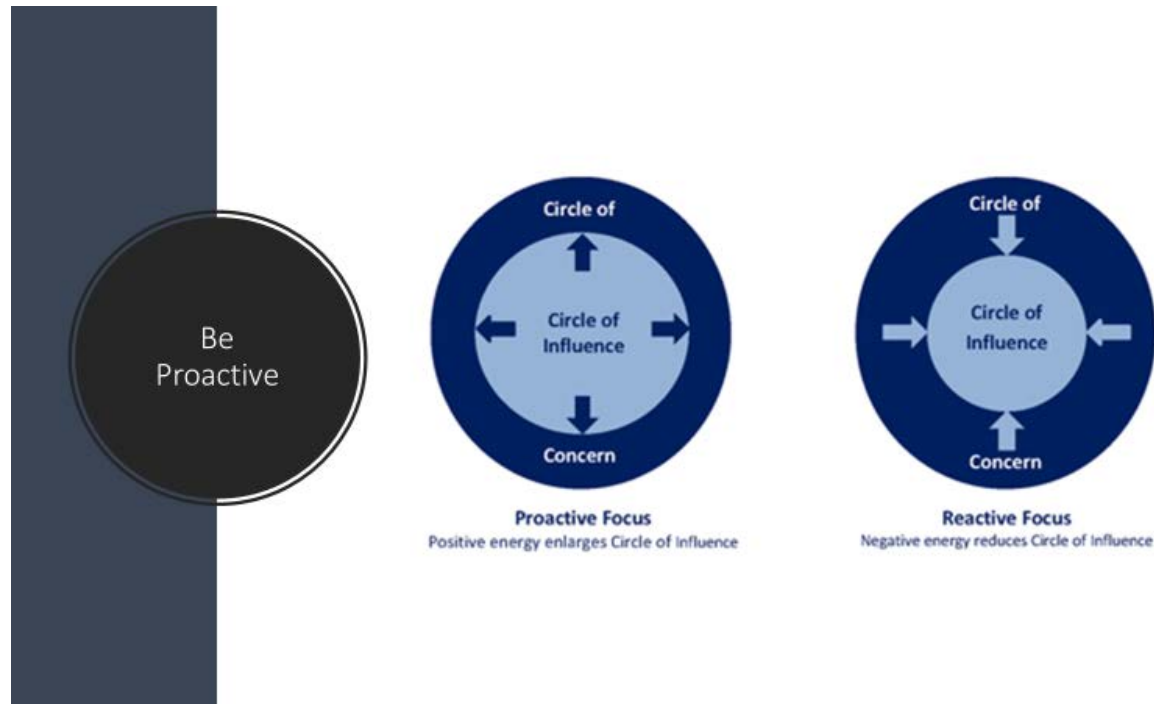
Figure 1. Being proactive means focusing on the Circle of Influence that lies within our Circle of Concern—concentrating on working on things that we can do something about.

▪ Habit 2: Realizing a Purpose for Your Business

Successful dairy farmers have taken the time to think about not only what they do but why they do it (Figure 2). Having a clear mission (reason for existing), vision (future goals), and core values for your farm will allow everyone on the farm to work more cohesively and move in the same direction toward a common goal. A farm's values guide every decision it makes and encompasses its purpose for farming. Before dairy farms, even small family owned/operated dairy farms, can truly be successful, they must first establish their core values and beliefs that make their farm unique. Common characteristics of progressive farms include being proactive, innovative, cow-focused, efficient, passionate, accountable and virtuous
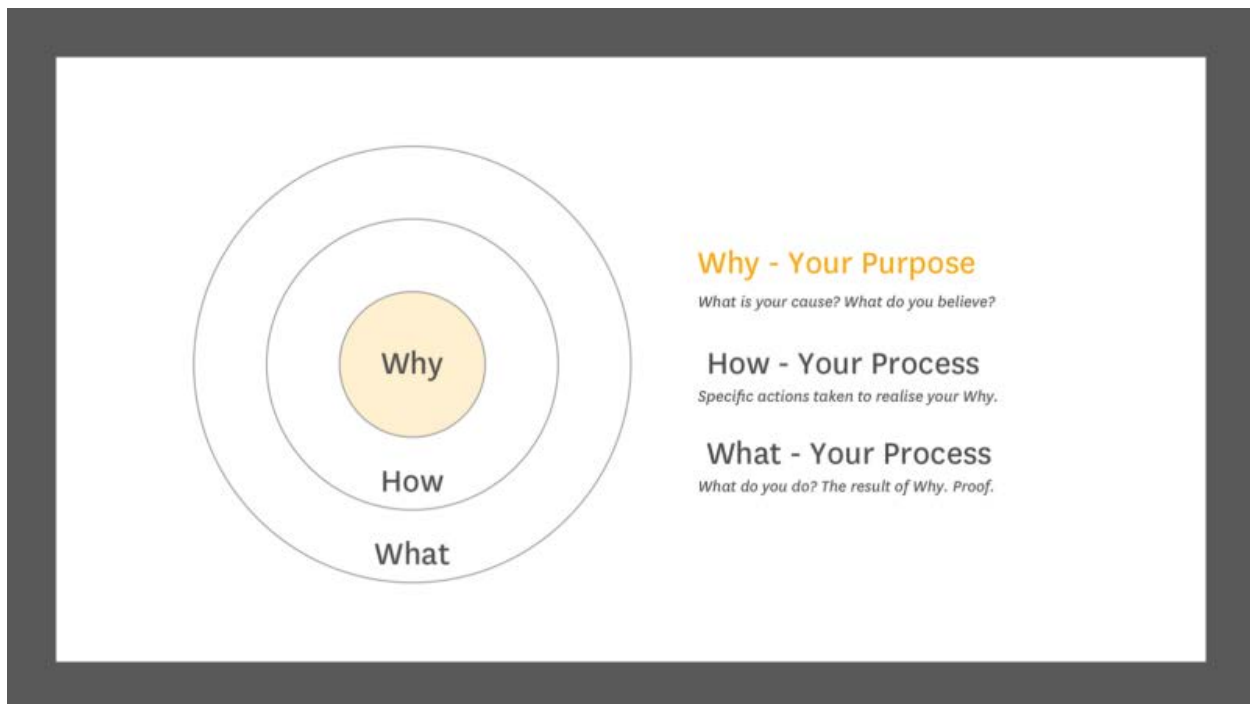
Figure 2. Realizing a purpose for your business relies on Simon Sinek's principle of the Golden Circle: Why, How, and What.

▪ Habit 3: Begin with the End in Mind

All things are created twice, first in theory and then in reality. This concept may new for a lot of farmers. Traditional farmers can get distracted by what they think are victories on their farm— they reach a certain number of cows or a certain amount of milk in the tank, or acquire that next piece of land—without asking if the things they are focusing on so intently are what really matter to them. Goal oriented farms always have the end in mind, with a clear vision of where they are going and how they are going to get there. This allows them to always make sure that the steps they are taking are in the right direction. They know where the farm will be 5, 10, and 15 years from now, who will be running it and how big the farm will be and have even defined clear goals to achieve. This will allow the farm to be sustainable into the future. Progressive dairy farms take the time to make sure everyone on the farm has a clear understanding of the vision for the farm and ensures that each employee feels a part of the team.

▪ Habit 4: Prioritize and Manage

Once you have an end goal, you can set priorities for what needs to happen to move in a straight line toward your goal managing all areas of your business to get there. Progressive farmers are excellent at prioritizing and managing their operations. They do everything well. The most sustained success comes from doing 20 things 5% better rather than doing one thing 100% better. The most successful farms can manage the day to day tasks and pay attention to all the details that will allow them to achieve their goals. These farms possess an attitude of excellence and concentrate on doing the little things better. Standardized protocols are in place for key areas of the farm, such as milking routine, calf feeding, and reproduction. The most productive farms can transfer both the passion and attention to detail to its employees through coaching and mentorship.

As time moves on and different opportunities arise, whether it is to build a new barn, buy more cows, or purchase a new tractor, successful farmers step back and ask the question "Is this in line with my purpose, vision and goals for my farm?" This removes the old impulsive way of doing business. They also

manage risk by evaluating 'but-what-if' scenarios and have contingency plans and processes in place for situations such as: what if the interest rate goes up, the price of milk drops, supply management disappears? By asking the tough and uncomfortable questions, progressive farms can identify the weaknesses in their business. These farms intend to succeed and are confident in their plan, but they still have an alternate plan just in case.

▪ Habit 5: Benchmark and Analyze Your Performance

It's not enough to know how you compare to the average farmer, but rather how you stack up against the top 25%. Progressive farms set performance goals and review them often. They use technological advancements to monitor their farm and make necessary adjustments along the way in order to benchmark their performance. Analyzing the different areas of the farm against its performance goals can highlight things that the farm needs to stop doing in order to become more profitable. For instance, if a farm is understaffed and puts in poor crops year after year, perhaps it is better off concentrating on milking cows and having a custom operator doing the crops.

▪ Bonus Habit: Sharpen The Saw

For farmers to be effective in reaching their goals as farm owners and operators, they must devote time to renewing themselves physically, spiritually, mentally and socially. Dairy farming can be a lonely job and there are days on end that don't go the way that the farmer planned. The most successful farmers take the time to recharge their battery and remember the important things in life. They have developed productive skills that allow them to cope with the stresses of farming.

Four dimensions of our nature exist and each must be exercised regularly in balanced ways without one dominating another. To renew our physical dimension, we must eat well, get sufficient rest and relaxation, and exercise on a regular basis. To renew our spiritual and mental dimensions, we must practice daily meditation, communicate with nature, and take time to read or listen to music. This may be as simple as riding your farm land with the radio on and not just getting from point A to point B as quickly as possible but rather spending time thinking about what your land means to you and how it fits in with your values. The social dimension implies the importance of developing meaningful relationships. When you can really connect with another person, it allows you to feel less alone when the barn cleaner or tractor has broken down for the third time in a week.
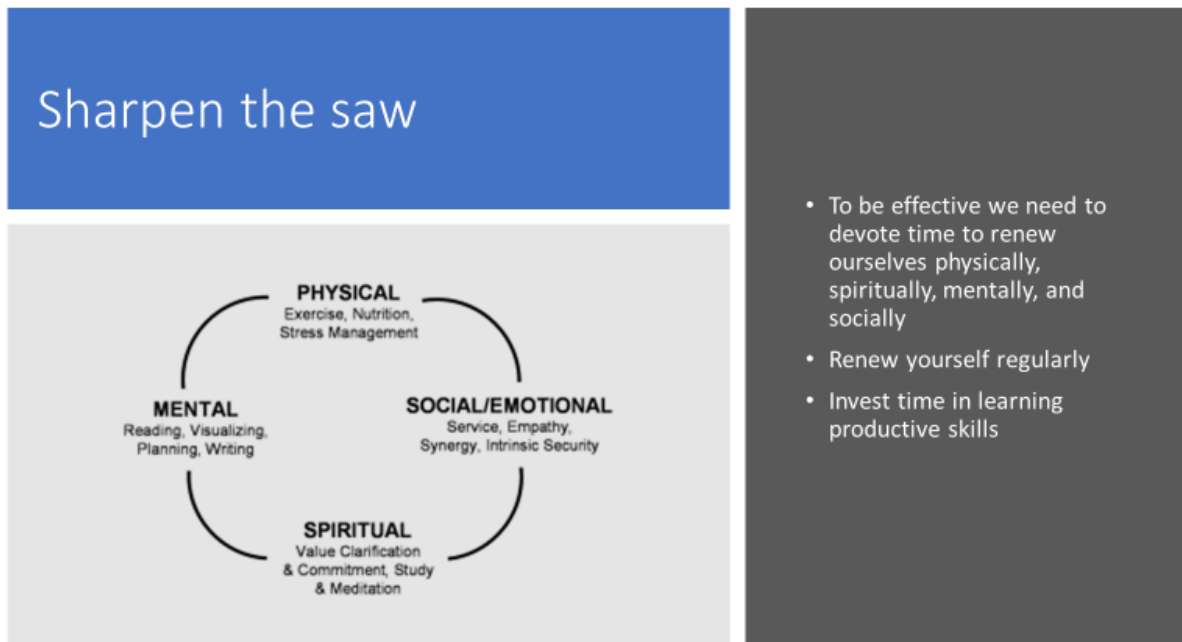
Figure 3. This key habit of renewing ourselves physically, mentally, and socially is what makes all the other habits possible.

▪ Conclusion

All the elements of highly effective farmers are called habits for a reason. They are challenging and need to be practiced every day. You must place the right employees in key areas on your farm (calves, parlour, breeding, and feeding) and give them the tools, support and knowledge to succeed. The five habits can be best achieved when you surround your farm with key advisors who can help you reach your goals.

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How to Do More for Mental Health in Agriculture

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▪ Take Home Messages

- Mental health affects the agriculture industry more prevalently than other industries.
- We need to begin to change the conversation and decrease the stigma surrounding mental health in agriculture.
- We can reduce the stigma by increasing our mental health literacy, learning how to have conversations, and understanding self-care and prevention.

▪ Understanding Mental Health

Mental wellness vs. mental stress vs. mental illness. The terms are often used interchangeably, but they have very different meanings. Mental wellness (or mental health) is defined as "a state of well-being in which the individual realizes his or her own abilities, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to his or her community (World Health Organization, 2018). Mental stress is a form of stress that occurs because of how events in one's external or internal environment are perceived, resulting in the psychological experience of distress and anxiety (Salomon, 2013), whereas mental illness is a health condition involving changes in emotion, thinking or behaviour or a combination of these (American Psychiatric Association, 2020).

It is normal to fluctuate between mental wellness and mental stress, often on a daily basis. We actually need stress in our lives; it helps us operate at peak effectiveness and assists us with problem solving and capacity for building resiliency. We need to ensure though that there is a fluctuation back to mental wellness, for when we do not have a reprieve from the stress we are at increased risk of developing a mental illness. The return to mental wellness can be done through self-care, by setting boundaries, and by increasing our knowledge of mental illness overall.

▪ Most Prevalent Types of Mental Illness

- Mood disorders: depression, bipolar disorder, suicide
- Anxiety disorders: anxiety, panic attack, post-traumatic stress disorder
- Substance-related disorders: alcohol, cannabis, opioids

In-depth training on recognizing signs and symptoms of mental illness is most appropriately gained through mental health first aid, but there are other ways to be conscientious about mental health. A prolonged and progressive change in a person's normal behaviour can be indicative of mental stress. Often, it is a loved one who may recognize this change before the person himself or herself recognizes a decline. Gaining the skills to open a conversation to address this is an important tool in your toolbox. Early recognition of undue stress can go a long way in maintaining our health, and here we will explore the nature and some solutions of being proactive this season.

▪ Total Health

When we talk about mental health, we also need to talk about total health. It is important to recognize the pillars to total wellness, which include mental, physical, spiritual and emotional health.

While we are living and working on the farm, our physical health needs are often met, but total health encompasses more than resistance training and elevated heart rates. Not only do mental, emotional and spiritual elements make up three quarters of our total health recipe, but mental stress and illness can have negative effects on our physical health, including headaches, gut health, back pain, adverse cardiovascular consequences and more.

Mental health is the overarching umbrella that encompasses mental wellness, mental stress, and mental illness.

Mental Wellness	⟷	Mental Stress	⟷	Mental Illness
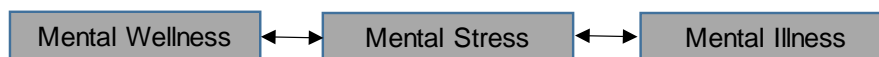
Unlike a physical fracture, in which a bone breaks, mends and is healed, mental health is a constant fluctuation of one's state of mind. It's important to know that every individual floats between mental wellness and mental stress, usually on a daily basis. One in five individuals will fluctuate to mental illness in their lifetime. Achieving a consistent return to mental wellness requires a balance of physical, emotional, spiritual and social attention.

Humans are social creatures and require quality interaction with others to stimulate their social wellness. Whether it's company in the combine, a game of cards in the barn alley waiting to see if #23 needs a calf pull, lunch around the kitchen table, or morning coffee at the local shop, spending some quality time on a social level with family and friends is a great step towards supporting one's mental wellness. Understandably, a lot of farming and ranching is done solo, so making meaningful interactions via technology, versus just scrolling through social reels, can help get us through.

When it comes to emotional wellness, we use the term 'feel your feels.' We have a wide array of natural emotions and it is important to experience and go through them versus stuffing them away. Whether you're feeling sad, mad, happy or glad, resolving to talk to someone or exploring those feelings yourself through reflection, journaling or your own means of exploration can make a big impact on your abilities to put perspective on a situation and help you cope with the stressors the seasons and agriculture overall undoubtedly bring.

Above all, give yourself permission to 'do you.' There are many tips and techniques that people use to manage stress but no one way works for every person. The key is exploration and finding what works for you, even if it's nothing you've ever been told before. Try it, you might like it, but if you don't, keep trying something else. The key is to keep searching for your own solutions, but if you feel that you have and are out of options, reach out. If you need help accessing resources, visit https://www.domore.ag/ where you can find national, provincial and regional supports to help you through a hard time.

▪ Impacts of Sleep Deprivation

Lack of sleep can contribute to physical and mental stress, and illness. Our ability to concentrate the longer we go without sleep can be affected and can cause an increase in accidents, irritability and memory lapse. Our overall stress can also increase from simply not sleeping. There are many times in agriculture that getting more sleep simply isn't an option, but by being aware of the impact lack of sleep can have you can at least be forgiving with yourself, thereby reducing stress in at least one area of your situation.

▪ Implementing a Community of Self Care

We can't change the conversation and stigma about mental health in agriculture without going to a grass roots level. At Do More Ag, our vision is to build up families and communities to have preventative wellness strategies; prevention is always an easier road than recovery. Understanding how different we are as human beings—personality differences and differences in resiliency levels—and approaching each other with empathy are the first steps to creating communities more resilient and adept to remaining in a place of wellness.

If you, or someone you know, needs help, you can visit https://www.domore.ag/resources for a list of resources by province or http://211.ca/find-help-211/ for live chat options on mental health. In the event of an emergency situation, please call 911.

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Transporting Cattle in 2020: Research and Regulation Update

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▪ Take Home Messages

- Young unweaned dairy calves are more prone to transport related stress, morbidity and mortality than are adult cattle.

- Approximately 60% of cull cows have compromised health and advanced age making them more vulnerable to transport stress.

- Cull cows have the greatest probability of becoming lame, non-ambulatory and dying at the end of a long haul (> 400 km) journey compared with other cattle.

- Cull dairy cows with a body condition score of < 2.75, that are < 100 or > 300 days in milk and suffering from digital dermatitis are at higher risk of increased lameness during transport.

- More welfare issues are observed when calves and cows are shipped for > 30 hours.

- Feed withdrawal for up to 30 hours is associated with more indicators of poor welfare than is water withdrawal.

- Animal condition at loading may be more important than provision of a rest in transports exceeding 16 hours.

- Optimal welfare in dairy calves and cows is highly dependent on the animals' condition at loading.

▪ Introduction

Transportation of animals by road continues to be a necessary part of the dairy industry related mainly to the marketing of surplus bull calves and cull cows. However, transportation of young and cull animals has been identified as an important welfare issue needing special consideration (Schwartzkopf-Genswein and Grandin, 2019). Dairy calves and cull dairy cows are among the most vulnerable types of animals regarding fitness for transport when overall condition, health and ability to cope with stress are accounted for.

Heightened public awareness and concern for animal welfare related to transport have increased the need for assessing and developing strategies to minimize potential for poor welfare outcomes. The OIE (The World Organisation for Animal Health, 2008) defines welfare as "how an animal is coping with the conditions in which it lives" and that good welfare "(as indicated by scientific evidence) means the animal is healthy, comfortable, well nourished, safe, able to express innate behaviour, and is not suffering from unpleasant states such as pain, fear, and distress."

Although many studies have documented behavioural and physiological changes in cattle during and after transportation (Schwartzkopf-Genswein and Grandin, 2019), few have assessed very young or old cattle. Behavioural and physiological changes are used as indicators of welfare that can be affected by both animal and non-animal factors. Animal related factors include age, health, body condition, experience, and temperament while non-animal factors include animal management before transport,

handling, loading density, mixing with unfamiliar animals, trailer environment (temperature and humidity, noise and vibration), location within the trailer, transport duration and feed, rest and water intervals (Schwartzkopf-Genswein and Grandin, 2019). Before strategies to improve welfare outcomes during transport can be developed, the impact of each factor indicated above, alone or in combination, need to be better understood for all types of cattle, but more urgently for young calves and cull cows.

The revised Canadian transport regulations (CFIA, 2019) come into effect February 20, 2020, nearly 4 decades after the last regulations were made. The new regulations are science informed, taking into account research studies that provide consistent evidence for areas of improvement. The main changes focus on reducing transport duration, and feed, rest and water intervals. These regulations will play a major role in how cattle transport is conducted in Canada and in animal welfare outcomes.

The objective of this paper is to provide a summary of past and current research relevant to the impacts of transport on Canadian dairy cattle. The following sections will discuss factors contributing to increased susceptibility for poor welfare outcomes during and after transport in unweaned calves and cull cows. In addition, the effects of transport distance; feed, rest and water intervals; and extreme environmental conditions on the same types of animals will be reviewed. Changes to the transport regulations will be outlined with a focus on how they will impact calf and cull cow transport. Finally, recommendations will be given regarding best management practice for these more vulnerable types of cattle. Continued concern and awareness of welfare-conscious transportation practices will improve the health and well-being of dairy cattle while increasing consumer confidence and sustainability of dairy-derived meat products.

▪ **Animal Factors Contributing to Poor Welfare**

The following section reviews transport studies with emphasis on dairy animals where possible. Recent beef calf and cull cow studies have also been included in this review as they may provide further insight into the impact transport has on young calves and older cows of reduced condition. The major difference between dairy and beef calf studies is the calves' age at time of transport. Beef calves are usually transported at 5–8 months of age and may be weaned a significant number of days before they are transported off farm whereas dairy bull calves are transported as early as 5–30 days of age and are usually not weaned at the time of transport. Most transport studies assessing cull cows do not distinguish between beef and dairy breeds but indicate a cow that is no longer economically viable.

Calves

Young calves are more prone to transport related stress, morbidity and mortality than are adult cattle (González, 2012c; Knowles, 1995), likely due to a combination of factors including reduced ability to thermoregulate (Eicher, 2001; Knowles et al., 1997; Knowles, 1999), minimal fat reserves for energy, an immature immune system (Knowles et al., 1997), and reliance on a milk-based diet that may alter metabolism (Schrama et al., 1992), thirst and hunger (compared to calves on solid feed). In addition, young dairy calves (between 1 and 30 days of age) are typically not weaned before marketing and may be transported to veal production units after assembly from several different farms (Wilson et al., 2000), increasing their exposure to pathogens and mixing with unfamiliar animals.

Cull Cows

The culling of cows is a humane and essential practice guaranteeing that the cows are not retained beyond their productive and healthy life, thus ensuring food safety and eliminating the chance that they may become unfit for transport (Rezac et al., 2014). According to the Canadian Dairy Information Centre (CDIC, 2019) the average culling rate for cow herds in 2018 was 31.6%. Although 18% of the culling total was attributed to reproductive and difficult calving issues, an even larger proportion (29.3%) of the total was due to health (mastitis, foot and leg problems, sickness, injury, displaced abomasum, milk fever, arthritis and pneumonia) and age-related issues. While not specifically listed in the CDIC report, cancer eye and poor body condition are common reasons for culling. For example, a U.S. benchmark study of

cull dairy cows arriving at a large slaughter facility found that 9% had one or more health problems (severe lameness, low body condition score (BCS), poor udder condition, prolapse, cancer eye, illness, wounds, active parturition, nervous system disorder, and non-ambulation) that would be considered a welfare problem (Edwards-Callaway et al., 2019). Similarly, Harris et al. (2017) found that 9% of dairy cows were very thin, 43% had some defect (i.e., swollen joint) and 23% were lame. Edwards-Callaway et al. (2019) noted that the majority of compromised conditions documented in those industry surveys would have been present prior to transport. Consequently, advanced age, reduced health and marginal energy reserves are the main reasons why cull cows are more vulnerable to transport stress.

The above findings are consistent with other studies documenting some compromise in cull cow condition prior to transport that could potentially lead to reduced welfare during transportation. For example, Dahl-Pedersen et al. (2018a) reported that close to 75% of cull dairy cows assessed immediately before shipping to market deviated from normal according to at least one clinical measure of compromise. They found that 31% of cows were lame, 20% showed signs of mastitis and 22% had non-severe wounds; however, very few were considered unfit for transport. In a related study, Dahl-Pedersen et al. (2018b) concluded that the clinical condition of cull dairy cows deteriorated during transport based on increased lameness and milk leakage following transport compared with that prior to loading onto the truck. Rezac et al. (2014) also found that the prevalence of gross pathologic lesions in cull cows (87% Holstein) assessed at slaughter was 18.5%, 10% and 10.3% for liver abscesses, ruminal lesions (associated with ruminal acidosis) and lung lesions (associated with bovine respiratory disease), respectively. Therefore, it is not surprising that cull cows can suffer a compromised physical state including weakness, hypothermia, recumbence and death during and after transport (Schwartzkopf-Genswein and Grandin, 2019). These conditions can be exacerbated by the fact that cows are transported long distances (> 400 km) from their farm of origin to processing plants or auction markets where they may be held until a sufficient number of cows are assembled to fill one truck. They may even be resold which means they could remain within the marketing system for an extended period of time, further deteriorating their condition. A recent Canadian study found that cull cows spent 79.6 ± 1.9 hours in the marketing system before being slaughtered (Stojkov and Fraser, 2019). Approximately 43% were in transit for 4–6 days and 4% for 7–9 days, which included delays at auctions or assembly yards. The same study reported a reduction of 0.4 in BCS between the farm and the time of slaughter. Although the prevalence of lameness did not change before compared to after transport, there was a large increase (33%) in milk accumulation and udder inflammation. Collectively, these studies show a clear need for extra vigilance by the producer and transporter to ensure that cull cows are fit for transport and that stressors are minimized as much as possible to avoid negative welfare outcomes.

▪ Non-Animal Factors Contributing to Poor Welfare

The transport process has the potential to impose significant challenges for cattle including physical and psychological stress, injury and even death (Schwartzkopf-Genswein and Grandin, 2019). The conditions of transport can vary substantially according to its duration, when the animals had last been rested, fed and watered, and the environmental conditions under which the transportation takes place. Although there are several other factors (driver experience, trailer compartment, animal handling, road conditions, loading density, and regulations) that can affect the quality of the transport, only the factors previously listed will be covered in this paper. The effects of these factors on animal welfare outcomes are not mutually exclusive, and multiple stressors can have an additive effect imposing even greater challenges for the cattle.

Transport Duration

The total amount of time (duration) that an animal is on a truck has been referred to as the transport continuum. This continuum comprises several events including loading, waiting to depart after loading, driving and stationary periods, waiting to off-load and any experienced delays in between (Schwartzkopf-Genswein and Grandin, 2019). A transport survey conducted by our research group found that cattle (all types) shipped within and outside of Alberta took on average 15.9 hours, and up to 45 hours to reach their

final destination (Gonzàlez et al., 2012a). However, only 1% of the cattle tracked in that study were classified as cull with no distinction based on breed or gender. An Ontario study reported average shipping times of 4.6 hours and up to 68.3 hours for all cattle types shipped to slaughter with no specific mention of young calves or cull animals (Warren et al., 2010). A recent study assessing cull dairy cow conditions at slaughter found that approximately 16% of cattle were transported 1,100 km (approximately 11 hours in transit based on a driving speed of 100 km/ hour not including other events within the transport continuum) from farm to abattoir (Stojkov and Fraser, 2017).

Numerous research studies have shown a strong association between increased transport duration and decreased animal welfare (Schwartzkopf-Genswein and Grandin, 2019). This is mainly due to the length of time animals are without feed, water and rest, which have obvious effects on weight loss (shrink), dehydration, hunger, fatigue and stress. The most significant weight loss occurs within the first 12 hours of transport resulting from the elimination of urine and feces as well as water through breathing and evaporation (Barnes et al., 2004; Coffey et al., 2001). Any weight loss occurring after that point in time is believed to be more detrimental to welfare since it is associated with mobilization of the animal's energy reserves. As stated earlier, dairy calves and cull dairy cows would be most affected by this stage of shrink because of their already low fat reserves compared with other cattle types.

Our previous studies showed that cull cows were at higher risk of poor welfare when transported > 400 km based on having the greatest likelihood of becoming lame, non-ambulatory or dying in transit compared with other cattle types (calves, feeders and fats; González et al., 2012c). Journeys > 400 km also caused greater shrink in cull cows compared with that in fat cattle transported the same distance (González et al., 2012b). A recent Danish study assessing cull cows transported an average of 187 minutes (ranging between 32 and 510 minutes) found that one-fifth of them became lame or got more lame during transport (Dahl-Pedersen et al., 2019b). There was also a significant increase in the proportion of lame cows after transport (41%) compared with before transport (31%). The risk factors associated with the increased lameness following transport included low BCS (< 2.75), early or late lactation (< 100 or > 300 days in milk (DIM)), digital dermatitis in the hind feet and pelvic asymmetry. The same study reported increased milk leakage (1% vs. 17%) and wounds (22% vs. 34%) before and after transport, respectively. Animals that were < 100 DIM and transported > 100 km were more likely to be observed with milk leakage. The authors concluded that even transports as short as 8 hours can affect welfare outcomes in cull dairy cows.

Transport durations exceeding 30 hours significantly increased the chance of cattle becoming lame, non-ambulatory or dying, regardless of breed or age (González et al., 2012d). Cattle losing 8% of their body weight also had an increased risk of death (González et al., 2012b). In addition, several studies showed that journeys > 24 hours increased physiological indicators of fatigue, dehydration and mobilization of energy reserves (Tarrant et al., 1992; Warriss et al., 1995) leading the authors to conclude that time on the truck should not exceed 1 day. The most recent National Animal Health Monitoring System (NAHMS) survey of the U.S. dairy industry reported 37% of farms sent their cows directly to slaughter, which reduced transport duration as well as the total time required to get to the abattoir (USDA, 2014). For example, 50% of slaughter-direct cows were transported < 80 km, 38% were transported between 80 and 400 km and 11% were transported > 400 km. On the other hand, 78% of non-direct (auction) cows travelled < 80 km and 22% were transported between 80 and 400 km. Following the trip to the auction, most of these cows would be transported to an abattoir. The survey indicated that cows were shipped an average of 6.7 hours, with maximum durations being > 24 hours. Edwards-Callaway et al. (2019) cautioned that the transport durations reported in that study only represented the final leg of the cow's journey, not any prior transports, and therefore are likely an underestimation of their actual transport continuum. Most studies assessing the effects of transport duration on young calves looked at the combined effect of transport duration, and feed, water and rest intervals; these will be discussed in the following section.

Feed and Water Deprivation

Currently, transport trailers used to haul livestock in North America are not equipped to provide animals with access to feed and water while they are on board. Consequently, when cattle are confined within the truck, they are subject to periods of feed and water deprivation dictated by the length of time they are transported. Although cattle could gain access to feed and water once they are off-loaded, it does not always mean they do (many auctions do not provide feed, but all provide water) which can further exacerbate any negative effects. In contrast, some European trailers (Pezzaioli, Carrrozzeria Pezzaioli Ltd., Monticharai, Italy) have been designed such that cattle can eat and drink during their journey. Until similar trailer designs are available for use in Canada, the effects of feed and water deprivation will remain an important factor in the welfare of all transported cattle.

The main effects of feed and water withdrawal, regardless of cattle type, are weight loss and the potential for hunger, dehydration and stress that can increase as the time between feeding or drinking opportunities increases. Feed withdrawal for 12, 24, 48, and 96 hours reduced live weight in beef calves by 6, 8, 12, and 14%, respectively (Schwartzkopf-Genswein and Grandin, 2019). In the case of young calves, some studies have shown that long journeys resulted in the mobilization of energy reserves (Mormède et al., 1982; Nielsen et al., 2011; Bernardini et al., 2012; Fisher et al., 2014) and low blood sugar (hypoglycemia) (Bernardini et al., 2012; Nielsen et al., 2011; Fisher et al., 2014). Feed and water deprivation can also alter normal patterns of feed consumption and digestion. For example, rumination was reduced and almost disappeared within 24 hours of feed and water removal from sheep (Welch and Smith, 1968). It has also been speculated that restricting access to feed and water reduces the fermentation capacity of the rumen for 5 days or more (Hutcheson and Cole, 1986). Collectively, these studies suggest that calves could be at greater risk of hunger, fatigue, weakness and injury during transportation as a result of restricted feed and water access.

Interestingly, numerous studies in young calves reported no signs of dehydration following prolonged periods of transport-associated water deprivation. For example, dairy calves ranging in age from 5 to 21 days that were transported and fasted between 18 and 30 hours showed no signs of dehydration (Kent and Ewbank, 1986; Todd et al., 2000; Fisher et al., 2014). This is particularly intriguing given that they consumed a milk-based diet before transport. More research needs to be conducted regarding these findings so the outcomes can be better understood.

Overall, feed withdrawal for up to 30 hours appears to result in more indicators of poor welfare than does water withdrawal. We are still not sure what level of energy mobilization can affect welfare outcomes. In addition, few studies have assessed feed and water withdrawal on cull cows.

Rest Intervals

Long-haul transport causes muscle damage (indicator of muscle exertion and fatigue) in young calves (Grigor et al., 2001; Bernardini et al., 2012; Fisher et al., 2014). This is a result of increased physical effort required to maintain balance during braking, cornering and accelerating over long periods of time. Although no studies have been conducted assessing fatigue in cull cows, it is very likely that they experience similar or even more severe effects because of increased health issues such as lameness, known to worsen over the course of a journey. Furthermore, cows are typically under loaded in trailer compartments compared with calves or feeder cattle (González et al., 2012d) (especially in the nose and top back compartment, known as the doghouse) due to axle weight restrictions, which may add further challenges to maintaining balance.

A rest period provided to cattle in the middle of a long journey allows them to lie down and consume feed and water, mitigating negative outcomes such as fatigue, hunger and dehydration. The need for the provision of a rest has been supported by studies showing young dairy calves (less than 30 days of age) increase lying time on the truck with increasing time in transit (Cockram and Spence, 2012) and they experience fewer losses of balance and trampling following a longer (12 hours) compared with a shorter

(1 hour) rest (Grigor et al., 2001). Edwards-Callaway et al. (2019) noted that cull dairy cows typically transported between 7 and 24 hours to slaughter would rarely stand for that length of time given the choice; unfortunately, the ability to lie down and rest on a truck is extremely limited. One study concluded that cows deprived of the chance to feed and rest for as little as 3 hours will select rest over feed (Metz, 1985).

Our research group has conducted two studies assessing the effects of different lengths of rest stop following 15 hours of transport under Canadian commercial conditions in newly weaned beef calves (Marti et al., 2017) and following 12 and 36 hours of transport in conditioned beef calves (Melendez et al., 2020). The studies looked at weight loss, indicators of stress, muscle damage, hunger and dehydration, and behaviour after off-loading. The first study found that rest periods ≥ 10 hours did not prevent short- and long-term stress after transport in newly weaned calves. The second study found that 36 hours of transport (compared with 12) had greater effects on the calves (weaned and vaccinated) including lower weight, average daily gain and intake, and increased shrink, inflammation and fat metabolism. Surprisingly, there were no effects on any indicators of welfare measured among calves given 0, 4, 8, or 12 hours of rest with the exception of fat metabolism, which was greater in calves provided no rest (no food). The study concluded that preconditioning (implying good calf condition) may have more impact on calf welfare than a rest period on journeys exceeding a total (combined time before and after rest period) of 16 hours. A study conducted at a commercial rest stop site in Canada reported that in the first hour after off-loading, cattle (all types) transported an average of 30 hours were observed to eat more frequently than rest, however, after the first hour they were observed to rest (lie down) more than eat (Ross et al., 2016). These studies help to provide guidance regarding optimal lengths of rest (relative to the distance transported) as well as which aspect of the rest is more important than others.

In order to fully understand the value of rest periods on dairy cattle welfare, all factors including loading and unloading, mixing with other animals, novel feed and water, and quality and accessibility of the feed, water and rest areas must be considered. More research assessing the effects of rest periods on newly weaned calves, unweaned calves, and cull cows is urgently needed.

Environmental Conditions

Unlike some European trailers, transport vehicles in Canada are not climate controlled (Schwartzkopf-Genswein and Grandin, 2019). Instead, temperature and humidity (microclimate) within the trailer is managed using passive air flow via perforations of varying sizes, dimensions and patterns along the sides and roof of the trailer. Therefore, extreme cold or hot environmental conditions can have direct impact on the trailer microclimate as well as the cattle held within them (Schwartzkopf-Genswein and Grandin, 2019). A survey conducted by our research group found that temperature extremes of -42°C and +45°C were recorded over an 18-month period when cattle were transported within and outside of Alberta (González et al., 2012a). Although cattle are homoeothermic (ability to adapt temperature change) a period of acclimation (days or even weeks) is needed for them to adjust because metabolic changes associated with acclimation take time. Consequently, abrupt changes in ambient temperature could have greater negative impacts (heat or cold stress) on cattle than consistently hot or cold conditions. This is even more critical for young calves because they have limited ability to thermoregulate. In addition, dairy calves and cull cows are usually housed indoors or with shelter such that exposure to extreme temperatures is minimized. Therefore, dairy cattle are more likely to experience abrupt changes in temperature when they are transported because their ability to adapt to high or low temperatures within a trailer is limited.

Cattle cope with heat and cold stress by adjusting their behavioural and physiological responses. In the case of heat stress, cattle respond by panting, sweating, seeking shade, and increasing respiration rate and peripheral blood flow (West, 2003). Likewise, cattle experiencing cold stress shiver to maintain their core body temperature and may seek shelter away from drafts. This increases their energy demand, further depleting energy reserves in cattle that are already feed and water deprived. Some or all of these coping strategies can be restricted during transportation making it more difficult for the animal to dissipate

or reserve heat, particularly if they are very young or very old (Grandin, 2001).

Trailer microclimate can vary substantially based on air temperature, humidity, loading density, use of bedding, airflow, and animal respiration, sweat, and excretions (Schwartzkopf-Genswein and Grandin, 2019). Although the effects of trailer microclimate have not been well studied in unweaned calves and cull dairy cows, there is some evidence that it can negatively impact animal welfare if too high or too low. For example, death in commercial cattle (all types) transported > 400 km increased significantly when ambient temperatures went below -15⁰C while the probability of becoming non-ambulatory increased when temperatures rose above 30⁰C (González et al., 2012d). In addition, ambient temperature during the summer had a greater impact on trailer microclimate than did loading density (Goldhawk et al., 2014).

Producers and transporters can employ various strategies to moderate cold environmental conditions including the use of bedding and boarding. Bedding provides insulation for calves and cows during cold weather and its use is required on journeys over 12 hours (CFIA, 2019). One study showed that young dairy calves increased the time they spent lying down when bedding was provided compared with when it was not (Jongman and Butler, 2014) suggesting bedding also increases calf comfort. Boarding is the use of solid pieces of plastic, fiberglass or plywood to block (either fully or partially) perforations within the trailer walls. Boards are used during cold weather to reduce air exchange between the outside (colder air) and inside of the trailer resulting in warmer trailer microclimates. A study assessing the effects of winter transport on cull beef cows found that boarding increased ventilation when trailers were in transit but decreased ventilation (increasing internal temperature and humidity) during stationary periods (Goldhawk et al., 2015).

Strategies to reduce heat stress include reducing stationary periods, transporting during the coolest rather the hottest part of the day, and parking in shade (Schwartzkopf-Genswein and Grandin, 2019). Future studies need to assess the effects of provision of water on trailers in reducing heat stress.

▪ Transport Regulations

As of February 20, 2020, the amendments to the Transportation of Animals requirements under the Health of Animals Regulations (CFIA, 2019) will take effect. The last changes to the regulations were made over 40 years ago in 1977.

The most significant changes for cattle include a reduction in the maximum allowable transport duration from 48 to 36 hours (with no rest stop) before they must be unloaded for food, water and rest, and an increase in the mandatory rest stop time from 5 to 8 hours (CFIA, 2019). An exception to this is for unweaned calves that cannot be transported for more than 12 hours before having a mandatory rest of 8 hours (CFIA, 2019). Another major difference between the old and new regulations is that transport duration includes the time required to load and unload the cattle since this is also time that the animals must remain on the truck without feed, water or rest.

The regulations also define animal fitness for travel. A compromised animal refers to "an animal with reduced capacity to withstand transportation but where local transportation with special provisions will not lead to undue suffering" (CFIA, 2019). Unfit cattle refer to "animals with reduced capacity to withstand transportation and where there is a high risk that transportation will lead to undue suffering" (CFIA, 2019). Unfit cattle may only be transported for veterinary treatment or diagnosis. Examples of unfit cattle include downer animals, and cattle with cancer eye or bone fracture. Currently there is little financial disincentive for farmers to stop shipping compromised dairy cows because slaughter plants stand to get significant financial returns if these animals survive their journey to the plant (Edwards-Callaway et al., 2019). Consequently, shipping compromised cull cows to slaughter remains common despite being a substantial welfare problem.

The current revisions to the transport regulations were based on several years of consultation with veterinarians, producers, livestock transporters, scientists and the public. The main goal of the revisions

was "to improve the well-being of animals during the entire transportation process, keeping in mind Canada's geographic size and the time required to travel between locations" (CFIA, 2019). The current amendments took into consideration several factors including public concern for lengthy transport durations, limited feed, rest and water intervals, incorporation of current research and increased pressure for compliance with international standards such as the OIE. Currently, the EU regulations specify a maximum of 14 hours of transport before a required 8-hour rest (European Commission, 2005) while the U.S. specifies a maximum of 28 hours (USDA, 1997) with no mention of a feed, water and rest period. Future changes to the transport regulations need to remain science-based rather than emotion-based to ensure animal welfare and not political pressure is the main consideration.

▪ Conclusions

Unweaned dairy calves and cull dairy cows have unique challenges regarding transportation because of their reduced condition, health and ability to cope with stress. Consequently, producers and transporters must be more vigilant about how these cattle are managed throughout the transport process so poor welfare outcomes are minimized. This requires that producers have access to science-based information regarding the welfare impacts of relevant transport related factors (i.e., animal type, transport duration, environment, regulations). The studies outlined above provide evidence that calf and cow welfare can be improved by ensuring the animals are in good condition at loading. In addition, longer duration transports with restricted access to feed, water and rest, and under extreme environmental conditions, increase indicators of reduced welfare. At this time little is known regarding what level of indicators represent a true welfare concern. This is one reason why animal outcome measures such as morbidity and mortality will remain important. Continued assessment of combined animal and non-animal factors will be necessary to further guide animal management aligned with industry and societal demands. It is important that research rather than emotion continues to drive any regulation changes going forward.

Acknowledgements

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Three Ways to Lose Money on the Farm: A View from the Udder

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▪ Take Home Messages

Three things to improve the bottom line for dairy producers:

- ‣ Properly stimulate teats before milking to reduce bimodal milk letdown.
- ‣ Maintain and properly operate automated cluster removers (automatic take-offs) to reduce overmilking.
- ‣ Use individual cow somatic cell counts rather than bulk tank or herd average somatic cell counts to monitor subclinical mastitis in a herd.. New infection rates and proportion of infected cows are key indicators, especially for early lactation cows.

▪ Milking Efficiency: Udder Prep and Bimodal Milking

A large majority of dairy farms routinely evaluate and maintain milking equipment; however, milking efficiency should be considered from two other points: the amount of time the milking cluster is attached to the udder (unit on time) and the percent of unit on time that milk flow is near maximum. When milk isn't flowing while the unit is attached, the unit is inefficient, and more importantly, it damages the teat tissue, which may increase the risk of mastitis and decrease milk yield. Two problems that lead to poor milking efficiency are milking routines that don't achieve consistent milk letdown and overmilking. Either one of these problems can leave cows 'high and dry' for a period of time, and expose teats to high vacuum levels. In this section, I will discuss poor milk letdown or what is called bimodal milking.

When teats are being stimulated before milking, nerves carry an 'electric signal' to the brain. The brain then releases oxytocin into the blood which travels to the udder. It takes about one to two minutes for oxytocin levels to increase in blood to optimally facilitate milk letdown toward the teats. The two important points for oxytocin release are sufficient stimulation (at least 10 seconds of actual physical touching) of the teats and the duration of the latency period, that is, the time interval between when teats are first stimulated until the cluster is attached. Unfortunately, with increasing herd size, the number of cows that can be milked through the parlour per hour, or parlour turnover rate, is thought by many dairy producers to be a choke point of profitability. Thus, parlour efficiency is emphasized at the expense of milking efficiency.

How would you know if your routine is minimizing bimodal milking? One method is to measure milk flow with digital vacuum recorders (VaDia®, Biocontrol NA). VaDia units record vacuum in the mouthpiece chamber (at the opening of the liner) and in the cluster. VaDia units don't measure milk flow directly, but give us a snapshot on milk flow. A simple way to interpret VaDia results relative to milk flow is:

High Milk Flow = Low vacuum in the liner or cluster
Low Milk Flow = High vacuum in the liner or cluster

VaDia units measure vacuum levels at four different places on the cluster simultaneously: front and rear liners, near the cluster, and in a short pulsation tube (Figure 1).
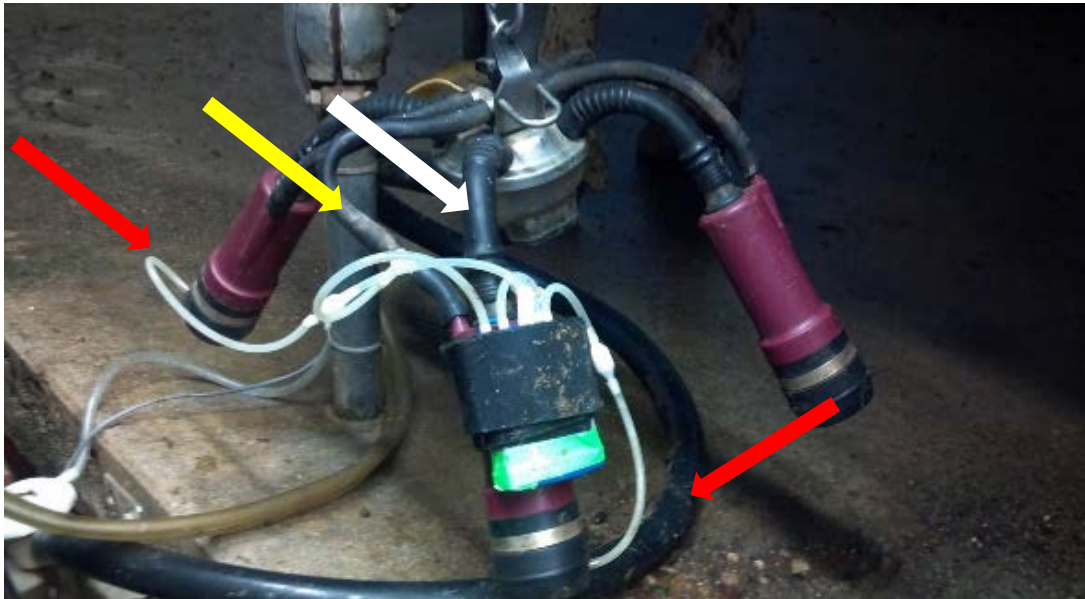
Figure 1: VaDia units measure vacuum in the mouthpiece of a front and rear liner (red arrows), near the cluster (short milk tube, white arrow) and in a short pulsation tube (yellow arrow).
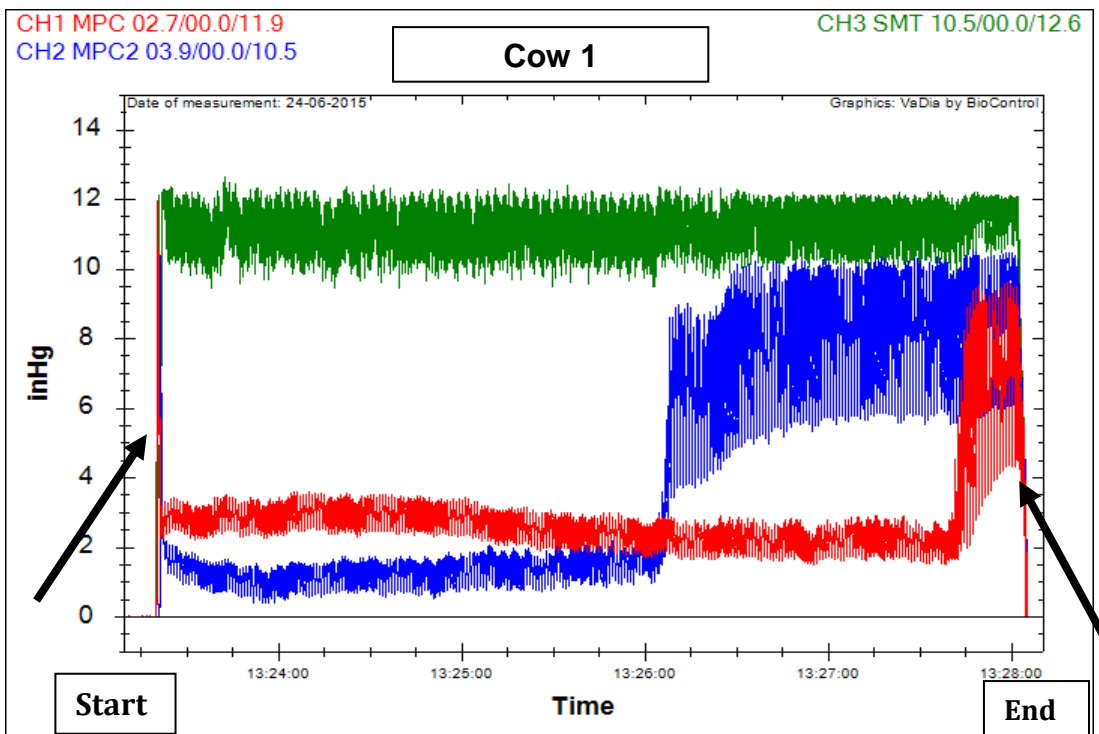
Figure 2: VaDia plot depicting that Cow 1 was ready for milking. Vacuum (red line - rear teat; blue line - front teat; green line - liner mouthpiece and cluster) in liner mouthpiece drops to low level immediately after unit is attached. Cow was ready to milk!!! Arrows on the left and right indicate start and end of milking, respectively.

Figure 2 shows a cow that was ready to milk. The vacuum in the liner mouthpiece near the teat (red and blue lines [lower lines]) dropped quickly (< 10 seconds after the unit was attached) and remained low until each teat was finished milking. The front quarter (blue line) finished before the rear quarter (red line).

What about cow 2 (Figure 3)? Vacuum in the liner mouthpiece and cluster (green line) decreased, but then increased to near maximum levels, and finally decreased again. This cow was not ready to milk, milk flow was low for more than a minute after the milking unit was attached, signifying bimodal milk letdown.
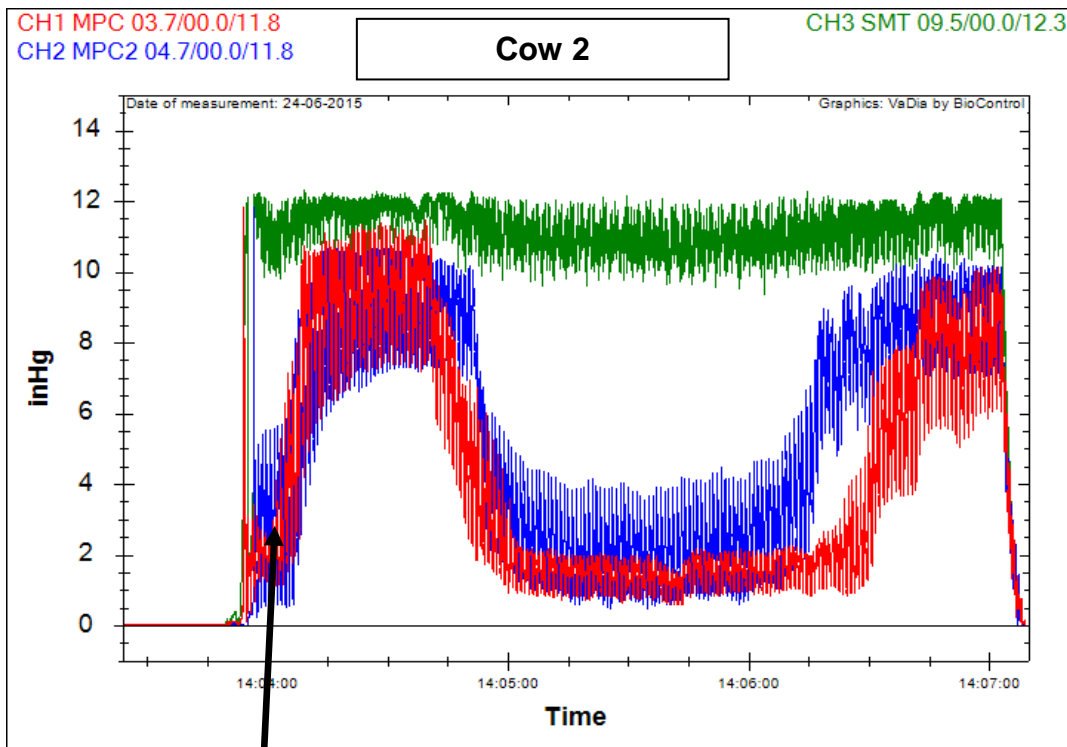
Figure 3: VaDia plot depicting that for Cow 2, vacuum (red line - rear teat; blue line - front teat; green line - liner mouthpiece and cluster) decreased, increased, and then decreased again. This is bimodal milking indicating poor milk letdown.

For information on how VaDia recorders describe milk flow, visit the Quality Milk Alliance article site listed below.

http://qualitymilkalliance.com/2016/01/07/let-the-cows-score-the-milking-protocols/

To view the graphs online, visit the QMA website at these links:

http://qualitymilkalliance.com/2018/05/01/how-is-your-milking-efficiency-part-1/

http://qualitymilkalliance.com/2018/11/13/how-is-your-milking-efficiency-part-2/

So how does bimodal milking relate to milking efficiency? For Cow 1, milk was flowing for about 4 minutes and 30 seconds of the total unit on time of 4 minutes and 45 seconds. Thus, the efficiency of this milking was 95%. For Cow 2, milk was flowing for about 2 minutes of the total 3 minutes and 15 seconds the unit was attached, or a milking efficiency of about 60%. Why does this matter?

When teats are subjected to high vacuum (as in the case for cow 2) blood is congested within the teat and the diameter of the teat canal decreases, which then decreases milk flow (Penry et al., 2018). This can be detrimental to the heath of the teat tissue. Additionally, the longer it takes for milk to start flowing after cluster attachment, the more milk is lost from that milking (Erskine et al., 2019). Cows that have bimodal letdown have the same unit on time as cows that have normal milk letdown. That is, cows that have a poor start to milking don't "catch up" with milk yield by taking longer to milk. This may seem illogical, but the changes that have occurred in the teats from high vacuum result in lower milking efficiency and less milk.

A delay of one minute causes more than a 3 kg loss of milk—in just one milking.

▪ Milking Efficiency: Overmilking

After a milking is completed, the vacuum should be turned off and the cluster removed from the cow as soon as possible. Removal of the units is usually done by automatic detachers (take-offs) that rely on sensors that record milk flow between the cluster and milk pipeline. When milk flow remains at a low level (about 0.2 kg/minute) for a few seconds, the vacuum shuts off and the cluster is detached from the cow. However, if herds lack detachers, or if operators intervene in deciding when a cow is done milking, units may be removed manually. Units should be removed no more than 15 seconds after milking is done and units that stay on more than 30 seconds are considered to be overmilking.

How do you recognize overmilking? A simple way is to hand strip the udder after the unit is detached. A cup of milk should be easily attained without overworking the teats. As mentioned previously, milk flow can also be estimated with digital vacuum recorders.

In the example of Cow 1 above, at the end of milking, although the front quarter was done milking for nearly two minutes before unit take-off, the rear quarter continued to milk until about 15 seconds before unit take-off and thus was not overmilked. Cluster vacuum (green line) continued to fluctuate in a range of about two inches of mercury, or about seven kPa during this time, suggesting milk flow for the cow was continuous.

What about Cow 3 (Figure 4)? Milk flow started soon after cluster attachment, but vacuum in both the front and rear quarters increased to near maximum and plateaued two minutes before unit take-off. Also, the cluster vacuum (green line) increased to maximum vacuum during this time with little variation, which suggests little or no milk flow. This cow was overmilked.
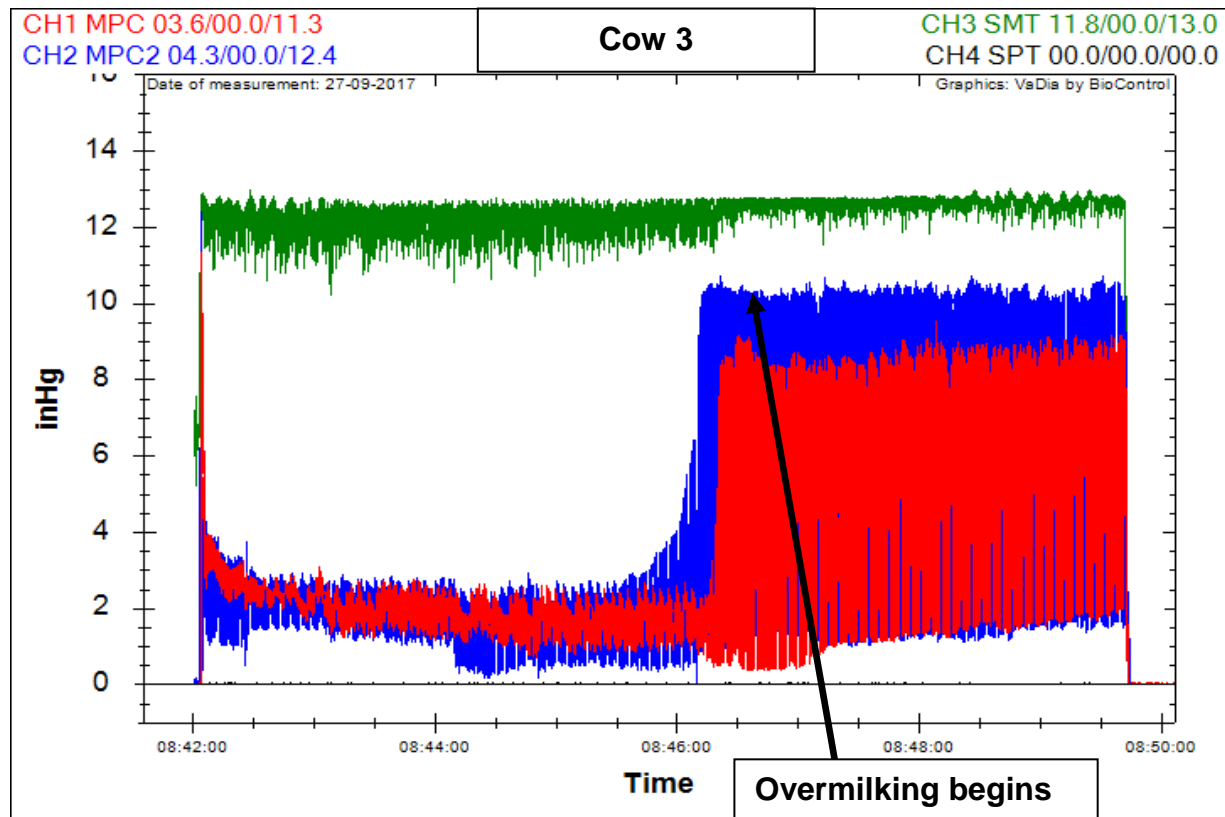
Figure 4: VaDia plot depicting that milk flow started soon after cluster attachment, but vacuum in front and rear quarters (red line - rear teat; blue line - front teat; green line - liner mouthpiece and cluster) increased to near maximum and plateaued 2 minutes before unit take-off. Cluster vacuum (green line) increased to maximum vacuum with little variation, suggesting little or no milk flow. This cow was overmilked.

How does overmilking affect milking efficiency? As described earlier for Cow 1, milk was flowing for about 4 minutes and 30 seconds of the total unit on time of 4 minutes and 45 seconds, or a milking efficiency of about 95%. For Cow 3, milk was flowing for about 4 minutes and 30 seconds of the total milking time of 7 minutes and 30 seconds, or a milking efficiency of about 60%. Why does this matter?

Unnecessary high vacuum is never good for teat health. Additionally, overmilked cows have long unit on-times. This reduces cow throughput in the parlour and extends the length of time needed to milk a herd. Also, slower parlour efficiency requires cows to stand in the holding pen and parlour for longer periods of time, reducing the time they spend resting and eating.

Reducing overmilking and decreasing unit on time by just a couple of minutes per cow decreases the duration of a milking shift by at least 45 minutes in a 400 cow dairy, or the total milking time by about two hours per day for a three times-a-day milking herd.

The most common causes of overmilking are lack of automatic detachers, automatic detachers that are not operating properly, or the tendency to have milking operators place the auto-detach mode to manual. This often occurs when operators are frustrated with detachers that aren't functioning well, or the mistaken belief that cows need to be milked out dry. Cow 4 (Figure 5) is a VaDia plot of overmilking that occurred from re-attaching the cluster after the cow was done milking. Note the drop in all vacuum lines while the unit was off the cow.
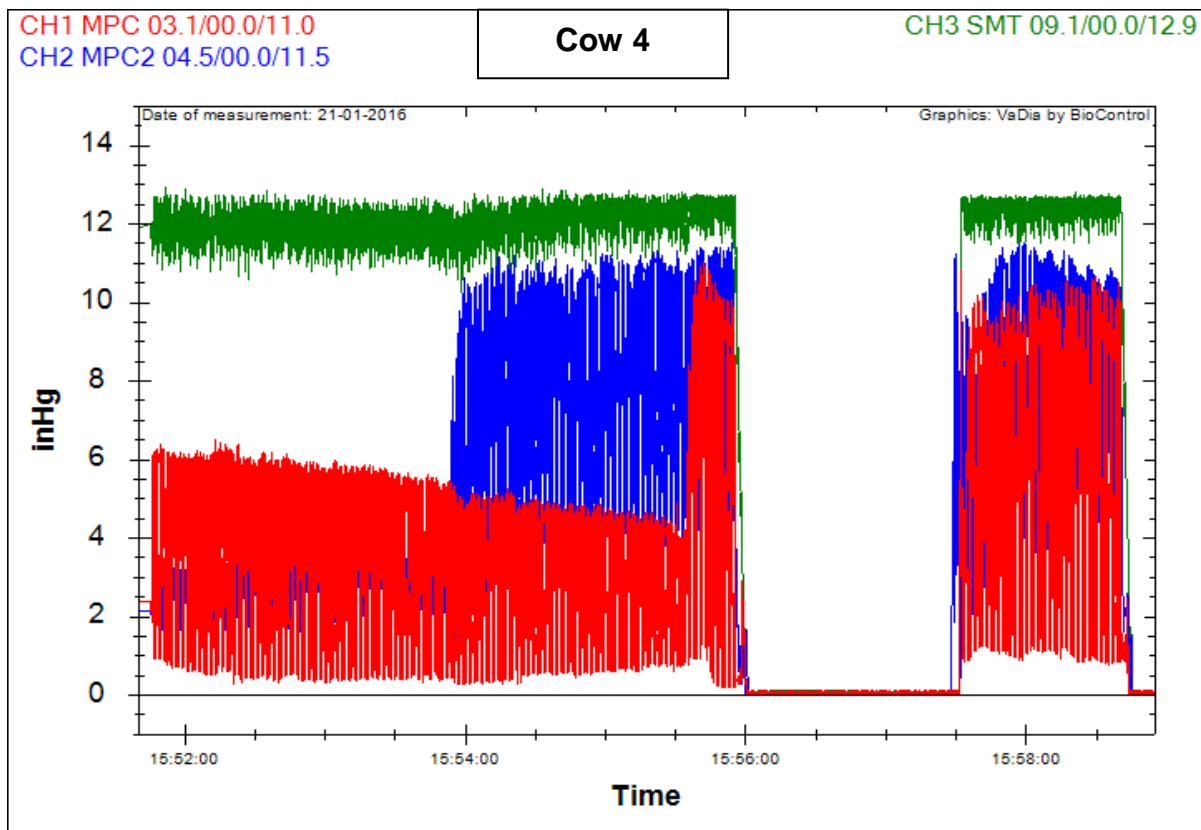
Figure 5: VaDia plot of overmilking that occurred from re-attaching the cluster after the cow was done milking. Note the drop in all vacuum lines while the unit was off the cow.

- ## Herd Average (Bulk Tank) Somatic Cell Counts: The Whole Mastitis Story?

Somatic cell counts (SCC) are excellent indicators of subclinical mastitis. Somatic cells are mostly leukocytes (white blood cells), and they increase in milk almost entirely because of microbial infections. Thus, SCC are used widely by dairy producers and processors as a measure of milk quality. Higher SCC in milk decreases shelf life in the grocery store and decreases yields of cultured dairy foods such as cheese.

Dairy producers routinely use bulk tank (or DHI average) SCC to track mastitis in their herd. There is a strong correlation between the higher proportion of infected cows (mastitis) and herd SCC, and an increase in herd SCC equates to lost production of milk. Herds with SCC that are consistently below 150,000 cells/mL will have less than 15% of their cows infected with subclinical mastitis, whereas herds with SCC consistently near 500,000 cells/mL will have nearly half of their cows with mastitis. But interpreting the level of mastitis in a herd using herd SCC from bulk tanks or DHIA records requires a little insight. The key words for this interpretation are consistent and average.

Herd SCC can vary from day to day and week to week. Additionally, bulk tank SCC are not indicative of the entire mastitis picture because treated cows with clinical mastitis may have their milk withheld from market because of drug residue concerns; thus, they do not contribute to the herd bulk tank SCC. Bulk tank SCC rely on two factors: the number of cells and the kg of milk that each cow yields into the milk supply. Thus, cows that are higher milk producers than their herd mates will contribute more to the herd SCC. High-producing cows with low SCC are beneficial for herd milk quality, but high producing cows with

mastitis will have a negative impact on herd milk quality.

Table 1 lists the percent of the total herd SCC (weighted DHI average) that is contributed by (1) the three highest SCC cows in each herd, or (2) the top 2% (highest SCC) of cows in each herd for 50 dairy farms from Michigan, Ohio, Indiana, and Pennsylvania. The data are further divided into herds with less than 200 cows (18 herds), 200 to 499 cows (20 herds), and 500 or more cows (12 herds). As would be expected in smaller herds, just three cows with high SCC cows can impact the herd SCC dramatically, averaging 32% of the SCC contribution from all cows in the herd.

As herds get larger, the impact from three individual cows is smaller. However, what does not change with herd size is the impact that just a small percent of the herd (2% of the milking cows) has on the total herd SCC. For example, even in herds with more than 500 milking cows, the highest 2% SCC cows contributed an average of 35% of the total herd SCC.

Whether you are milking 70 or 3,000 cows, herd milk quality is affected by a small proportion of animals.

Table 1. Contribution to total herd SCC (weighted DHIA average) by small populations of cows within herds (n=50 herds). None of the herds had SCC more than 400,000 cells/mL.

Herd Size (# of milking cows)	Percent of total herd SCC	
	Highest 3 SCC cows in herd	**Highest 2% SCC cows in herd**
< 200	32	30
201 – 499	22	32
≥ 500	13	35

Consistency must also be considered when using herd-level SCC. Somatic cell counts vary considerably and are affected by daily changes in the herd relative to proportions of fresh cows to cows that are culled or dried off and younger to older animals, cows with infections of long duration, cases of clinical mastitis, and to some extent sampling and laboratory variation.

Care should be used to assess meaningful changes in herd average SCC based solely on one or two months of records. As described in Table 1, all herds can see deviations in SCC from the infection dynamics of just a small percentage of cows. When considered over longer periods of time, herd average SCC correlate well with the number of infected cows within the herd. However, a better indicator of trends in subclinical mastitis is the distribution of individual cow SCC rather than the average herd SCC.

In general, cows with linear SCC scores of 4 or greater are likely to be infected. Thus, a key indicator for milk quality might be to follow the proportion of cows with linear SCC scores of less than 4, or non-infected cows.

Table 2 is a six-month history of a herd with a DHIA herd average SCC that exceeded 400,000 cells/mL. Overall, the herd average SCC trended downwards over the period. However, the overall increase in the percentage of non-infected cows (linear score < 4) was very modest, only 65 to 67%. Additionally, the apparent decline in subclinical mastitis that occurred from month 2 to 3 (as measured by herd SCC decreasing from 334,000 to 281,000 cells/mL), resulted in an increase of infected cows by 5%. Thus, dairy managers and veterinarians should track the distribution of SCC scores as well as average SCC to monitor quality milk.

Table 2. Herd average SCC and individual cow linear SCC score over an 8 month period.

		Linear SCC Score			
Month	**Herd SCC** (cells/mL)	0-3* (< 142,000)	4 (142-283,000)	5 (284-565,000)	6-9 (>565,000)
---	439,000	65	12	7	16
1	272,000	69	10	9	12
2	334,000	69	11	7	13
3	281,000	64	14	11	11
4	169,000	72	15	8	5
5	243,000	74	11	8	7
6	280,000	67	12	9	12

*Uninfected by DHIA SCC standards

Two key factors determine the percent of infected (mastitis) cows in a herd: 1) the rate of new infections, and 2) the rate of removal of infections from a herd. Herds remove infections by treating cows with antibiotics, culling chronically infected cows, treating cows at dry off, or in some cases, stop milking the affected quarter….choosing to milk the "3 quarter" cow. Drying off a chronically infected quarter is a viable option for removing poor quality milk from the food supply and reduces the risk for the infected quarter to spread the infection to other cows. Drying off a chronically infected quarter is also preferable to repeating antibiotic therapy, which will not likely result in a cure…treating the "chronic offenders". However, is culling cows, drying off quarters, or treating mastitis with antibiotics the best way to maintain lower herd SCC, or is this a game of "whack-a-mole"? If the new infection rate for subclinical mastitis is not lowered while removing infected cows or quarters from the herd, the percent of infected cows essentially remains the same.

Figure 6 shows a scattergram of over 120 herds in the Midwest (mainly from Michigan) where the DHI herd average SCC is plotted against the new infection rate (percent of cows) for each herd. In this case, a new infection was defined as a cow that had a linear SCC score of less than 4 during the previous test date but had a SCC score of 4 or greater at the current test date. As expected, there is a strong correlation between new infection rate and herd average SCC. Within the "blue rectangle" are herds with test date herd average SCC between 100,000 cells/mL and 175,000 cells/mL, a SCC that is below the U.S. national average and an indicator of good milk quality. However, the range of new infection rates in these herds varies from 2% to 15%, a more than seven-fold difference. How can this be happening when the SCC for all these herds is considered to be very good?
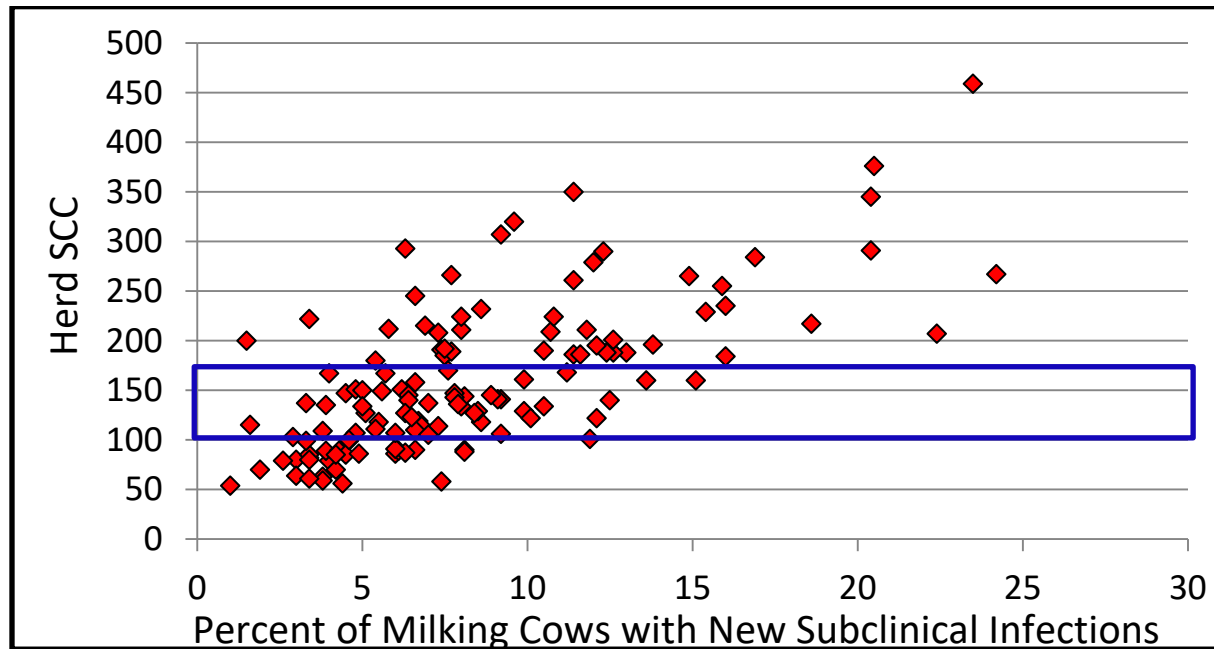
Figure 6: Scattergram of over 120 herds. The DHI herd average SCC is plotted against the new infection rate for each herd.

The answer is that the herds with the higher infection rates are maintaining lower SCC by culling cows, drying off quarters or treating clinical mastitis cases as they appear. These are all sound management options, but which herd, a herd with a new infection rate of 4% or a herd with a new infection rate of 15%, is spending more labour and money reacting to the problem, instead of preventing it? Which herd is likely using more antibiotics, losing potential genetic value in culled cows, and losing milk production in 3 quarter cows?

The greatest potential opportunity to use individual cow SCC is as a tool for fresh cow mastitis. Cows with chronic mastitis, measured by increased consecutive DHI test days with SCC ≥ 100,000 cells/mL, in early lactation have higher milk losses than cows with new infections later in lactation (Hadrich et al, 2018). Additionally, DHI records from > 166,000 cow-lactations in the western U.S. found that cows with a first test date SCC ≥ 200,000 cells/mL produced 718 kg less milk than cows with first test date SCC < 200,000 cells/mL, and were two to three times more likely to have clinical mastitis in early lactation and be culled by 60 days in milk (Kirkpatrick and Olson, 2015).

Herd average SCC are a great monitor for milk quality, but if maintaining a low SCC is done through reactive management strategies, rather than proactive strategies, the productivity of the herd is decreased despite having a desirable herd average SCC or bulk tank SCC. An ounce of prevention is worth a pound of cure.

Four useful measures of individual cow SCC to monitor the prevalence of subclinical mastitis are:

1. **Proportion of milking cows in a herd with subclinical mastitis:** The percent of milking cows with a linear SCC score (LSCC) of 4 or greater (or ≥ 200,000 cells/mL).

2. **The monthly new subclinical mastitis rate:** The proportion of cows that did not have subclinical mastitis on the last test date (LSCC < 4) but are now infected on the current test date.

3. **Dry cow subclinical mastitis rate:** The proportion of cows that did not have subclinical mastitis (LSCC ≥ 4) at the last test date of the previous lactation but have subclinical mastitis at the first test date of the current lactation.

4. **Proportion of fresh cows with subclinical mastitis:** The percent of cows that have a LSCC of > 4 on the first test date for the current lactation.

For examples of herd goals and articles about individual cow SCC, visit the following weblinks:

http://qualitymilkalliance.com/2015/01/17/is-cell-count-a-good-measure/

http://qualitymilkalliance.com/2013/07/01/herd-somatic-cell-counts-the-complete-story/

http://qualitymilkalliance.com/2018/07/25/revisited-article-you-are-here-on-the-somatic-cell-count-map/

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Kirkpatrick, M. A., and J. D. Olson. 2015. Somatic cell counts at first test: More than a number. Proc. of the 54th Ann Mtg Natl Mast Council, p. 53–56.
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STUDENT RESEARCH PRESENTATION COMPETITION

ABSTRACTS

Early Identification of Cows at Risk of Metritis Using Calving Factors and Activity Monitors

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The aim of this study was to identify cows at risk of metritis using calving factors and changes in activity. Initially healthy Holstein cows (N=542) were followed from 3wk before to 3wk after calving. Cows were monitored continuously by a leg-mounted pedometer (AfiMilk®, Afitag™) and cameras in the prepartum and calving pens were used to record calving duration. Calving assistance was recorded. Metritis was diagnosed based on vaginal discharge and body temperature was measured at 6 and 12 DIM. Body condition score (BCS) and gait score (GS) were measured 3wk before calving, 6DIM, and 12DIM. Duration of labour was estimated as time from the appearance of the amniotic sac until the calf was expelled (minutes; min). Cows diagnosed with lameness, milk fever, retained placenta, or displaced abomasum were excluded from analysis. Cows assisted during labour had a higher odds of developing metritis (OR=2.5; P<0.02). As duration of labour increased, the odds of developing metritis increased (OR=1.0; P<0.01). The duration of labour ranged from 11min to 173min (mean±SE = 57.5±2.4) in unassisted cows and 49min to 232min (mean±SE = 118.6±5.5) in assisted cows. Probability of metritis in unassisted cows was not impacted by duration of labour (slope=0.12%; P=0.14). The probability of metritis was greatest at the shortest (49min; 84.5%) and longest (232min; 100%) durations of labour, but the lowest probability was found at 128min (28.2%; P=0.02). Parity, BCS, GS, days dry, gestation length, and calving location were not significant. During the first 5DIM, cows later diagnosed with metritis had lower a duration of lying bouts (56.9±1.6 vs. 67.5±2.9 min; P<0.001). Activity, restlessness, and rest time were not significant. Assisting cows too early or too late during parturition can increase the probability of developing metritis. Measuring lying behaviour during the transition period could contribute to early identification of cows at risk of metritis. Implications: Appropriate timing of calving assistance can reduce the impact of calving on metritis. Changes in lying behaviour could contribute to early identification of cows as risk of metritis.

Effects of Feeding Hay and Calf Starter as a Mixture or as Separate Components to Holstein Calves on Intake and Growth

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Hay consumption in dairy calves fed a high plane of milk can increase average daily gain (ADG), gut development, and starter intake, but the optimum way to present hay to young calves has not been evaluated. We hypothesized that feeding hay and calf starter as a mixture would promote solid feed intake and increase ADG. To evaluate the effects of providing hay mixed with calf starter on dry matter intake (DMI), growth, and hormone concentrations, 40 Holstein heifer calves were fed calf starter and chopped grass hay as separate components (CONT) or mixed at a 90:10 ratio on an as-fed basis (MIX) ad libitum from 14 to 90 d of life. Calves were fed a milk replacer (28% CP and 15% fat) at 1,105 g/d at the maximum. Treatment did not affect solid feed intake or growth performance during the pre-weaning or weaning periods, but MIX calves had lower ADG (1.20 vs 1.31 kg/d; P < 0.01) in the post-weaning period. This is possibly because MIX calves were provided a fixed amount of hay (10%) in a mixture leading to lower overall DMI (3,292 vs. 3,536 g/d; P < 0.05) due to earlier rumen fill compared to CONT calves that could easily choose desired feed components. Although MIX calves sorted against (P < 0.05) long particles (primarily hay), they had lower starch intake (966 vs. 1,098 g/d; P < 0.05), indicating reduced calf starter intake compared to CONT calves. In addition, MIX calves had lower plasma concentration of glucagon-like peptide 2 (0.46 vs. 0.77 ng/mg; P < 0.05), a hormone stimulating gut development and nutrient absorption. As such, mixing 10% hay with calf starter is not recommended for post-weaned calves as it may limit DMI, ADG, and perhaps gut development of calves.

Dairy Production Performance Replacing Corn and Barley Silages with Whole Crop Faba Bean Silage in Western Canada

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The aim of this study was to determine the effect of partial (50% and 75%) and complete (100%) replacement of barley and corn silages with low tannin (snowdrop variety) whole crop faba bean silage at late pod stage (97 days old) on high production dairy cows in terms of milk yield, efficiency, digestibility of primary nutrients and rumen fermentation characteristics. A double 4 × 4 Latin square was used as experimental design. Statistical analyses were performed using PROC MIXED procedure of SAS 9.4 with significance declared at $P < 0.05$. The results showed that T100 (30.60 % whole crop faba bean silage) produced higher ($P < 0.05$) fat corrected milk (3.5% FCM) and higher ($P < 0.05$) energy corrected milk (ECM) than control diet T0 (18.37% corn silage + 12.23 % barley silage) (+4.35 and +3.48 kg/cow/d respectively), but produce similar FCM and ECM than T50 (9.18% corn silage + 6.12 % barley silage + 15.30 % whole crop faba bean silage) and T75 (4.59% corn silage + 3.06 % barley silage + 22.95 % whole crop faba bean silage). Additionally, the fat yield was higher ($P < 0.05$) when whole crop faba bean silage was included in the diet (2.11 vs. 1.89 kg/cow/d). Efficiency (DMI/FCM) was higher ($P < 0.05$) when animals consumed T75 than T0 (2.21 vs. 1.91), while the digestibility of starch was similar ($P > 0.10$) among T50, T75 and T100 but were lower ($P < 0.05$) than in T0 (92.65 % vs. 96.13 %). Ammonia, volatile fatty acids and pH were similar ($P > 0.10$) among all the treatment. In conclusion, the inclusion of whole crop faba bean silage at late pod stage improve fat corrected milk, energy corrected milk, milk fat yield and efficiency without negatively affecting the intake of dry matter. Consequently, this study showed that whole crop faba bean silage is a highly nutritive alternative feed to improve the performance of dairy cows in western Canada.

The Effects of Concentrate Feeding Level and Rate of Increase When Offered Through an Automatic Milking System on Fresh Cow Performance

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The objective of this study was to determine if the amount of pellet offered through an automated milking system (AMS) and the rate of increase for the pellet allocation affects dry matter intake (DMI), milking frequency, and milk and milk component yield of fresh cows housed in a guided traffic barn. Fifty-nine cows were fed the same partial mixed ration (PMR, 19.3% starch DM basis) and assigned to 1 of 3 AMS pellet (39.6% starch DM basis) allowances. Cows received (DM basis) either a low allocation (3 kg/d, LOW; n=19); or 1 of 2 high allocations (HIG; 8 kg/d). Pellet allocations for the high treatments increased at moderate (MOD; increased from 3 to 8 kg over 15 d) or rapid (RAP; increased from 3 to 8 kg over 5 d) rates. Intake of AMS pellet and PMR, milk yield, and milking frequency were recorded daily with milk samples analyzed weekly. Average DMI of the AMS pellet was 2.76, 4.06 and 4.01 kg/d for LOW, MOD, and RAP. The standard deviation in pellet intake was less for LOW as compared to HIG (0.43 vs. 0.83 kg/d). PMR intake did not differ between LOW and HIG (15.7 kg/d; $P = 0.33$); however, PMR intake was greater for MOD than RAP (16.8 vs. 14.1 kg/d; $P = 0.03$). There were no treatment differences for total DMI (18.8 kg/d; $P = 0.11$), milking frequency (3.1 milkings/d; $P = 0.66$), or milk yield (39.7 kg/d; $P = 0.33$). Milk fat yield tended to be greater for LOW when compared to HIG (1.64 vs. 1.48 kg/d; $P = 0.08$), but there was no difference for milk protein yield (1.30 kg/d; $P = 0.44$). Take Home Messages: Offering a high amount of pellet through the AMS increased variation in pellet intake without affecting DMI, milk yield, or milking frequency; however, there may be negative impacts on milk fat yield. Overall, the data suggest there is no benefit to offering an increased amount of pellet through the AMS in a guided traffic barn in early lactation.

Impact of Concentrate Allowance on the Behavior and Production of Dairy Cows Milked in a Free Traffic Automated Milking System.

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Automated milking systems (AMS) have been used for over 30 years, however, research on optimizing feeding strategies in these systems is ongoing. Concentrated feed is typically used within the AMS to motivate cows to voluntarily visit the milking unit, and the type and amount of concentrate provided may be modified for each cow. The objective of this study was to determine if the allowance of AMS concentrate affects partial mixed ration (PMR) eating behavior, milking activity, and production of dairy cows milked in a free-traffic AMS. Fifteen Holstein cows (124±53 DIM) were used in a cross-over design with 28-d periods and were provided a basal PMR; with treatments consisting of a pelleted AMS concentrate allowance of either 3.0 kg/d (L-AMS) or 6.0 kg/d (H-AMS) on a dry matter (DM) basis. As designed, cows on the H-AMS had greater AMS concentrate provision (5.9 vs. 3.0 kg/d; $P<0.01$). The standard deviation for mean daily concentrate intake increased from 0.49 to 1.3 kg/d ($P<0.01$) with greater AMS concentrate allocation. When on the H-AMS, PMR intake was reduced (24.7 vs. 26.3 kg /d; $P=0.03$) and meal size was smaller (3.2 vs. 3.5 kg /meal; $P=0.02$). The reduction in PMR intake was offset by greater AMS concentrate provision; thus, cows on the H-AMS tended to have greater total dry matter intake (30.6 vs. 29.3 kg/d; $P=0.06$). When on the L-AMS, cows spent 19.3 min/d more time ruminating ($P<0.01$), tended to have greater lying bout length (93.7 vs. 85.9 min/bout; $P=0.07$), and lost body condition (-0.04 vs. +0.13 BCS units; $P=0.02$). Milk yield was numerically greater when cows were on the H-AMS (46.0 vs. 44.4 kg/d), however milking activity (3.9 milking/d) was not affected by treatment. Overall, these data suggest that allocating a greater amount of concentrate to the AMS, in a free traffic setup, may promote greater total dry matter intake, but may also result in greater day-to-day variation in AMS concentrate intake.

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Bio-Security Management – Producer Panel

Three dairy producers discuss different aspects of bio-security management on their farms.

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———————————— ❧ ————————————

Managing Calf Health and Performance in utero

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▪ Take Home Messages

- Late gestation interventions on the cow can alter the performance and health of the developing calf after birth.

- Supplementation of the dam with specific nutrients such as choline may be required for optimal growth and immune function of the calf

- Disease outcomes, such as fever, in late gestation of the cow may alter the calf's responses to similar stimuli after birth.

- Environmental challenges, such as heat stress, program deficits in calf growth and health, and those impacts are transgenerational.

▪ Introduction

There is now little argument about the importance of good calf nutrition and management to ultimate productivity in the milking herd, especially in the early weeks before weaning. This knowledge has resulted in significant improvements in calf feeding and health monitoring because those are viewed as an investment that yields strong returns in productivity and health down the line. One example is that most farms have a specific protocol to ensure that newborn calves receive sufficient volumes of high-quality colostrum within the first 24 hours of life, with the initial feeding within 4 hours of birth. Because calves are born with a naïve and immature immune system, colostrum intake is critical to calf health and survival, and ultimately productivity. Recognition that calf lean mass accumulation progresses at a maximal rate early in life led to re-examination of feeding regimens to accelerate growth, with significant improvements in productivity at maturity relative to traditional rates of milk replacer and milk feeding. Of course, appropriate housing with particular attention to ventilation is essential to reduce disease and avoid cold stress. A well-designed vaccination protocol also should be in place to improve resistance to disease as calves transition through weaning.

While the aforementioned improvements to nutrition and management of calves have resulted in greater performance as those animals move through early life and as they enter the production string, less attention has been paid to factors that alter in utero calf development. Emerging evidence suggests that significant influences of in utero insults to the calf as it develops are associated with limits to performance and health after birth that might persist into adulthood. Because developmental trajectories are particularly plastic in the developing fetus, the concept of fetal programming or epigenetic imprinting has been promoted as a mechanism whereby nutrient or environmental factors can affect the fetus and influence that animal for life (Reynolds et al., 2019). More importantly, these epigenetic effects can be transmitted to the offspring of the affected animal and thus impact future generations. Below we consider some examples of nutrient deficiencies, pathogen exposure and environmental insult that may alter fetal development and, if ignored, may negatively impact performance and health of the calf for life.

▪ In utero Nutritional Deficits

Poor nutrition of the dam can lead to energy or protein related limitations to fetal growth, and severe deficiencies will reduce birthweight and negatively impact growth. Indeed, stunted calves may never catch up with normal herd mates. In contrast to systems in which cattle are managed extensively, significant gross nutritional limitations are not typically observed in dairy systems because of the nutritional requirements for milk yield concurrent with pregnancy. While gross nutrient deficiencies may be slightly more likely during the dry period, it is possible that the dam will compensate and favour the developing fetus over her own needs. Specific nutrient deficiencies, however, may occur and alter development.

Choline is a nutrient that may be limiting in many situations, even when other nutrient needs are met in the dry cow Supplementation to the cow in late gestation can significantly improve health and growth of the calf after parturition. As a collateral benefit, choline improves lactational performance of the dam. In a recent study, cows were supplemented with 60 grams of rumen protected choline (12.9 grams of choline ion) for 3 weeks before through 3 weeks after calving (Zenobi et al., 2018a,b). The objective was to test whether increased choline availability would improve aspects of calf health and performance, particularly those related to colostrum physiology. Calves were born to dams that were or were not supplemented with choline, and then were fed colostrum from either supplemented or non-supplemented dams such that four treatments were established: 1) choline in utero and no choline supplemented colostrum, 2) choline in utero and choline supplemented colostrum (positive control), 3) no choline in utero and no choline supplemented colostrum (negative control), and 4) no choline in utero and choline supplemented colostrum. Using that design allowed for assessment of the effects of in utero choline to be determined separate from the effect of ingestion with colostrum.

With regard to health, choline supplementation increased the survival of calves relative to that of non-supplemented calves, with the in utero and colostrum supplemented calves having the highest overall survival to 24 days of age. Calves that received in utero or colostrum choline were intermediate to the positive and negative controls. Part of that effect may have resulted from improved immunoglobulin G (IgG) uptake in the calves that received colostrum from supplemented dams, regardless of their in utero treatment. In contrast, in utero choline reduced the incidence of fever relative to the absence of choline. Calves from choline supplemented dams also showed less severe responses to challenge with lipopolysaccharide (LPS), which is a commonly used stimulator of immune responses. Collectively, these results suggest that choline supplementation to the dam improves immune status in the calf, especially when the calf is fed colostrum from the supplemented dam.

In addition to the impacts on calf health, choline treatment increased average daily gain through 300 days of life. This is likely because of an increase in consumption of milk in choline treated calves; however, choline also improved the intake of starter grain as calf age advanced. That improvement in starter intake should buffer the transition off milk at weaning, which may then reduce the lag associated with the shift from a liquid to a solid diet. More efficient feed utilization may also result from the modulation of immune sensitivity discussed above, wherein nutrients are partitioned to productive purposes in calves that received choline in utero compared with non-supplemented calves.

▪ Disease Challenge of the Dam in Late Gestation

As discussed previously, challenge with the outer coat of the gram-negative bacteria *E. Coli* (i.e., LPS) is a commonly used proxy for an animal's response to a pathogen stimulus. Using this approach, Burdick Sanchez et al. (2017) and Carroll et al. (2017) investigated the effects of a late gestation LPS challenge of the dam on calf performance and the calf's response to similar LPS challenge after birth. Beef cows received a single, moderate dose of LPS or saline approximately 50 days before calving and were then monitored until parturition. Calf birth weights were not affected by in utero LPS exposure, but preweaning average daily gain and weaning weight were increased for calves exposed to in utero LPS compared with the control calves..

Heifer calves delivered by the LPS challenged and control cows were raised as a group under the same conditions and then subjected to an LPS challenge at ~240 days of age. A number of variables related to immune status were monitored in the heifers during the acute phase of the response to LPS to determine if in utero exposure would alter responses after birth. Basal vaginal temperature was not affected by in utero exposure to LPS. But the response of those heifers to LPS challenge did differ. Specifically, the heifers exposed to LPS in utero had a longer duration of fever after LPS challenge relative to the control animals. The extension of the fever was associated with a similar increase in sickness behaviour of the prenatal LPS heifers compared with the controls, which may indicate an increased risk for extended performance loss in calves.

In response to LPS, the body typically secretes signalling factors called cytokines to induce movement and activity of immune cells toward a pathogen insult. After LPS challenge, all heifers increased secretion of tumour necrosis factor-α (TNF-α), a potent cytokine, but the increase was similar for both groups. However, in utero LPS-exposed heifers secreted more interleukin-6 (IL-6) relative to the control heifers, suggesting greater sensitivity to a similar stimulus. Both TNF-α and IL-6 are important stimulators of fever responses, so the greater IL-6 secretion in in utero LPS heifers is consistent with the extended fever in those animals vs. controls. Whereas there was no treatment effect on total numbers of white blood cells or the proportion of monocytes to polymorphonuclear cells after LPS challenge, there were differences in certain cell markers that indicate an enhanced activation of leukocytes in the heifers that had been exposed to LPS in utero. Because energy is required for immune surveillance, greater sensitivity of the immune system may partition energy away from growth and other more productive endpoints while not enhancing disease resistance. Indeed, Burdick Sanchez et al. (2017) indicated in utero LPS-exposed heifers had significant reductions in energy and protein efficiency after the acute LPS challenge compared with control animals.

▪ In utero Heat Stress Impacts on Health and Growth

Heat stress in late gestation significantly reduces productivity of the dam in the next lactation, but perhaps of greater interest are the negative effects of in utero heat stress on multiple aspects of health, growth and performance of the developing fetus (reviewed in Dahl et al., 2017). In an effort to increase the capacity for heat exchange from the uterus to the external environment in cattle, heat stress increases uterine vascularity but not in a manner that increases nutrient and oxygen exchange with the fetus. Indeed, in utero heat stress compromises growth, likely because perfusion of the placenta is reduced. In addition, gestation length is shorter, thereby decreasing time for growth and possibly development in general. Thus, it is no surprise that calves born to heat stressed dams have lower bodyweights at birth.

 For calves that experienced in utero heat stress, lower bodyweight persists through weaning and puberty relative to that of calves born to cooled dams. No difference in bodyweight is observed at maturity, which suggests that compensatory growth likely occurs from year 1 to 2 of life. But is also likely that composition of gain is not the same in heat stressed vs. cooled calves. Phenotypic observations that support the notion of differences in body composition include greater stature in cooled calves vs. those that endured in utero heat stress. Additionally, there are metabolic adaptations that favour energy partitioning to peripheral tissues in in utero heat stressed calves including elevated concentrations of insulin early in life, more rapid clearance of glucose following glucose challenge and slower clearance of insulin after an insulin challenge, the latter two being indicators of greater potential for movement of nutrients into peripheral tissue.

The negative impacts of in utero heat stress are not limited to growth and metabolism (reviewed in Dahl et al., 2019). Heat stressed calves have lower transfer of IgG compared with cooled calves, but there is little evidence of an effect on colostrum quantity of IgG. When the effects of in utero heat stress were compared with those of cooling by feeding both types of calves colostrum from the same source, IgG transfer remained lower in heat stressed calves. When calves born from cows that were housed under cool conditions were fed colostrum from either a heat stressed or a cooled dam, there was no difference in absorption of IgG, which provides further confirmation that it is not a colostrum-mediated effect.

Rather, more recent studies indicate that in utero heat stress accelerates gut closure, so there is less time for IgG transfer to occur. Within the first 24 to 36 hours of life, the initial layer of enterocytes lining the calf intestine rapidly degrades and sloughs off of the intestine, and the cells are replaced by new enterocytes that have tight junctions with the adjacent cells to limit transfer of large molecules across the gut; this is known as gut closure. Thus, we can use the relative rate of enterocyte death as an indicator of the speed of gut closure. In calves that experience in utero heat stress, this process appears to occur at a more rapid pace than that in calves born to cooled dams. That translates to an acceleration of closure that then reduces the amount of time for IgG uptake by the calf regardless of the source or quality of colostrum. Unfortunately, that also suggests that there is no way to manage that lower IgG uptake after birth.

The effects of lower immune status in heat stressed calves before weaning translate into poorer health as those calves grow. Indeed, calf loss from birth through first calving increases with in utero heat stress, indicating that health is compromised. There are also reductions in milk yield in the first lactation, but this is not growth-related because bodyweight at first parturition is the same regardless of in utero heat stress or cooling (reviewed in Dahl et al., 2019). However, as discussed earlier, the in utero heat stressed calves are likely less efficient from a milk production at maturity and have a higher fat composition of body mass. The heat stress effect continues to reduce yields in the second and third lactation relative to calves from cooled dams, suggesting that the heat stress impacts are permanent for that calf. There are significant changes in the methylation patterns that accompany in utero heat stress, which is a hallmark of imprinting or 'fetal programming' (Skibiel et al., 2018). Methylation is a mechanism whereby the efficiency of genetic signalling is altered without any change in the actual coding sequence of the gene. More importantly, methylation patterns are transmitted to those animal's offspring, and we have now observed that the reductions in performance are passed on to at least two subsequent generations (Almeida et al., 2019). This means that the effect of in utero heat stress continues to be a drag on performance and health long after the actual stressor has been removed.

▪ Conclusions

The preceding examples highlight the dramatic impact of changes within normal ranges of temperature, nutrient supply and disease exposure that alter performance and health outcomes long after the initial stimulus is gone. It is important to note that all these effects occurred during the last trimester of gestation, when it might be reasonable to expect less impact from a developmental standpoint on the developing fetus because of the older gestational age. Therefore, these studies highlight the importance of dry period management for positive outcomes on the dam, but perhaps more important and less explored, positive effects on the calf for life.

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New Concepts in Calf Nutrition: The First Week of Life

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▪ Take Home Messages

- The high rates of calf morbidity and mortality during the first weeks of life are largely due to digestive disorders and diseases; therefore, implementing a sound colostrum and milk feeding program is critical.

- Early (< 2 hours after birth) feeding of colostrum via nipple bottle or esophageal tube feeder is recommended; IgG absorption will not be compromised if a large volume of colostrum (~3 L) is tube-fed.

- Feeding colostrum earlier in life not only increases blood concentrations of IgG but may also have a positive effect on beneficial gut bacterial populations compared with feeding colostrum later than 6 hours after birth.

- Colostrum contains more than just IgG: it also contains high concentrations of prebiotic oligosaccharides, fatty acids, insulin, and antimicrobial compounds.

- Transition milk contains elevated levels of beneficial compounds and feeding transition milk can lead to improved intestinal development.

- The majority of farms only feed up to 4 L of milk per day during the first week of life, resulting in compromised body weight gain (up to an extra 400 g/d) compared with calves fed 8 to 10 L of milk per day.

- Calves can be fed up to 4 L of milk per meal two times per day without compromising insulin sensitivity provided that feeding large volumes of milk starts during the first week of life.

- Milk replacer is high in lactose and low in fat compared with whole milk. Research is needed to evaluate how this may affect gut health and development when large volumes of milk replacer are fed.

▪ Introduction

Preweaned calves suffer from the highest rates of mortality (5 to 6.4%) and morbidity (34%) during the first two months of life (Urie et al., 2018; Winder et al., 2018). Specifically, digestive disorders are the most common reported cause of morbidity and mortality, accounting for 48% of sick calves and 32% of deaths, with most cases occurring before 2 weeks of age (Urie et al., 2018). This has severe consequences for the Canadian dairy industry because these disorders not only cause concern from an animal health and welfare standpoint but can also be costly to producers. Digestive disorders can often be prevented and mitigated through well-developed nutritional and health management programs. Although preweaned calf management has improved over the past decades, mortality and morbidity incidence has only decreased by 4.5% and 2.8%, respectively, since 2007 (NAHMS), demonstrating that we still have much to learn as to how early life nutrition can mitigate the incidence of preweaning sickness and death. Therefore, this paper will focus on how calf gut health is influenced by colostrum, transition milk, and whole milk or milk replacer (MR) feeding during the first week of life.

▪ The First Days of Life

Colostrum and Passive Transfer

The structure of the bovine placenta prohibits passive transfer of IgG from the dam to the calf *in utero*. Consequently, the neonatal calf is born without a fully developed immune system and, thus, relies on immunoglobulin-rich colostrum to establish immunity. To ensure passive transfer, it is important to feed newborn calves an adequate volume of colostrum (~3 to 4 L) containing more than 50 g of IgG/L and a total bacterial count less than 100,000 cfu/mL (McGuirk and Collins, 2004). Timing of colostrum feeding is also critical because the absorption of IgG decreases linearly as the calf ages, with calves fed later than 12 hours after birth being at high risk for illness and death (Stott et al., 1979). Although these four 'golden rules' of colostrum feeding are well-known and practiced by approximately 67% of Canadian dairy producers (Winder et al., 2018), failure of passive transfer (FPT, serum IgG < 10 g/L) still occurs on 6.4 to 12.1% of farms (Shivley et al., 2018; Winder et al., 2018). High rates of FPT are associated with increased calf morbidity and mortality, costing up to C$87 per calf (Raboisson et al., 2016). Moreover, feeding inadequate amounts of colostrum to a calf results in reduced milk production during her first and second lactations (DeNise et al., 1989; Faber et al., 2005). Multiple factors are associated with FPT, including relying on the dam to provide colostrum, not assessing colostrum quality before feeding, and infrequently walking the barn during the night causing colostrum feeding to be delayed by at least 6 hours after birth (Vasseur et al., 2010).

The majority of studies examining the timely feeding of colostrum and its effect on passive transfer were conducted more than 25 years ago. To our knowledge, a study by Fischer et al. (2018a) was the first to determine how a delay in colostrum feeding using current colostrum recommendations affects passive transfer in the neonatal calf. Importantly in this study and in contrast to past research, colostrum IgG concentration and volume fed were standardized by feeding a pooled colostrum source that contained 62 g of IgG/L at 7.5% of birth body weight. It was hypothesized that delaying the colostrum meal would linearly reduce passive transfer. As expected, calves fed within 1 hour of birth had a 28% increase in the maximum blood IgG concentration (25.5 mg/mL) reached compared with calves fed at 6 hours and 12 hours (18.4 mg/mL). However, contrary to expectations, calf blood IgG concentrations did not differ between calves fed at 6 or 12 hours. These results suggest that there may be a critical time point between birth and 6 hours of life whereby the ability of the small intestine to absorb IgG diminishes to a 'point of no return'. Interestingly, calves fed colostrum at 12 hours after birth still achieved successful passive transfer in this study. This is likely because of the consumption of adequate volumes of high-quality colostrum; calves received an average of 3.2 L of colostrum or a total mass intake of 197 g of IgG. Colostrum IgG concentrations can widely vary on farm, from 7.1 to 159 mg/ml, with 16 to 22.6% of samples containing less than 50 mg/ml (Quigley et al., 2013; Shivley et al., 2018). Therefore, although all calves in this study (Fischer et al., 2018a) had adequate passive transfer, the authors do not recommend waiting until 6 hours after birth or later to feed colostrum because the on-farm variation in IgG will increase the risk for FPT and, consequently, preweaning morbidity and mortality.

In addition to the importance of the quickness, quality, quantity and cleanliness of colostrum feeding, the method by which colostrum is fed can also impact the success of passive transfer. Specifically, calves fed colostrum directly from suckling the dam, a nipple bottle, or an esophageal tube feeder had FPT rates of 61%, 19%, and 10%, respectively (Besser, 1991). Tube feeding on-farm is an appealing strategy because it takes only a matter of minutes (~1 to 5 minutes for 3 L of colostrum; Hare et al., unpublished data; Desjardins-Morrissette et al., 2018) compared with bottle feeding that can average 18 minutes per meal (Desjardins-Morrissette et al., 2018). Although tube feeding is a time efficient method, there is a concern that colostrum entering the rumen via tube feeding impedes emptying of IgG into the small intestine for absorption thereby reducing the efficiency of IgG absorption and serum IgG concentration. Decreased serum IgG concentrations have been observed when small volumes of colostrum are tube-fed; however, these studies did not assess abomasal emptying rate (the rate at which the colostrum meal empties into the intestine from the abomasum). To investigate this, Desjardins-Morrissette et al. (2018) fed 3 L of colostrum, containing acetaminophen as a marker for abomasal emptying, via esophageal tube or nipple

bottle and found that abomasal emptying rates and blood IgG concentrations of newborn calves did not differ between feeding methods. The authors suggested from their results and others (Godden et al., 2009) that feeding a small volume (e.g., 1.5 L) of colostrum results in a larger proportion (approximately 26%) of the meal remaining in the rumen and decreases IgG absorption compared with feeding a large volume (e.g., 3 L) feeding, where only 13% of the meal would remain in the rumen and thus there would be negligible differences in passive transfer. Therefore, if a calf is tube-fed a sufficient volume (≥ 3 L) of good-quality colostrum, this method should result in adequate passive transfer of immunity.

Colostrum and Gut Development

While it is well known that colostrum feeding greatly influences IgG concentrations, it can also stimulate the secretion of gut hormones. Of the many hormones stimulated, glucagon-like peptide (GLP)-1 and GLP-2 are of high interest. Glucagon-like peptide-2 is known to stimulate gut development, while GLP-1 stimulates insulin release, resulting in increased uptake of glucose for energy use by peripheral tissues. Prior to the study conducted by Desjardins-Morrissette et al. (2018), no research had reported GLP-1 and GLP-2 concentrations in newborn calves. No effect on GLP-1 or GLP-2 was found by feeding colostrum via bottle or tube-feeder, indicating that both feeding methods were effective in promoting the release of gut hormones. Nutrients, such as fats and carbohydrates, stimulate secretion of GLP-1 and GLP-2 within the intestine. Therefore, feeding colostrum, which is high in fat, caused large increases in the concentrations of these hormones in the blood (Desjardin-Morrissette et al., 2018). In addition, Inabu et al. (2018) demonstrated that GLP-1 and GLP-2 concentrations were lower in calves that were not fed colostrum until 12 hours after birth compared with those in calves fed immediately after birth. These findings demonstrate that the release of beneficial gut hormones is stimulated by colostrum feeding and that delayed feeding suppresses the amount of GLP released, potentially compromising intestinal development in the neonate.

In terms of macronutrients, protein initially appears to be the predominant energy source in colostrum for the calf, but it is unlikely that the majority of protein in colostrum is fully digestible because of the presence of compounds in colostrum that inhibit protein digestion (McGrath et al., 2016). Additionally, the newborn calf gut is relatively inefficient at digesting and absorbing protein. We recently conducted a review of our lab's data and determined that fat supersedes protein as the major energy source in colostrum, providing an estimated 63% of the approximate digestible energy supply compared with protein supplying an estimated 25%. Fat in colostrum is essential for fueling the metabolism of the newborn calf and for thermoregulation. It is also involved in hormonal signalling and inflammatory and immune responses. Furthermore, supplementing the colostrum fed to neonatal calves with fish and flax oils, which are high in omega-3 fatty acids (FA), has prolonged benefits in terms of antioxidant status and immune response (Opgenorth et al., 2019). These compounds are naturally elevated in colostrum compared with whole milk (Hare et al., 2019), highlighting that colostrum contains numerous factors apart from IgG that will promote calf health.

The newborn gut is a complex environment and hosts numerous microbial species. Gut microbiota fundamentally influence early life gut development and maturation, including the metabolism of otherwise indigestible compounds, the development of the immune system, and the overall physiology of the calf. The establishment of a healthy microbial community within the gut is also associated with overall calf health and disease outcomes, with certain fecal bacteria positively correlated with weight gain and negatively correlated with diarrhea incidence (Oikonomou et al., 2013). Feeding colostrum is critical in establishing beneficial gut microbiota populations and not feeding colostrum can result in a decreased abundance of total bacteria in the small intestine (Malmuthuge et al., 2015). For this reason, the study in which colostrum feeding was sequentially delayed after birth (Fischer et al., 2018a) also investigated the effect of this practice on gut microbial populations. Calves fed colostrum at 12 hours tended to have lower amounts of *Bifidobacteria* and *Lactobacillus,* which are well known for their beneficial role in the newborn gut microbiome, associated with the colon mucosa at 2 days of life. These results indicate that preventing the immediate establishment of beneficial early life bacteria by delaying the first colostrum feeding may have an impact on calf intestinal microbiota. Unfortunately, this study cannot answer whether or not this

affects the ability of the gut to respond to future pathogenic challenges later in life and requires further research.

An abundance of bioactive molecules is present in colostrum, but these molecules have taken a backseat to the widely discussed IgG. One the key families of bioactive compounds are oligosaccharides (OS), which are considered to be one of the major prebiotic compounds in colostrum that aid in establishing beneficial gut microbiota after birth. Oligosaccharides are small polymers of indigestible simple sugars composed of a lactose core. In bovine colostrum and milk more than 70% of OS contain sialic acid, a nine-carbon sugar with an acidic charge. This is in contrast to humans, where 50 to 70% of OS in colostrum and milk contain fucose, a neutral six-carbon sugar, and only 5 to 15% contain sialic acid. To date, over 50 bovine OS have been detected, with 3'siayllactose (3'SL) being the most abundant OS and present in colostrum at concentrations 15 times greater than in whole milk (Fischer-Tlustos et al., 2020). Recently, Fischer et al. (2018b) demonstrated that bovine heat-treated (HT; 60°C for 60 minutes) colostrum had higher concentrations of free OS compared with fresh colostrum, likely due to their cleavage from glycoconjugate structures during the HT process. Subsequently, when calves were fed HT colostrum, they had a higher prevalence of *Bifidobacteria* in the small intestine at 6 hours of life compared with calves fed fresh colostrum (Malmuthuge et al., 2015). The correlation between high concentrations of free OS in HT colostrum and *Bifidobacteria* in the calf gut suggests that OS may be a key compound in mediating the early establishment of beneficial bacteria. Bovine OS have also been shown to inhibit common pathogens implicated in calf diarrhea and positively influence the immune system. Furthermore, the sialic acid portion of bovine OS may enhance the uptake of IgG by the intestine (Gill et al., 1999). This finding may explain the high abundance of sialylated OS in bovine colostrum because the uptake of IgG is one of the most important factors in promoting the health and survival of the neonatal calf. Due to the high rate of digestive disorders in dairy calves and societal pressure to reduce antibiotic use in the agricultural industry, the potential benefits of these naturally produced compounds on calf health warrant further research. However, studies at the calf level are lacking and future research should explore the specific mechanisms by which OS exert beneficial effects on the newborn calf gut.

In addition to the aforementioned bioactive molecules, colostrum contains high levels of growth factors, hormones, cytokines, enzymes, nucleotides, and antimicrobial components (Blum and Hammon, 2000; McGrath et al., 2016). These components enhance the calf's ability to fight infection, as well as promote growth and gut development. For example, Blum and Hammon (2000) reported that insulin concentrations in colostrum are 65 times greater than in whole milk. Insulin has positive effects on the development of the neonatal gut, including promoting gastrointestinal cell proliferation and increasing intestinal mass and enzyme activity. Similar to insulin, insulin-like growth factor 1 (IGF-1) in colostrum can also stimulate intestinal cell proliferation, while antimicrobial compounds, such as lactoferrin and lactoperoxidase, help to maintain a healthy gut environment. Therefore, although the multitude of potentially beneficial compounds in colostrum have been overlooked during the past few decades, it is clear that colostrum has a much larger role in calf development than simply providing IgG.

Transition Milk

Many of the aforementioned bioactive compounds are not only elevated in colostrum but are also present at higher concentrations in transition milk (TM; defined as milkings 2 to 5) than in whole milk (Table 1). For instance, the major bovine OS, namely 3'SL, 6'sialyllactose (6'SL), and 6'sialyllactosamine (6'SLN) are higher in TM than in whole milk (Fischer-Tlustos et al., 2020). Furthermore, TM has elevated proportions of omega-3 and omega-6 FA (Hare et al., 2019), nucleotides (Gill et al., 2011), IGF-1, and insulin compared with whole milk (Blum and Hammon, 2000). Unfortunately, after feeding colostrum many producers transition calves directly onto whole milk or MR, which is a stark contrast to calves naturally consuming TM from the dam. Due to this common practice, most dairy calves miss out on the potential benefits of TM. Research has demonstrated that calves fed TM after the initial colostrum feeding have lower odds of being assigned a poor eye/ear score (Conneely et al., 2014). Similarly, calves that consume a 1:1 colostrum:whole milk mixture (to simulate TM) after the initial colostrum feeding may have increased production of GLP-1 (Inabu et al., 2019) that, as previously discussed, can have beneficial effects on

energy use. Furthermore, a study by Pyo et al. (2020), in which the same simulated TM was used, determined that calves fed colostrum or the 1:1 colostrum:whole milk mixture for 3 days after birth had increased small intestinal surface area and cell proliferation in certain intestinal segments compared with calves consuming only whole milk, suggesting that TM feeding promotes intestinal development. Importantly, the simulated TM promoted intestinal development to the same degree as providing solely colostrum for 3 days, despite the nutrient and bioactive compound concentrations being lesser. Therefore, feeding fresh or frozen TM to calves is a possible strategy producers can implement to promote gut development. An additional solution is to feed a mixture of colostrum and whole milk, or even whole milk mixed with a colostrum replacer product, to achieve this goal. Unfortunately, research regarding the feeding of TM to calves is lacking and future studies should investigate the roles of potential bioactive compounds in TM that may assist in proper gut development.

Table 1. Levels of bioactive molecules in colostrum (milking 1), transition milk (milkings 2 to 5) and whole milk (milking 12)[1].

Bioactive compound[2]	Milking					
	1	2	3	4	5	12
IgG, g/L	94.1	39.3	13.9	6.1	3.4	1.2
Fat content, g/milking	371.2	335.4	376.2	441.8	511.6	523.4
Omega-6 FA, %	4.2	3.1	3.5	3.1	3.0	2.7
Omega-3 FA, %	0.63	0.43	0.48	0.40	0.38	0.35
Omega 6:3 ratio	7.1	7.4	7.6	7.9	8.2	7.8
3'SL, μg/mL	592.4	304.9	171.2	99.3	67.0	41.2
Total SA-OS, μg/mL	1065.2	569.3	317.2	186.0	134.5	76.0
Nucleotides, μmol/dL	258.7	86.4	174.4	-	133.8	15.6

[1]IgG, fat content, 3'SL, and total SA-OS concentrations are reported in Fischer-Tlustos et al. (2020); Omega-3 and -6 concentrations and the omega 6:3 ratio are reported in Hare et al. (2019); nucleotide concentrations are reported in Gill et al. (2011).
[2]FA = fatty acids; 3'SL = 3'sialyllactose; SA-OS = sialylated oligosaccharides.

▪ The First Week of Life

Milk Feeding

After consuming colostrum and TM for the first 1 to 3 days of life, calves begin consuming whole milk or MR. Typically, calves are either fed large (≥ 8 L, 67% of Canadian producers) or small (≤ 6 L, 33% of Canadian producers) volumes of milk (Winder et al., 2018). Conventional feeding programs aim to encourage early starter intake by limiting milk consumption to 10% of body weight (BW), which is roughly 4 to 6 L of milk/day or 600 to 750 g of MR powder/day. By decreasing milk intake, early starter intake is promoted and rumen development is enhanced (Khan et al., 2016). In turn, calves are thought to be less susceptible to health and production challenges during weaning. However, research has shown that calves suffer from hunger when milk is restricted, demonstrating compromised animal welfare. In contrast, feeding an elevated plane of milk nutrition (20% of BW; ≥ 8 L of milk or 1.2 kg of MR powder per day) improves animal welfare because starvation-associated behaviours are reduced. Recent studies show positive outcomes from feeding larger volumes of milk, including increased BW gain, the potential to produce more milk during lactation, improved mammary development, and reduced age at first calving (Vasseur et al., 2010; Soberon et al., 2012). Yet, producers still limit calves to only 10% of BW (4 to 6 L) per day during the first week of life and gradually transition calves to higher amounts of milk (8 to 10 L) throughout weeks 2 and 3 of life. A Quebec survey (Vasseur et al., 2010) showed that the majority of farms feed only 4 L of milk per day during the first week after birth. At this time, starter intake is negligible and all metabolizable nutrients are consumed directly from milk. Maintenance requirements alone equal ~3 L of milk per day; therefore, feeding only 4 L largely restricts energy for growth. This is typically why we see depressed weight gain (e.g., only up to 400 g/day) when calves are limit-fed milk at 10% BW. Haisan

et al. (2019) showed that all calves (n = 26) offered large volumes of milk were able to consume over 8 L/day and up to 10 L/day using an automated calf rail during the first week of life, resulting in an average daily gain (ADG) up to 800 g/day, whereas calves limit-fed 5 L/day only gained up to 400 g/day during this period. While feeding up to 10 L/day of milk during the first week may seem daunting to producers because of the perceived economic inefficiency, it is a feasible strategy to incorporate an elevated plane of nutrition and can be considered as an investment in the replacement herd's future productivity.

One of the major concerns centred around feeding larger volumes of milk is that it is difficult to implement on-farm because of labour constraints unless an automated feeding system is used. Producers who aim to provide more milk without automated feeding often feed large volumes of milk per feeding, generally in two meals daily. With this, there is concern regarding abomasal overflow of milk into the rumen, abomasal inflammation and lesions, and reduced insulin sensitivity due to a large amount of glucose being supplied during a short period of time. However, a recent study from Norway (Ellingsen et al., 2016) demonstrated that calves allowed free access to milk consumed between 5 to 7 L per meal without any overflow into the rumen. Regarding insulin sensitivity, Bach et al. (2013) showed that calves fed 8 L of milk/day from 2 weeks of life onward released more insulin to control blood glucose than calves fed 6 L of milk/day. In contrast, MacPherson et al. (2018), fed calves 8 L per day over two or four meals, beginning at the first week of life, and found no differences in insulin sensitivity between groups during a glucose tolerance test. Calves fed only two meals per day had a decreased rate of abomasal emptying indicating that the slower delivery of nutrients, namely glucose, from the abomasum to the intestine may have regulated insulin response. It may be important to begin feeding large volumes of milk during the first week of life because this may be a critical developmental window in which the calf adapts to consuming high levels of milk; however, the long-term effects of this practice on calf development and metabolism are unknown.

Whole milk vs. Milk Replacer

Another controversial topic in terms of milk feeding are the benefits and disadvantages of feeding whole milk vs. MR. Producers generally feed MR because the calf receives a known and consistent nutrient supply, and it is clean and convenient. However, the macronutrient composition of the majority of MR do not resemble that of whole milk, which is plausibly more suited to the calf's needs. Most MR today contain more lactose (45 vs. 35%) and less fat (18 vs. 30%) compared with whole milk (Figure 1). Considering that approximately 60% of calves are fed MR or a combination of MR and whole milk (Urie et al., 2018), more research on how this practice affects calf gut health is needed. High lactose inclusion in MR could negatively affect glucose homeostasis, resulting in high blood glucose and insulin that may eventually lead to the development of insulin resistance. Additionally, high lactose concentrations increase the osmolality of MR (400 to 600 mOsm/L) relative to whole milk (300 mOsm/L; Figure 1). The high osmolality of MR can increase intestinal permeability, potentially disturbing gut mucosal structure and function (Wilms et al., 2019). However, recent work by Welboren et al. (2019b) found that feeding high lactose MR tended to decrease intestinal permeability, which may decrease the risk of pathogens or toxins entering the body. From this conflicting research, it is clear that more research is required to identify calf metabolic and intestinal development responses as calves are progressively fed larger volumes of milk replacer.
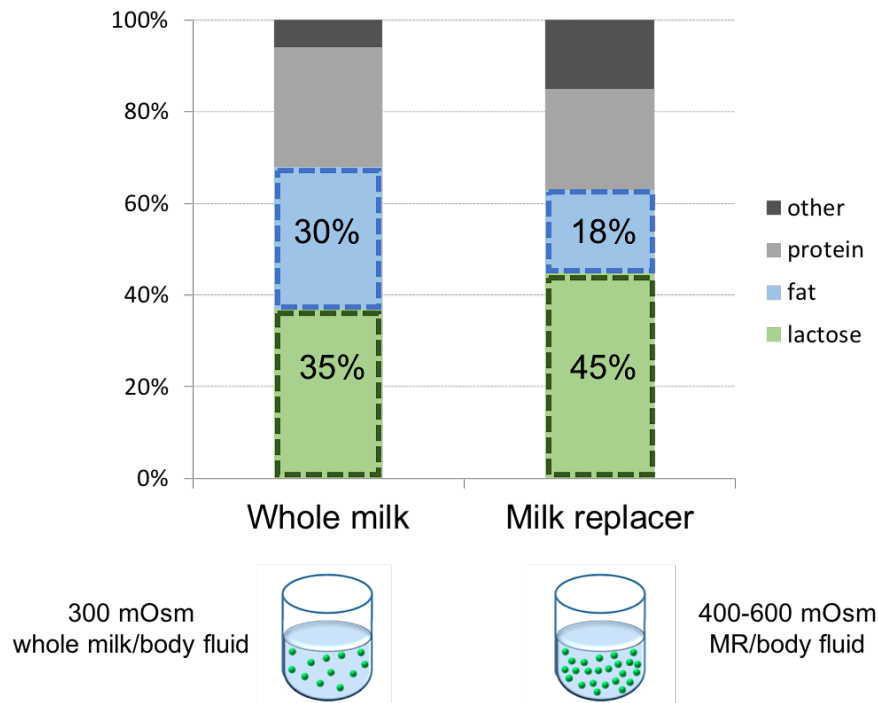
Figure 1. The macronutrient composition (%DM) and osmolality (mOsm) of whole milk and milk replacer (MR).

In addition to high amounts of lactose in MR possibly disturbing calf development, the low levels of fat may also be of concern. High fat consumption is essential for calves during the first week of life because it is crucial for providing energy and assists in thermoregulation. Moreover, increased fat inclusion in liquid feed decreased mortality in preweaned calves (Urie et al., 2018), further demonstrating that this is a critical macronutrient promoting young calf health and survival. Welboren et al. (2019a) showed that feeding 6 L of MR with low lactose and high fat content (HF) twice daily during the first week of life tended to delay abomasal emptying compared with feeding a high lactose and low fat (HL) MR. This may be beneficial in delaying the digestion of protein and fat from MR to allow for better absorption of nutrients and may have positive effects on glucose regulation. The calves fed the HF MR also experienced a lesser rise in glucose and insulin concentrations, although insulin sensitivity was unaffected. Unfortunately, milk fat is not commonly used in MR for economic reasons, with animal-based fats being widely used, including tallow and lard, as well as plant-based coconut, canola, and palm kernel oil. Whole milk lipids appear as globules that are emulsified in the aqueous phase of milk and are coated with bipolar materials, called the milk fat globule membrane (MFGM). The lipid droplets in the fat mixtures used in MR do not contain MFGM but are instead coated with casein and whey molecules from skim milk – a stark contrast to the bipolar molecules that make up whole milk MFGM. The MFGM structure in whole milk potentially plays a role in digestion, lipid metabolism and delivery of lipids to the gut, where they may play a critical role in protection and maturation. Providing MR void of MFGM may have consequences on calf gut development and maturation but has not been thoroughly investigated. Furthermore, milk fat contains medium- to long-chain saturated FA, while many of the plant-based fats used in MR contain high levels of polyunsaturated FA. Feeding high polyunsaturated FA sources can result in poorer growth and nutrient digestibility, and increased occurrences of diarrhea compared with feeding MR that more closely resembles the FA profile of whole milk (Jenkins et al., 1985). To date, there is little research investigating how current MR macronutrient composition, namely the high inclusion of lactose and low amount of fat, affects calves fed elevated planes of nutrition. Future research is needed to evaluate the specific mechanisms by which MR formulations directly affect calf gut barrier function, development, and overall health.

▪ Conclusion

Nutritional management during the first week of life can largely affect calf health, gastrointestinal development and growth performance, and may influence future productivity. Aside from ensuring passive transfer, it is clear that feeding colostrum and transition milk can exert beneficial effects on gut development and maturation. This may occur directly through the action of bioactive molecules, such as OS, fatty acids, and antimicrobial compounds, and indirectly by stimulating the production of gut hormones. In addition, maximizing nutrient intake from whole milk or milk replacer during the first week of life is essential to support growth because starter intake is negligible. There is currently a large knowledge gap as to how the typical macronutrient composition of MR affects calf gut development and health when larger volumes are provided. More research is needed regarding the potential benefits and long-term effects of colostrum, transition milk, and whole milk or MR feeding in order to make confident and informed decisions to promote optimal calf growth, health and productivity during the calf's first week of life.

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Rethinking Ruminal Acidosis in Dairy Calves

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▪ Take Home Messages

- Subacute ruminal acidosis is a costly disease in cows associated with extended bouts of low rumen pH; calves are assumed to be similarly responsive and susceptible to subacute ruminal acidosis.
- Little is known about rumen pH dynamics in calves.
- Calf rumens (pH dynamics) respond to increased diet fermentability differently than mature rumens do.
- In calves, low rumen pH does not appear to reduce growth, so increasing rumen pH may not be necessary.
- Low rumen pH may aid in development of the rumen papillae.
- Optimal rumen pH in calves is unknown but likely is different from that in adult cows.

▪ Introduction

Raising of replacement heifers represents 10–20% of all on-farm costs (Bailey and Cullin, 2009), yet performance in calf nutrition is poor. Morbidity rates before weaning remain stubbornly high at 20–25% of all calves (Windeyer et al., 2014), with gastrointestinal problems being the principal reason calves are treated with antibiotics. In a survey of Canadian dairy producers, researchers, and veterinarians, animal health was the top priority across all surveyed categories (Bauman et al., 2016). Therefore, looking at major gastrointestinal problems such as subacute ruminal acidosis (SARA) and evaluating them in calves is crucial.

In the last decade, research showed that calf nutrition and management impact lifetime performance of the animal. As a result, there has been a resurgence of interest in the young calf. Often, calves are treated as 'mini cows' as soon as they are weaned, and it is assumed that they will respond the same way to nutritional and management signals as mature cows do. Because calves undergo a massive transformation of their rumen, and gastrointestinal tract in general, in the first few months of life, assumptions that calves should be managed as though they are miniature cows require a re-evaluation.

The conundrum in calves is the high fermentability of calf starter and the drive to increase calf starter intake. Fermentation of calf starter drives rumen papillae development, so calf starters often have starch contents of 30% or more, very high by mature cow standards. High starch diets are a strong contributor to SARA and depressing health and productivity of cows (Plaizier et al., 2008). Though SARA is a complex disorder, one of the key indicators that a cow is experiencing SARA is an acidic rumen with pH below 5.8 (Aschenbach et al., 2011). Because our knowledge regarding SARA in calves is very limited, the same threshold of rumen pH 5.8 is used in calves. However, a rumen pH below 5.8 might not be a bad thing in calves whose rumens are undergoing rapid development.

▪ Rumen Development

The newborn calf has an undeveloped rumen that is non-functional. As the consumption of highly fermentable calf starter increases, butyrate produced from fermentation drives a strong increase in papillae development, which can be seen by the naked eye (Figure 1). For that reason, maximizing starter intake as a measure of readiness to wean is key to calf management. The NRC (2001) recommended weaning calves as soon as their starter intake is 680 g/day (1.5 lb/day), though this number has recently been revised to 1000 g/day (Stamey et al., 2012).

Figure 1. Development of the young calf's rumen via consumption of calf starter. At 28 days, calves fed milk only (LEFT) show little development, whereas calves fed milk and grain (RIGHT) show darker, more vascularized tissue with more prominent papillae. These papillae increase the absorptive surface area of the rumen. Modified from https://extension.psu.edu/photos-of-rumen-development

Increasing calf starter is primarily linked to decreased milk intake. In one study (de Passille et al., 2011), calves were fed a high plane of nutrition and a medium plane of nutrition and weaned at either 5 weeks or 8 weeks. Regardless of plane of nutrition, calf starter intake increased in earnest during the weaning transition when milk provision was reduced. When calves are fed a lower amount of milk replacer powder (~750 g/day), calves are so short on milk supply that they reach 680 g/day of calf starter intake by 7 weeks of age (Laarman and Oba, 2011). Limiting milk or milk replacer is therefore the most effective way of maximizing calf starter intake, and the reason why limit-fed programs were popular for decades.

In the past decade, this mantra has been overturned largely due to the discovered links between calf performance and lifelong productivity. In pre-weaned calves, average daily gain and body weight at weaning are associated with greater milk production in first lactation (Soberon et al., 2012). Recently, increases in calf starter intake were also linked to improved first lactation performance (Rauba et al., 2019). Specifically, for every Mcal of metabolizable energy intake from calf starter before weaning, cows will produce an extra 1.43 kg of milk in their first lactation (Rauba et al., 2019). Given the importance of early life nutrition and health, the current focus of calf management is on successful rumen development, driven by improving intakes of milk and calf starter.

One of the principal outcomes of starter intake is morphological development of the rumen, which was long seen as synonymous with absorptive capacity of the rumen. As a result, starter intake is often used

as an indicator of morphological development. The issue is that morphological development does not necessarily mean the rumen is capable of absorbing enough nutrients to meet the calf's energy needs post-weaning. Recent studies have shown that volatile fatty acid (VFA) absorption in the rumen can change independent of morphological changes (Laarman et al., 2016) and that pre-weaned calves with developed rumen papillae have the same VFA absorption rates as calves with an undeveloped rumen (Yohe et al., 2019). These results imply that rumen development includes more than just the morphological development (i.e., papillae formation) of the rumen epithelium. The cells that make up the rumen epithelium are also developing and may have a much larger impact on nutrient metabolism than previously thought.

▪ Regulation of Rumen pH in Calves

The rumen epithelium is continuously exposed to the rumen environment; therefore, fluctuations in rumen pH and the regulation of rumen pH are important to the barrier integrity and cellular survival of the rumen epithelium. Once rumination behaviour begins, rumen pH regulation is assumed to follow that of the mature cow: Increased fermentability in diets increases VFA production and rumen acidity, decreasing pH. Maximizing VFA production while mitigating pH drops is a decades-long effort that constantly requires research and updating (Plaizier et al., 2008). Underlying this research is the assumption that the relationship between diet fermentability, VFA concentrations, and rumen pH is constant, which may not be the case in young calves.

During the early phases of rumen development, rumen pH is considerably lower (more acidic) than that of a healthy rumen in a mature cow. For a long time, research on rumen fermentation dynamics in calves showed differences in rumen pH in calves fed starters using different starch sources (Khan et al., 2008). In many of these studies, calves younger than 50 days of age had rumen pH well below the threshold of SARA (in mature cows), despite having access to forage ad libitum (Table 1). These data suggest that optimal rumen pH in young calves may be lower than in adult cows, where a rumen pH above 5.8 is desirable for optimum feed digestion.

Table 1. Rumen pH in young calves at various ages

Calf Age (days)	Rumen pH	Forage	Source
35	5.19 – 5.49	Ad libitum	Khan et al., 2008
50	6.27 – 6.42	Ad libitum	Laarman and Oba, 2011
50	5.46 – 5.79	Ad libitum	Khan et al., 2008
64-69	5.72 – 5.83	Ad libitum	Laarman et al., 2012
70	5.66 – 6.16	Ad libitum	Khan et al., 2008
70	5.09 – 5.31	Variable	Suarez et al., 2007

Rumen pH is the product of several pressures that depress pH (e.g., increased dry matter intake and diet fermentability) and increase pH (e.g., increased inclusion of physically effective neutral detergent fibre (peNDF), and buffers, increased passage rate). In adult cows, managing forage intake to ensure inclusion of peNDF at levels above 12.5% (Plaizier et al., 2008) helps to keep rumen pH above the SARA thresholds. In calves, forage is generally fed either ad libitum (Laarman et al., 2012b, McCurdy et al., 2019) or not at all, despite calves being on bedding (Bach et al., 2007). In calves, the ability to regulate rumen pH is likely underdeveloped, leaving calves more vulnerable to an upset in rumen pH. Indeed, following a rapidly fermentable meal, rumen pH drops much more quickly in calves than it does in cows (Figure 2).
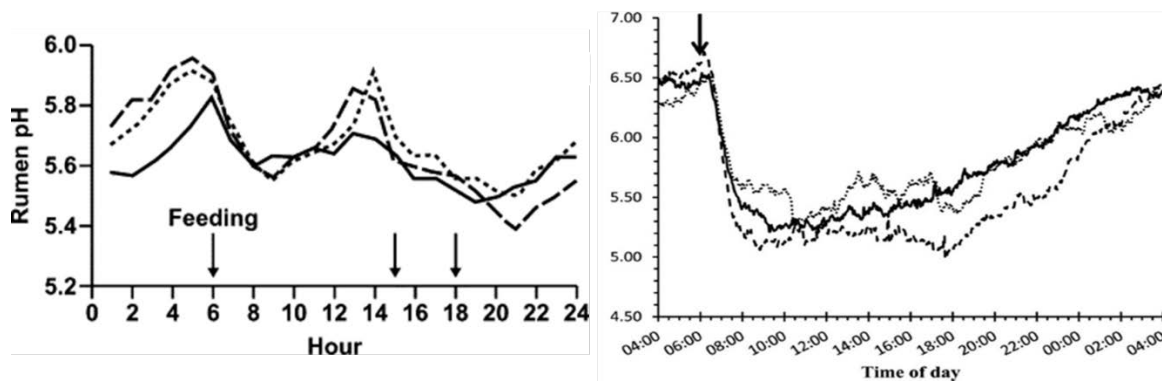
Figure 2. Changes in rumen pH following a rapidly fermentable meal (arrows) in cows (LEFT) and weaned calves (RIGHT). Cows reach their lowest pH point approximately 8 hours after a meal, whereas calves reach the lowest point in 2 hours. From Yang and Beauchemin (2006) and Laarman et al. (2012)

A recent study showed that the differences between cows and calves in terms of rumen pH dynamics are much greater than the rate of decrease only. In a recent study (McCurdy et al., 2019), calves were divided into pre-weaning and post-weaning groups (Figure 3). The pre-weaning group was fed either milk replacer only (PRE-M) or milk replacer, starter and hay (PRE-S). The post-weaning group was weaned during weeks 7 and 8 by reducing milk replacer provision to 900 g/day and 600 g/day, respectively. In week 9, milk replacer was cut-off completely and calves were harvested (slaughtered) one week later. During weeks 7 and 8, calf starter was either not supplemented (POST-B) or supplemented with butyrate at 1% w/w (POST-S). In week 9, all calves were fed non-supplemented calf starter and calves were harvested at the end of week 9.

Figure 3. Schematic of milk feeding and weaning in a recent study (McCurdy et al., 2019) examining the effects of calf starter intake on VFA concentrations, rumen pH, and growth; each box represents one week of life. Two groups of calves were harvested before the start of the weaning transition (PRE-M & PRE-S), with PRE-M being fed milk only, and PRE-S being fed milk, starter, and hay. Two additional groups were harvested one week after completion of weaning (POST-S & POST-B), with both being fed milk, starter, and hay before the weaning transition and starter and hay. During weeks 7 and 8 (yellow boxes), POST-B calf starter was supplemented with butyrate at 1% inclusion, while POST-S calf starter was not supplemented.

Prior to the beginning of the weaning transition, increased calf starter intake tended to increase VFA concentrations but did not impact rumen pH (Table 2), in line with other studies (Laarman and Oba, 2011; Yohe et al., 2019). During the weaning transition, calf starter intakes increased by 2000 g/day and VFA concentrations increased 4-fold, yet rumen pH remained unchanged (Table 2). After weaning, the POST-B calves (received butyrate during the 2-week weaning transition only) had a calf starter intake 800 g/day

higher than POST-S calves (did not receive supplemented calf starter during weaning transition). Post-weaning, one week after POST-B calves were returned to their non-supplemented calf starter, ruminal VFA concentrations tended to be lower and the POST-B calves had a strong drop in rumen pH (McCurdy et al., 2019). Despite these variable responses to rapidly fermentable diets, average daily gain was not adversely impacted. On the contrary, the POST-B group with the highest starter intake and lowest rumen pH had the highest average daily gain (McCurdy et al., 2019).

This study suggests calf rumen pH dynamics behave differently than in adult cows. Increased intake of calf starter did not impact rumen pH until after calves were weaned. Further, increased calf starter intake did not increase VFA concentrations after weaning. Lastly, productivity in calves appears to be linked to starter intake, not rumen pH. Higher calf starter intake caused lower pH and higher average daily gain, suggesting that, unlike cows, calves are able to thrive despite low rumen pH. Altogether, these findings paint a picture of rumen pH regulation in young calves that is distinct from mature cows and changing in various phases of life. A freshly weaned calf is not yet a mature ruminant in terms of its ability to regulate rumen pH. When rumen pH dynamics in calves begin to resemble those of a mature cow is unclear but appears to be well after dairy calves are typically weaned.

Table 2. Changes in rumen pH pre-weaning ((PRE), 42 days of age) or post-weaning ((POST), 63 days of age). Calves were fed milk only (PRE-M) or milk, starter and hay (PRE-S) and slaughtered at 42 days of age; or were fed milk, starter and hay, then weaned over a period of 14 days from 42 until 56 days of age, with either no supplementation during the two-week weaning transition (POST-S) or supplementation with sodium butyrate (POST-B). From McCurdy et al., 2019.

					P Value[1]		
					PRE-M vs. PRE-S	PRE-S vs. POST-S	POST-S vs. POST-B
	PRE-M	PRE-S	POST-S	POST-B			
Age, days	42	42	63	63	N/A[2]	N/A[2]	N/A[2]
Starter intake, g/day	0	77 ± 165	2247 ± 171	3102 ± 171	N/A[2]	< 0.01	< 0.01
Total VFA[3], mM	11.9 ± 11.8	35.6 ± 11.4	154.4 ± 11.8	131.0 ± 11.8	0.08	< 0.01	0.09
Mean pH	6.17 ± 0.21	6.25 ± 0.22	6.40 ± 0.22	5.83 ± 0.21	0.78	0.66	0.05
Duration pH < 5.8, min/d	485 ± 188	280 ± 178	209 ± 201	730 ± 188	0.44	0.79	0.07

[1]P value of 0.05 or less indicates significant difference
[2]No statistical comparison was made
[3]Ruminal volatile fatty acid concentration

▪ How Does pH Affect Papillae Development?

Rumen epithelial development and the formation of papillae are the result of division and differentiation of cells that make up the rumen epithelium. Over time, papillae become visible as cell division continues. The primary driver of cell division and papillae development is butyrate, which is a bioactive molecule produced by ruminal fermentation of carbohydrates. Part of the reason calf starter is a great nutritional stimulus is its propensity to raise butyrate concentration in the rumen (Laarman and Oba, 2011, Yohe et al., 2019). Cell division is then increased, leading to increased development of the rumen epithelium, which becomes visible as papillae begin to develop.

Butyrate is a bioactive VFA involved in many processes critical to rumen development, including VFA transport (Laarman et al., 2012a), and cellular changes involved in epithelial development (Baldwin et al., 2012). Butyrate, however, has different effects in the lab than it does in an animal. In calves,

supplementing butyrate appears to be effective only in the first week of life and during the weaning transition (Górka et al., 2018). We don't know why. Since butyrate is bioactive, the impact of butyrate on calf performance is likely involved in the functioning of the cells that make up the rumen epithelium.

Cells in the rumen epithelium must tightly regulate homeostasis, the maintenance of intracellular conditions that allow them to function. In much the same way that the rumen only functions effectively in a certain pH range, so too do epithelial cells only function in a narrow pH range. However, the physiological pH range in epithelial cells is extremely narrow, from 7.0–7.4, whereas the physiological pH in the rumen ranges from 5.8–6.8, considerably lower than the intracellular pH of the epithelial cells.

By itself, low rumen pH is not a problem for the rumen epithelium. In a recent study, rumen tissue that was exposed to pH of 5.2 without VFA present did not exhibit breakdown of the rumen epithelium that occurs in cows with rumen pH of 5.2 (Meissner et al., 2017). Only when VFA was added at 100 mM did the integrity of the rumen epithelium break down (Meissner et al., 2017). At low pH, passive diffusion of VFA increases (Sehested et al., 1999), which represents an unregulated flow of VFA into the cells, acidifying the interior of the cells. Therefore, the production of VFA during the fermentation of calf starter puts a constant acidotic pressure on cells in the calf's ruminal epithelium.

Acidotic pressure may be a good thing for the developing rumen through the promotion of epithelial remodelling, which is what turns the undeveloped rumen at birth into the developed rumen with papillae at weaning (Figure 1). Epithelial remodelling requires a breakup of the bonds that hold cells to each other, normally preventing cells from floating away and preventing bacteria from passing around the cells and into the bloodstream. When the cell interior acidifies, the bonds that hold cells to one another to form a protective barrier, the backbone of epithelial integrity, begin to come apart (Duffy et al., 2004). The breaking of these bonds allows the cells to migrate to new positions (Streuli, 1999), allowing for the development of rumen papillae. Once the cells have migrated, the bonds holding cells together will re-form, and epithelial integrity will be re-established. In other words, low intracellular pH in calves may boost the epithelial remodelling process that forms papillae in early life.

▪ Is SARA a Problem in Calves?

In calves, the impact of SARA may be different than in cows. In cows, SARA is a well-studied problem where rapid increases in non-fibre carbohydrate intake is linked to reduced rumen pH, gut inflammation, epithelial barrier breakdown, and reduced productivity (Plaizier et al., 2008; Aschenbach et al., 2011). In calves, that linkage is not established; however, in the absence of studies on SARA in calves, the impact of low rumen pH on gut health and animal productivity is assumed to be the same in calves as in cows. Calf research in the past 10 years casts doubt on that assumption.

In young calves, SARA does not necessarily happen when calves eat large amounts of non-fibre carbohydrates (Figure 4). Before and during weaning, increases in non-fibre carbohydrates intake do not appear to impact rumen pH (McCurdy et al., 2019; Yohe et al., 2019). Likewise, the microbiome, responsible for the actual fermentation, is also developing and changes dramatically over the course of weaning (Meale et al., 2016), and through 1 year of age (Li et al., 2012). The young rumen's microbiome may therefore be a contributor to the uniqueness of rumen pH dynamics in young calves, though the linkage between rumen microbiome and rumen pH in young calves requires more research.

Weaning	Pre-	Peri-	Post-	Mature
NFC	⬆	⬆	⬆	⬆
VFA	⬆	⬆	⬇	⬆
Rumen pH	=	=	⬇	⬇

Figure 4. Impact of changes in non-fibre carbohydrate (NFC) intake on VFA concentration and rumen pH in calves pre-weaning (Pre-), during weaning (Peri-) and post-weaning (Post-), compared with that in a mature cow. Until completion of weaning, calves are highly able to manage rumen pH despite drastic changes in NFC intake, whereas weaned calves and mature cows are far less able to. Based on Laarman and Oba (2011), McCurdy et al. (2019), and Plaizier et al. (2008).

While calf rumen pH prior to weaning is not impacted by calf starter intake, it is impacted by forage intake. In a recent study (McCurdy and Laarman, unpublished), our team fed calves 1200 g/day of milk replacer, unlimited calf starter, and either unlimited long-stem forage or limited (90 g/day) long-stem forage. All calves were housed on sand to limit fibre intake to the forage source only. While there was no difference in starter intake between the two treatments, calves fed limited amounts of forage tended to have a lower rumen pH (5.98 ± 0.23 vs. 6.38 ± 0.16, P = 0.09). The duration of SARA, where rumen pH fell below 5.8, increased from 261 min/day to 796 min/day (P = 0.03; Table 3). Despite these differences in rumen pH, no difference in average daily gain was noted. The similar growth rates in all these studies suggests that the performance of these calves is unaffected when rumen pH drops.

Table 3. Performance of pre-weaned calves, housed on sand, fed high amounts of milk replacer, unlimited calf starter, and a long-stem forage source either free-choice or limited to 90 g/d.

	Free Choice	Limited Forage Provision	*P* Value
Starter intake, g/d	617 ± 90	763 ± 106	0.33
Average daily gain, kg/day	0.91 ± 0.03	0.89 ± 0.04	0.61
Total VFA, mM	87.7 ± 13.1	62.1 ± 16.7	0.16
Average pH	6.38 ± 0.16	5.98 ± 0.23	0.09
Duration pH <5.8, min/day	261 ± 133	796 ± 145	0.03

▪ **Conclusion**

In the young calf, the immature rumen is physiologically distinct from that of a mature cow, where an increase in rapidly fermentable carbohydrates increases fermentation rates, leading to increased VFA concentrations and a decreased rumen pH. The young calf, however, responds quite differently, and rumen pH appears much more stable through weaning with ad libitum feeding of calf starter and forage. Rumen pH can be manipulated before and immediately after weaning without compromising productivity, and inducing SARA in young calves may be a useful tool for promoting gut development. At this stage, our team is working on research to define the conditions and timeframe under which SARA may have beneficial effects on rumen development in young animals. Despite uncertainty regarding optimal rumen

pH in calves, recent research results indicate optimal rumen pH in calves is likely to be very different from that of mature cows.

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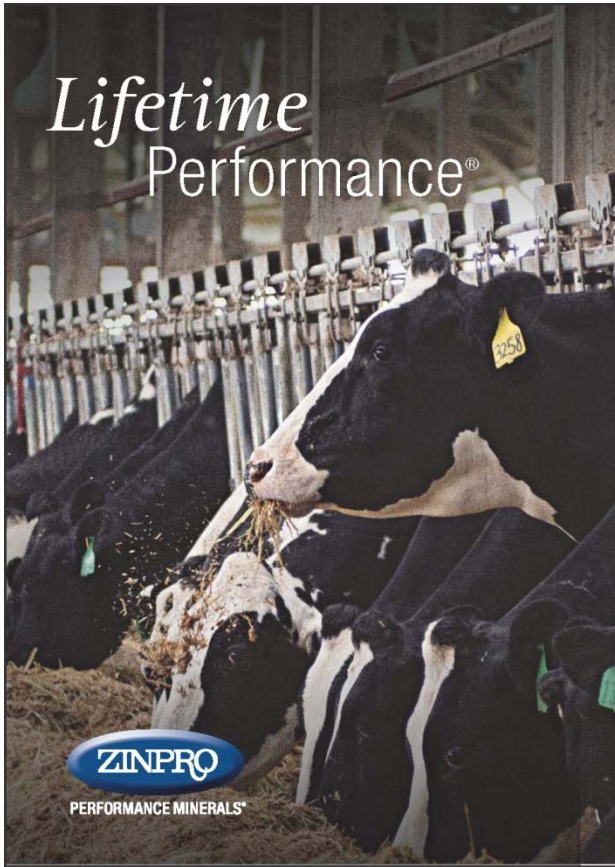
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Economics of Raising Dairy Replacement Heifers in Canada

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▪ Take Home Messages

- The estimated cost for raising a Holstein replacement heifer for Canadian dairies is C$2890.

- The highest cost per day occurs during the milk feeding phase and the second highest cost per day is during the immediate prepartum period.

- The cost of raising heifers from birth to first calving should reflect the impacts of initial calf value, mortality and culling, and the opportunity cost of these expenditures.

- The final cost reported here was similar but slightly higher than the cost estimated currently for a similar U.S. dairy after accounting for the currency conversion of C$1= US$0.76. Key differences included a higher labour cost, a higher cost per artificial insemination service, and higher cost/kg for calf starter grain and calf grower grain than those evaluated in the US.

- Individual results may differ, but in general, it appears that the costs of raising heifers in Canada are similar to the costs in the U.S. and in both cases are much higher than current market prices for springing or fresh heifers.

▪ Introduction

Dairy replacement heifers, much like dry cows, are often overlooked, undermanaged, and lamented as a large cost centre because there is little to no income generated from them until they enter the milking herd. While it is true that replacement heifer programs usually rank as the second or third largest cost of producing milk (trailing only feed costs and perhaps labour), these costs should more properly be viewed as an investment toward the future, and though raising costs can be significant, managing this process efficiently can yield large returns on this investment. Much like any other investment, money is spent up front for a return that will not be realized until much later, i.e., after the heifer calves and enters lactation. Careful attention to the correct kind and approach to this investing can influence the anticipated future returns.

Within the dairy heifer growing period, the highest daily expense is during the preweaning period and is a consequence of the liquid diet and the higher labour and housing costs associated with this time period. As a result of the large up-front costs, most producers historically have adopted management and feeding strategies that appeared to save money up front, but resulted in diminished performance and greater lost opportunity costs in the future. This traditional feeding approach provided low levels of milk replacer, commonly 3.5 to 4 L per day of a 20/20 milk replacer mixed to achieve 12% solids. Calf starter was usually fed ad libitum and contained only 16–18% crude protein on a dry matter basis (Drackley, 2008). This feeding approach encouraged earlier and higher levels of calf starter grain intake, thereby reducing the total amount and cost of liquid feed provided. As expected, this resulted in a lower daily feed cost but a longer total rearing time because of a slower rate of gain in height and weight leading to a delay in reaching breeding size.

Intensive feeding and management programs have received a lot of attention in the last decade or so with several studies showing that delivering more nutrients preweaning has been associated with improved health via reduced morbidity and mortality, greater weight and frame growth, earlier age at first service,

earlier age at first calving, and increased milk yield during the first lactation (Jasper and Weary, 2002; Davis Rincker et al., 2011; Van Amburgh et al., 2011; Soberon et al., 2012; Soberon and Van Amburgh, 2013). Consequently, many farms have begun more aggressive nutritional approaches by providing more volume and more nutrient dense liquid feed, either by providing more saleable whole milk or pasteurized waste milk, or higher volumes of milk replacer, often mixed at higher solids levels. Typical milk replacers used for Holsteins in these intensive programs are 25–28% protein and 15–20% fat and are fed at 12–15% milk solids with a total of 4–10 L of fluid volume per day, depending upon the size and age of the calf and management preferences. Pasteurized waste milk or saleable whole milk also work well to improve calf health and growth. Feeding higher levels of nutrients will allow 0.8–1.1 kg/d (1.7 to 2.5 lb/d) or more of body weight gain, depending upon environmental conditions, volume of milk provided, and the quality and quantity of the calf starter grain mix consumed. Additionally, the higher level of nutrients can allow calves to withstand more environmental stressors without resulting in weight loss or spikes in morbidity.

Dairy farms often fall somewhere in between a completely conventional approach and a fully intensive one. The most successful programs that have impact well beyond weaning usually include starter rations, grower rations and subsequent rations that provide high levels of metabolizable protein to support rapid and efficient lean tissue gain and frame growth without promoting fattening (Van Amburgh et al., 2008; Van Amburgh et al., 2011; Soberon et al., 2012; Stamey et al., 2012; Van Amburgh, 2017).

The downside of the intensive approach is that the feed cost during the liquid feeding period is significantly higher than that of the conventional approach and calves sometimes are slow to begin eating calf starter. The weaning process is critical when feeding higher volumes of milk and calves must be properly transitioned from milk to feed. As calves grow and move through the various diet and pen changes, they are provided with rations that continue to be higher in metabolizable protein than comparable conventional rations; these larger heifers eat more feed per day because of their larger body size and higher growth rates. Consequently, the increased feed costs continue through the entire replacement rearing period. However, these well-fed heifers usually experience the advantages of a reduction in both morbidity and mortality, reduced impact of cold weather stress, an earlier age at first service and first calving, and improved feed efficiency because total days on feed is reduced but rate of gain is increased. Because of the faster rate of gain, there should be a reduction in the total inventory of heifers on farm at any given time due to the reduced time from birth to calving. Also, because fewer heifers are expected to experience disease or mortality due to the improved nutritional management, fewer calves need to be placed into the system to meet the targeted number for future replacement needs. Finally, there is also an improvement in the expected net present value of heifers at calving due to the projected improvement in production that has been associated with improved nutrition and management as heifers (Soberon et al., 2012).

The size and health status of heifers at first calving are highly associated with first lactation productivity and survivability. Heifers immediately postpartum should weigh approximately 85% of mature cow weight in order to achieve their genetic potential for first lactation milk production (Van Amburgh, 2017). Heifers prioritize growth during their first lactation in an effort to achieve approximately 92% of mature size by the start of second lactation. Undersized heifers at first calving will partition disproportionately more energy intake towards growth vs. production relative to heifers that are closer to the size goal. From a lifetime perspective, more milk in the first lactation should also generate greater lifetime productivity assuming the normal expected increases in projected 305d milk from first to second, second to third, and third to fourth lactation.

The objective of this presentation is to estimate the current cost of raising Holstein replacement heifers using current estimated Canadian economic values within a modelled approach representative of many commercial dairies today that have adopted a moderately aggressive feeding and management system from birth through calving.

▪ **Economic Modelling**

An existing partial budget model was modified to incorporate current Canadian economic values to estimate the cost of raising Holstein dairy replacement heifers (Overton and Dhuyvetter, 2017). Unless otherwise stated, all values used as inputs or reported as outputs are reflected as Canadian currency (C$). The model is divided into age groups based on feeding, housing and management needs and consists of six different stages. Each stage has its own unique features regarding nutrition, housing, vaccination, morbidity, and mortality. The feeding strategy within each stage is designed to provide sufficient metabolizable protein and metabolizable energy to ensure appropriate frame growth and weight gain without promoting excessive body condition. An age appropriate vaccination protocol covers the heifers from birth to calving. Mortality risk varies by stage but reflects typical levels expected in average to above average Holstein herds. Morbidity includes diarrhea in the first stage and pneumonia across all stages. Each disease has its own treatment protocol using age appropriate therapy with dosage based upon body weight of the heifer being treated. Housing type for each stage varies but usually represents a 50/50 blend of two different indoor housing options. In all cases, the estimated investment cost for housing is amortized over the projected lifetime of the facility in question and then, bedding costs are added to better represent the true economic cost and not just cash flow needs.

1) Stage 1: birth to 2 months of age

   a. An initial wet calf value of $75 is assumed

   b. All calves receive two feedings of colostrum

   c. A 28/20 milk replacer, mixed to 14% solids, and fed at the following rates: 5 L/day for 7 days; 7 L/day for 42 days; 3.5 L/day for 7 days

   d. Ad libitum 22% calf starter grain

   e. Calves are housed in individual calf hutches until 60 days of age

2) Stage 2: 2 to 4 months of age

   a. Post-weaning period with 20% grower grain and calf hay

   b. Heifers are housed indoors in small, bedded group pens

3) Stage 3: 4 to 10 months of age

   a. Total mixed ration (TMR)-based feeding

   b. Heifers are housed in either larger, bedded group pens (50%) or freestalls (50%)

4) Stage 4: 10 months through completion of breeding

   a. TMR-based feeding

   b. Six 21-day cycles of breeding opportunity with conventional semen assuming an average conception risk of 56% and an average insemination risk of 68%

   c. Heifers are inseminated starting at 57% of mature weight

   d. Artificial insemination (AI) cost/service is $40 and includes the semen, supplies and insemination fee

   e. 7% of the heifers that enter the breeding program are culled because of reproductive failure

   f. Heifers are housed in either larger, bedded group pens (50%) or freestalls (50%)

5) Stage 5: gestation and growth

   a. TMR-based feeding

   b. Heifers are housed in larger, bedded group pens (50%) or freestalls (50%)

6) Stage 6: springers (final two months prior to calving)

    a. TMR-based feeding

    b. 0.76 kg/d daily gain

    c. Heifers are housed in either bedded packs (50%) or freestalls (50%)

The growth curves used to generate the predicted daily gains from birth to calving were fit from multiple sources. Preweaning feed intake and growth was adapted from two published studies and an unpublished study from a large, private commercial herd that has been using a more intensive feeding system for 10 years or more (Raeth-Knight et al., 2009; Hengst et al., 2012). Postweaning growth curves were modelled from multiple sources with 50% of the contribution coming from unpublished commercial data, 25% of the source data adapted from the 75th percentile of older, published growth data from Penn State University, and the remaining 25% of the source data based upon the 95th percentile of the Penn State data set (Jones and Heinrichs, 2004). The 75th and 95th percentiles were used from the Penn State data instead of the median because the original growth data were captured prior to widespread adoption of more intensive feeding approaches.

Throughout each of the cycles, modelled costs include the upfront purchase cost or initial value of each heifer; the feeding, housing, equipment, reproductive management, labour, and health management costs of each heifer; and the interest or opportunity costs associated with mortality or alternative investment opportunity. All costs, including the costs attributed to the rearing expenses of the calves that die, are adjusted to the net present value expected at calving using an interest rate of 6% and are distributed over the heifers that actually survive to calving. In other words, all expected costs for every calf that enters the rearing enterprise is redistributed over the surviving heifers.

▪ **Results and Discussion**

The details for mortality, culling, costs, and gains for each stage are shown in Table 1. The cumulative mortality risk was 7.3% and together with the removal of heifers that experienced reproductive failure, the total removal risk from live birth through calving was 13.8%, resulting in a heifer completion risk of 86.2%. The costs incurred for each heifer that died or was removed was shifted onto the surviving heifers within each stage as the losses occurred. The value of heifers culled because of reproductive failure was set at $1.54/kg of live weight based on suggestions from my colleagues in Canada. Of course, individual results will vary and the value received for culls will also vary by region. Most herds will cull some heifers along the raising process based upon health and growth performance, but that cost was not modelled here. Based upon current economic values of cull heifers, the diversion of heifers at any stage from the dairy replacement population to a beef operation (culling) results in significant losses for each heifer removed. These losses must be shifted back onto the surviving heifers and result in an even greater cost per heifer calving.

Table 1. Summary of estimated mortality, costs (Canadian dollars), and weight gain by growth stage from birth to calving for Canadian Holsteins as modelled for a hypothetical dairy using strictly indoor housing.

Stage	Hutch	Post Wean	Growing	Breeding	Post-breeding	Close-up
Age in months	Birth to 2	2 to 4	4 to 10	10.0-16.0	16.0-21.6	21.6-23.6
Mortality	3.50%	1.75%	1.00%	0.50%	0.30%	0.25%
Culled (sold)	0.0%	0.0%	0.0%	0.0%	7.0%	0.0%
Colostrum*	$19	0	0	0	0	0
Milk*	$160	0	0	0	0	0
Starter*	$30	0	0	0	0	0
Grain*	0	$126	0	0	0	0
Hay*	0	$5	0	0	0	0
Feed (TMR)*	0	0	$261	$352	$410	$193
Total Feed*	$209	$131	$261	$352	$410	$193
Labor*	$169	$64	$69	$68	$69	$131
Vet Med/ Health*	$11	$3	$8	$3	$3	$16
Breeding & Culls*	$0	$0	$0	$73	($51)	$0
Housing and Other*	$29	$18	$56	$64	$80	$44
Interest*	$2	$5	$25	$39	$55	$24
Total Cost*	$421	$221	$419	$598	$565	$408
Cost/ Day*	$7.01	$3.58	$2.29	$3.28	$3.29	$6.71
Entering Weight (kg)	39	87	148	319	477	614
Exit Weight (kg)	87	148	319	477	614	661
Average daily gain (kg)	0.81	0.98	0.94	0.87	0.80	0.76
Cumulative ADG (kg)	0.81	0.90	0.92	0.90	0.88	0.87
Cumulative from birth						
Total Cost*	$421	$649	$1,075	$1,679	$2,375	$2,792
Cost/ Day*	$7.01	$5.33	$3.53	$3.45	$3.61	$3.88
Cost Including Wet Calf*	$499	$730	$1,159	$1,766	$2,472	$2,890

*Adjusted for death loss, with costs incurred by heifers that die within each stage shifted onto the surviving heifers.

Table 2 shows the estimated morbidity and treatment cost per heifer by stage. The cost per heifer was calculated as the total cost for each heifer treated within each stage divided by the total number of heifers present. The disease levels shown here are reflective of disease rates commonly recorded in on-farm records but likely underrepresent the true disease levels occurring on farms.

Table 2. Modeled morbidity risks and costs: these costs represent the cost per heifer present in each stage and were calculated by dividing the cost per case treated by the total population of heifers present.

Stage 1		Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
24 hrs – 2 mos		2 – 4 mos	4 – 10 mos	10 mos - breeding	Post breeding	Close-up (final 2 mos)
Diarr Dz.	Resp Dz.	Resp Dz	Any Tx	Any Tx	Any Tx	Any Tx
16.0%	10.0%	7.5%	3.0%	1.5%	0.8%	0.6%
$5.07	$1.53	$2.74	$1.54	$1.27	$0.80	$0.67

Diarr Dz = diarrhea; Resp. Dz = respiratory disease, Any Tx = any treatment

As expected, the highest cost per day was during the preweaning period (Stage 1), with an estimated cost of $7.01. During this phase, the milk feeding, housing approach, and labour requirements result in the highest feed, housing, and labour costs/day than at any other stage. The second highest cost/day is during the final stage where prepartum heifers are fed more costly TMR, and the labour and total vaccination costs are much greater.

Heifer growth across the entire growing period was curvilinear in nature. Growth was modest at 0.81 kg/day in the preweaning period, was maximized in the early postweaning period at 0.98 kg/day, and then declined slowly across the raising period for an average of 0.87 kg/day. Note, many herds are able to achieve much higher rates of gain across the entire raising period but higher rates of gain require even higher levels of nutritional management. The goal of this project was to mimic a current system used by a representative dairy herd, thus, the growth performance was more moderate in nature.

Within this model, attempts have been made to represent the true estimated costs using Canadian inputs and Canadian dollars where possible. While no model is perfect, valuable insights can be gained through modelling efforts. Usually, producers are surprised to learn of the estimated costs associated with raising replacement heifers. Some of the surprise comes from a failure by many to account for losses incurred because of culling or mortality. Some of the added expenses previously unaccounted for result from my attempt to capture the opportunity costs of capital invested in the raising period. Finally, another large source of cost often not fully evaluated are the labour and housing costs. In this model, housing costs are estimated by amortizing the initial construction cost for a facility over 25 years and adding in projected daily costs for bedding. Labour was estimated by use of survey results by the author across different herd sizes.

The final cost reported here was similar but slightly higher than the cost estimated currently for a similar U.S. dairy, after accounting for the currency conversion of C$1 = US$0.76. Key differences included a higher labour cost, a higher cost per AI service, and higher costs for calf grain than those used in my U.S. model. However, the TMR costs used here for older heifers were slightly less than my U.S. values. All Canadian inputs were provided by individuals working within the Canadian dairy industry and may or may not fully represent the true current values across Canada. Of course, individual results may differ, but in general, it appears that the cost of raising heifers in Canada are similar to the costs in the U.S. and in both cases are much higher than current market prices for springing or fresh heifers.

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Trouble-Shooting Reproduction Issues

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▪ Take Home Messages

- The best metric to quantify the overall herd reproductive performance is the pregnancy rate. In Holstein herds, it should be ≥ 25%.

- When the pregnancy rate is below expectation, the first step is to look at the insemination rate. In Holstein herds, it should be ≥ 61%.

- An insemination rate below expectation is generally associated with a long interval between calving and first insemination, poor heat detection, a nonpregnancy diagnosis that occurs too late or a long interval between a nonpregnancy diagnosis and the subsequent re-insemination.

- If the insemination rate is appropriate, the next step is to look at the conception rate of the herd. In Holstein herds, it should be ≥ 42%.

- If the conception rate is below expectation, it is important to determine if it affects all inseminations or only the first insemination. Common causes affecting all inseminations can include poor hormonal protocol compliance, poor insemination technique and semen handling, elevated milk urea nitrogen, and infectious diseases. Common causes affecting only the first insemination can include a high prevalence of postpartum diseases (hyperketonemia, endometritis, prolonged anovulation) and poor hormonal protocol compliance.

▪ Introduction

Dairy producers know that efficient reproduction management is important to maximize productivity and profitability of their herd. Although the mathematical demonstration of this relationship can be complex, one of the main benefits resulting from efficient reproduction management is the reduction of days open, because they are costly.

On a daily basis, reproduction management can be done by a dairy producer at two different levels. The first level is the individual management of each cow. In other words, the farmer will make decisions regarding a cow in order to optimize specifically her performance. For example, a cow is diagnosed open by a veterinarian at the herd health visit and a strategy to rebreed her will be implemented to maximize her chance of becoming pregnant. It could be based on individual characteristics of this cow such as having or not having a corpus luteum.

The second level involves making decisions at the herd level (or group level). In other words, herd-level strategies will be established to improve the group performance although sometimes some cows might not benefit from it. For example, a cow is diagnosed open by a veterinarian at the herd health visit and she is automatically enrolled in an ovulation synchronization protocol (e.g., Ovsynch) no matter what structures she has on her ovaries. This strategy is implemented on this farm to organize and facilitate farm staff work. Although it depends on what day of their estrous cycle the cows are at, most cows will benefit from this strategy (i.e., have a high chance of conception) but some will not (if they were at a specific time in their cycle when they will not optimally respond to the synchronization protocol).

Although it may seem counter-intuitive, doing all you can to maximize the performance of each individual cow does not always lead to good herd-level performance. In reproduction, maximizing cow-level management generally implies maximizing the chance of conception of each cow. The potential problem with this approach is that sometimes producers might choose to delay insemination in order to obtain a potentially better conception probability. It may seem like a good idea for this cow to wait for the perfect time to breed her and optimize her probability of conception. However, not breeding her for many days will give you zero chance of conception. This will likely result in an increase of days open for this cow. This is probably not a big deal if you only do it for her, but it will soon be costly if you apply this strategy to a certain proportion of cows in your herd.

When troubleshooting reproduction issues in a herd, it is important to use herd-level metrics to focus on problems that affect a large proportion of cows. In other words, if a cow is having a reproduction problem, she will not be the cause of your low pregnancy rate at the herd level. Something else is going on. If we look at it the other way, it can also imply that a herd with an excellent pregnancy rate may have some cows that are still not pregnant at 300 days in milk (DIM).

Although troubleshooting reproduction issues may sometimes seem complex, one should keep in mind that some problems are common in your region and some others are less frequent. Therefore, the first focus of your investigation should be based on common causes instead of focusing on the not-so-frequent causes.

▪ What Metrics to Use to Assess a Herd Reproductive Performance?

The best metric available to quantify the reproductive performance of a herd is the pregnancy rate (PregR). This metric is commonly used on farms and can be computed on a 21-day basis, a monthly basis or a daily basis. Most software programs used for daily management on Canadian farms provide a PregR and their calculation is relatively similar. The PregR refers to the proportion of cows that became pregnant out of all the cows eligible for pregnancy during a certain time period. All cows that have completed the voluntary waiting period and that are not yet pregnant or do not have a 'do not breed' status will be computed as eligible cows for pregnancy. Globally, the PregR reflects the speed at which eligible cows become pregnant over time.

If you only have one number to look at to figure out if your herd reproductive performance is good or not, it has to be the PregR because it reflects the overall performance. A good farmer always knows his average bulk tank milk production per cow. The same holds true for somatic cell count. I highly recommend adding the PregR to this list of unavoidable numbers.

Other important metrics in reproduction management are the insemination rate (InseR) and conception rate (ConcR). The InseR refers to the proportion of cows that were inseminated out of all the cows eligible for pregnancy during a certain time period. This metric reflects the speed or intensity of insemination within the population of eligible cows. The ConcR refers to the proportion of cows that became pregnant out of the all the ones that were inseminated during a certain time period. It reflects the proportion of success at insemination. To investigate further, the ConcR can be stratified to look at conception rate at the first insemination (ConcR1st) or at the second insemination or more (ConcR2+).

▪ Investigation Steps

The purpose of the current manuscript is to provide some guidelines to investigate reproductive performance in a herd. To help you when troubleshooting your herd, I have some suggestions of questions to look at. This approach is designed to help identify the common causes of reproductive problems and not to summarize all the potential causes. These questions should help you figure out the main bottlenecks in your herd and give you some insights on potential solutions. Make sure to discuss with your veterinarian and other advisors to validate that this approach is relevant for your herd. It may need to be adjusted based on your location in the country. Many numerical objectives will be presented

over the next pages; these targets are based on what research suggests or on what the best herds can generally achieve. Keep in mind that these numbers are only suggestions and are based on Holstein herds.

Question 1: Is your PregR ≥ 25%?

The PregR is the overall reproductive performance metric, so it is the one to look at first. Based on currently available data in Québec, the median PregR rate was 18% in 2018. It must be similar in the rest of Canada because it has been in previous years. The median PregR has been increasing steadily in Québec and Canada over the last decade. Top herds will generally have PregR between 30 and 35%.

If your herd's PregR is below 25%, it means that there are opportunities for improvement. Two main metrics can guide you to identify the bottlenecks in your herd: InseR and ConcR. The first one informs you on the intensity of insemination whereas the second one informs you on the success of these inseminations. Because cows need to be inseminated to become pregnant, it is logical to look into InseR first.

Question 2: Is the InseR ≥ 61%?

If your herd's InseR is below 61%, it means that the intensity of insemination in eligible cows could be improved. Based on currently available data in Québec, the median InseR rate was 45% in 2018. It must be similar in the rest of Canada because it has been in previous years. The median InseR has been increasing steadily in Canada over the two last decades; this increase is largely responsible for the PregR increase over the same time period. Top herds will generally have InseR of 70% or more.

Four key points to get a high InseR include:

‣ Early insemination after the end of the voluntary waiting period

‣ Good heat detection

‣ Early identification of nonpregnant cows after last insemination

‣ Short interval between diagnosis of nonpregnancy and subsequent insemination

The first key point ensures that no time is lost at the end of the voluntary waiting period. The three other key points ensure that cows that did not become pregnant at the insemination will be identified early and re-inseminated quickly.

Question 3: Is the average days in milk at first insemination ≤ 66 days?

This question helps to figure out if cows are bred early enough after the end of the voluntary waiting period. Similar to any plot graph interpretation, two important things need to be investigated while looking at the graph: 1) the average (or central tendency), and 2) the dispersion of the dots. Ideally, the average of this metric would be low and the dispersion of cows would be homogenous (similar between cows). If it is heterogenous (largely different) between cows, it may imply that the potential gain could come from avoiding to have a certain proportion of cows that are inseminated late or very late.

In Québec, the average DIM at first insemination is 72. Top herds will manage to have all of their cows inseminated within 21 days (a cow cycle) after the end of the voluntary waiting period. For instance, a herd with a 50-day voluntary waiting period would imply that all the cows are bred for the first time no later than 71 DIM, which would likely lead to an average of 60 DIM at first insemination.

The following plot graph (Figure 1) is an example of an opportunity for improvement in a herd. Two problems can be observed. The first one is that all cows are inseminated for the first time after 80 DIM, which can be considered late. The second one is that 53% of cows (8/15) are inseminated after 100 DIM,

which is considered very late. Some solutions could be: 1) find ways to avoid cows being inseminated for the first time after 100 DIM (the use of ovulation or estrus synchronization protocols could help, and 2) start inseminating all cows as soon as 50 DIM to reduce the proportion of cows being inseminated late or very late.
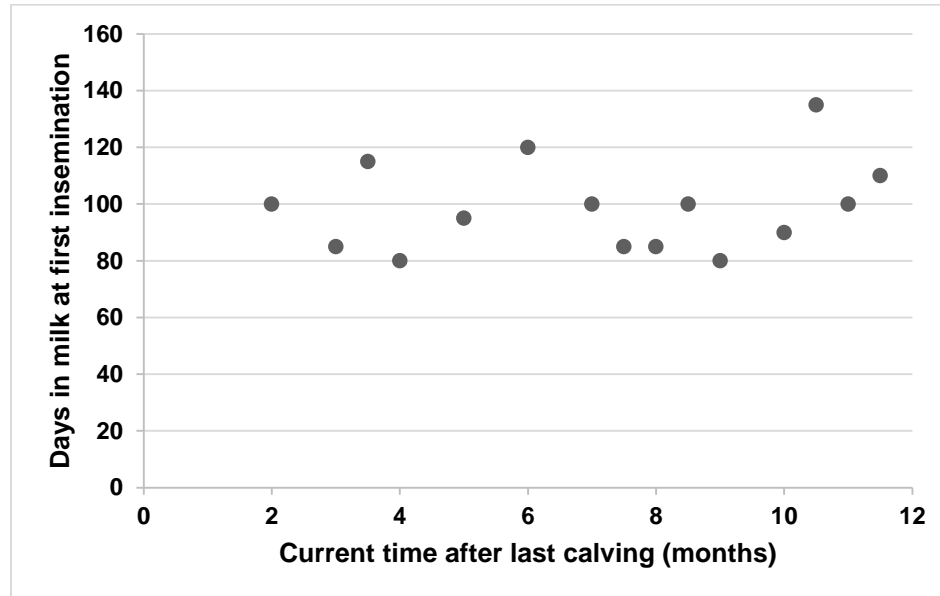
Figure 1. Plot graph of days in milk at first insemination of a herd (each dot represents a cow)

Question 4: Is the average interval between inseminations ≤ 36 days?

This question helps to figure out if cows that are not pregnant after the insemination will be identified early enough to minimize unproductive days. Again, the plot graph (Figure 2) can be looked at to investigate the average and the dispersion. All dots before 30 days after insemination generally reflect the intensity of heat detection by farm staff. After 30 days, the dots reflect the intensity to diagnose open cows and re-inseminate them in a timely fashion.

In Québec, the average interval between inseminations is 42 days. Top herds are generally able to get this interval to 30 days or less.

Figure 2 shows a herd with some opportunities for improvement. Only 23% of the cows (7/30) were re-inseminated before 30 days, likely by heat detection. This might be improved by promoting heat detection through the use of activity monitors or pedometers for instance. The dispersion of dots is quite large (up to almost 70 days), which could reflect that cows are not diagnosed open early (number of days after insemination at which cows are examined by veterinarian) or frequently (frequency of veterinary visits). It could also reflect that cows are diagnosed open early but there is a long delay between this exam and the subsequent re-insemination. Such a situation could be caused by a farmer who wants to wait for the next heat or by prostaglandin injections that did not result in cows being seen in estrus over the next couple of days. The use of ovulation synchronization protocols in such cases might be useful.
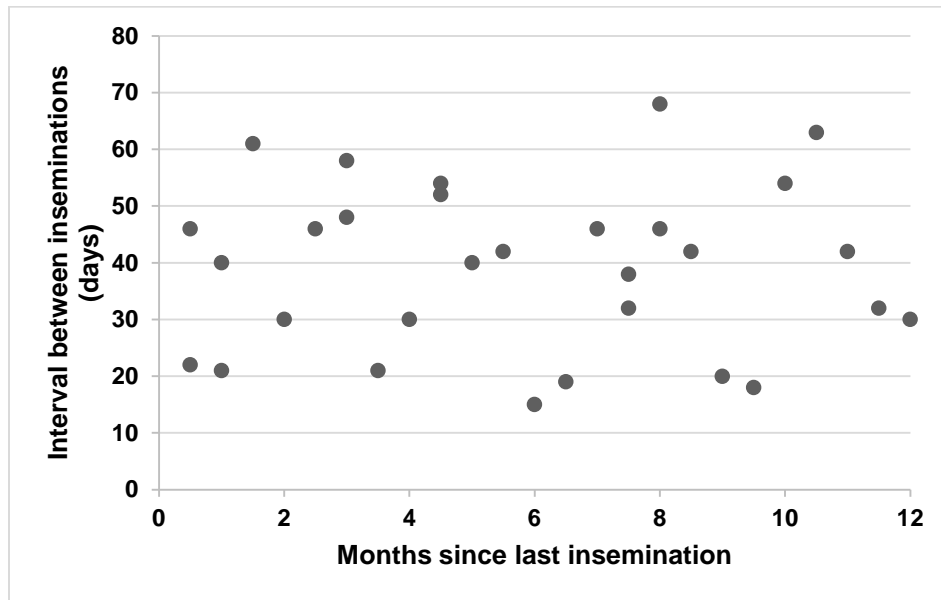
Figure 2. Plot graph of interval between inseminations of a herd (each dot represents a cow)

In summary, for a herd that has a PregR below 25%, the last questions were designed to identify if the InseR is low and what may cause such a situation. In cases where the InseR is ≥ 61%, the intensity of insemination is high; thus, it is likely that the problem comes from a low ConcR.

Question 5: Is the overall ConcR ≥ 42%?

If your herd's ConcR is below 42%, it means that the success of the insemination could be improved. Current Québec data suggest that the median ConcR was 38% in 2018. It must be similar in the rest of Canada. Although it has been slowly increasing over the last decade, the magnitude of this increase remains small compared to the InseR increase over the same period of time. Top herds will generally have ConcR at 45% or more.

Question 6: Is ConcR1st similar to ConcR2+?

If the overall ConcR is low, you must figure out if it is because it affects only the first insemination or if it affects all inseminations. When it affects all inseminations, the causes involved are likely to be different from a situation where the decrease in ConcR is only attributable to a decrease of ConcR1st.

When ConcR is Low for All Inseminations

If your overall ConcR is low and ConcR1st is similar to ConcR2+, various common causes can be involved. In herds where a lot of ovulation synchronization protocols are used, poor compliance with injection schedule might be an issue. Farmers need to keep in mind that compliance implies that the right cow gets the right product at the right dose at the right moment. It may seem easy to do but here are some examples of inappropriate compliance:

‣ Cow 42 got the injection instead of cow 22 (tag reading problem)

‣ Cow 54 got a 1-mL dose of a product that requires a 2-mL dose

‣ Cow 61 got the wrong product by mistake

‣ Cow 19 was not found this morning in the pen so she got her dose tonight instead

Herds with good compliance generally have ≥ 95% of their injections given correctly. In a herd using a protocol involving up to six injections (e.g., Double-Ovsynch), it is possible that only 70% of the cows got the ovulation synchronization protocol as initially planned (5% errors × 6 injections = 30% incorrect protocols). This could reduce the ConcR significantly.

Other causes to consider in such situations are poor insemination technique and semen management. It may seem easy to inseminate cows but it is hard for an advisor to know what is really done on a farm. Collecting data about individual ConcR of breeders may help to identify a breeder who struggles with his technique. However, caution should be taken when interpreting these data. For instance, if two breeders work on the same farm and each one selects which cows they prefer to inseminate, this may lead to a selection bias. In other words, one could only select the best cows (best heat) whereas the other breeder might end up with the cows that are not in such good heat. In such case, the farmer should probably not expect a similar ConcR from both breeders.

If all breeders have the same low ConcR, the ConcR may be related to poor semen storage (inappropriate temperature), poor semen handling and thawing (inadequate cleanliness of equipment and inappropriate temperature of bath), or extended time between semen preparation and insemination (ideally less than 10 minutes if kept at body temperature). Sometimes, the problem may be that semen handling is fine but it takes a long time between the first and the last cows bred, such as in a freestall barn with no headlocks in which the breeder keeps trying to catch cows without assistance. Therefore, do not thaw too many semen straws at a time; the number allowed will depend on how fast the cows can be bred.

Although it remains controversial for some, data suggest that high milk urea nitrogen (MUN) could be involved in low ConcR. The explanation would be from a variation in uterine pH, which could interfere with embryo development and survival. Research data showed that cows with MUN ≥ 18-20 mg/dL are at greater risk of embryo loss (Raboisson et al., 2017). Such a situation could occur when the ration is improperly balanced for degradable protein and non-fibre carbohydrates.

Infectious problems such as infectious bovine rhinotracheitis (IBR) virus, bovine viral diarrhea (BVD) virus, and some other infectious agents can also cause a decrease in reproductive performance of a herd. If herds are vaccinated with an appropriate vaccination schedule and adequate compliance, this situation becomes less likely.

When ConcR is Low Only for the First Insemination

If the overall ConcR is low because the ConcR1st is low, it is important to target causes that are probably linked with the postpartum management of the herd. For instance, a herd with a high proportion of cows affected by an excessive negative energy balance status would likely have a high proportion of cows with hyperketonemia (subclinical ketosis), prolonged anovulation (cows not cycling), and uterine disease (endometritis). A surveillance strategy for these diseases could be implemented to quantify if the proportion of affected cows is high or not. Here are some ways to perform such surveillance with the support of your veterinarian. To have a representative sample, 12–20 cows should be sampled (Oetzel, 2004):

‣ Testing blood samples to quantify beta-hydroxybutyrate (BHBA) within the first two weeks in milk. Less than 15% of cows should have blood BHBA ≥ 1.2 mmol/L (Dubuc and Denis-Robichaud, 2017).

‣ Testing blood samples to quantify non-esterified fatty acids (NEFA) within the last week before calving. Less than 15% of cows should have blood NEFA ≥ 0.27 mmol/L (Ospina et al., 2013).

‣ Testing cows for clinical (metricheck device) and subclinical endometritis (esterase testing) at 30 DIM. There should be less than 10% of cows with clinical endometritis and less than 20% of cows with subclinical endometritis (Dubuc and Denis-Robichaud, 2017).

▸ Testing cows for prolonged anovulation (absence of a corpus luteum) at 30 and 45 DIM (ideally two exams at an interval of two weeks). Less than 21% of cows should be anovular at both exams (Dubuc and Denis-Robichaud, 2017).

In addition to postpartum diseases, it is not uncommon that poor compliance to the ovulation synchronization protocol is involved in such situations, especially when a lot of protocols are used for the first insemination but not many after that.

▪ Conclusions

In summary, if the herd's PregR is below 25%, examine the InseR. Common causes of low InseR (below 61%) are generally associated with a long interval between calving and first insemination, poor heat detection, a nonpregnancy diagnosis that occurs too late or a long interval between nonpregnancy diagnosis and subsequent re-insemination. If the InseR is appropriate, the next step is to validate if the ConcR is low (below 42%). In such a situation, identify if it is the case for all inseminations or only the first insemination. Causes affecting all inseminations will likely involve poor hormonal protocol compliance, poor insemination technique and semen management, elevated milk urea nitrogen or infectious diseases. Causes affecting only the first insemination will likely include a high prevalence of postpartum diseases such as hyperketonemia, endometritis and prolonged anovulation. When troubleshooting a reproductive problem in a herd, it is important to consider common causes before less frequent causes are considered.

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Potential Impact of Viral Diseases on Conception Rates in Cattle

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▪ Take Home Messages

- Many viral diseases are endemic in cattle populations. Their impact on production losses is established, but effects on fertility are often underestimated.

- Viral infections can disturb normal ovarian function, inhibit early embryo development and cause abortions and fetal malformations later in pregnancy. They also suppress immunity and so increase the risk of cows developing uterine disease due to bacterial infections after calving.

- Three common viruses—BVDV, BoHV-1 and BoHV-4— all reduce conception rates.

- Knowing which viruses are present in a herd and taking proactive measures to reduce the risk of viral infections through vaccination and good biosecurity measures should improve general herd fertility.

▪ Introduction

Many viral diseases remain prevalent in the cattle population worldwide while new ones threaten to emerge. Some of these diseases are controlled in different countries through various vaccination and slaughter programs, but trade globalization, increasing herd size, and environmental change have all contributed to their spread. They are known to cause major financial losses to the dairy industry (Richter et al., 2017). With respect to fertility, the ability of some viruses to cause abortions and fetal malformations is the most evident manifestation and has received the most attention (Ali et al., 2012). The outcome will, however, depend on the stage of pregnancy when the initial infection occurs. Conception rates may be reduced and this in turn will increase the risk of culling through failure to conceive in a timely fashion. These effects are much harder to quantify because many other factors relating to the health and management of the cows are also influential, so it is hard to separate out their respective contributions. This means that the potential impact of viral disease on fertility is generally underestimated. This paper describes some of the ways by which viruses can reduce conception rates using three common viruses found in cattle as examples: bovine viral diarrhea virus (BVDV), bovine herpes virus 1 (BoHV-1) and bovine herpes virus 4 (BoHV-4). These have varied mechanisms of action and are all currently endemic in the Canadian cattle population.

▪ Factors Affecting Reproductive Efficiency

Many factors affect the reproductive performance of individual cows and consequently herd fertility. These can be categorized under the following three broad headings: 1) the interval from calving to resumption of ovulation and regular estrous cycles, 2) estrous detection efficiency and submission rate, and 3) conception rate following service. In general, about 80% of dairy cows ovulate for the first time within the first 4 weeks after calving, with around 10% of cows remaining acyclic at 6 weeks (Wathes et al., 2007). The main cause of prolonged anestrum is negative energy balance (NEB), which impacts the ability of the ovary to grow and ovulate a mature dominant follicle. Other events in the peripartum period such as an abnormal calving or early disease episodes (e.g., mastitis, metritis, displaced abomasum) are also influential.

Once cycles have resumed, the subsequent pattern of estrous cycles can be monitored through the use of milk progesterone profiles (Wathes et al., 2007). If a cow has ovulated within 4 weeks of calving, she can experience one or two cycles before she needs to be bred, which increases the likelihood that she will conceive at the appropriate time. However, only about half of high yielding dairy cows achieve this normal pattern. Apart from a delayed resumption of cyclicity, some cows develop a persistent corpus luteum. This occurs more often in multiparous cows and is particularly associated with uterine disease. Opsomer et al. (2000) reported incidences of 15% in first parity cows, 24% in parities two and three, and 32% in parities ≥ 4. Other cows (5-10%) experience an irregular pattern of fluctuating progesterone levels, which may be associated with the development of luteinized cysts (Opsomer et al., 2000; Wathes et al., 2007). All of these irregularities in cycle pattern make reliable heat detection more difficult. Because they reflect underlying issues relating to the health and physiology of the individual cow, they will also affect the likelihood of a successful response to a timed insemination regime.

The actual conception rate is determined by the initial fertilization rate minus any subsequent losses due to death of the embryo (Table 1). Providing that adequate numbers of spermatozoa are used from bulls with high fertility and that cows are inseminated at the correct stage of the cycle, then available evidence indicates that fertilization rates are usually around 90%. Calving rates to a particular service are, however, much lower and this is largely attributable to high rates of early embryo loss (Diskin et al., 2016; Table 1). Calving rates to a particular service of around 65% are achievable in dairy heifers, reducing to 30% or less in high-producing cows. If fertilization rates are 90%, this implies that around 35–70% of all potential pregnancies do not result in birth of a live calf. Of these, approximately 40% of bovine embryos die in the first 3 weeks after service or insemination, with cows returning to estrus at the normal time at about 21–24 days. A further 5–25% of embryos are lost between days 24–60 of gestation. Greater loss rates at this later stage have been associated with higher levels of milk production, episodes of mastitis or high ambient temperature. The later embryo losses are numerically fewer than the earlier ones, but they have a greater impact on the fate of the individual cow. By the time they are detected it may be too long after calving to rebreed the animal, so many are culled. Once the pregnancy progresses beyond two months then abortion rates are usually quite low, around 5%. Abortions are often associated with specific diseases that include opportunistic bacterial infections (e.g., *Arcanobacterium pyogenes*), zoonotic bacteria (e.g., *Leptospira spp*), fungal infections (e.g., *Aspergillus fumigatus*), protozoa (e.g., *Neospora caninum*) and viral infections (e.g., BVDV, infectious bovine rhinotracheitis). However, in over half of cases submitted for diagnosis no infectious agents are identified (Givens and Marley, 2008).

Table 1. Summary of pregnancy losses in cattle from insemination until calving

Stage of pregnancy	Reason	Reported range of lost pregnancies	Number of cows still pregnant	
			Worst case	Best case
Insemination of dam			100	100
Fertilization of oocyte	Fertilization failure, wrong time AI	0-20%	80	100
0-24 days post AI	Early embryo mortality	30-50%	40	70
25-60 days post AI	Late embryo mortality	5-25%	30	67
61 days until term	Abortion	3-10%	27	64

▪ **Establishment of Pregnancy**

Following a successful fertilization, the embryo should enter the uterus on days 4–6 post mating and then form a spherical blastocyst, initially surrounded by the zona pellucida that enclosed the original oocyte.

The blastocyst then hatches out of the zona pellucida on about day 8 and develops into an ovoid and then tubular conceptus that begins to elongate on around day 15 into a filamentous form which should extend to occupy the entire length of the uterine horn. This elongation is critical to enable production of sufficient interferon tau (IFNT), which is synthesised by the outer trophectodermal layer of the conceptus from around day 8 (Dorniak et al., 2013). Interferon tau is the pregnancy recognition signal in cattle and is required to prevent regression of the corpus luteum and the continued production of the pregnancy hormone progesterone. It also induces the endometrium to produce a large number of other molecules that act to remodel the endometrium to a receptive environment and promote further development of the conceptus. This is essential to enable successful implantation and subsequent formation of the placenta.

It can be seen from the above that anything which perturbs the early stages of embryo development will also reduce reproductive efficiency. Some embryos fail to develop normally because of genetic abnormalities that were present from fertilization. The environment within the cow's reproductive tract is equally important because it must provide everything required to support blastocyst growth (Dorniak et al., 2013). The uterine environment is strongly influenced by a variety of factors that include the ovarian steroid hormones estradiol-17β and progesterone, the nutritional status of the cow, and inflammatory agents including prostaglandins and cytokines. Amongst these, the timing of the rise in progesterone after ovulation is of key importance, while any infectious diseases contracted earlier in the postpartum period, particularly those causing endometritis, can result in long term inflammatory changes in the endometrium.

Specific examples with references are given below, but in broad terms viral diseases influence many of the processes outlined that are required to achieve a successful conception and establish pregnancy. Fever during the follicular stage of the estrous cycle can inhibit ovulation via actions at the level of the brain. Viruses can also affect the ovary directly, inhibiting follicular development and steroid hormone production. Based on in vitro culture systems, direct effects on the early stages of embryo development are sometimes apparent although viruses may be unable to cross an intact zona pellucida. Many viruses have, however, evolved mechanisms to evade or suppress the normal immune mechanisms that the host uses to repress them in order to promote their own survival. Immunosuppression can then make the animal more susceptible to any other disease agents to which it is exposed. Some of these immune mechanisms are also essential components of pregnancy recognition and implantation, so these may also be prevented.

▪ Examples of Viral Diseases Affecting Fertility

Bovine Viral Diarrhea Virus (BVDV)

BVDV is a pestivirus belonging to the family *Flaviviridae*, comprising a single-stranded, positive sense RNA genome that is classified by sequence differences as type 1 or 2 (BVDV-1 or -2). It is endemic in many countries, with a prevalence of 40–90% of individual cattle and 28–66% of herds and is known to cause major economic losses (Richter et al., 2017). It exists as either cytopathogenic (cp) or non-cytopathogenic (ncp) biotypes, with the ncp biotype causing the majority of field losses (Ridpath, 2003). A third atypical pestivirus, BVDV-3, is also in circulation.

If a pregnant cow becomes infected with ncp BVDV between about 1 to 4 months of gestation, before the fetus develops its own immune competence, the result is either early embryonic death(abortion) or birth of an immunotolerant calf that is persistently infected with BVDV. Persistently infected calves shed virus continuously, so if they are undetected at birth or are purchased, they are a major source of infection within a herd. Those herd members that subsequently become infected acutely should be able to eliminate the virus within 10–14 days. During this period, they transmit virus to other animals they are in contact with, mainly via secretions from the mouth and nose, but direct transmission to the reproductive tract via semen from an infected bull or embryo transfer is also possible. Some animals carry transmissible virus for several months after they have apparently recovered. During such an acute infection BVDV causes immunosuppression through its ability to inhibit production of type 1 interferons (IFN), so delaying the host's responses and enabling the virus to complete its own replication cycle

(Oguejiofor et al., 2019).

With respect to fertility, the effects on abortion, fetal deformity and birth of persistently infected calves are the best known consequences and a meta-analysis of vaccinated cows found a reduction of nearly 45% in the abortion rate and of 85% in fetal infection in comparison with unvaccinated cohorts (Newcomer et al., 2015). BVDV can, however, impact on most stages of the reproductive cycle, depending on when an individual cow or heifer becomes infected. A number of primary studies using either experimental infections of naïve animals or field reports have attempted to quantify conception rates and have reported reductions of up to 44% if an animal develops an acute infection in an approximate 4-week window covering the period immediately before or after insemination (Fray et al., 2000). No such effect was found when persistently infected animals were introduced to naïve heifers 7 weeks before breeding, presumably because they then had sufficient time to recover (Rodning et al., 2012). Vaccination against BVDV increased the likelihood of cows becoming pregnant by about 5% (Newcomer et al., 2015).

BVDV infections can reduce fertility via effects on the ovary, uterus and early embryo. The ovaries may become inflamed, affecting follicular development including growth of the dominant follicles. The timing of ovulation can be delayed and there may be a longer interval from ovulation to the time of the subsequent progesterone rise followed by reduced luteal progesterone production. Several studies have investigated the effects of BVDV infection on early embryo development in vitro. The effects were dependent on the strain used (Oguejiofor et al., 2019). In general, infection with ncp BVDV type-1 or -2 did not adversely affect embryo development but cp BVDV caused cell death when the zona pellucida was not present, while BVDV-3 reduced both cleavage and blastocyst rates. From this it appears that BVDV type-1 present in the uterus probably does not have a direct negative impact on the development of embryos prior to hatching because the zona pellucida is protective. The risk of transmission of BVDV to host cows via embryo transfer is also low as long as correct washing procedures (as recommended by International Embryo Transfer Society guidelines) are applied.

Virus can be found in the uterus for at least 3 weeks after initial infection and there is good evidence that this may reduce fertility in two ways (Oguejiofor et al., 2019). Firstly, the fact that BVDV causes immunosuppression will increase the likelihood that the cow may develop endometritis if she becomes infected with BVDV in the peripartum period. This is already a high-risk time because the uterus is normally colonized with many bacterial species following calving. Healthy cows should be able to respond rapidly using local immune defence systems that involve epithelial and stromal cells together with specialized white blood cells that are also present in the endometrium. If this early response is sufficient, then the bacterial infections can be cleared and the cow will not go on to develop uterine disease.

Up to 50% of postpartum dairy cows do, however, develop metritis, endometritis and/or cervicitis, which may in turn result in long term chronic inflammation of the uterine wall that continues to have adverse effects on the intrauterine environment even after the initial infection has passed. We have shown using in vitro culture techniques that experimental infection of endometrial cells with ncp BVDV markedly reduced their ability to mount an immune response to a challenge with the bacterial wall component lipopolysaccharide. Amongst the changes observed, the ratio of prostaglandin produced by the endometrium switched from the luteolysin $PGF_{2\alpha}$ to more PGE_2 (Oguejiofor et al., 2019). In the reproductive tract $PGF_{2\alpha}$ acts as an immune enhancer while PGE_2 is an immune suppressor and also helps to maintain the corpus luteum. The relative increase in PGE_2 would therefore not only promote inflammatory changes within the endometrium itself but would also increase the likelihood of a cow developing a persistent corpus luteum, which is one of the detrimental consequences associated with uterine disease.

The second way in which BVDV can reduce conception rates involves its ability to inhibit immune pathways. As mentioned above, a normal response to an infection includes increased local production of type 1 interferons. These then act through interferon receptors in the endometrium to up-regulate many interferon stimulated genes that play an important part in local defence mechanisms. However, the cow uses IFNT produced by the conceptus to signal that she is pregnant. IFNT is also a type 1 interferon that

acts through the same interferon receptors as those involved with the normal immune response to induce local changes in the endometrium. In the case of IFNT these include remodelling of the uterine wall, increased blood flow, production of uterine secretions and alteration of the local immune response to prevent the dam from rejecting the conceptus (which is genetically dissimilar to her own tissues). All of these steps are essential to promote sufficient nutrient supply to the pre-attachment conceptus and then to enable it to establish a functional placenta. Again, using in vitro techniques, we have shown that endometrial cells infected with ncp BVDV have not only a reduced ability to synthesize type 1 interferons, but their ability to respond to IFNT by production of interferon stimulated genes is also seriously impaired (Oguejiofor et al., 2019). This is likely to be the main mechanism whereby BVDV infection around the time of estrus or in the early luteal phase can prevent the successful establishment of a pregnancy.

Bovine herpesvirus-1 (BoHV-1)

Bovine herpesvirus-1 is a herpes virus that also impacts on fertility. Following an initial infection, the body is usually unable to eliminate members of the *Herpesviridae* family completely and the viruses are able to remain in an inactive, latent state in some parts of the body including neural tissue and macrophages. The virus can then be reactivated in times of stress, or if the body is faced with a subsequent disease challenge. In the case of BoHV-1, it initially causes infectious bovine rhinotracheitis (IBR), an acute and highly contagious inflammation of the upper respiratory tract that is endemic in many parts of the world, including the UK and Canada. Bovine respiratory disease (BRD) is the main cause of mortality and morbidity in dairy calves between 1–5 months of age, and BoHV-1 is one of a variety of pathogens that contribute to this (Johnson et al., 2012). BoHV-1 can also cause conjunctivitis, encephalitis and abortion. In those animals surviving an initial BoHV-1 infection, latent virus can be reactivated by glucocorticoids (Rock et al., 1992). Because glucocorticoid levels increase as part of the normal parturition mechanism and the cow is subsequently likely to suffer from both bacterial infections and negative energy balance, the postpartum period is clearly a high-risk time for the virus to reactivate.

BRD is extremely common in dairy herds, with numerous epidemiology studies showing that up to 46% of calves are affected (Johnson et al., 2011). The consequences of this have been reviewed (Wathes et al., 2014). Surviving heifers have reduced growth rates, which in turn delay the age at which they are ready to be bred and so the age at first calving. For those affected in their first 3 months of life, first calving was delayed by around 6 months and subsequent calving intervals were increased by 12%. The most severely affected animals, which experienced four episodes of BRD before calving, were 1.9 times less likely to complete their first lactation compared with their healthy compatriots. Because BRD is associated with a variety of infectious agents, not all cases will be associated with BoHV-1 infection. However, the importance of BoHV-1 in these effects on fertility was supported by a study of seasonally calving Irish herds that measured antibodies against BoHV-1 in bulk milk tanks and found that the 3-week calving rate was significantly lower in herds that tested positive compared with BoHV-1 negative herds (Sayers, 2017). Further epidemiology studies in Ethiopia found that cows that were seropositive for BoHV-1 had significantly higher rates of uterine infection and more retained fetal membranes than cows that tested negative (Asmare et al., 2018).

Further support for the role of BoHV-1 in reducing fertility has come from a number of studies that investigated the effects of treating cattle with modified live IBR vaccine at estrus or within the subsequent 4 weeks (Chase et al., 2017). The main effects noticed involved the ovary, which developed regions of necrosis affecting both follicles and the corpus luteum. Vaccination at estrus was followed by reductions in circulating progesterone levels and conception rates. The manufacturer therefore recommends that the vaccine be given one month before breeding. To our knowledge, possible actions of BoHV-1 on the immune function of the uterus, such as those described for BVDV, have not been investigated to date, but would not be unexpected.

Bovine herpesvirus-4 (BoHV-4)

Bovine herpesvirus-4 is another herpes virus that is highly prevalent in some dairy herds and also remains latent in the body following initial infection in several cell types including macrophages (Gagnon et al., 2017). In common with BoHV-1, there is in vitro evidence that BoHV-4 can be reactivated by glucocorticoids. In addition, in vivo evidence measuring seroconversion rates has shown that it can reactivate during the peripartum period. BoHV-4 infects the uterus and its presence there has been associated with both metritis and endometritis (Donofrio et al., 2008). Evidence that it can reduce fertility includes studies showing that BoHV-4 positive cows were less likely to be inseminated in the first 80 days postpartum, they required more services per conception, and they had a reduced likelihood of conceiving again within 200 days of calving in comparison with uninfected cows (Chastant-Maillard, 2015).

The causal role of BoHV-4 in uterine disease is somewhat unclear as it can also be found in cows that did not have uterine infection and its presence in the uterus is usually associated with well-recognized bacterial pathogens including *Trueperella pyogenes* and *Escherichia coli* (Klamminger et al., 2017). Available evidence now supports the view that BoHV-4 acts as a co-factor with such bacterial pathogens to increase the likelihood that an infected animal will develop endometritis (Donofrio et al., 2008). It does this by increasing local production of the chemokine IL8, which then acts to attract immune cell migration into the uterus and activate a variety of inflammatory cytokines including interleukin (IL)1, IL8 and tumour necrosis factor alpha. In common with BVDV, the presence of BoHV-4 in the uterus therefore alters the normal immune response to bacterial infection. Unlike ncp BVD however, BoHV-4 is cytopathic, and can kill endometrial epithelial and stromal cells. Because the uterine epithelium is normally protective, this increases the vulnerability of the underlying stromal cells to direct attack by intra-uterine pathogens.

▪ Evidence for the Importance of Viral Diseases in Dairy Cow Fertility

In summary, the three viruses used here as examples are all in circulation in Canadian dairy herds. An acute ncp BVDV infection experienced during the breeding period can reduce cow fertility by causing estrous cycle irregularities, early embryo mortality and immunosuppression. Later infections will increase abortion rates or result in birth of a persistently infected calf. Many dairy calves experience BRD, and this is often associated with BoHV-1 infection. BRD slows growth, delays first calving and increases the subsequent likelihood of culling. There is good evidence that BoHV-1 impairs ovarian function and is associated with uterine infection, lower conception rates and abortion. Furthermore, latent virus remains present following the initial infection and can be reactivated if the animal is stressed. BoHV-4 infections alone are probably insufficient to cause clinical uterine disease, but if the virus is already present then it can also be reactivated from latency following calving and then act together with bacterial pathogens to disrupt the normal immune response, so increasing the risk of endometritis and delaying uterine repair mechanisms (Figure 1).
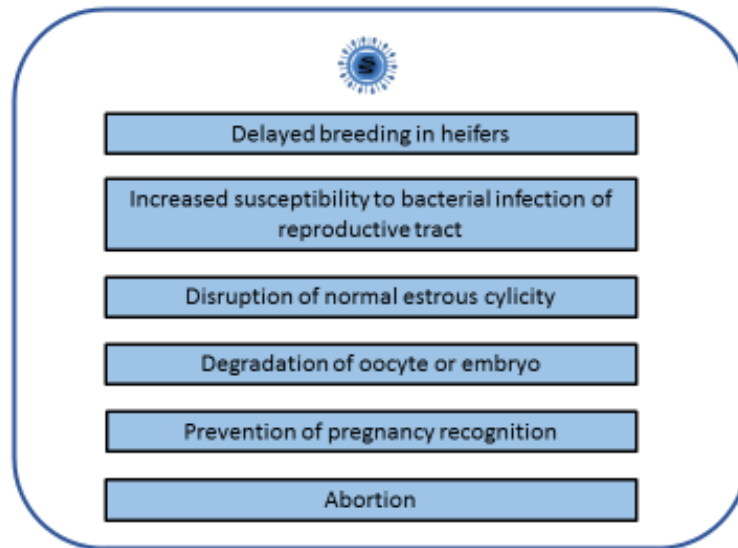
Figure 1: Summary of possible ways in which viral diseases reduce fertility in dairy cows

These three examples show that some common viruses can act through a diverse range of mechanisms to have adverse effects on the ovary, endometrium and the conceptus, so reducing fertility. Their presence in the herd may be identified if there is an issue with high rates of abortion or fetal abnormalities. Nevertheless, they are not usually top of the list of considerations when herds are experiencing suboptimal conception rates. In practice, fertility rates in the field are influenced by many factors that each make a small contribution. It is often hard to identify their relative impact because many potentially relevant factors are not recorded in an easily accessible format to link to the success of a particular insemination. For example, previous disease and vaccination history, changes in feed constituents and the current metabolic status of individual cows are all influential.

In addition to the work cited above, we have recently acquired new evidence that viral disease may indeed play an influential role on conception rates. Peripheral blood is becoming widely used to determine gene expression profiles associated with a variety of infectious and non-infectious diseases of both humans and domestic livestock. Such blood contains a population of white blood cells that circulate between disease sites and lymphoid organs in the body. As part of a recent EU sponsored study, GplusE (www.gpluse.eu), we acquired blood samples from 176 multiparous Holstein cows from six herds, each based in a different country. These samples were taken at 2 weeks after calving, when cows are often in a state of negative energy balance. We then measured both the circulating metabolic hormone insulin-like growth factor-1 (IGF-1) and the global gene expression profile in the white blood cells, using the technique of RNA sequencing. We have shown previously that the IGF-1 concentration at this time is strongly related to the energy balance status and is also highly predictive of future fertility in that lactation. We found that multiparous cows whose circulating IGF-I concentrations exceeded 25 ng/mL in the week after calving were 11 times more likely to conceive to first service than those with lower IGF-1 concentrations (Wathes et al., 2007). In the recent GplusE study we then used a technique of data analysis known as weighted correlation network analysis to identify clusters of genes whose expression pattern in white blood cells was highly correlated both with each other and also with the circulating IGF-1 concentration. This analysis showed that the genes in the cluster that had the strongest overall negative relationship with the IGF-1 concentration are known to be important in innate immune responses, in particular relating to viral infection. As outlined above, the IFN system represents the first line of defense against a wide range of viruses, with the rise in IFN activating antiviral responses using at least five main effector pathways. We found that key genes involved in each of these five pathways were up-regulated in cows in which the circulating IGF-1 concentration was low. This evidence is strongly indicative that such cows were responding to a viral infection at this time, although none was specifically identified in their

clinical records. We believe that the most likely explanation for these observations is that a pre-existing but latent virus had been reactivated in the peripartum period in those animals with a low energy balance status. This would then represent a chicken-and-egg situation, as mounting an effective immune response is energetically demanding and so would further deplete the available body reserves.

▪ Conclusion

A wide variety of viral diseases are present in cattle populations. Although they are acknowledged to cause major production and economic losses, few have been controlled effectively at a global scale and there is always a real danger that new strains or completely new diseases may emerge. Detecting these requires a vigilant screening process combined with robust epidemiological studies to inform both national and international animal health policies. Applying rigorous quarantine procedures can reduce the risk of disease spread. This applies not only between countries but also at a farm level when any newly acquired animals are introduced into a naïve herd. This precaution will however be ineffective against some vector borne diseases such as Schmallenberg and Bluetongue virus. Various schemes have been implemented to incentivize farmers to increase their use of regular testing and appropriate vaccination. Disease monitoring may in future benefit from the development of more rapid and reliable diagnostic procedures. Amongst other benefits, reducing the prevalence of viral diseases in dairy herds should improve conception rates and increase cow longevity.

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New Strategies to Maximize Pregnancy Outcomes

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▪ Take-Home Messages

- Periparturient clinical disease reduces fertilization rates, embryo quality, embryo growth and pregnancy outcome.
- Presynchronization of estrous cycles before first postpartum AI improves pregnancy per AI (P/AI) compared with applying only the Ovsynch timed AI program.
- Using Presynch ($PGF_{2\alpha}$)-Ovsynch to setup first postpartum AI increased P/AI by exposing cows to the entire program compared with inseminating cows detected earlier in estrus after Presynch $PGF_{2\alpha}$ treatments.
- Incorporating GnRH into Presynch programs (G-6-G, PG-3-G, or Double Ovsynch) before Ovsynch increased pregnancy outcomes at first AI services compared with standard Presynch 'PGF_{2a}' programs, especially in herds with larger percentages of anovular cows.
- Double Ovsynch increases P/AI at first service in primiparous cows compared with Presynch ($PGF_{2\alpha}$)-Ovsynch, whereas no increase in fertility is observed in multiparous cows.
- Resynchronization programs that include administering GnRH 7 days before starting the Resynch-Ovsynch program seem to improve pregnancy outcomes.
- Incorporating PGF_{2a} as part of a presynchronization program before Resynch-Ovsynch facilitates estrus expression and reduces the proportion of cows requiring timed AI, whereas GnRH as part of a presynchronization Resynch program inhibits estrus expression and increases the proportion of cows requiring timed AI.
- Incorporating progesterone via intravaginal inserts increased pregnancy outcome at day 60 after timed AI and reduced pregnancy loss in cows receiving their first postpartum AI.
- Administering post-AI treatments of human chorionic gonadotropin or GnRH may induce ancillary luteal tissue, increase progesterone concentrations, and improve pregnancy outcomes by 3.0 to 3.5 percentage units.
- Applying a pregnancy-associated glycoprotein pregnancy test is a viable alternative to a weekly transrectal ultrasound or palpation pregnancy diagnosis to allow earlier detection of nonpregnant cows.

▪ Introduction

Although overall fertility of lactating dairy cows in North America and elsewhere followed a downward trend since the 1950s (Butler and Smith, 1989), annual U.S. milk yield per cow increased 4.4 times from 2,409 kg in the 1950s to 10,500 kg in 2018. Based on a sample of comparatively less-productive dairy cows in the United Kingdom, fertility also decreased from 1975 to 1982 and from 1995 to 1998 (Royal et al., 2000). During that period, conception rates after first services decreased from 56 to 40% despite similar intervals to first service, whereas calving intervals increased from 370 to 390 days.

Since 2000, however, an increasing upward trend in daughter pregnancy rate (DPR) has occurred (Wiltbank and Pursley, 2014). For every unit increase or decrease in DPR (a value derived from days open), the 21-day pregnancy rate of a sire's daughters increased or decreased by 1%. During the past decade, while milk yield continued to increase, a dramatic increase of approximately 5% in DPR has occurred, which should translate into a reduction of 20 days open for their daughters when they become cows. Although the association between milk production and reproductive performance of dairy cows

based on some metrics (i.e., days open, calving interval) is generally believed to be antagonistic, the antagonism between milk production and reproductive performance is not universal. In fact, recent work (Bello et al., 2012) supports a highly heterogeneous association between milk production and reproductive performance, whereby heterogeneity is partitioned across several scales and driven by many contributing factors, both physiological and managerial. It is clear in many herds that sound management practices overcome this potential negative relationship to achieve acceptable rates of reproductive efficiency.

Reproductive inefficiency of dairy cattle causes great frustration and potential lost income for dairy producers (Call and Stevenson, 1985). Even under optimal conditions, the reproductive process is less than perfect because of multiple factors involved in producing a live calf. To manage the complexities of the estrous cycle, understanding of many interrelated physiological functions is critical. Further, reproductive efficiency involves successful management of not only cows, but also the people who milk, feed, house, inseminate, and care for them.

Although benefits of improving reproduction are apparent, specific causes of poor reproductive performance are difficult to identify and not resolved easily. To improve reproductive efficiency, the limiting factors or bottlenecks must be addressed (Senger, 2001). Some potential bottlenecks over which dairy management teams can exert major control include: 1) estrus-detection efficiency, 2) reducing estrus-detection errors, 3) improving inseminator skills, 4) reducing errors in semen thawing and handling to prevent 'cold shock' of semen, 5) selecting for AI bull fertility and calving ease, 6) strategic use of sexed semen, 7) reducing heat stress, 8) addressing health and poor footing conditions, 9) improving health of transition cows, and 10) optimizing frequency of 'open' checks of potentially pregnant cows When management focuses on these bottlenecks, improvement in reproductive efficiency is possible.

▪ Voluntary Waiting Period

Several physiological changes including uterine involution and recurrence of ovarian follicular waves and normal estrous cycles must occur early postpartum to facilitate good fertility at first AI. Many factors affect these outcomes including, but not limited to, body condition, energy balance (milk yield and dry matter intake), parity, season, and disease (Crowe et al., 2014).

Results from Germany (Tenhagen et al., 2003) demonstrated that lengthening the voluntary waiting period (VWP) by 3 weeks from 53 ± 3 to 77 ± 3 days in milk (DIM) in low milk-producing cows or from 77 ± 3 to 98 ± 3 DIM in high milk-producing cows increased fertility at first AI when applying a timed AI program. Extending duration of VWP from 60 to 88 DIM increased P/AI to first service (VWP60 = 41%; VWP88 = 47%; Stangaferro et al., 2018). The greatest benefit of extending VWP on first-service P/AI was detected in primiparous cows (VWP60 = 46%; VWP88 = 55%) because P/AI did not differ in multiparous cows (VWP60 = 36%; VWP88 = 40%). Physiological status more conducive to pregnancy—characterized by improved uterine health, greater body condition score, reduced systemic inflammation, and to a lesser extent, more time to resume ovarian cyclicity—explained the increment in P/AI to first service. Despite having greater P/AI to first service, cows with the longer VWP had delayed time to pregnancy during lactation and greater risk of leaving the herd, particularly for multiparous cows. This shift in pregnancy timing led to an overall extension of the lactation length (+ 13 days), which resulted in greater total milk yield per lactation (+ 491 kg) but not greater milk yield per day of lactation.

▪ Postpartum Disease

Approximately 50% of the dairy cows in the U.S. suffer from at least one disease event during the first 60 DIM. Transition from pregnancy (no lactation) to lactation (not pregnant) presents the greatest risk of culling and death for a dairy cow. During this transition period, a number of metabolic and endocrine adaptations must occur to keep cows healthy. Most cows face a negative energy balance during early lactation because the cow cannot consume adequate energy to meet the demands of milk yield until later in lactation when daily dry matter intake peaks, often after peak in milk in older cows.

Calving-related disorders and diseases that affect the reproductive tract are major contributors to poor fertility. Dystocia (calving difficulty), metritis, and clinical endometritis were observed in 14.6, 16.1, and 20.8% of postpartum dairy cows in large U.S. confinement herds, respectively (Ribeiro et al., 2013). Cows that have one of the aforementioned disorders were 50 to 63% less likely to resume estrous cycles by the end of the VWP, and were 25 to 38% less likely to become pregnant following the first AI-breeding compared with healthy cows.

Direct effects of clinical disease on reproductive traits have been reported (Ribeiro et al., 2016). Embryos collected on day 5 to 6 after AI were evaluated for fertilization and grade quality. It was clear that cows suffering from at least one case of clinical disease had reduced fertilization rates and compromised embryo quality as early as 5 to 6 days after insemination. When conceptuses (embryos and their developing placenta) were collected on day 15 after AI, fewer cows with clinical diseases were pregnant. In fact, when day 15 conceptuses were evaluated, 83.9% of those collected from cows without a diagnosis of a clinical disease were elongated as expected for a day 15 bovine conceptus. In contrast, only 28.6% of them were elongated when they originated from cows with a diagnosis of clinical disease event during the first 60 DIM. When one clinical disease was diagnosed during the first 60 days, percentage of cows pregnant on day 15 after AI decreased from 49.4 to 29.8%. Uterine disease reduced the percentage of cows pregnant by more than half (49.4 to 20%). This carryover effect of disease on reproductive responses, embryo survival and maintenance of pregnancy in lactating dairy cows was independent of the category of postpartum health disorder (Mohtashamipour et al., 2019).

▪ Timed AI Programs for First Services

Ovsynch

The most common timed AI system is Ovsynch (injections of GnRH 7 days before (G-1) and 56 hours after $PGF_{2\alpha}$ (G-2), with AI administered at 72 hours after $PGF_{2\alpha}$ or 16 hours after G-2 treatment (Figure 1). Some variations have included a 5-day program with G-1 administered on day 0 and doses of $PGF_{2\alpha}$ given on day 5 and 6 with timed AI on day 8 (at the time of G-2). In the peer-reviewed literature (Wiltbank and Pursley, 2014), hundreds of articles have cited the original Ovsynch article and numerous articles use the term Ovsynch in the title (n = 6,870). Obviously, the 'Ovsynch' program has become an integral part of dairy reproductive programs during the 20 years since the original publication. A recent review of ovulation synchronization for management of reproduction in dairy cows provides insights into current methods and limitations (Bisinotto et al., 2014).
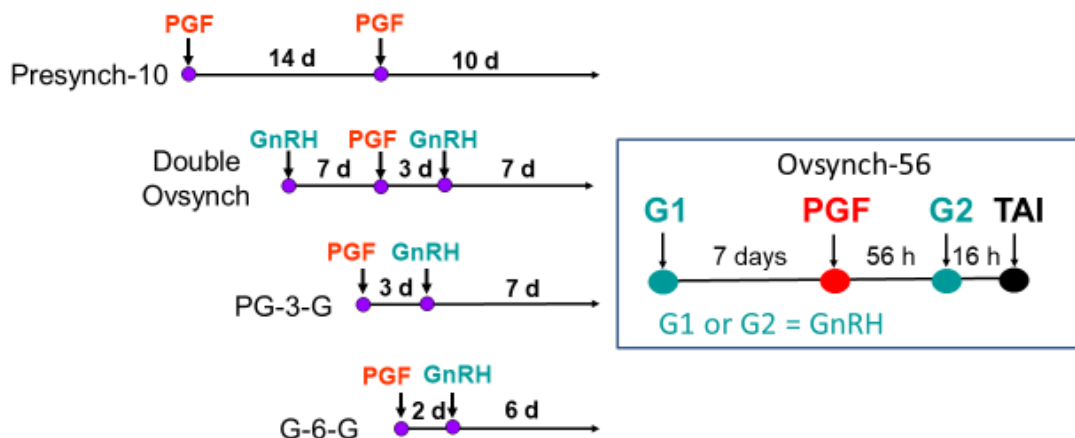
Figure 1. Standard presynchronization '$PGF_{2\alpha}$' – Ovsynch or Presynch-Ovsynch (e.g., Presynch-10 shown here or the interval from the last PGF injection to the start of Ovsynch can be 14, 12, or 11 days) and presynchronization 'GnRH' options (e.g., Double Ovsynch, PG-3-G, or G-6-G).

Presynch 'PGF$_{2\alpha}$' Programs

Early studies indicated that pregnancy outcomes at first AI service after calving were improved when cows were at specific stages of the estrous cycle before initiating a timed AI program. Cows beginning the timed AI program on days 5 through 12 of the estrous cycle had greater ovulatory responses to G-1 and greater fertility than cows at other stages of the estrous cycle (Vasconcelos et al., 1999). The first Presynch 'PGF$_{2\alpha}$' program tested was the Presynch-12 program. Permutations of the standard Presynch have been applied (e.g., Presynch-14, Presynch-11, and Presynch-10) where the two injections of PGF$_{2\alpha}$ were consistently administered 14 days apart, but the interval from the last injection to the onset of the timed AI program was either 14, 11, or 10 days, respectively (Figure 1).

Presynch 'GnRH' Programs

The major limitation to Presynch 'PGF$_{2\alpha}$' programs is their inability to improve fertility in anovular cows (i.e., those not having initiated estrous cycles since calving), which may represent up to 41% of dairy cows at the end of the VWP (Bisinotto et al., 2014). Including GnRH with PGF$_{2\alpha}$ in a Presynch program increased the odds for pregnancy by 1.65 times (Bisinotto et al., 2013) and resulted in new presynchronization programs that add GnRH to PGF$_{2\alpha}$ (e.g., Double Ovsynch, PG-3-G, and G-6-G; Figure 1).

Presynch 5-Day Programs

Ovulation to G-1 of Ovsynch improves synchronization of the estrous cycle and reduces the period of follicular dominance; both factors are associated with greater pregnancy outcomes (Vasconcelos et al., 1999; Santos et al., 2010). As a result, studies comparing 5-day vs. 7-day Ovsynch programs tested in dairy (Santos et al., 2010) and beef cattle (Bridges et al., 2008) sometimes produced greater pregnancy outcomes in the 5-day programs because proestrus is prolonged and follicular dominance is reduced in more cows. One limitation to the 5-day program is the inability of a single dose of PGF$_{2\alpha}$ to induce complete corpus luteum (CL) regression (particularly the 'new' CL that resulted from GnRH-induced ovulation). Therefore, two doses of PGF$_{2\alpha}$ are required (one on day 5 and another on day 6) to optimize luteal regression by either PGF$_{2\alpha}$ or by one of its analogues (Riberio et al., 2012a). Even larger single doses administered on day 5 were less effective in the 5-day program when the one 50-mg dose (dinoprost or Lutalyse) was applied, but both PGF$_{2\alpha}$ dose-frequencies (one 50-mg dose or two 25 mg doses [24 hours apart]) effectively induced complete luteolysis in the 7-day program (Stevenson et al., 2018).

▪ Comparative Fertility of First-AI Programs

Ovsynch

A meta-analysis examined 71 treatment and control comparisons extracted from 53 research papers (Rabiee et al., 2005). Programs evaluated included Ovsynch, natural breeding, single, double, or triple prostaglandin injections, Select Synch, Heat Synch, and modified Ovsynch. Pregnancy rates for Ovsynch programs did not differ from those any of those breeding programs. What these studies did not evaluate was that all cows submitted to a timed-AI program, which is independent of expressed estrus as a prerequisite for AI, were inseminated at one time, and reduced the variation and postpartum interval to semen exposure at first AI. The findings demonstrated that the Ovsynch program could benefit dairy operations because it allows for timed AI of lactating cows without detection of estrus. There was, however, little or no significant improvement in pregnancy rates using Ovsynch over other programs and the costs of labor and hormone administration should be considered when selecting this form of reproductive technology for routine use.

Presynch 'PGF$_{2\alpha}$' (Presynch-Ovsynch) Programs

Studies demonstrated that presynchronization of estrous cycles to days 5 to 12 after estrus before applying Ovsynch could improve fertility at the timed AI. Two early studies (Moreira et al., 2001; El-Zarkouny et al., 2004) tested whether estrous cycles could be staged in cows to meet this ideal by applying two injections of PGF$_{2\alpha}$ (named Presynch-12) administered 14 days apart and then initiating the timed AI program 12 days after the second PGF$_{2\alpha}$ injection. In the preceding two studies and in another study employing the Presynch-14 program (Navanukraw et al., 2004), the Presynch programs were superior to Ovsynch alone for timed AI pregnancy outcome.

In nearly all published studies, these Presynch 'PGF$_{2\alpha}$' systems have produced greater pregnancy outcomes in cows than in those submitted to the timed AI program at random stages of the estrous cycle without Presynch. Furthermore, greater pregnancy outcomes were reported in cows treated with Presynch-11 than with Presynch-14 before a timed AI program (Galvão et al., 2007), probably because more cows were at the ideal stage of the cycle after Presynch-11 treatment (Figure 1). Further shortening the interval between Presynch and Ovsynch to less than 10 days may reduce pregnancy outcomes (Colazo et al., 2013). Overall, lactating dairy cows exposed to Presynch 'PGF$_{2\alpha}$' programs for presynchronization have 42% greater odds of pregnancy compared with cows receiving only the timed AI program (Bisinotto et al., 2014).

These Presynch 'PGF$_{2\alpha}$' programs also have the flexibility of choosing to inseminate cows detected in estrus after PGF$_{2\alpha}$ (Melendez et al., 2006; Chebel and Santos, 2010; Chebel et al., 2010) but it may come at a cost. A meta-analysis examined the question whether to inseminate cows that were detected in estrus after PGF$_{2\alpha}$ and before cows completed the entire Presynch 'PGF$_{2\alpha}$' program (Borchardt et al., 2016). Cows from another 20 experimental groups including 8,124 cows submitted to first AI using a Presynch-Ovsynch protocol were used. The overall proportion of P/AI for cows inseminated early (EDAI + timed AI) was less than that of cows that completed the program and received the timed AI (Figure 2). Information regarding pregnancy loss at 60 days after AI was available for 5,200 cows and did not differ between groups(Figure 2).
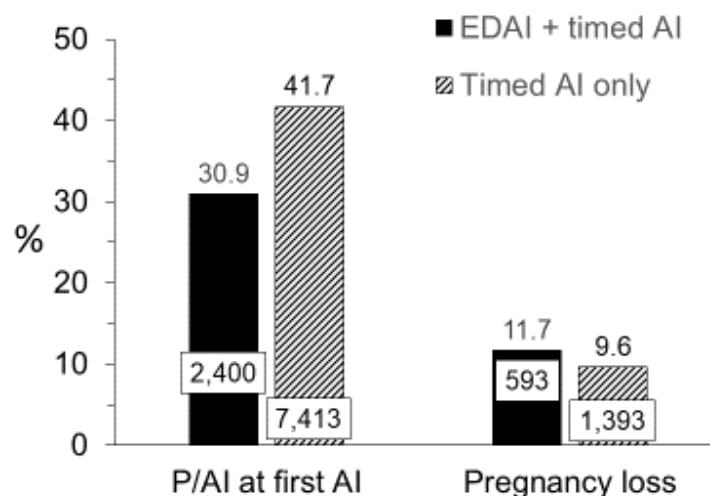
Figure 2. Pregnancy outcomes (assessed at days 28 to 42 after AI) in cows exposed to standard Presynch-Ovsynch program before first postpartum AI for cows inseminated after the second PGF$_{2\alpha}$ treatment upon estrus detection or that received the timed AI (ED + timed AI) compared with cows only receiving the timed AI (timed AI only). Pregnancy losses were assessed at days 42 to 74 after AI.

Presynch 'GnRH' Programs

Comparisons of various Presynch programs showed that G-6-G produced greater pregnancy outcomes than Presynch-11 when Cosynch-72 (combining timed AI and G-2 at 72 hours after $PGF_{2\alpha}$) was used (Ribeiro et al., 2011). When Presynch-12 was applied before timed AI, pregnancy outcome was similar in Ovsynch-56 vs. Cosynch-72 (Bisinotto et al., 2010). Comparison of two Presynch programs and two times for administering G-2 and timed AI (56 vs. 72 hours; Ribeiro et al., 2012b) produced no significant difference in pregnancy outcomes at day 30 after timed AI. At day 65 after timed AI, however, fewer cows treated with Presynch-10 and injected with GnRH and inseminated at 58 hours were pregnant compared with those injected with GnRH and inseminated at 72 hours. In contrast, no such differences were detected when cows were exposed to Double Ovsynch before the timed AI program.

In some cases, Double Ovsynch (Souza et al., 2008; Herlihy et al., 2012) and PG-3-G (Stevenson and Pulley, 2012) may produce greater pregnancy outcomes than the two standard Presynch '$PGF_{2\alpha}$' variants (Presynch-12 or Presynch-10). Another recent meta-analysis including a total of 25 articles with 27 experimental groups from 63 herds including 21,046 cows submitted to first timed AI using either a Presynch-Ovsynch or a Double-Ovsynch protocol were reviewed (Borchardt et al., 2017). Information was available for P/AI for 7,400 primiparous cows and 10,999 multiparous cows. Primiparous cows treated with Double Ovsynch had greater P/AI than cows exposed to Presynch '$PGF_{2\alpha}$' programs, whereas no differences were detected in multiparous cows (Figure 3). The overall proportion of pregnancy loss was 11.3% and 11.7% on day 60 after AI for Presynch-Ovsynch and Double-Ovsynch, respectively.
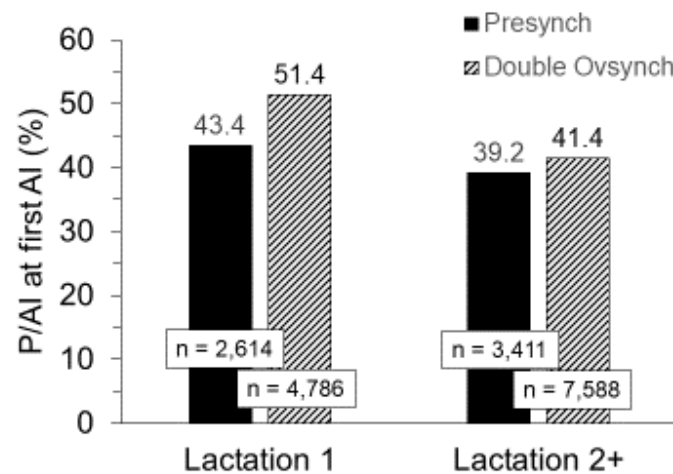
Figure 3. Pregnancy per AI (assessed at days 28 to 42 after AI) in primiparous (lactation 1) and multiparous (lactation 2+) cows exposed to Presynch-Ovsynch or Double Ovsynch before first postpartum AI. Pregnancy losses were assessed at days 42 to 74 after AI.

The PG-3-G program produced greater timed AI pregnancy outcomes in four herds during summer than did the Presynch 10 program and tended to improve P/AI compared with that in cows inseminated early before completing the entire program (Figure 4; Stevenson and Pulley, 2012). During cooler and cold seasons of the year, both presynchronization programs produced greater P/AI than that in cows inseminated early.
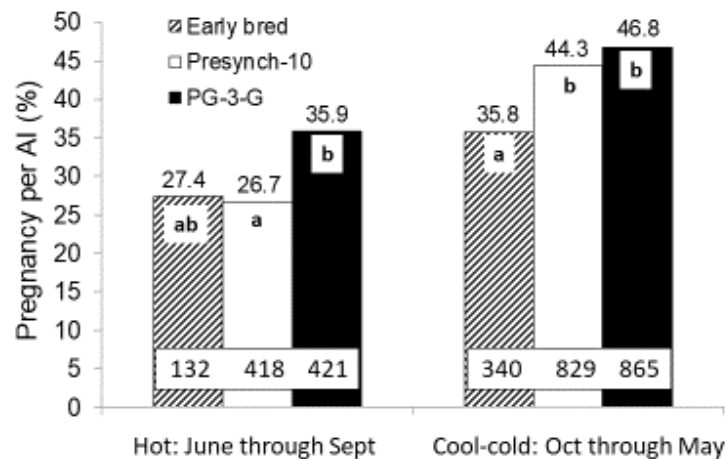
Figure 4. Pregnancy per AI (assessed at days 32 to 38 after AI) in cows exposed to Presynch-Ovsynch (Presynch-10) or PG-3-G before first postpartum AI during summer and cooler seasons. Early bred (EB) cows were inseminated upon detected estrus before the scheduled timed AI. Pregnancy loss at days 60 to 66 did not differ for EB, Presynch-10, and PG-3-G treatments (4.0, 6.8, and 9.3%, respectively).

Programs Including Progesterone

A review of the literature (meta-analysis) indicates that use of a single CIDR (controlled internal drug release) containing progesterone administered during the period between G-1 and the $PGF_{2\alpha}$ of the timed AI protocol increased pregnancy outcome at day 60 after AI by 18% compared with untreated controls (34.2% vs. 29.6%; Bisinotto et al., 2015b). The benefit from progesterone supplementation was similar for cows with and without a CL at G-1. Nevertheless, P/AI for cows without CL treated with a single insert was 10.5% less than that of untreated cows that had a CL at the initiation of the timed AI program (29.0% vs. 32.0%). Incorporating a single progesterone insert as part of a timed AI program increased fertility in cows that lacked a CL at the first GnRH injection, but did not restore fertility to the same level as those cows starting the timed AI program in diestrus (i.e., having a CL), likely related to the amount of progesterone released.

Several recent studies have attempted to target potential problem cows with progesterone as part of timed AI programs. Ovaries were examined for the presence of a CL by ultrasound at the beginning of Ovsynch after earlier treatment with two injections of $PGF_{2\alpha}$ 14 days apart (Presynch, Stevenson et al., 2008). Cows without a CL were treated with Ovsynch with or without progesterone via a CIDR insert. Cows with a CL served as a control. Cows without a CL had greater P/AI at 33 days after timed AI when treated with progesterone (CIDR) compared with control cows with no CL or cows having a CL at the onset of Ovsynch (Figure 5; left panel). In two other reports (Bisinotto et al., 2013, 2015a), cows without a CL at the onset of a 5-day or 7-day Ovsynch program were treated with two CIDR inserts and P/AI was compared with contemporary no-CL cows and cows in diestrus (bearing a CL). Pregnancy per AI at 32 days after timed AI did not differ between cows with a CL and those treated with two CIDR inserts but was greater than that in untreated, no-CL cows (Figure 5; middle and right panel).
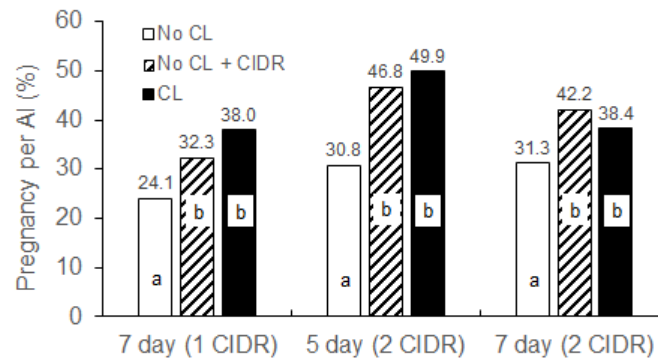
Figure 5. Pregnancy per AI for cows at first postpartum AI after treatment with either a 5-day or 7-day Ovsynch timed AI program. Cows with no corpus luteum (CL) were either controls or received an intravaginal progesterone-releasing insert (CIDR) and were compared with cows starting the Ovsynch treatment with a CL.

Progesterone supplementation during a timed AI program tended to reduce the risk of pregnancy loss between day 32 and 60 of gestation, which corresponded to 2.5 percentage units based on data of a meta-analysis, from 11.6 to 9.1% (Bisinotto et al., 2015b). The benefit of progesterone supplementation in reducing pregnancy loss was not affected by service number, use of presynchronization, or detection of estrus during the timed AI program. Reduced risk of pregnancy loss in response to progesterone supplementation was not affected by the presence of a CL at the initiation of the synchronization protocol.

- **Post Insemination Treatments**

A recent meta-analysis examined the effects of either GnRH or human chorionic gonadotropin (hCG) administered during the luteal phase after AI (Besbaci et al., 2019). No difference in P/AI was detected between cows treated with hCG or GnRH. Compared with no treatment, treatment with GnRH vs. control (45.9 vs. 43.8%) or hCG vs. control (42.0 vs. 39.1%), respectively, improved the chances of P/AI in cows with very poor (< 30%) and poor (30.1 to 45%) fertility, whereas treatment did not benefit cows with very good fertility (> 60%). Moreover, treatment with GnRH and hCG improved the chances of P/AI in primiparous cows, especially those with very poor fertility. Treatments with GnRH on or before day 10 post-AI did not differ from controls (41.1 vs. 40.4%), but GnRH administered after day 10 increased P/AI (51.2 vs. 47.7%) compared with controls, respectively. Treatment with hCG at doses greater than 2,500 IU was associated with increased chances of P/AI compared with smaller doses.

- **Repeat Service (Resynch) Programs**

Early Not-Pregnant Diagnosis

Increased efficiency of reproduction occurs when the not-pregnant diagnosis (NPD) in previously inseminated cows is determined as soon as possible (Fricke, 2002). Means to detect not-pregnant status relatively soon after insemination include:

- Monitoring of blood concentrations of progesterone or commercially available kits to measure blood or milk pregnancy-associated glycoproteins (PAG)
- Presence of estrus determined by visual heat detection or heat-detection aids, or by employing other electronic methods (pressure-sensitive, rump-mounted transmitters or physical activity monitors)
- Absence of uterine fluid, CL, and embryo via transrectal ultrasonography

Early detection of NPD facilitates earlier re-insemination of open cows. Although detection of pregnancy status earlier is associated with improved overall herd fertility measures, more embryo loss is observed

than when pregnancy status is detected later. Methods to facilitate re-insemination of open cows are described hereafter.

Presynchronization before Resynch (No or Limited Estrus Detection)

In some herds, cows diagnosed not pregnant are started on a timed AI program on the day of NPD. In other herds, in order to re-inseminate cows sooner after a NPD, the first GnRH injection (G-1) of the timed AI is administered to all cows eligible for the next pregnancy diagnosis (unknown pregnancy status) either 5 or 7 days before NPD depending on which timed AI program is used (i.e., 5-day vs. 7-day Ovsynch).

Applying a presynchronization treatment such as GnRH or hCG to ovulate a follicle and initiate a new follicular wave in cows with unknown pregnancy status has been tested for its profertility effects. Simple Resynch-Ovsynch programs initiated at day 32 or 39 after previous AI were compared with treatments that included a presynchronization GnRH or hCG injection administered 7 days before Ovsynch. At both initiation times, the pre-GnRH or pre-hCG injection increased pregnancy risk by 4 to 5 percentage units (Table 1).

Table 1. Pregnancy per AI (P/AI) of nonpregnant dairy cows exposed to a 7-day Resynch-Ovsynch timed AI program initiated at a not-pregnant diagnosis at either 32 or 39 days after previous AI with or without presynchronization injections of either GnRH or hCG.

Program	P/AI 32 to 39 days after AI, %	No. of studies
Control Resynch[1]	29.0 (3,657)[3]	11
Pre-GnRH[2] or pre-hCG[2] + Resynch	33.5 (2,996)	8

[1]Ovsynch was started at the non-pregnant diagnosis (NPD).
[2]GnRH or hCG injection administered 7 days before NPD and starting Ovsynch-56.
[3]No. of cows.

Presynchronization before Resynch (Estrus Detection)

In the previously cited summary (Table 1), detection of estrus was not applied and all cows received only the timed AI. In herds in which cows are housed in dry lots or free stall barns with or without turnout dry lots detection of estrus is often applied to cows in addition to using timed AI programs for cows not detected in estrus. In subsequent studies, a pre-GnRH injection was applied as a presynchronization treatment and cows were inseminated when detected in estrus. The timed AI program was discontinued when cows were re-inseminated at estrus, or in the absence of estrus, the complete timed AI program was carried out.

In two such studies (Table 2), fewer cows were detected in estrus and inseminated before the timed AI program was initiated when the pre-GnRH injection was applied compared with the control Resynch (37.4 vs. 50.3%; calculated from Table 2 data), even though resulting P/AI was not different. In the face of similar P/AI after insemination, cows detected in estrus and inseminated became pregnant earlier than timed AI cows.

Table 2. Pregnancy per AI (P/AI) risk of nonpregnant cows exposed to a 7-day Resynch-Ovsynch timed AI program initiated at not-pregnant diagnosis at either 32 or 39 days after previous AI: Insemination at estrus or at appointment (timed AI).

Program	P/AI 32 to 39 days after AI, %	No. of studies
Control Resynch	32.3 (1,501)[2]	
Estrus	39.1 (756)	2
Timed AI	25.4 (745)	2
Pre-GnRH + Resynch[1]	33.9 (1,398)	
Estrus	41.2 (523)	2
Timed AI	29.6 (875)	2

[1] GnRH injection administered 7 days before Ovsynch-56.
[2] No. of cows.

Subsequent studies endeavoured to presynchronize estrous cycles with $PGF_{2\alpha}$ to facilitate estrus expression and earlier re-insemination after a NPD. In most cases fertility was not improved (Chebel et al., 2013), but more cows detected in estrus were inseminated earlier than cows assigned to a timed AI program. In general, when applying Resynch programs to dairy cows at NPD, employing presynchronization $PGF_{2\alpha}$ facilitates estrus expression, whereas using a pre-GnRH or Pre hCG injection suppresses estrus expression. Another study demonstrated that when $PGF_{2\alpha}$ was administered at NPD or 3 days after NPD compared with a pre-GnRH-Resynch, less than 24% of the cows received the timed AI scheduled 7 or 14 days after NPD because cows already were inseminated upon detected estrus compared with 78% of GnRH-Resynch cows receiving the timed AI (Bruno et al., 2013).

Use of Progesterone in Resynch Programs

Addition of progesterone in the form of a CIDR to nonpregnant cows in designed studies in 7-day Resynch-Ovsynch programs initiated at the NPD failed to increase pregnancy risk of cows, whereas when tested in a 5-day Ovsynch program initiated at day 32, progesterone increased pregnancy outcome (Bisinotto et al., 2010). As previously noted, the greatest pregnancy advantage accrues from applying progesterone to cows without a CL rather than those with a CL.

We conducted a recent study in three herds using a shortened version of Ovsynch program that excluded GnRH-1 to resynchronize ovulation in cows bearing a CL after a NPD. In addition, we included progesterone supplementation with the Ovsynch program for cows without a CL to determine if shorter inter-insemination intervals and P/AI in either treatment would differ from that of cows treated with the OVS treatment (Sauls-Hiesterman et al., 2019). Cows (n = 1,584) were enrolled in the study and assigned to one of three treatments at NPD (32 ± 3 days after AI [day 0]). Cows with a detected CL were assigned randomly to: 1) a modified Ovsynch (OVS: G-1 — 7 days — $PGF_{2\alpha}$-1 — 24 h — $PGF_{2\alpha}$-2 — 32 h — G-2 — 16 h — AI) or 2) Short Synch (SS: $PGF_{2\alpha}$-1 — 24 h — $PGF_{2\alpha}$-2 — 32 h — G-2 — 16 h — AI). Cows with no CL were assigned to OVS plus a progesterone insert (CIDR) administered between G-1 and $PGF_{2\alpha}$-1. Mean and median inter-insemination intervals were less in SS cows (mean = 34.3 ± 0.05 days [median = 35 days] than in OVS cows (40.2 ± 0.05 days [42 days]), but that in OVS cows did not differ from OVS + CIDR cows (41.4 ± 0.05 days [42 days]). Herd technicians were more accurate in visually detecting a functional CL than a non-functional CL (81.2 vs. 61.1%). Pregnancy per AI at 32 days after AI was less for SS (16.5% [n = 115]) than OVS (29.3% [n = 133] when a functional CL was inaccurately detected, but did not differ when a functional CL was detected accurately (27.6% [n = 561] vs. 30.3% [n = 508]). Pregnancy per AI did not differ between OVS and OVS + CIDR cows regardless of CL status. Short synch is an alternative to the entire modified Ovsynch program to produce similar P/AI when the CL status was detected accurately, and regardless of functional CL status, SS reduced inter-insemination intervals by 7 days.

▪ Timing of GnRH before Timed AI

Success of timed AI programs depends on adequate duration of proestrus and proper timing of insemination relative to ovulation. For the standard Ovsynch program, with 7 days between the initial GnRH and $PGF_{2\alpha}$ injections, administering the final GnRH 56 hours after $PGF_{2\alpha}$ and performing AI 16 hours later seems to optimize P/AI in dairy cows (Pursley et al., 1998). Conversely, extending the proestrus longer than 56 hours and inseminating cows concurrently with the final GnRH injection may reduce fertility in dairy cows (Sterry et al., 2006; Brusveen et al., 2008). Allowing 56 h of proestrus provides additional growth of the ovulatory follicle and increased exposure to estradiol (Peters and Pursley, 2003), which is thought to be needed to avoid short estrus cycles after induced ovulation. For 7-day Ovsynch programs, optimal timing of the second (or breeding) injection of GnRH (G-2) is approximately 56 hours after $PGF_{2\alpha}$ (Ovsynch-56) with AI occurring approximately 16 hours later (or 72 hours after $PGF_{2\alpha}$ compared with timed AI at either 48 or 72 hours, concurrent with the second GnRH injection (Table 3).

For 7-day Ovsynch programs, which were preceded by Presynch-11, optimal timing of the second or breeding injection of GnRH is approximately 56 h after PGF_{2a} (Ovsynch-56) with AI occurring approximately 16 hours later or 72 hours after PGF_{2a} (Brusveen et al., 2008). When GnRH + AI at 72 hours after PGF_{2a} occurred (Cosynch-72), pregnancy outcomes at 31 days after timed AI were less than when GnRH was administered at 56 h (27.5 vs. 45.2%). In contrast, when GnRH + AI occurred at 48 hours after PGF_{2a} (Cosynch-48), pregnancy outcome at 31 days post-timed AI did not differ from Ovysnch-56 (38.2 vs. 45.2%), respectively.

Table 3. Pregnancy per AI (P/AI) of dairy cows exposed to a 7-day Ovsynch timed AI program and various times of GnRH injection relative to $PGF_{2\alpha}$.

Program	P/AI 28 to 40 days after AI, %	No. of studies
Cosynch-48[1]	28.4 (1,640)[4]	6
Cosynch-72[2]	29.0 (1,582)	7
Ovsynch-56[3]	33.7 (1,729)	5

[1]Cosynch 48 = GnRH (G-2) and AI at 48 hours after $PGF_{2\alpha}$.
[2]Cosynch72 = GnRH (G-2) and AI at 72 hours after $PGF_{2\alpha}$.
[3]Ovsynch-56 = GnRH (G-2) at 56 hours after $PGF_{2\alpha}$ and AI 16 hours later.
[4]No. of cows.

▪ Frequency of $PGF_{2\alpha}$ Treatments in Ovsynch

A few studies have tested the addition of a second dose of $PGF_{2\alpha}$ administered 24 hours after the standard $PGF_{2\alpha}$ treatment in Ovsynch (Figure 1) in cows inseminated at first postpartum AI. Luteolytic risk (progesterone < 0.5 ng/mL at 72 hours after the first or only $PGF_{2\alpha}$ treatment) increased from approximately 83% with one standard dose of $PGF_{2\alpha}$ to 88% with one double dose of prostaglandin $F_{2\alpha}$ to 97% with two standard doses administered 24 hours apart (Wiltbank et al., 2015; Stevenson et al., 2018). In the former study consisting of 11 herds, the increase in P/AI was only 3 percentage points for cows receiving two vs. one $PGF_{2\alpha}$ treatment.

▪ Using PAG Testing in Reproductive Management

Using blood or milk testing of PAG in lieu of transrectal ultrasound or palpation to detect pregnancy is commonplace. It allows any size herd to conduct a weekly pregnancy diagnosis when the herd veterinarian is unavailable and serves the purpose of finding nonpregnant cows sooner than later. Most DHI labs offer this service for samples beginning at 28 days after AI. Based on PAG profiles in plasma

and milk samples collected weekly, the optimal time to conduct a first pregnancy diagnosis is approximately 32 days after AI when plasma and milk PAG concentrations are at an early peak (Ricci et al., 2015). Because of the occurrence of pregnancy loss, all pregnant cows should be submitted for a confirming pregnancy diagnosis 74 days or more after AI when relative PAG concentrations in plasma and milk of pregnant cows have rebounded from their nadir.

▪ Conclusions

A magic program does not exist that will result in pregnancy success without the hard work of animal care that includes cow comfort, good health, balanced diets, and excellent insemination skills. One must exercise caution when considering changes to current programs and operating procedures that are not broken. A critical point to remember when considering the value of reproductive changes is that profit gains follow the law of diminishing returns. In other words, gains are greater when improvements are made from a very low starting point, but the exact value will vary depending on economic conditions. For example, using an example with U.S. dairy milk prices and 21-day pregnancy risk (21-day PR), the economic benefit from increasing 21-day PR from 16 to 18% (approximately $40/cow) is much greater than attempting to move it from 26 to 28% (< $10/cow). Whatever the milk price, increasing the 21-day PR results in diminishing economic returns. This example is for a herd producing 12,250 kg (305-day mature equivalent basis) with $20 per cwt. milk, a 60-day VWP, a market cow value of $0.55 per cwt, lactating feed cost of $0.05 per kg of dry matter, dry cow feed costs of $2.75 per day, newborn calf values of $200 for heifers and $50 for bulls, a replacement heifer cost of $1,800, labor cost of $15 per hour, a cost per A.I. service of $21, and an annual nonfeed transition management cost of $300, including the predicted cost of fresh cow disease.

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Genetics and Economics of Using In-vitro Produced Embryo Transfer in Dairy Herds

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▪ Take Home Messages

‣ In-vitro produced embryo transfer (IVP-ET) in dairy herds greatly reduces the genetic lag with service sires. The value of this decreased genetic lag may overcome the high cost of IVP-ET.

‣ The most profitable use of AI and IVP-ET is often a combination of the two. More IVP-ET should be used when the value of surplus calves is high and the cost of IVP-ET is low, among many other factors.

‣ In the future, use of IVP-ET will increase by more accurately identifying superior donors and recipients, reducing the generation interval, and achieving greater efficiency in embryo production.

▪ Introduction

Artificial insemination (AI) and in-vitro produced (IVP) embryos for embryo transfer (ET) are two reproductive technologies that result in genetic gain by propagating offspring from animals with greater genetic merit. The National Association of Animal Breeders (NAAB, 2019) reported 23,196,413 units of dairy semen sold in the U.S. in 2017. The number of transferable IVP embryos of dairy breeds produced in North America during 2017 was 311,867, of which 95.5% (≈ 298,000) were produced in the U.S. (Viana, 2018). Adding units of semen sold to transferable IVP embryos in the U.S. during 2017 shows that approximately 1.3% of dairy breedings were with IVP embryos. Use of IVP-ET is growing fast in North America; the number of IVP embryos doubled between 2013 and 2017.

Genetic gain has been accelerating since 2010 when genomic testing became widely used to select service sires. The 5-year moving average rate of genetic gain in predicted transmitting ability (PTA) for the economic selection index Lifetime Net Merit (NM$) is now greater than $70 per year for sires born between 2013 and 2017 (CDCB, 2019). This rate of genetic gain was just $28 per year for sires born between 2003 and 2007. Dairy farms that use only AI make genetic gain in their herds because of genetic gain in marketed AI sires. The Council on Dairy Cattle Breeding (CDCB) data also show that the genetic merit of cows is less than that of service sires. The difference is constant as long as the rate of genetic gain in service sires is constant. Genetic merit of cows lags behind the genetic merit of service sires.

Value of the level of genetic merit in a dairy herd should be based on the difference (genetic lag) in genetic merit between the average cow in the herd and the best available sires (the genetic nucleus; Dechow and Rogers, 2018). This genetic lag is an opportunity cost: each cow consists of 'old' sire genetics. For example, when only AI is used and no selection occurs within the herd, the average cow in the herd may be 3.5 years old. If we assume an annual increase of $50 per year in PTA for NM$, then service sires 3.5 years ago had a $175 lesser PTA than today's service sires. The genetic merit of a cow, however, can be thought to consist of 50% her sire + 25% her dam's sire + 12.5% her granddam's sire + 6.25% of her great grand dam's sire, etc. If the generation interval stays the same between generations, then the genetic lag of the average cow in the herd with the genetic nucleus would be $350 PTA of NM$

(200% × 3.5 × $50). This is a doubling of the genetic lag of the first generation. The genetic lag increases with a greater rate of genetic gain in service sires. If the annual increase in PTA of NM$ is $70 per year, then the genetic lag between the average cow and the genetic nucleus is $490 PTA of NM$ (200% × 3.5 × $70). This math is a simplification of reality, but illustrates the important principle of genetic lag.

Selection of superior females in the herd reduces the genetic lag with service sires. For example, use of female sexed semen in younger animals or selection of surplus heifer calves based on genomic test results produces dairy calves that are on average better than the average unselected dairy calf from the herd. The result is a decrease in the genetic lag with the best available service sires. Use of IVP-ET can greatly decrease this genetic lag as will be illustrated later. Use of technologies such as AI, sexed semen, IVP-ET, and selection of surplus animals all contribute to a reduction in genetic lag.

A greater rate of genetic gain means differences in genetic merit resulting from age become greater. In other words, the difference in genetic merit of the best heifers in the herd compared with the genetic merit of the average cow in the herd is becoming greater. As a result, capturing and propagating the best genetics in the herd is becoming more valuable.

From the perspective of a typical herd, the genetic merit of available service sires is a given factor that cannot be controlled. When the rate of genetic gain of service sires is constant over time, and the reproduction and selection program for females in the herd are constant over time, it follows that use of technologies like IVP-ET in a herd do not accelerate the rate of genetic gain (that is, they do not increase the annual change) as is often thought. They do reduce, however, genetic lag with service sires compared with use of AI.

What is the opportunity cost of genetic lag? Using again simple math, a genetic lag of $350 PTA of NM$ is equivalent to a genetic lag of $700 estimated breeding value (EBV) of NM$ (2 × $350 because EBV = 2 × PTA). We use EBV to express the genetic merit of the female herself, whereas PTA is the genetic merit transmitted to her offspring). The $700 is expressed per lifetime, which is 2.8 lactations, or approximately 3 years. Thus, the opportunity cost of this genetic lag of $350 PTA of NM$ is $700 ÷ 3 = $233 per cow per year. Using a program that would reduce the genetic lag by $50 is worth approximately 2 × $50 ÷ 3 = $33 per cow per year. One dollar reduction in genetic lag is worth $0.67 dollar per cow per year (simplified). This math does not include any discounting for time value of money, differences in actual lifespan, and phenotypic response to selection, and assumes that NM$ is the ideal measure of profitability.

The toolbox of technologies such as AI, sexed semen, beef semen, IVP-ET, genetic evaluations, genomic testing, and fertility programs all affect genetic lag. In addition, these technologies have various direct costs and may affect the phenotypic performance of the herd, such as conception risk. For example, the cost to produce a pregnancy with an IVP embryo is much greater than the cost to produce a pregnancy with AI, but the genetic lag using IVP-ET is smaller. The net benefit of using IVP-ET over AI is not immediately clear. Another question is how much IVP-ET use in a herd is optimal, if not to create 100% of pregnancies. The goal of this paper is to provide some insight into these questions.

▪ General Principles of an IVP-ET Program

An IVP-ET program consists of three components: 1) selection of an appropriate ovum pick-up (OPU) protocol, 2) selection of donors, and 3) selection of recipients. Ovum pick up (egg or oocyte collection) is the transvaginal retrieval of oocytes from ovaries of donor females (Hansen, 2017) after a hormonal treatment. These oocytes are then fertilized with sperm outside the body in a laboratory (in vitro is Latin for 'in glass') to produce embryos. Approximately 1-week-old embryos are then transferred into nonpregnant recipients to create pregnancies. Typically, donors have reached puberty, but commercial interest in oocyte collection from prepubertal animals is increasing (Moore and Hasler, 2017). Oocytes also can be collected from animals that are up to 4 months pregnant (Hansen, 2017). The efficiencies of IVP-ET programs vary, but a reasonable number is four transferable embryos per one OPU occurring every 14 days. In other words, an average donor may produce on average one transferable embryo every

3.5 days.

Candidate donors are those animals eligible for OPU and those that will create embryos with the greatest genetic merit. To identify such donors, it is useful to rank candidates using a genetic selection index, such as the PTA for NM$. Reliability of PTA based on traditional parent averages (dam and sire of the candidate donor) is low, especially for young animals (≤ 35%; Weigel, 2011). Low reliabilities imply that the difference between PTA (what we know) and true transmitting ability (what it is) of genetic merit of a trait can be large, which might result in selection of donors of low genetic merit. Therefore, genomic testing with much greater reliability (≥ 70%) is routinely used to identify candidate donors and give more certainty that donors with high true transmitting abilities are selected.

Figure 1 shows genomic PTA of NM$ for 1,247 animals at the University of Florida Dairy Unit. The genetic evaluation was made in 2017. Animals range from a few weeks after birth to more than 2,800 d after birth. The animals were impregnated by conventional and sexed semen, but not IVP-ET. Figure 1 shows a typical distribution of PTA of NM$ in any herd. Younger animals have greater genomic PTA of NM$ than older cows, but variation exists within the same age. The top young heifers have genomic PTA of close to $800, whereas the average genomic PTA of cows that are 2,500 days old is approximately $0. The genetic trend in these data is approximately $70 PTA per year. This is a greater rate of genetic gain than that in the sires of these females at the time when the females were conceived. The greater rate occurs because of more emphasis on sire selection in the last 4 years. Therefore, the genetic lag is being reduced. If IVP-ET were to be used in this herd in 2017, 1-year-old donors would have genomic PTA of approximately $600.
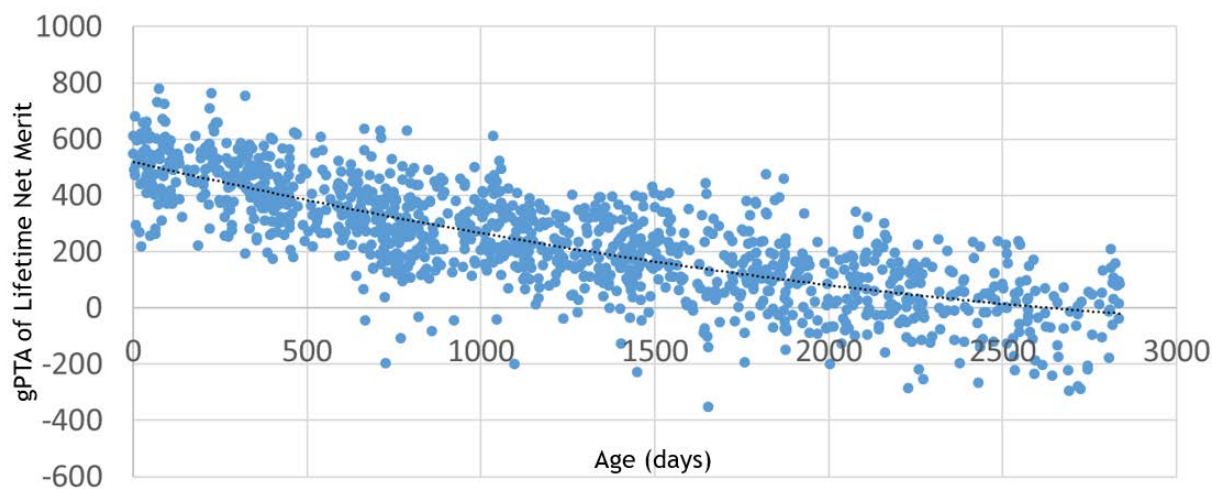
Figure 1. Genomic predicted transmitting abilities (gPTA) of Lifetime Net Merit by age for 1,247 animals at the University of Florida Dairy Unit (2017).

Candidate recipients are non-pregnant animals that have a high likelihood of bringing the transferred embryo to term and producing a live calf. Recipients must be approximately on day 7 of their estrous cycle when an embryo is transferred. Fertility, low risk of abortion, and stillbirth are important selection criteria for recipients because of the high cost of IVP embryos. On the other hand, recipients should be animals of relatively lesser genetic merit because they forego the gestation of their own calf. Foregoing the production of their own calf is an opportunity cost. An opportunity cost is the loss of potential gain from other alternatives when one alternative is chosen. Genomic testing also helps more accurately identify recipients.

The breeder's equation (Lush, 1937) predicts change in a trait resulting from selection using a simple statistical model. The four factors that determine genetic change per unit of time are genetic variation, selection intensity, accuracy of selection, and generation interval. An IVP-ET program has a high selection intensity because a small number of genetically superior animals provide many calves for the next generation. It also has a short generation interval because donors are typically young (heifers). Use of genomic testing for both the selection of donors and recipients increases the accuracy (square root of reliability).

In vitro-produced embryos for ET allows for rapid multiplication of the best genetics in the herd, but is also more expensive than AI. There is often an economically optimal amount of IVP-ET to be used, depending on, for example, the value of the calves, cost of the IVP-ET procedure, accuracy of identifying the best dams, and alternative options such as sexed and beef semen. Only a few studies are available that looked at the economics of the use of IVP-ET in dairy herds.

▪ Economics and Genetic Lag of IVP-ET vs. AI Programs

In an economic study that did not include genetic merit, Ribeiro et al. (2012) calculated the cost of a female pregnancy to be $329 more for IVP-ET than for AI using sexed semen. In Denmark, Thomasen et al. (2016) reported that the greatest increase in economic value of genetic gain in a closed population was obtained when juvenile IVP-ET was used along with genomic selection in the bull-dam part of the population. Combining IVP-ET with genomic testing was profitable in almost all evaluated scenarios when the cost of producing a calf (future sire) by IVP-ET ranged from $500 to $1,500. This study looked at the whole population, including the making of service sires. These authors did not study the cost of IVP-ET to improve the female performance in a closed herd. Recently, Sanches et al. (2019) concluded that IVF is becoming an economically viable practice after they reviewed the current use of IVF by large-scale dairy programs.

Several years ago, we built and validated a detailed simulation model that mimics the genetic, technical, and financial performance of a dairy herd over time (Kaniyamattam et al., 2016). The purpose was to investigate how a herd would respond over time to the use of various assisted reproductive technologies such as AI and IVP-ET and genetic selection strategies. We wanted to do this as realistically as possible.

In our model, a dairy herd consisted of individual cows and heifers. Each animal had 12 genetically correlated traits that were present in the 2014 NM$ index, such as milk yield, daughter pregnancy rate, and productive life. An animal's performance (milk yield, fertility, risk of involuntary culling etc.) was the result of her true breeding value (TBV) for each trait, and permanent and environmental effects. Animals also had EBV that were correlated with the TBV for each trait, depending on the reliabilities of the EBV.

Service sires were not part of the herd and followed a genetic trend of $76 PTA of NM$ per year. Therefore, matings with eligible heifers and cows resulted in calves that had PTA depending on those of the dam and the sire and Mendelian sampling (random variation). Heifer calves that were raised likely became cows. Cows already in the herd had a daily risk of culling. Over time, the herd improved genetically as matings with genetically improved sires produced superior dairy calves. The herd, consisting of individual animals, was followed daily and technical results (such as conception risk, milk yield, average TBV, etc.) and financial results (such as milk sales, profitability) were collected over 20 years.

The following general settings were used to study the economics and genetic performance of various AI and IVP-ET strategies: Annual cow cull rate was set at 34% and the herd had 1,000 milking cows. All dairy heifer calves were genomically tested, which gave high reliabilities and therefore high correlations between EBV and TBV. When more dairy heifer calves were born than were needed to replace culled cows, young heifers were ranked based on the EBV of the trait of interest (often NM$) and heifers with the least desirable EBV were sold. Consequently, retained dairy heifers had more desirable TBV on average than unselected dairy heifer calves (similar to analytic results in Weigel et al., 2012) and the

genetic lag with the service sires was decreased. This also resulted in greater profitability.

The herd started with using only AI for the first 5 years. The first two inseminations in the top 50% of heifers were done with sexed semen. All other inseminations were done with conventional semen. After 5 years, the IVP-ET program was implemented (Kaniyamattam et al., 2017. 2018) and all or some pregnancies were made with IVP embryos. The herd was then followed for another 15 years.

The performance of an IVP-ET system depends on many factors. We assumed that 4.25 transferable embryos were produced per OPU, independent of the age of the donor. Donors for OPU were selected based on rankings for the desirable EBV (e.g., high NM$). The time between OPU of the same donor was 2 weeks. Heifer donors could be collected for a maximum of 4 times between 11 months of age and start of the breeding period. Once a heifer was confirmed pregnant (from AI), she was eligible for 3 more collections. Cows were eligible for a maximum of five collections. Embryos harvested at day 7 after conception were transferred to recipients on day 6, 7 or 8 of the estrous cycle. Recipients were selected based on reverse ranking for the trait of interest (e.g., low NM$), so that the lowest ranked animals had the first chance to receive a randomly chosen IVP embryo.

We assumed that the conception risk was similar for AI and IVP-ET. Conception risks depended on TBV and environmental effects for the traits, daughter pregnancy rate (DPR) and cow conception rate, as well as parity and breeding number. Risk of abortion and stillbirth was at least twice as high in calves made by IVP as from AI. A recent review on post-transfer consequences of IVP embryos in cattle revealed lower conception risks compared with AI (Ealy et al., 2019).

▪ First Study

In the first study (Kaniyamattam et al., 2017), we compared four scenarios with exclusive AI use with four scenarios with exclusive IVP-ET use (100% of pregnancies from IVP-ET). Selections of donors and surplus heifer calves were based on one of four selection criteria: EBV of either milk yield, DPR, or NM$, or random selection. Both AI and IVP-ET scenarios produced surplus dairy heifer calves. The lowest ranking surplus calves based on EBV were sold after genomic testing at approximately 3.5 months of age. Surplus calves were either sold at $500 each (3 to 4 months old), or for IVP calves for a higher price that included a premium based on the EBV of NM$. The idea here was that surplus IVP calves had greater genetic merit and may be worth more than surplus calves from AI when sold. Cost of production and transfer of one IVP embryo was set at $165. For the IVP-ET scenarios, the top 2% of females were selected as donors. Half of the donors produced oocytes in 1 week, whereas the other half was not involved in oocyte collection that week. Oocytes were fertilized with female sexed semen.

Figure 2 shows the average TBV ($2 \times$ PTA) of NM$ in sires and cows from year -4 to +15 after implementation of the 8 scenarios in year 1. The genetic lag between sires and the average cow in the herd before year 1 was approximately $500 PTA of NM$ when no selection among females occurred and only AI was used. The genetic lag started to decrease after year 3 when the first cows started to produce that were conceived after selection criteria were implemented.

The scenarios using IVP-ET and selection of females based on NM$, milk, and DPR all reduced the genetic lag more than the scenario based on AI with selection on NM$ did. The IVP-ET scenario based on NM$ reduced the genetic lag to $150 PTA of NM$ (= $300 TBV in Figure 2). This constant genetic lag with the service sires was reached approximately in year 13 after the first use of IVP-ET. Thus, from year 3 to year 13 the genetic gain in the females was greater than that in the service sires, but this was the result of moving from the old genetic lag of $500 PTA of NM$ to the new genetic lag of $150 PTA of NM$. In year 15, the AI scenarios produced approximately 30% surplus dairy heifer calves and the IVP-ET scenarios approximately 54% surplus after years of genetic improvement in reproductive traits. This was only 8% in year 0.
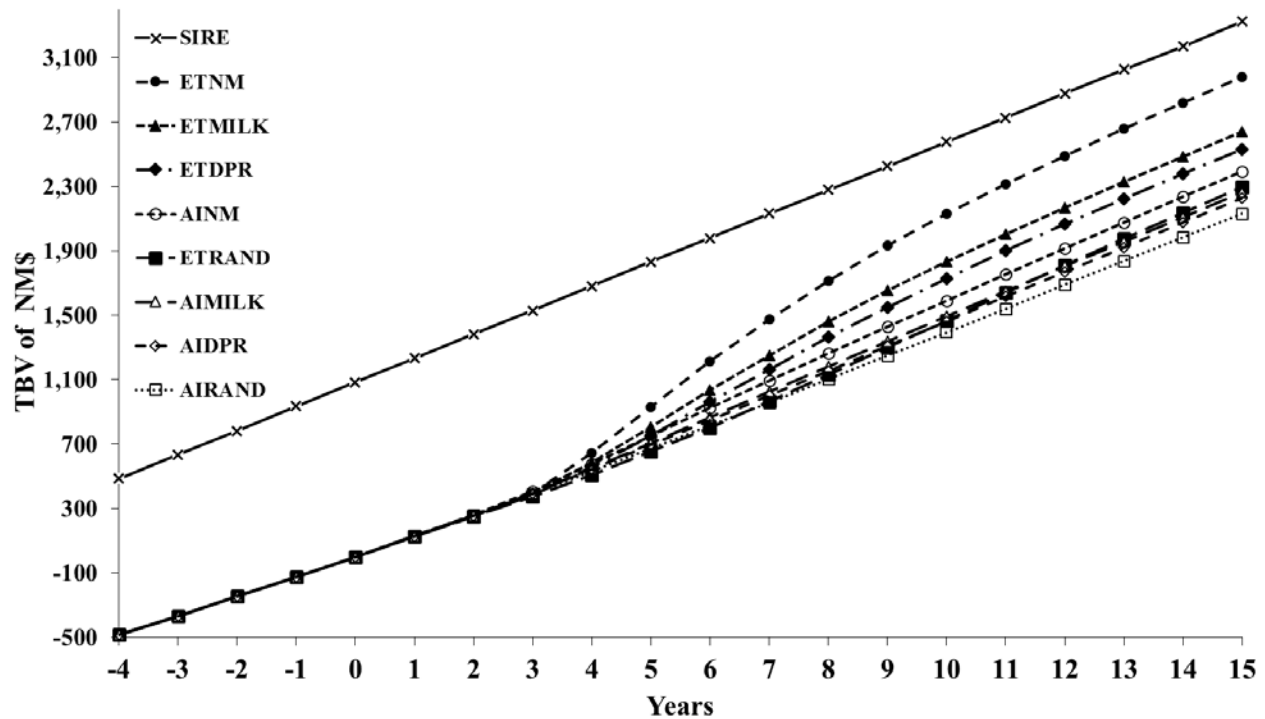
Figure 2. Average true breeding values (TBV) of Lifetime Net Merit (NM$) in sires and cows from year -4 to +15 for 8 scenarios. Name of the scenarios: AI = exclusive artificial insemination program, ET = exclusive in-vitro produced embryo transfer program. Eligible animals were ranked either randomly (RAND) or based on their estimated breeding value of NM$, milk yield (MILK) or daughter pregnancy rate (DPR; Kaniyamattam et al., 2017).

Figure 3 shows profit per cow per year. Change in profitability over time is the combined result of increases in genetic merit and cost of implementing the IVP program from year 1 on. We assumed that there is no inflation. Profitability of the IVP-ET scenarios decreased immediately after year 0 because of the high cost of making IVP embryos. The increased genetic merit of these embryos did not start to pay back until these embryos had become cows (and a little bit as better young stock with improved heifer conception rate).

By year 9, the AI and IVP scenarios with selection based on NM$ started to have similar profitability and by year 15 they differed only by $8 per cow per year (Figure 3; advantage IVP scenario) when the greater surplus calf prices for IVP calves were included. In year 15, the break-even price for an IVP embryo was $168 per transfer, so it was very similar to the input price of $165 (Kaniyamattam et al., 2017).

The AI scenarios were more profitable than the IVP-ET scenarios when the surplus calves were sold for the same price, independent of their genetic merit. With selection on NM$, the break-even price for an IVP embryo was $89. This low break-even price is below current market prices for IVP-ET. The advantage of the AI scenario was $185 per cow per year. The 3 other AI scenarios with selection only on milk yield, DPR, or random selection resulted in greater advantages of AI over the IVP scenarios.
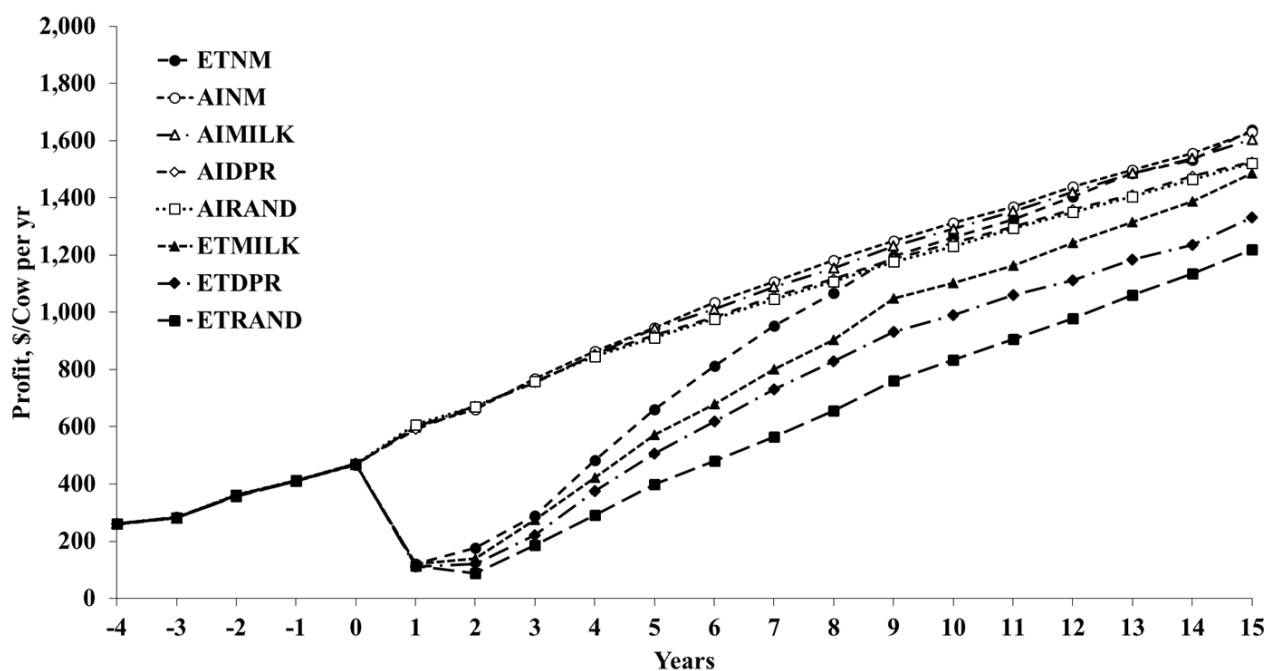
Figure 3. Profit per cow per year from year -4 to +15 for 8 scenarios. Premium pricing of surplus heifer calves is assumed. Name of the scenarios: AI = exclusive artificial insemination program, ET = exclusive in-vitro produced embryo transfer (IVP-ET) program. Eligible animals were ranked either randomly (RAND) or based on their estimated breeding value of Lifetime Net Merit (NM$), milk yield (MILK) or daughter pregnancy rate (DPR). The profit of the 4 IVP-ET scenarios decreases rapidly after the start of the IVP-ET program in year 1 because the embryo transfer cost are greater than the AI cost (Kaniyamattam et al., 2017).

The large decrease in profit per cow in year 1 for IVP-ET program was the result of an immediate transition from AI to IVP-ET where costs were assigned as soon as embryos were transferred. A more gradual use of IVP-ET (< 100%) would avoid this large sudden decrease in profitability, but also delay the reduction in genetic lag and delay in future profitability. This first study showed that 100% IVP-ET programs were typically less profitable than 100% AI programs, even though the genetic lag with service sires was much reduced by the IVP-ET programs.

- **Second Study**

In the second study (Kaniyamattam et al., 2018), we varied the fraction of pregnancies made with IVP-ET from 0% to 100% with intervals of approximately 20%. The best amount of IVP-ET could be less than 100% of pregnancies because the donors would be more superior (fewer are needed) and genetically good animals (that are not donors) would carry their own calves instead of carrying slightly superior but much more expensive calves from IVP-ET. In addition, avoiding recipients that have low conception risks after embryo transfer might be beneficial.

Figure 4 shows that the genetic lag with the service sires decreases with greater use of IVP-ET, as expected. The rate of decrease in the lag was greatest when IVP-ET use was small. In other words, the more IVP-ET was used, the less the genetic lag changed. It took approximately 10 years to transition from the old constant genetic lag based on AI only to the new constant genetic lag based on some use of IVP-ET.

Table 1 (found at the end of the report) shows the results for combinations in: 1) surplus base heifer calf price, 2) premium paid for genetically better surplus heifer calves, 3) IVP-ET price, and 4) the fraction

pregnancies from IVP-ET (3 × 2 × 4 × 6 = 144 combinations). As expected, more IVP-ET use was optimal with a greater surplus base heifer calf price, a premium paid for surplus heifer calves, and a lower IVP-ET price. For six of the 24 combinations in prices, the 100% IVP-ET program was optimal. All had embryo prices of $100 or less and required a premium paid for genetically better surplus heifer calves. Differences between 0% IVP and 100% IVP could be hundreds of dollars per cow per year when embryo transfer prices were low. When the use of IVP-ET was somewhere in the middle, profitability increased by tens of dollars per cow per year compared with no IVP-ET use or 100% IVP-ET use for the same price assumptions. Figure 5 shows the trend in profitability over time for four scenarios.
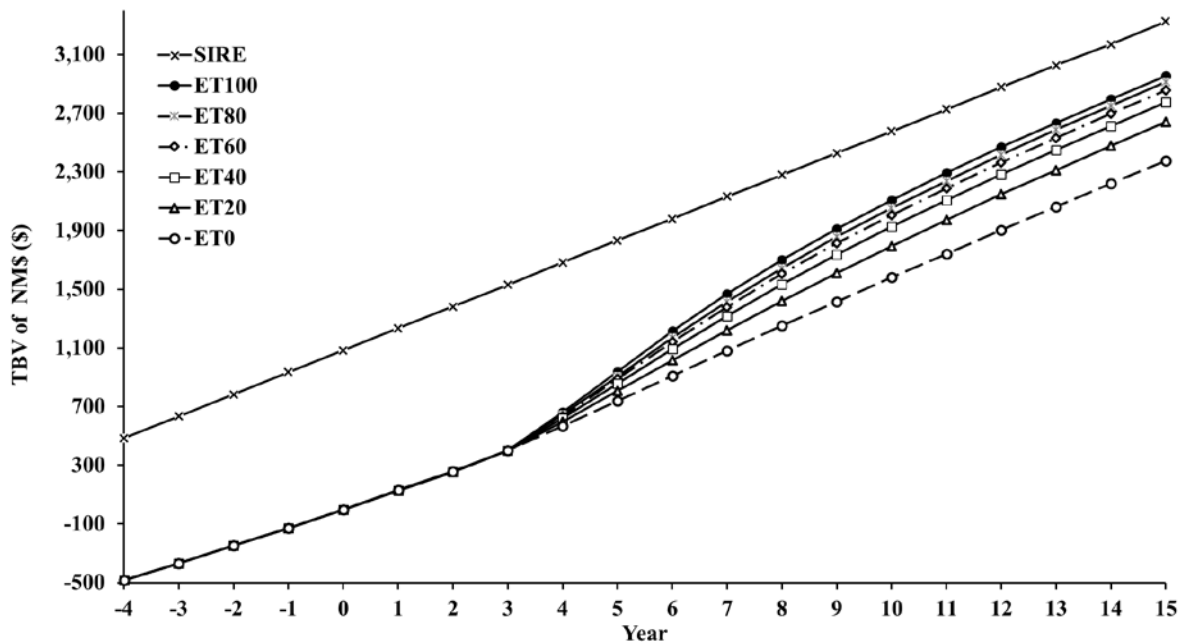
Figure 4. Average true breeding values (TBV) of Lifetime Net Merit (NM$) in sires and cows in year -4 to +15, in scenarios which used in-vitro produced embryo transfer 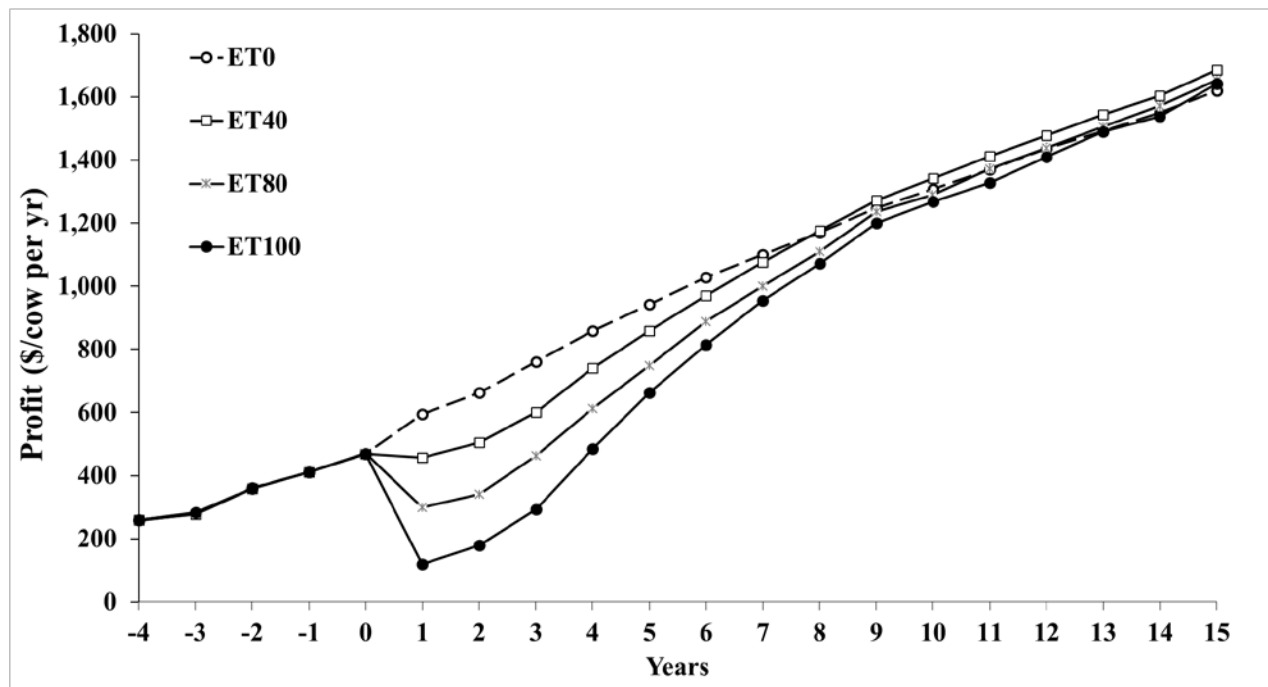(IVP-ET) to obtain varying proportions of conceptions from IVP-ET, ranging from 0% (ET0) to 100% (ET100) from IVP-ET (Kaniyamattam et al., 2018).

In this second study, the selection of donors was based on rankings for PTA for NM$ after genomic testing. We did not assume any prior knowledge about the ability of donors to produce transferable embryos. Various factors that determine the production of transferable embryos for an animal are heritable. In one study, heritability estimates for IVP factors in a sample 628 IVP-ET records ranged from 1% to 21%, but were not significantly different from zero (Parker Gaddis et al., 2017). Better understanding of factors that affect the production of transferable embryos should lead to fine-tuning of donor selection.

Further in this second study, we prioritized non-pregnant, non-donor heifers as first eligible to receive IVP embryos. The rationale was that these heifers had greater conception risks than candidate cow recipients and that this was important because of the high IVP-ET prices ($50 to $200). On the other hand, recipient heifers pregnant after IVP-ET have greater opportunity costs of not carrying their own calf compared with recipient cows. Among recipient cows, we gave the highest priority to cows with high PTA for DPR and high PTA for cow conception rate. These cows were expected to have the greatest conception risks, but might not have the lowest PTA for NM$. Again, opportunity cost for the value of their own calf was not considered in selection of cow recipients.

Figure 5. Profit per cow per year in year -4 to +15, in scenarios that used in-vitro produced embryo transfer (IVP-ET) to obtain varying proportions of conceptions from IVP-ET, ranging from 0% (ET0) to 100% (ET100) from IVP-ET. The cost of the fresh embryo was $165 and the sale price of a 3.5-month old surplus dairy heifer calf was $500 in addition to a premium price calculated based on the difference of the estimated breeding value of Lifetime Net Merit of sold dairy heifer calves from the IVP-ET scenario compared to the ET0 scenario (Kaniyamattam et al., 2018).

Recipients were selected on the same day the donors were selected. We also ranked candidate recipients independently of their stage in the estrous cycle and looked for estrus daily in the simulation model. If estrus was observed in a selected recipient, the animal was scheduled to receive an IVP embryo on day 6, 7, or 8 after estrus, depending on availability of a fresh embryo. Use of estrus detection instead of estrus synchronization likely resulted in a less than ideal use of candidate recipients, but also at lower direct costs. All eligible animals that were not selected as recipients received AI.

We also assumed that the expected phenotypic performance of calves born from IVP or AI was on average the same if they had the same genetic merit. This may not be the case in practice. For example, in one study, mortality of IVP calves produced by reverse female-sorted semen was greater than in calves produced by AI (Sequeira et al., 2017). Calves born from IVP-ET also have greater risk of large offspring syndrome, which may increase incidences of dystocia and retained placenta (Bonilla et al., 2014). Stillbirths and calf deaths also may increase in IVP calves (Bonilla et al., 2014).

In summary, selection of recipients could be improved by better integration of all factors that determine the profitability of an IVP-ET program. These factors include conception risk, abortion, still birth, value of the IVP-ET calf once born, and the foregone value of the recipient's own calf. An index that integrates these factors is not too difficult to put together.

▪ **Outlook**

In these two studies by Kaniyamattam et al. (2017, 2018), we assumed that all IVP embryos were made with female sexed semen, and some sexed semen was used in AI for heifers. Consequently, we had a surplus of dairy heifer calves being born and we used genomic testing to help select and sell surplus dairy heifers calves. All dairy bull calves were also sold.

Alternatively, an economically better strategy may be to use AI with beef semen so that crossbred calves are made. Market prices for crossbred calves are approximately $100 greater than those for marketed dairy calves. In contrast, this strategy would lead to fewer surplus dairy heifer calves, and would limit the genetic gain in retained dairy calves because the heifer selection intensity would be lower. A good strategy might involve a combination of IVP-ET, and AI with sexed semen, beef semen, and even conventional semen (Weigel, 2019). We are currently working to identify such promising strategies.

The USDA's NM$ is a general economic selection index that is useful for a wide range of herds. Other economic selection indexes may be more appropriate in certain markets, such as the Fluid Merit, Cheese Merit and Grazing Merit (VanRaden et al., 2018). A reformulation of the components of the NM$ index using financial investment methods has led to two new economic selection indexes that cause some reranking of service sires (Schmitt et al., 2019). In theory, these new indexes are better at identifying most profitable donors and recipients.

Further reduction in the generation interval will increase the rate of genetic gain in a nucleus population, for example, in the production of service sires. In-vitro breeding is an emerging technique that greatly reduces the generation interval. It also combines genomic selection with derivation of embryonic stem cells and in vitro differentiation of germ cells from pluripotent stem cells (Goszczynski et al., 2019). With this technique, the generation interval can be reduced to 3 to 4 months. This technique may be soon within reach (Goszczynski et al., 2019).

Individual dairy farms that rely on marketed service sires to produce IVP embryos will continue to have a rate of genetic gain that in steady state will be the same as that of the service sires. These farms should strive to reduce the genetic lag. Improvements in the ranking of donors and recipients, as outlined above, and improved efficiencies and reduced costs will strengthen the economic viability of IVP-ET programs. IVP-ET programs will become more economically competitive with AI programs and eventually they might become clearly more profitable. The best use of IVP-ET on commercial dairy farms remains an interesting puzzle with many variable factors.

Acknowledgments

Table 1. Sensitivity analysis for 6 surplus dairy heifer calf prices and 4 embryo prices and the optimal proportion of conceptions to be achieved by in-vitro produced embryo transfer (IVP-ET) such that profit per cow is maximized (Kaniyamattam et al., 2018).

Dairy heifer calf sale price[1]		Embryo price ($)	ET Conceptions (%)[2]						Optimal ET[4] %	Maximum added profit[5] ($)
Base price ($)	Premium		0%	21%	42%	63%	82%	100%		
			Additional profit per cow in year 15 ($)[3]							
300	No	50	0	64	90	80	61	47	46%	91
300	No	100	0	39	42	7	-36	-75	33%	45
300	No	150	0	13	-6	-66	-133	-197	19%	14
300	No	200	0	-12	-55	-139	-231	-319	3%	0
300	Yes	50	0	91	155	187	209	241	100%	241
300	Yes	100	0	66	107	114	112	119	100%	119
300	Yes	150	0	41	58	41	15	-3	42%	58
300	Yes	200	0	16	10	-32	-82	-124	28%	18
500	No	50	35	107	148	150	144	142	69%	158
500	No	100	35	82	99	77	47	21	41%	99
500	No	150	35	57	51	4	-50	-101	28%	59
500	No	200	35	32	3	-69	-147	-223	8%	37
500	Yes	50	35	135	213	258	293	337	100%	337
500	Yes	100	35	110	164	185	196	215	100%	215
500	Yes	150	35	84	116	112	99	93	62%	116
500	Yes	200	35	59	67	39	2	-29	36%	69
700	No	50	70	150	205	221	228	238	84%	238
700	No	100	70	125	157	148	131	116	48%	158
700	No	150	70	100	108	75	33	-5	36%	110
700	No	200	70	75	60	2	-64	-127	12%	77
700	Yes	50	70	178	270	328	376	432	100%	432
700	Yes	100	70	153	222	255	279	311	0%	311
700	Yes	150	70	128	173	182	182	189	79%	191
700	Yes	200	70	103	125	109	85	67	25%	129

[1]Base female calf sale prices of $300, $500 or $700 at 105 days of age. The dairy heifer calf rearing cost since birth at 105 days was $375.
[2]Scenario and actual proportion of pregnancies from IVP-ET: ET0 (0%), ET20 (21%), ET40 (42%), ET60 (63%), ET80 (82%), ET100 (100%).
[3]Additional profit per cow in year 15 for varying proportions of conceptions from IVP-ET compared to the scenario with no conceptions from IVP-ET (ET0).
[4]The economically optimal proportion of conceptions obtained from IVP-ET.
[5]The maximum additional profit per cow per year at the optimal proportion of conceptions from IVP-ET compared to the scenario with no conceptions from IVP-ET.

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When (Before) Disaster Strikes: Preparing for a Disease Outbreak

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▪ Take Home Messages

- Diseases can have significant effects on productivity, profitability, and market access.
- The threat of foreign animal disease is growing in frequency and impact.
- On-farm planning can help to dramatically lower the risks associated with disease through emergency plans, contingency plans, and enhancements to biosecurity.
- Zoning may be an option in some cases and may allow parts of the country to continue trading while other parts of the country are managing a disease issue. Trading partners must recognize the zone and be willing to trade in that situation.
- Zoning can also be applied at the farm level as part of a contingency plan. This requires designation of certain areas of the farm as either restricted access, controlled access, or unrestricted. Establishing these zones in advance can help to ensure ongoing deliveries and pickup services in the midst of a disease outbreak with minimal risk of transmitting the disease off farm.
- Biosecurity programs that are established to prevent or reduce transmission of disease on-farm serve equally well in relation to serious foreign animal diseases, where these programs provide the last line of defence.

▪ Introduction

There is no doubt major disease outbreaks can have significant economic consequences due to the loss of production that can result from the disease itself and from the loss of infected animals in situations where it is beneficial or mandated to cull animals as a means of controlling the disease. This problem is further exacerbated in situations that result in closure of international markets, particularly in export dependent countries that rely on foreign markets for significant portions of their production chain. Figure 1 outlines some of the risks and impacts associated with a range of disease, pest, and natural disasters.
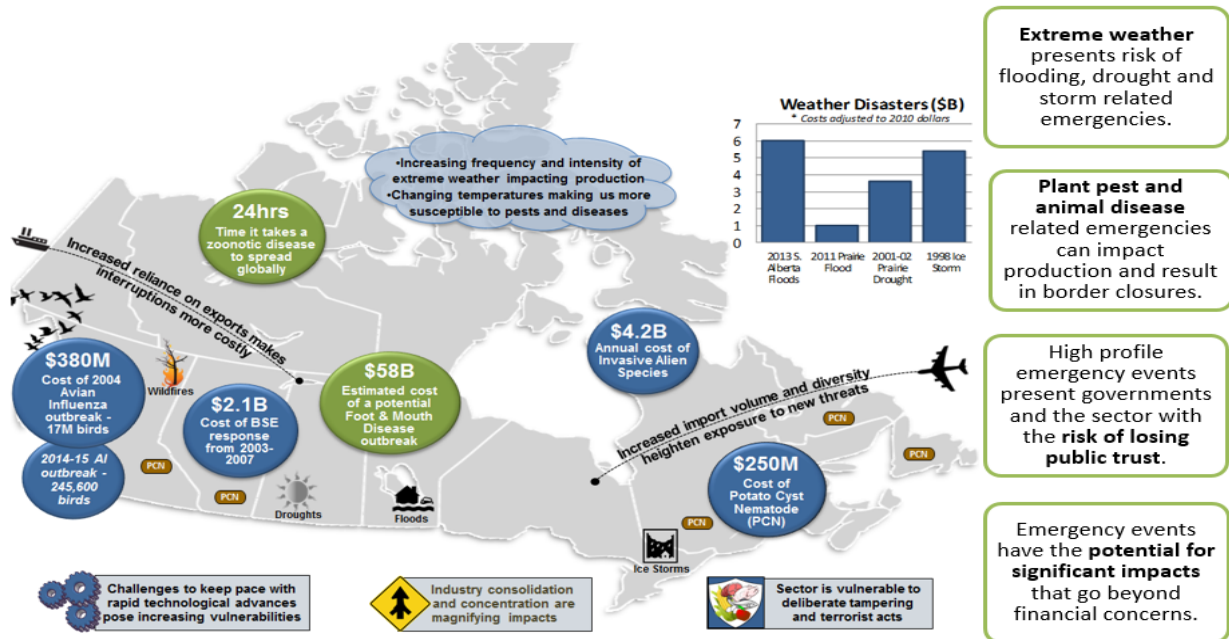
Figure 1. Risks and impacts of disease, pests, and natural disasters. (Courtesy of the Canadian Plant and Animal Health Strategy)

The most significant cattle disease related incident in recent Canadian history involves the detection of Canada's first native case of bovine spongiform encephalopathy (BSE) in May 2003. Several articles documented the financial impacts of this crisis and several estimates were circulated in the following years. The National Farmed Animal Health and Welfare Council, through contractors working on the "Animal Health Canada" initiative, has looked back on some of these reports to understand the financial impacts that these events can have to justify the need for an updated approach to management of large scale disease events.

In that summary, the contractors noted four reports relating to BSE, with each looking at various aspects of the issue including lost cattle and beef sales, loss of genetics, costs associated with managing the disease, and labour costs. While the figures reported have a wide range (from $541 million for the first month after detection to $5.5 billion over the first two years), the key takeaway is that the numbers can be extremely large.

In other disease outbreaks in Canada and around the world, the findings are similar. One Canadian Food Inspection Agency (CFIA) report from 2002-03 noted that the potential cost of a foot and mouth disease (FMD) outbreak in Canada would be $30 billion while a Serecon report from 2002 looked at three possible scenarios and estimated the cost between $8.3 billion and $46 billion.

▪ Prevention, Preparedness, Response, and Recovery

Discussions around large-scale disease outbreaks often focus on four key elements: prevention, preparedness, response, and recovery.

Prevention items can include steps taken internationally, nationally, regionally, and at the farm level. Internationally, risk assessments, inspection, and evaluations of foreign country veterinary services help to establish which countries have sufficient measures in place to ensure low risk trade potential.

Nationally, import policies and border inspections address the majority of the preventative measures taken. Regionally and at the farm level, biosecurity programs become the last line of defence.

Preparedness involves a wide range of activities. The first, and perhaps most obvious to some, comes in the form of plans for responding to a disease incursion. This can include government response plans, industry organization plans or farm level plans. Government plans often include policy decisions, which may have been made through consultation with industry in advance of the crisis, and provide strategic approaches in key areas of the disease management process. This can include decisions on the type of approach or desired outcome (eradication vs. management) and mechanisms to achieve that outcome (cull vs. vaccination). Government preparedness also includes development of infrastructure items like traceability and surveillance programs that can help understand the spread of the disease or help to establish disease control zones. Another infrastructure item involves the establishment of vaccine banks and agreements for accessing them if developed in partnership with other countries or regions.

Industry plans may include items that are critical early in the incident such as communications planning to keep members informed and engagement plans to remain in contact with government. This becomes helpful in building a collaborative approach to managing the disease and ensuring goals and outcomes of the respective stakeholders are well understood. Farm plans often include information for farmers regarding possible movement controls, enhancement of biosecurity measures, quick reference guides, risk assessment tools, and contact lists. They may also include recommendations on contingency plans that farmers can complete in advance of a crisis in order to improve their level of preparedness.

Other important elements that go along with these plans are training and exercises, both of which improve the level of understanding of the plans and how they will be implemented. Unfortunately, it is impossible to predict how a disease will enter Canada, when it will be detected, how far it will have spread prior to detection, and what market impacts might occur. It is for this reason that plans cannot be written with a completely prescriptive process for managing the disease. Training and exercises help to identify some of the different scenarios that may occur.

Some may suggest that the response plans, training, and exercises fall within the response category, while others acknowledge the importance of having them as well developed as possible prior to the need to respond. Based on that notion, response is considered here as the actual component that follows detection of a disease situation. The response will include a wide range of actions including emergency management and leadership, communications, policy development to address situations that were not anticipated, and actual disease control activities including inspections, testing, containment (movement restrictions, zoning), culling, disposal, vaccinations if applicable, cleaning and disinfection, support programs (financial, mental health), and business continuity planning.

The late stages of response often merge into recovery as ongoing surveillance and monitoring activities start to confirm eradication or control of the disease, which then provides reassurance to trading partners that it is safe to resume trading and accepting products from the affected country. Significant work must go into the recovery stage as well; both in terms of the negotiations with those foreign trading partners and the recovery work with the industry to start rebuilding any lost animal populations and genetics established over extended periods as well as identifying what a changed industry might look like.

▪ Zoning for Control of Disease

Zoning is an internationally accepted mechanism to control disease and to potentially enable trade from an unaffected portion of a country while the remainder of the country works to control or eradicate the disease. The World Organization for Animal Health provides guidance on what is required for zones to be established and recognized, and gives recommendations on application of zoning for different diseases.

Two key elements are involved in establishing a disease control zone: movement restrictions and surveillance. The movement restrictions ensure that the disease is not transmitted outside of the zone

and include controls on anything that can potentially carry that disease agent. The carriers can include animal products and the infected animals themselves. Surveillance is necessary outside of the zone to ensure that the disease agent is being effectively contained within the zone.

Implementation of a zone early in a disease outbreak is challenging. The disease situation must be understood before embarking on this process so that the size of the zone captures the diseased population. An understanding of animal movement (traceability) can provide clues in advance of an outbreak as to how the disease might be contained. However, if animal products or feed are important in spreading the disease, it is important to understand more than just the animal movement.

The initial declaration of a zone should be larger rather than smaller, with the potential to shrink the size of the zone as more information becomes available. Starting with a smaller zone and needing to expand it may be viewed as a lack of control of the zone and result in trading partners losing faith or trust in the infected country to effectively manage the disease.

A newly established zone may have three components to it. At the centre, there will be an infected zone. Surrounding the infected zone is the buffer zone that provides an area to monitor for spread of disease (surveillance) beyond the infected zone. Vaccination may also occur within that buffer zone as a means to prevent the disease from moving outside the infected zone. Beyond the surveillance zone lies the disease-free zone from which trade can potentially occur.

This establishment of a zone can be applied at a smaller scale if the disease is caught early or has low rates of transmission. If there are very few infected farms and they are not located in proximity to each other, it may make more sense to apply an infected zone around the infected farms rather than capture them all in one zone. Each zone might include all farms within a certain radius of each infected farm. A larger radius would then be used to identify farms on which surveillance and/or vaccination would occur (the buffer zone) and beyond that radius, no action taken (the disease-free zone).

The concept of zoning can also apply at the individual farm level from a preparedness perspective. During peace time, farms should consider identifying a restricted access zone (RAZ) and a controlled access zone (CAZ) so that they can be established or implemented quickly. Figure 2 provides an example of how these zones could be applied. The RAZ should contain the animal holding areas, representing the area where you need to keep disease out of, or in the case of introduction of disease, where you need to keep it contained. The CAZ represents a buffer area around the RAZ and allows access to areas that are essential to ongoing animal production without direct access to the animals. This can include such things as feed delivery, milk retrieval, or animal loading areas. Being able to access these areas while minimizing the risk of coming into contact with the disease agent in the case of an outbreak of disease in the animal holding area is critical.

In addition to having access zones defined on the farm prior to an outbreak, producers should also have plans developed that will allow them to continue to function as well as possible in the face of imposed widespread movement controls. Movement controls in the early stage of an outbreak are a critical tool to allow disease investigators to determine risks for disease spread off the farm, where spread may have occurred based on those risks, and how widespread the disease might be.

During the initial stages of a disease outbreak, producers should be prepared to deal with keeping animals on farm longer than normal and have destruction and disposal contingency plans for when movement control periods become extended. These plans can also be helpful in dealing with animals that are unfit for transport, although the volume of animals may become larger when dealing with prolonged movement restrictions.
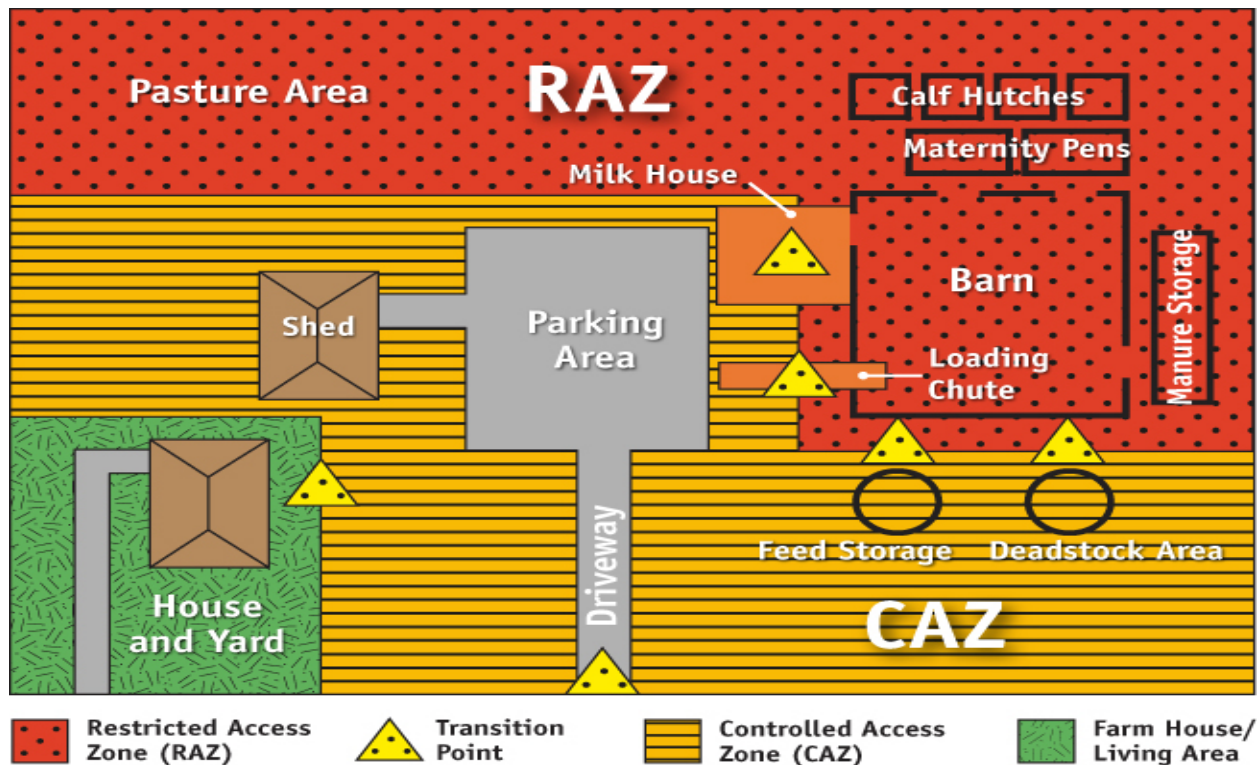
Figure 2. Diagram of possible restricted and controlled access zones. (Courtesy of the Canadian Food Inspection Agency)

▪ Vaccination

Canada has long considered vaccination a less favourable option in the face of a disease outbreak. However, advances in vaccine and testing technology that allow differentiation of vaccinated and infected animals, decreasing social acceptance of mass culling, and better understanding resource requirements for mass culling are making vaccination increasingly important in disease control strategies. Unfortunately, vaccines are not available for all diseases.

Foot and mouth disease is one disease where vaccination is being strongly considered within control strategies in Canada. Foot and mouth disease still provides a challenge in that there are several serotypes of the virus around the world and there will be a need to identify the serotype before being able to order and apply vaccination strategies. Currently, there are roughly 65 different strains identified.

Canada is working on vaccine banks for FMD with other countries around the world. Currently, Canada, the United States and Mexico participate in a North American Foot and Mouth Disease Vaccine Bank (NAFMDVB). However, the quantity of vaccine is insufficient and given the likelihood of more than one of these countries becoming infected should the disease enter North America, we will be all drawing from the same source. As a result, Canada is also pursuing options to participate in other banks with the United Kingdom, Australia, and New Zealand. Challenges with vaccine banks include their expense and the fact that the material (viral antigen) has a limited shelf life, which further adds to the expense through routine replacement or updating.

▪ On-farm Biosecurity

While on-farm biosecurity has been partly addressed in the discussion around RAZ and CAZ, there is much more to include for on-farm measures. These items can be discussed generally or conceptually, but

looking at them with a specific disease in mind might provide more value. Based on this, a disease that is provincially reportable in Alberta will be used.

Salmonella dublin (SD) is a bacterial disease that can cause pneumonia, diarrhea, and reproductive losses in cattle. Animals that recover from the disease can become persistent carriers and intermittent shedders of the bacteria. There are reports of increasing prevalence of this condition and given this as well as the fact that it can also cause illness in people, it is reportable in Alberta. The province is interested in monitoring SD trends and despite limited numbers of reports, it is suspected that there are more cases occurring. In terms of response, the Alberta government works with the herd owner and their veterinarian to assist with testing and making recommendations for disease management options in each case.

Salmonella Dublin is an opportunistic pathogen, taking advantage of stressful moments such as the period close to parturition. As a result, anything that can be done to support the cows during that time is beneficial. This can include provision of high-quality feed, adequate space, and dedicated, clean maternity areas.

The first consideration for SD, like with many other diseases, is to prevent introduction into the herd. Having programs that effectively mitigate risks associated with visitors, incoming animals, feed purchasing, and load out procedures for animals leaving are key areas to focus on.

In terms of dealing with introduction of disease or implementing practices to minimize the chance of SD becoming established on a farm, it is possible to look at various stages of production in a systematic fashion. This can provide a view to areas of risk and interventions that may be of greatest benefit.

The first stage is calving. Minimizing the number or density of cows, having a clean calving facility, keeping sick cows out, minimizing the time calves spend with mothers, and keeping calves from suckling cows can help reduce calf exposure.

The second stage is the post calving/weaning times. Feeding non-pooled colostrum, heat treating colostrum, physical separating calves from cows, separating out sick calves, and maintaining clean calf rearing areas and equipment, tools, boots, etc. further reduce calf exposure.

Manure contamination is a significant risk factor, given the ability of SD to remain viable in the environment for extended time. While this is a factor in many of the recommendations in the previous two stages, it is a primary driver for recommendations in the post weaning and heifer rearing stages. Minimizing contact with cattle of other age groups, pastures where other age groups have resided or where manure has been spread, and reducing the size and density of calf groups all help to minimize the risks of infection.

▪ Conclusion

Biosecurity and emergency preparedness are critical elements in current livestock production. While the farm represents a significant investment itself, the implications for the broader industry are significantly greater and have the potential to considerably affect market access and economics of livestock production. Many of the principles that apply to keeping herds free of endemic production-limiting diseases are highly relatable to also providing a last line of defence against foreign animal diseases. Completing a risk assessment at the farm level and outlining programs to address gaps and establish contingency plans should be strongly considered prior to incursion of disease.

▪ **Resources**

Biosecurity for Canadian Dairy Farms: National Standard: https://www.inspection.gc.ca/animals/terrestrial-animals/biosecurity/standards-and-principles/dairy-farms/eng/1359657658068/1359658301822

OIE – World Organization for Animal Health Zoning and Compartmentalization: https://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/current/chapitre_zoning_compartment.pdf

Salmonellosis in Cattle – A review https://www.vetmed.wisc.edu/dms/fapm/fapmtools/7health/Salmorev.pdf

Serecon Report https://www.ifama.org/resources/Documents/v8i1/Pritchett-Thilmany-Johnson.pdf

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Investigating Fetal Calf Loss

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▪ Take Home Messages

- Fetal calf loss (abortion) is economically significant to the dairy industry. A background reproductive loss of approximately 1% is expected. A rate higher than this may warrant investigation.

- Abortion diagnosis can be frustrating and costly. The chance of diagnosing the cause of a single abortion is relatively low (25–50%). However, the likelihood of diagnosis is increased if there are multiple cases and if the cause is infectious.

- The chance of diagnostic success is maximized if the producer, veterinarian and diagnostic laboratory collaborate. The most important part of this is collection and submission of correct tissue samples.

- Tissue sampling as part of an abortion investigation is not haphazard. Collection of an inadequate tissue set or preserving samples inappropriately can potentially cost a diagnosis.

- Any abortion investigation should begin with collection and examination of the aborted fetus and placenta. The placenta should always be included in a diagnostic submission. The diagnostic value of the placenta is equal to or greater than that of the fetus, yet it is frequently overlooked.

- Producers can play an important role in collecting data about aborted fetuses and placentas that may otherwise be lost. This is not difficult or time-consuming, and is explained in this paper.

- Abortion investigation is a complex, detailed and systematic procedure. Knowing what it involves will help producers understand its inherent limitations.

▪ General Introduction

Fetal calf loss is an important cause of economic loss in the dairy cattle industry. The significance of a cow aborting a calf is not always clear. It may be part of the normal expected background reproductive loss of 1%, a sporadic case of disease, or the start of an abortion outbreak or storm. Each may require a different investigative approach (Holler, 2012; Taylor and Njaa, 2012).

Determining a specific cause for abortion can be difficult, costly and frustrating. Despite spending several hundred dollars working up a case, the final diagnosis may be 'abortion; cause undetermined.' This should not discourage investigations of later abortions, especially when fetal losses are unexpectedly high in number, clustered in time, and at a similar stage of gestation. Identification of a specific cause may allow implementation of management strategies that reduce or prevent further losses.

This paper discusses: a) the common causes of bovine abortion in Western Canada, b) what information can be obtained from examination of the fetus and placenta, c) when to contact the veterinarian, and d) how the producer and veterinarian can work together to maximize the chance that a cause of abortion can be determined.

- ## Is Fetal Calf Loss Different from Abortion or Stillbirth?

For the purposes of this paper, fetal calf loss is not different from abortion or stillbirth. I chose the term 'fetal calf loss' for my title because it encompasses both abortion and stillbirth. Abortions happen in early gestation and stillbirths happen in late gestation. Specifically, abortion refers to fetal death and expulsion from the uterus before the fetus is viable. Even if it is alive at the time of expulsion, an aborted calf is not developed enough to survive outside the uterus. In contrast, stillbirth refers to delivery of a fully formed, but dead, calf. Had the calf been born alive it stood a chance of surviving (Holler, 2012). For practical purposes there is very little difference in the diagnostic workup of an aborted calf versus a stillborn calf. Most producers and veterinarians use the term 'abortion' in a wider sense to mean death and expulsion of a calf any time during gestation. This is the sense in which I use this term for the remainder of this paper.

- ## What are the Common Causes of Bovine Abortion?

The simplest way to subdivide bovine abortion is into infectious and non-infectious categories. Infectious causes of abortion are typically easier to diagnose through laboratory testing than are non-infectious causes because diagnostic laboratories are well set up to screen tissue samples for viruses, bacteria, protozoa and other infectious agents. Identification of non-infectious causes such as faulty management practices, toxins in feed, or genetic abnormalities typically requires a more in-depth investigation. Although there are numerous causes of abortion in cattle, very few of them leave tell-tale gross lesions. Identification of a specific cause, therefore, usually requires a detailed investigation by a veterinarian working in conjunction with a diagnostic laboratory. This does not mean that there is no value in gross examination of an aborted calf and placenta, just that this is only one step in a larger work-up (more on this later).

Infectious Causes of Bovine Abortion

How Infectious Agents Reach the Fetus

During pregnancy it is relatively uncommon for infectious agents to reach the uterus by ascending through the cervix. Instead, the majority of infectious agents that reach the pregnant uterus arrive hematogenously, via the cow's bloodstream (Anderson, 2012; Holler, 2012).

After reaching the pregnant uterus, many agents colonize and proliferate in the placenta, causing a placentitis (inflammation of the placenta), without necessarily spreading further downstream into the fetus. Placentitis alone may be enough to cause abortion because an inflamed placenta is less effective at the exchange processes needed to support a fetus. A fetus aborted because of a primary placentitis may lack gross or histologic lesions, and infectious agents may not be detectable; therefore, the placenta, if available, should always be submitted for testing along with the fetus.

After colonizing and proliferating in the placenta, infectious agents may spread secondarily to the fetus. Some agents do this by entering the amniotic fluid around the fetus, where they are ingested and inhaled, leading to lesions in the fetal lungs, digestive tract, and skin. Other agents enter the fetus via the umbilical blood vessels, leading first to lesions in the liver and later to widely disseminated lesions in many organs, including the brain. The distribution of fetal lesions provides some information about the route of pathogen entry and may hint broadly at a cause (e.g., viral versus fungal).

Viral Agents of Bovine Abortion

Viral agents include bovine herpesvirus type 1 (BHV-1) and bovine viral diarrhea virus (BVDV). Both agents are familiar to producers and should be controlled with an appropriate vaccination program. Bluetongue virus (BTV) and bovine parainfluenzavirus type 3 (PI-3) are less common viral causes of bovine abortion.

Bacterial Agents of Bovine Abortion

Bacterial agents that cause bovine abortion are numerous, and most are opportunistic environmental or mucosal microbes that infect the cow, gain entry to the maternal bloodstream and spread hematogenously to the pregnant uterus. The cow may or may not show signs of illness, and abortions tend to be sporadic rather than occur as outbreaks. Opportunistic bacteria include *E. coli*, *Trueperella pyogenes* (formerly *Arcanobacterium pyogenes*), *Listeria monocytogenes, Pasteurella* species, and *Histophilus somni*. Species of bacteria that are more commonly associated with maternal illness or have a predilection for the reproductive tract include *Salmonella* species, *Leptospira* species, *Ureaplasma diversum*, and *Campylobacter fetus*. Neither list is exhaustive and determination of a specific bacterial cause for abortion always requires testing of samples by a diagnostic laboratory.

Fungal Agents of Bovine Abortion

Like bacterial agents of abortion, most abortigenic fungal agents are opportunistic environmental microbes that gain entry to the maternal bloodstream (and hence, uterus) via the respiratory or digestive tracts. They include *Aspergillus* and *Zygomyces* species.

Protozoal Agents of Bovine Abortion

The most important abortigenic protozoal species in dairy cattle are *Tritrichomonas foetus* and *Neospora caninum*. *T. foetus* is spread venereally and is therefore more common in herds where cows are bred naturally rather than by AI; bulls may be inapparent and lifelong carriers. *N. caninum* (spread by canids such as dogs and coyotes, or acquired congenitally *in utero* without canid involvement) may cause lifelong, inapparent infection in cattle. Infection in cows is efficiently spread to the fetus resulting variably in abortion, a live calf with neurologic disease (rare), or a live, infected calf with no clinical signs that may go on to pass the parasite to its own offspring.

Non-Infectious Causes of Bovine Abortion

Non-infectious causes of bovine abortion are also numerous, but are less easily diagnosed than infectious causes. The majority consist of chromosomal abnormalities that cause early fetal death and resorption; these typically go undiagnosed. A number of ingested plant and fungal toxins may also cause abortion. These include ergot toxins derived from the grass fungus of the genus *Claviceps*, an increasing cause of abortion in Western Canadian cattle. Other recognized abortigenic plant toxins are derived from pine needles, juniper plants and locoweed (*Oxytropis* species). Toxins in hemlock (*Conium* species) can cause fetal malformation and deformity. Management issues, including vitamin A, vitamin E or selenium deficiency, may play an underappreciated role in bovine fetal loss.

In general, non-infectious causes for bovine abortion are more difficult to identify than infectious causes. An abortion investigation typically rules out common infectious causes first, and then focuses on a more complex investigation, which may include feed sample analysis, fetal and maternal tissue and blood mineral level testing, genetic analysis, farm walk-throughs, and observation of management and handling procedures.

▪ How often is the Specific Cause of Bovine Abortion Determined?

Diagnosis of bovine abortion can be challenging: a cause for abortion is found in only 25–50% of cases (Table 1). The chance of achieving a specific diagnosis is decreased if: 1) the abortion is a single case, rather than an outbreak, 2) its cause is non-infectious, or 3) incorrect samples are submitted to the diagnostic lab (Foster, 2012). The converse is true—the chance of successfully obtaining a specific diagnosis is increased in an outbreak situation caused by an infectious agent and where appropriate diagnostic samples are collected and submitted.

Table 1: Causes of sporadic failure of pregnancy in cattle (modified from Table 18-3 from Foster, 2012)

Cause	%
Unknown; no diagnosis	60
Non-infectious	3
Infectious	37

▪ How can the Producer Increase the Chances of Diagnostic Success?

Abortion investigation is a team effort with six players: 1) the cow, 2) the fetus, 3) the placenta, 4) the producer, 5) the veterinarian, and 6) the diagnostic laboratory. The producer can and should play an active role in abortion investigation in the ways described below.

Decide When to Call the Veterinarian

The term 'abortion storm' is not precisely defined. In theory it should reflect a certain percentage of abortions above an expected background level of 1%; for example, 4% of the herd aborting in a short time period. In practice it tends to be three abortions, regardless of herd size. However, there are no firm rules about what constitutes an abortion storm or how many abortions need to occur before calling the veterinarian.

Prepare for the Veterinarian's Arrival

Preparing in advance for the veterinarian's arrival saves time and money.

- The affected cow should be easily accessible for physical examination and blood sampling.
- The aborted fetus and placenta should be bagged and refrigerated, if possible.
- Information that may be requested by the veterinarian as part of the investigation should be easily accessible. This will include information about the whole herd, the affected cow(s), nutrition, vaccination history, any therapies used, the farm environment and possible toxin exposure.

For example, animal information requested could include the following questions (Holler, 2012; Taylor and Njaa, 2012):

- What is the age of affected cows: Older cows? First pregnancy cows? Mixtures of ages?
- What stage of pregnancy is abortion occurring—first, second or third trimester? Term?
- What percentage of cows pregnant have aborted?
- Are aborting cows otherwise clinically ill (beyond the abortion event)?
- Are abortions clustered by time of year or age group of cows?
- Are the abortions sporadic or are there many in rapid succession (that is, an abortion storm)?
- Is the herd closed or have there been new introductions?
- If new animals were introduced, when?
- Are cows bred naturally or by artificial insemination?
- Is there other evidence of disease in the herd besides abortion or neonatal loss?
- Is there a history of similar problems in previous years? If so, what were the findings and were management changes made?

‣ Have congenital defects been seen before in this herd?

In cases of suspected vaccine failure, some of the requested information will be very specific. It may include vaccine serial numbers and expiration dates, information about storage (refrigeration) prior to use, and any modification to the vaccination protocol. It is helpful to have this information recorded and on hand.

Collect (and Examine) the Aborted Fetus

Necropsy (post mortem) examination of the fetus and collection of specific tissue and fluid samples are integral to the diagnostic investigation. These procedures are generally handled by the veterinarian on site or by the diagnostic lab pathologist after submission. However, the producer has earliest access to the fetus and can collect important information that may otherwise be lost. This is especially true for the first and second abortions in an abortion outbreak, when fetuses may be discarded rather than submitted, and the veterinarian is not called. Photographs, gross inspection findings and crown-rump length measurements of aborted fetuses are easily collected before disposal. The following sections describe what a producer should look for and record. When a fetus is submitted to a diagnostic lab the pathologist will conduct a similar examination along with a more detailed internal examination of the carcass.

Obvious Causes for Abortion

Obvious causes for abortion are rare, and diagnosis generally relies on laboratory testing and histopathology. However, fetal malformations sometimes occur, which are easily seen and photographed. These may suggest a heritable, viral or toxic cause. The more common external malformations to look for include (Foster, 2012; Schlafer and Foster, 2016):

‣ Cleft palate or lip (palatoschisis or cheiloschisis)

‣ Overshot or undershot jaw (prognathism or brachygnathism)

‣ Domed, possibly fluid-filled skull (hydrocephalus)

‣ Abnormal contraction of the limbs (arthrogryposis)

‣ Multiple limbs (polymelia)

‣ Arched back with intestines on the outside of the body (schistosomus reflexus)

‣ Fetal monster (amorphus globosus)

‣ Spinal deviation causing a hunchback (scolioisis and kyphosis)

‣ Incomplete closure of the spine along the midline (spina bifida)

‣ Distended, fluid-filled fetus (anasarca or ascites)

Crown to Rump Length and Body Weight

The crown to rump (C-R) length is the length of the fetus from the dome of the head, just behind the eyes, to the junction of the sacrum and tail head (Figure 1). The fetus should be straightened out as much as possible before taking this measurement, with the muzzle pointed down. Note that the measurement is NOT 'tip of nose to tip of tail.' The measurement is taken using a flexible tape measure or a length of string and compared against readily available charts to estimate the gestational age of the aborted fetus. Search the internet for terms such as 'bovine fetal age calculator' or 'fetal age assessment for cattle.' An example is included below (Table 2).
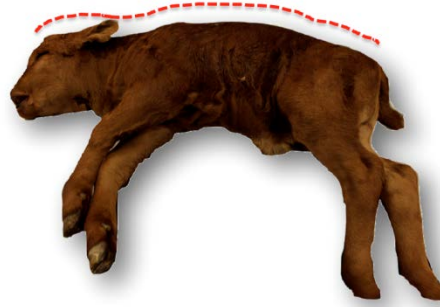
Figure 1: Measurement of fetal crown to rump (C-R) length, as indicated by the dashed line.

Table 2: Gestational age estimates for bovine fetuses (Modified from Njaa, 2012).

Gestational Age (months)	Crown to Rump Length (cm)	Weight (kg)	External Features
3	13–17	0.2–0.4	Hair on lips, chin and eyelids
4	22–32	1–2	Fine hair on eyebrows; claws developed
5	30–45	3–4	Hair on eyebrows and lips; testes in scrotum; teats developing
6	40–60	5–10	Hair on inside of ear and around horn buds, tail tip and muzzle
7	55–75	8–18	Hair on distal extremities and beginning on back; long hair on tip of tail
8	60–85	15–25	Fine short hair all over body; incisor teeth not erupted

State of Fetal Autolysis at Time of Expulsion

Autolysis refers to the softening of tissues that occurs after death (the reason carcasses are hung for a period after slaughter). It occurs as dead cells leak internal enzymes that cause self-digestion. Even tissues kept in a sterile environment will undergo autolysis. Autolyzed fetuses are soft and dark red, and their tissues ooze watery red fluid when incised. Autolysis is distinct from the putrefaction that occurs when dead tissues are colonized by bacteria, producing foul-smelling gas and green discoloration.

The state of autolysis of an aborted fetus is useful information because it may suggest the type of condition responsible for abortion. Note that the state of autolysis must be assessed at the time of expulsion, not after two days in a dead pit when bacterial putrefaction will make assessment inaccurate. This is a good reason for a producer to examine an aborted calf as soon as it is found.

Normal calves initiate their own birth at full term by signalling the cow through hormonal surges. A calf that dies suddenly (acutely or peracutely) does not have time to send these hormonal signals to the cow. As a result, the dead calf will remain undetected in the uterus for some period, during which autolysis will occur; this is accelerated by the cow's core body temperature. When it is eventually expelled, the aborted calf will be in an advanced state of autolysis. Conditions that can cause peracute fetal death include certain overwhelming fetal bacterial and viral infections.

In contrast, fetuses that are stressed for a period and die slowly have time to signal the cow and initiate their own birth. Thus, they are expelled in a fresh, non-autolyzed state shortly after they die. Conditions that cause fetal distress and hormonal signals to initiate birth include non-specific maternal illness,

chronic fetal illness, hypoxia (caused, for example, by placental disease), or maternal hyperthermia.

Indications of Dystocia or Fetal Distress

Sometimes it may not be clear whether a near-term calf was truly aborted, or died during an unobserved difficult delivery. The presence of fluid swelling (edema) of the subcutaneous tissues of the jaw, head and neck can indicate that the calf spent a prolonged period in the pelvic canal while still alive. Edema cannot develop after the heart stops, and an aborted calf will not develop regional edema even if compressed. Similarly, yellow staining of a calf's hair coat by meconium indicates prolonged delivery during which the stressed calf defecated into its own fetal sac. A calf that was already dead before delivery began cannot defecate and will not be meconium-stained.

Other ways to differentiate abortion from death during or after birth rely on a necropsy examination. Specifically, the lungs will be assessed for flotation in fluid, and the internal stumps of the umbilical arteries will be assessed for hemorrhage, both indicating that the calf was alive at the time of birth.

Skin Lesions

Although rare, in utero infection by fungal organisms may result in abortion of a calf with plaque-like skin lesions.

Collect (and Examine) the Placenta

As with the aborted fetus, gross examination of the placenta and collection of tissue samples are integral to the diagnostic investigation. Although the placenta is often the most important piece of diagnostic material it is frequently overlooked and rarely submitted. I would like to emphasize here that if the placenta is available it should always be collected and submitted. It may look irrelevant as it lies in the manure spreader but please collect and save it. Its diagnostic value is equal to, or greater than, that of the fetus. Placental examination and sampling are generally handled by the veterinarian on site or by the diagnostic lab pathologist after submission. However, the producer's early access to the placenta means that it can be collected and refrigerated as soon as it is found, reducing the chance that it will be lost or decompose further.

Placental Anatomy and Gross Examination

Gross examination of the placenta is often unrewarding, since very few diseases leave tell-tale pathognomonic lesions. Its diagnostic value comes from microbial testing and histopathologic examination. Nevertheless, gross placental examination can provide some basic information if the appearance of the placenta is abnormal. Appreciating this requires an understanding of normal placental anatomy (Figure 2). The placenta consists of two of the three so-called fetal membranes: the fused chorion and allantois, or chorioallantois. (The third fetal membrane, the amnion, is the translucent whitish membrane covering the calf at birth; it is not collected or examined). The several dozen, circular, raised tufts on the chorioallantois are the cotyledons. These are the site of exchange between the fetus and the cow's uterus. The thin, translucent areas between cotyledons are called the intercotyledonary zones. No exchange between the fetus and cow takes place here (Dyce et al., 2009).

Healthy intercotyledonary zones are translucent; as a rule of thumb, it should possible to read a page of printed text through the intercotyledonary zones when the placenta is draped over it. Bacterial, protozoal or fungal placentitis may result in exudate (pus and fibrin) covering the placenta, giving it a grey-yellow, thickened, leathery appearance and making the intercotyledonary zones opaque. Although this change is non-specific, it does suggest that the abortion was caused by a non-viral infectious microbe (bacterial, fungal or protozoal) rather than other non-infectious causes.
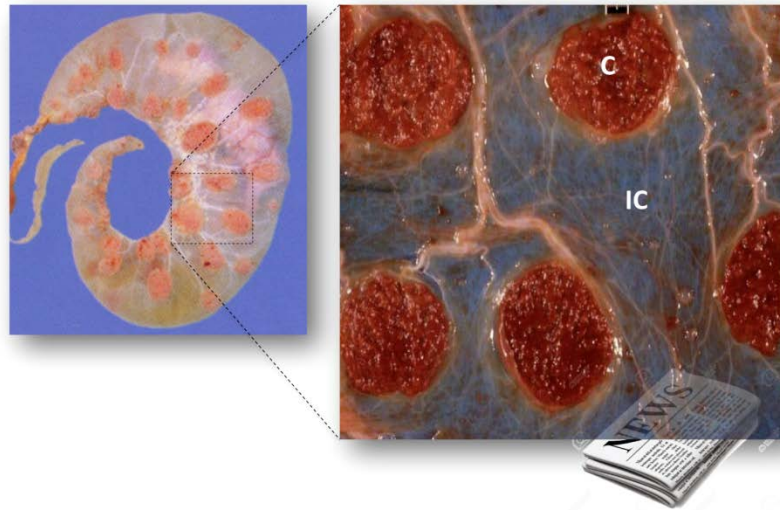
Figure 2: Normal bovine placenta. Exchange between the fetus and cow takes place at specialized tufts called cotyledons (C). No exchange takes place between cotyledons in the intercotyledonary (IC) zones, which should be nearly translucent; you should be able to read a newspaper through them. Both sites—cotyledonary and intercotyledonary—should be sampled and submitted (Modified from figure 5-67 in Dyce et al., 2009).

▪ How do the Veterinarian and Diagnostic Laboratory Work Up Abortion Cases?

It should be emphasized once again that any abortion workup should always begin with the collection, examination and storage of appropriate samples from the aborted fetus and placenta. These procedures can be conducted by the producer, as described in previous sections. Even if producer-collected samples are eventually discarded without being examined by a veterinarian or submitted to a diagnostic laboratory, they still provide important information that can and should be saved for later. At the very least, every aborted fetus should be weighed, measured and photographed.

It is frustrating for veterinarians and diagnostic laboratories to receive samples from a fourth abortion case accompanied by a history stating that nothing was recorded about the first three. Were the aborting cows clinically ill? Were the fetuses at a similar stage of gestation? Were the fetuses fresh or autolyzed? Were there lesions or malformations? Did the placentas look normal? Were any tissues from the first three cases stored and frozen? It is worth re-emphasizing that the likelihood of diagnosis of an abortion submission is fairly low but is increased by the submission of multiple abortions. If earlier tissues are still frozen and available, and the producer has collected information that suggests a common gestational age and cause, it is possible to pool tissues from multiple cases. This increases the chance of successful detection of microbes, toxins, mineral deficiencies, etc. by the diagnostic laboratory, generally at no extra cost.

Subsequent steps in an abortion investigation generally occur in the diagnostic laboratory. A pathologist conducts a necropsy examination of the calf during which internal organs and fluids are sampled for further testing. Sampling is very detailed and different samples have different destinations and testing utility. For example, a cube of fetal liver that has been placed in formalin to preserve it is ideal for microscopic examination but is worthless for bacterial and fungal culture (formalin kills bacteria and fungi). The converse is true; a cube of fresh liver that has been frozen is ideal for bacterial and fungal culture, but is not suitable for microscopic examination (freezing causes cells to expand and rupture, destroying microscopic architecture). In fact, four separate cubes of liver must be collected, with one placed in formalin (for histopathology), one kept chilled (for fungal and bacterial culture), and two frozen (one for

virology and molecular techniques and the other for nutritional/toxicological testing).

The necropsy procedure is fairly specialized, both in terms of the tools required and the experience needed to interpret tissue changes. Similarly, sampling is laborious and time-consuming and requires a large number of separate sterile containers, as well as access to formalin and freezing. For these reasons, few veterinarians conduct this part of the examination themselves although this forms part of their training. Generally, the veterinarian will submit the frozen or chilled intact fetus and placenta to the diagnostic laboratory. This is not always the case, however, and diagnostic labs sometimes receive a 'necropsy in a bottle,' where the veterinarian has performed the necropsy him or herself and collected tissue samples. As long as tissues are sampled and packaged appropriately this can save the producer money.

The chart below shows how veterinary students at the University of Calgary are taught to sample bovine abortion cases. This is too much information to memorize and we encourage students (and practicing veterinarians) to bring this chart with them as they sample. It is available for download from the University of Calgary's Diagnostic Services Unit (DSU). Most diagnostic labs produce similar guidelines. It is worthwhile for producers to understand what samples are collected and how complex an abortion investigation can be. As frustrating as it is never to receive samples from abortion cases, it is equally frustrating for diagnostic laboratories to receive inappropriate or inadequate samples, or samples that have been preserved incorrectly (e.g., formalin-fixed or frozen tissues only rather than both).

▪ Sampling Protocol for Bovine Abortions

Reproduced with permission from Dr. Jennifer Davies, Director of the University of Calgary Diagnostic Services Unit. (https://vet.ucalgary.ca/dsu)

1. **Examine the placenta for any abnormalities**

    a. Collect **multiple** sections of <u>cotyledonary</u> and <u>intercotyledonary</u> areas for histopathology (formalin fixed), bacteriology (fresh-frozen), and virology/PCR (fresh-frozen). Critical in the diagnosis of some mycotic and bacterial infections!

2. **External examination of the fetus for any outward congenital malformations, meconium staining, or skin lesions**

3. **Estimate/verify the gestational age (refer to aging chart)**

    a. Weigh the fetus

    b. Measure the crown to rump length

    c. Note fetal characteristics

4. **Determine the state of preservation**

    a. Fresh, autolyzed, mummified, macerated

5. **Classify the fetal death**

    a. Abortion, stillbirth, non-viable neonate (lungs partially inflated)

6. **Perform a routine necropsy and note any gross abnormalities**

    a. Section femur longitudinally (or send to lab) to look for growth disturbances

7. **Collect the following tissues for ancillary testing. Remember to maximize sampling initially. Samples can always be discarded later!**

    a. **Histopathology and immunohistochemistry** (10% neutral buffered formalin; 10:1 formalin to tissue ratio)

      i. Eyelid, parotid salivary gland, tongue, thyroid, thymus, lung, heart (t-section), diaphragm, liver, kidney, adrenal gland, spleen, ileum, colon, mesenteric lymph node, skeletal muscle, half of the brain, placenta, any lesions

      ii. IHC is available for many infectious agents

   b. **Bacteriology/mycology via culture or PCR (fresh-frozen)**

      i. 5 mL abomasal content, lung, liver, placenta

      ii. Collect stomach content in a syringe with a large gauge needle

      iii. <u>Package each specimen separately in sterile containers</u>

   c. **Virology and molecular techniques (PCR)** (fresh-frozen)

      i. Lung, liver, kidney, spleen, placenta, half of the brain, ear notch

      ii. <u>Package each sample separately in sterile containers</u>

   d. **Nutrition/Toxicology** (fresh-frozen)

      i. Liver (2- 5 grams of tissue required)

   e. **Serology** (refrigerated or frozen)

      i. Fetal fluids- heart blood, thoracic fluid, abdominal fluid

      ii. Collect in sterile red top tubes

▪ What Happens if I Receive a Diagnosis of 'No Diagnosis'?

Submitting veterinarians and producers should be aware that cases that yield negative findings do not equate with diagnostic failure. Rather, negative test results for the most common infectious causes of abortion indicate that the diagnostic focus should be directed toward other possible causes, such as genetic abnormalities, nutritional deficiencies or excesses, environmental factors and management practices.

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Use of Big Data to Monitor Herd Health

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▪ Take Home Messages

- Increased collection of data on farms from the cow, pen, and farm level may provide opportunities to improve management of herd and animal health, especially during the transition to lactation period.

- Data-based diagnostics may be a valuable and less invasive way to improve management and disease detection systems compared with blood-based diagnostics.

- Broad implementation of data-based diagnostics can allow for the generation of large-scale field data to identify patterns of risk or success.

- Improved large datasets for research may uncover novel genomic risk factors or metabolic pathways of interest.

- Integration of data streams available on farm (milk production and components, genomics, etc.) may improve the utility of management resources.

▪ Introduction

Recent and ongoing advances in dairy cattle management, nutrition, genetics, and reproduction have led to improvements in our understanding and knowledge of dairy cattle biology. Additionally, improvements in data availability and timeliness of data from farms on an individual cow, herd, and farm system level have increased. For example, the routine analysis of milk samples started as a way to monitor milk fat and protein but has progressed to other complex traits and monitoring of herd health indicators. Other data are now available to farms from numerous sources that range from feeding systems, rumination sensors, activity monitors, robotic milking systems, and weather stations to name a few (Wathes et al., 2008; Wolfert et al., 2017). Furthermore, management systems incorporating advanced technology can be accessed by off-farm management team members and send real-time alerts.

There is no doubt that there is an immense amount of information nested within data that is available from farms; however, there is also a need to sort, validate, and collate these data to format them in a way that is useful to the producer. For those that focus on animal health—producers, veterinarians, and researchers—a clear potential for this data is the potential use for detection of health events. Health diagnostics serve many different roles and range from confirming a clinical case of a suspected disorder to a screening for sub-clinical disorders that have non-visible symptoms. These tools can also be at a herd-level, which provides information about how well we are doing at preventing or managing a disorder, or at an individual cow-level, which can give information regarding the necessity of treatment. These tools can be integrated into management programs that allow them to complement each other.

▪ What Makes a Good Diagnostic Tool?

Before we dive into progress of diagnostic tools based on big data, we must first understand what makes a good diagnostic tool and what special or additional considerations must be made for 'big data' tools. Generally speaking, accuracy is used to describe how well the diagnostic or model fits the actual data. More specifically, we often need to know the ability of the diagnostic tool to appropriately identify a case, which we can calculate as sensitivity and specificity. Sensitivity is the ability of the diagnostic to identify

positive cases as positive. Specificity is the ability of the diagnostic to correctly identify a negative case as a negative. In general, our goal for these are 90%, but it is important to realize that when we optimize to improve one metric (i.e., sensitivity), the other metric (i.e., specificity) tends to decrease. In research, we try to maximize sensitivity and specificity simultaneously when developing a diagnostic tool. In reality, which metric we want to maximize also depends on the consequences of misdiagnosis. For example, low specificity results in more false positives (cows that do not have the disorder but are identified as positive). These cases could inappropriately receive pharmaceutical treatment, so in a scenario such as mastitis where a treatment entails antibiotic use and discarded milk, this can be a costly misdiagnosis. Conversely, low sensitivity results in more false negatives (cows that do have the disorder but are not identified) and could result in the disorder getting more severe before identification and treatment.

Additional considerations are needed when we are building diagnostics on 'big data'. Data generation needs sufficient controls and validation and should come from representative data sets. Diagnostic tools should be built on data sets that are sufficiently large and robust to remain accurate when applied to novel data. To this last point, prediction models or diagnostics must be validated on data sets that the tools weren't built on to ensure that the models can be applied to other data sets.

▪ Sub-Clinical Ketosis Detection as an Example of Data Integration

Of particular interest to us and others is monitoring animal health during the transition to lactation period. The transition to lactation period is a time of increased risk for metabolic disorders such as ketosis (hyperketonemia), milk fever (hypocalcemia), and fatty liver. Commonly categorized as sub-clinical ketosis (SCK) or clinical ketosis, hyperketonemia is defined as elevated blood ketone body concentrations. The reference test for SCK diagnosis is the quantification of blood β-hydroxybutyrate (BHB) concentration via enzymatic assay (Iwersen et al., 2009; Mcart et al., 2013). The establishment of current blood BHB thresholds based on the increased risk of unfavourable animal health and performance outcomes has been thoroughly reviewed (Mcart et al., 2013; Overton et al., 2017), with thresholds of BHB ≥ 1.2 mmol/L or BHB ≥ 1.4 mmol/L being the most frequently used.

Detection of SCK on farm has largely moved to using hand-held BHB meters that can be used cow side, such as the Precision Xtra and PortaCheck meters (Iersen et al., 2009; Sailer et al., 2018). Diagnostic protocols may involve screening cows between 4 and 18 days in milk (DIM) either twice within the first week of the postpartum period, or once a week, but in either scenario the goal is to test each cow twice. These detection protocols are based on our understanding of the risk period and causes for SCK and are customizable from farm to farm. Blood BHB testing is costly and labour-intensive, making it difficult to apply on farm (Denis-Robichaud et al., 2014; McArt et al., 2014). Furthermore, there are real on-farm challenges with dedicating skilled labour and resources to weekly blood analysis, and thus, many farms struggle to implement a blood-based detection protocol consistently.

If one challenge of appropriate SCK detection is the intensiveness of blood collection, the obvious question is: what readily available data or samples could allow for SCK detection? One potential answer involves closely examining milk samples using techniques that are non-invasive and can be incorporated into milking routines, and realizing that concentrations of milk ketone bodies are correlated to blood concentrations (Marstorp et al., 1983; Andersson, 1984; Denis-Robichaud et al., 2014). Additionally, many farms routinely collect milk samples for analysis as a part of dairy herd improvement. Early uses of milk data to monitor postpartum herd-health were through use of the milk fat:protein as an indicator of SCK. At a herd-level, the proportion of fresh cows above a ratio threshold was used as an indicator but this is not tightly correlated to blood BHB (Figure 1). Despite this tool not being as accurate as others available now, it represents our progress in using milk data to predict or detect postpartum health events.
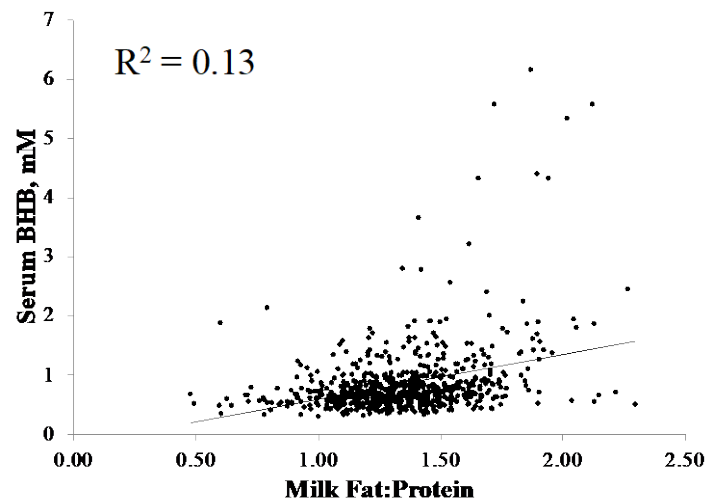
Figure 1. Relationship between milk fat:protein and serum BHB. Adapted from Chandler et al., 2018.

Although milk analysis historically only provided information on major milk components (fat, protein, lactose) or somatic cell count, advances in milk analysis have provided recent opportunities to learn more from these samples. Many milk analysis labs now incorporate Fourier transform infrared (FTIR) spectrometry (Rutten et al., 2009; 2011) that provides a practical method of predicting milk ketone body concentrations. This method is currently not as accurate as laboratory analysis, but it can be implemented on a larger scale.

Predicted concentrations of milk acetone or BHB from FTIR are correlated (r = 0.80) to chemically analyzed milk concentrations (de Roos et al., 2007); however, the prediction of milk acetone or BHB alone may not be sensitive or specific enough to detect individual cases of SCK. Across a range of cutoffs determined for milk ketone body concentrations for SCK diagnosis, milk BHB and acetone alone lack sufficient accuracy, sensitivity, and specificity for individual-cow diagnosis (Enjalbert et al. 2001; van Knegsel et al., 2010; van der Drift et al., 2012b). Predicting blood BHB from FTIR-predicted milk BHB (BHB ≥ 1.2 mmol/L as the diagnostic threshold) resulted in an overall sensitivity and specificity of 81% and 92%, respectively (Renaud et al., 2018). Similar sensitivity and specificity were achieved when FTIR-predicted BHB and acetone were analyzed in primiparous, but not multiparous, Holstein and Jersey cows (Chandler et al., 2018).

Based on understanding risk factors for SCK onset, we may find benefits for incorporating some of these factors into the prediction models, especially since these data are often collected during routine milk analysis and would be readily available. Incorporation of performance variables, including dry period length, gestation length, and lactation number, into milk analysis results (fat, protein, FTIR-predicted acetone and BHB) improved accuracy of models to predict SCK, but still lacked the sensitivity to provide an individual cow diagnostic tool (Chandler et al., 2018). Regardless of the lack of sensitivity of these models compared with enzymatic blood BHB quantification, there is a role for these models in herd-level diagnostics and monitoring (Denis-Robichaud et al., 2014; Chandler et al., 2018). Routine (i.e., monthly) implementation of these herd-level SCK predictions can aid farms in monitoring prevalence and identifying patterns and farm-specific risk factors. This is key since we know monthly prevalence of SCK changes across the year within a farm. For example, based on the economics of blood diagnostics and farm goals, a farm may only be able to justify postpartum blood sampling when prevalence is greater than 10%. If the farm's current prevalence is less than 10% and the farm stops sampling, the farm may miss an increase in prevalence to 30% because it doesn't have an indicator of the change. Figure 2 shows a real example of SCK prevalence over 12 months and demonstrates that while there are several months the farm may not want to do postpartum blood sampling, it could easily miss an increase in SCK events if it didn't have a monitoring protocol or tool in place. This is one role of a milk-based herd-level diagnostic

tool that can give a monthly indicator of prevalence to monitor these changes. There can also be additional benefits of observing herd health consequences from intentional or unintentional management and environment changes such as changes in forage, new or changing personnel, or implementation of a new protocol related to prepartum or postpartum cow management or nutrition.
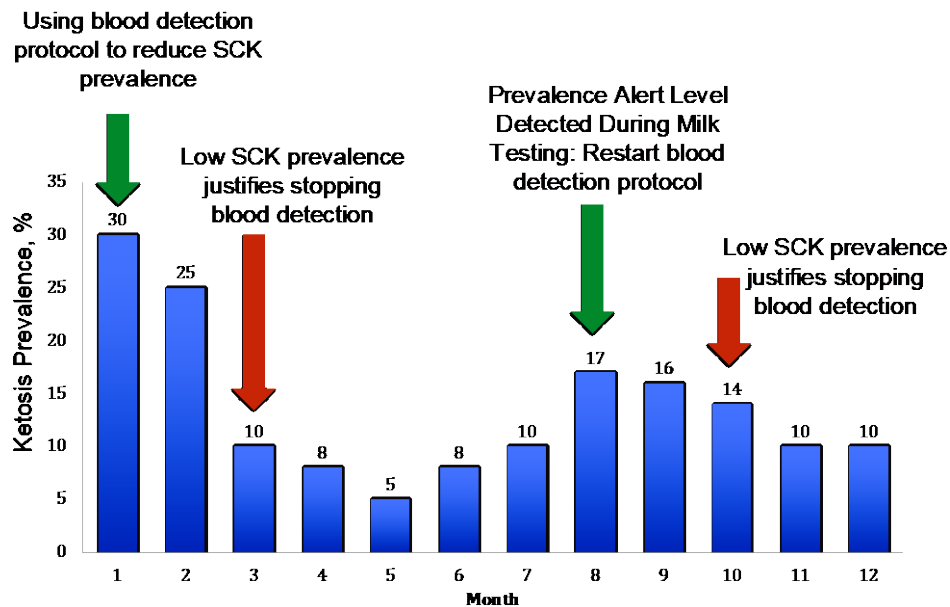
Figure 2. Example of real variance in sub-clinical ketosis (SCK) prevalence on a dairy farm over a 12-month period. Use of a herd-level milk-based diagnostic tool can give the farm an indicator of what the monthly prevalence is, whether the farm is doing blood-based diagnostics or not. Using this information, the farm could choose which months to do blood-based diagnostics based on economics, labour availability, and farm goals.

Ongoing attempts to increase reliability of model predictions have continued and have involved the use of more advanced statistical modelling techniques, larger sample sets, and use of raw FTIR data. Using a dataset of more than 3,600 samples, artificial neural network models based on milk analysis (i.e., milk fat, protein, FTIR-predicted acetone and BHB) and raw FTIR data resulted in improved SCK prediction models compared with models using either FTIR data or milk analysis data alone, or models using other statistical methods (Pralle et al., 2018). Despite failing to reach the diagnostic accuracy of the cowside enzymatic blood tests such as the Precision Xtra and PortaCheck meters (Iwersen et al., 2009; Sailer et al., 2018), these models are considered to have 'very good' diagnostic capacity based on general thresholds of diagnostic accuracy (Šimundić, 2009). When we recall that the goal of using FTIR milk analysis to diagnose SCK is to reduce the cost and labour necessary to employ blood-based diagnostics on farm, we may be willing to accept slightly lower diagnostic capacity in exchange for the reduced intensity and cost of FTIR-based diagnostics. A positive case of SCK is likely to be treated with oral propylene glycol, which represents a low-cost, no milk withdrawal treatment (McArt et al., 2012; 2014), resulting in minimal consequence to treating a false-positive case. Given this, future work could also optimize the prediction models in favour of sensitivity to increase the likelihood of identifying all positive cases, even if some negative cases are identified as false positives (decreased model specificity). These 'real-world' adjustments to research models could maintain cost-effectiveness and promote animal welfare and should be further considered. Additionally, as datasets from milk-based diagnostics increase in size, we can use this information to identify epidemiological type patterns regarding SCK. Growing data sets from milk analysis labs will be able to give information about SCK risk factors, patterns over time (within and across years), and patterns that are unique to region or management system. These data will never indicate causes, but if explored could provide insight that could guide future research into nutrition or management influences that might decrease SCK prevalence.

▪ Integration of Genetic and Genomics into Ketosis Management and Detection

Examining genetic selection for SCK susceptibility has predominately relied on voluntary records provided by dairy producers. Each farm has its own system of detection and recording for health events including SCK and therefore, this database includes innate variance. If a farm is actively implementing a blood detection method, it may to appear to have more SCK recorded compared with a farm that is only recording clinical cases that are obvious. This can result in misclassification of SCK cases as controls (or non-events) when they are actually just un-diagnosed positive cases; this presents an issue in using voluntary records, especially when considering subclinical cases and the variation in SCK diagnosis criteria (Oetzel, 2004; Pryce et al., 2016). Regardless, ranges of heritability can be learned from this data. Threshold model heritability estimates have ranged from 0.02 to 0.17 in Holstein cows (Kadarmideen et al., 2000; Zwald et al., 2004; Gaddis et al., 2014; Klein et al., 2019). Weigel et al. (2017) explored the heritability of SCK based on intensive sampling of blood BHB concentration (four timepoints between 5 and 18 DIM). The estimated heritability for the diagnosis of SCK (positive or negative diagnosis) was 0.07, which was within the range reported for voluntary records.

Incorporation of SCK susceptibility genetic and genomic evaluations into management through animal breeding programs is intuitive. A potential concern for directly breeding against SCK susceptibility is the unintentional elimination of compensatory metabolism (i.e., ketogenesis), at least with respect to the subclinical phenotype. An alternative, unexplored use of genomics is as a tool for precision health management. Genome-guided management of SCK would be like personalized medicine, where estimated breeding values for SCK alone or coupled with other on-farm data streams, such as test-day prediction models (Chandler et al., 2018; Pralle et al., 2018), could be used to stratify cows into risk groups for group-specific management (Weigel et al., 2017).

▪ Opportunities to Further Integrate Data-Based Diagnostics On-Farm

Although the collective body of research has generated diagnostic tools, on-farm integration and implementation remains a challenge. A few key challenges present hurdles and deserve our vested interest. One key challenge for consideration from the farm perspective is the nature of 'monthly' DHI milk testing on farm. Historically, DHI milk sampling has been done about every 4 weeks on privately-owned dairy farms (Weigel et al., 2017). Based on the at-risk period and duration for SCK, a farm would only test half of the fresh cows within the peak risk period (3 to 18 DIM; McArt et al. 2012b) and those cows would only be tested once. The two intuitive ways to address this challenge are to: 1) produce equations based on in-line measurement systems and 2) implement weekly milk testing of fresh cows. Current in-line systems include Herd Navigator (DeLaval International AB, Tumba, Sweden) and AfiLab (Afimilk, Kibbutz Afikim, Israel); however, only the former provides milk BHB predictions for monitoring SCK (Blom et al., 2015). For farms that do not have in-line measurement systems, the second option provides a viable option. In this system, all early postpartum cows would be milk sampled weekly, which results in fresh cows being sampled twice during the at-risk period. Use of prediction models on these samples would allow for either treatment of cows predicted positive or generation of a list of cows that warrant blood BHB testing. Beyond detection of SCK, these weekly milk samples could have additional benefits. Reporting back SCC, or differential SCC, could provide valuable information in this postpartum period to help identify cases of mastitis earlier (Fourdraine et al., 2019). As the technology continues to improve (e.g., differential SCC), it may also be a means to flag cows with increased inflammatory markers. Furthermore, if a group of cows are being milk tested weekly, it is not beyond reason that other sub-groups would be selectively milk sampled that day, which opens the door further for using milk FTIR to determine energy balance (McParland et al., 2015; Grelet et al., 2016), evaluate likelihood of conception (Ho et al., 2019), test for pregnancy in mid-lactation cows (Lainé et al., 2017; Toledo-Alvarado et al., 2018), or monitor cows within treatment pens. Milk analysis does not occur without cost, but analysis from postpartum cows may provide more valuable information than from cows in late lactation, allowing us to envision that DHI programs of the future may shift to more frequent testing of cows in periods of interest.

Acknowledgements

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Antibiotic Therapy: It's Not the Drugs, It's How We Use Them

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▪ Take Home Messages

- Prudent antibiotic use improves animal welfare, food quality, public health and herd productivity.
- Mastitis is the single biggest cause of antimicrobial use in dairy cows.
- Picking the wrong fight is the biggest reason for antimicrobial failure when treating clinical mastitis.
- If you change the rules of labeled drug use, you will likely reduce the efficacy of treatment and increase residue risks.
- Prevention and monitoring of disease is profitable, reacting to a sick animal with antimicrobial therapy is not.

▪ Mastitis Therapy—To Treat or Not to Treat

Mastitis is the most common reason for antimicrobial use for cows on U.S. dairy farms—about 80% of all antimicrobial use was for treatment or prevention of mastitis, which included dry cow therapy (Pol and Ruegg, 2007). Of the approximate 9 million cows in the U.S., 16.4% were treated for this disease (USDA-APHIS, 2008), or about 1.5 million mastitis cases treated annually. In a Canadian study, intramammary administration of antimicrobials (IMM) accounted for 35% of all antimicrobial use on dairy farms, which was lower than systemic administration (38%; Saini et al., 2012). However, the proportion of systemic administration that was for the treatment of mastitis was not recorded; systemic use also included that for calves. Although antimicrobial therapy improves animal health and well-being, economic losses from additional labour and discarded milk can be considerable. Culled dairy cows account for 67% of residue violations among all marketed livestock in the U.S., and 83% of the residues in culled dairy cows were antimicrobial drugs (USDA, 2018).

To date, the risk of emerging antimicrobial resistance among bovine mastitis pathogens has been low, particularly for drugs with high therapeutic value in human medicine (Lindeman et al., 2013). Nonetheless, prudent use of antimicrobials is good for the farm and good for the public. Thus, it is important to know the basics of mastitis therapy.

- Know the enemy—the bacteria causing the infection.
- Know which cows and quarters are at greater risk not to respond.
- Strictly follow a treatment protocol in consultation with your veterinarian.
- Follow the basic rules of pharmacology.

Milk Culture to Identify the Enemy

Microbial culture of milk is a practical tool to identify mastitis-causing bacteria. On-farm culture of milk before treatment of mild clinical mastitis cases (Hess et al., 2003) reduces the percent of treated cows by 70 to 80%. This occurs because treatment is only given for cases that are caused by bacteria that are most likely to respond to antimicrobial therapy, especially Gram-positive bacteria (Lago et al., 2011a). Typically, treatment is not given for 24 hours until results of milk culture are known. Cows that do not yield a bacterium on culture or have a pathogen that is resistant to treatment (e.g., coliforms, Gram-negatives, yeasts) are not treated. Not only does the 24-hour delay reduce antimicrobial use, but this 'wait and see' approach doesn't cause any difference in long term milk production, somatic cell count (SCC) or likelihood of a repeat case of clinical mastitis compared with that in cows who are treated without waiting for culture (Lago et al., 2011b).

At the very least, milk samples should be regularly sampled from a portion of clinical mastitis cases in a dairy herd to help guide therapy and target management for prevention. Example flow charts for using culture to direct mastitis therapy are given below (Figures 1 and 2). While culture of severe clinical mastitis cases should also be done when possible, therapy should be administered immediately along with supportive care such as fluids.
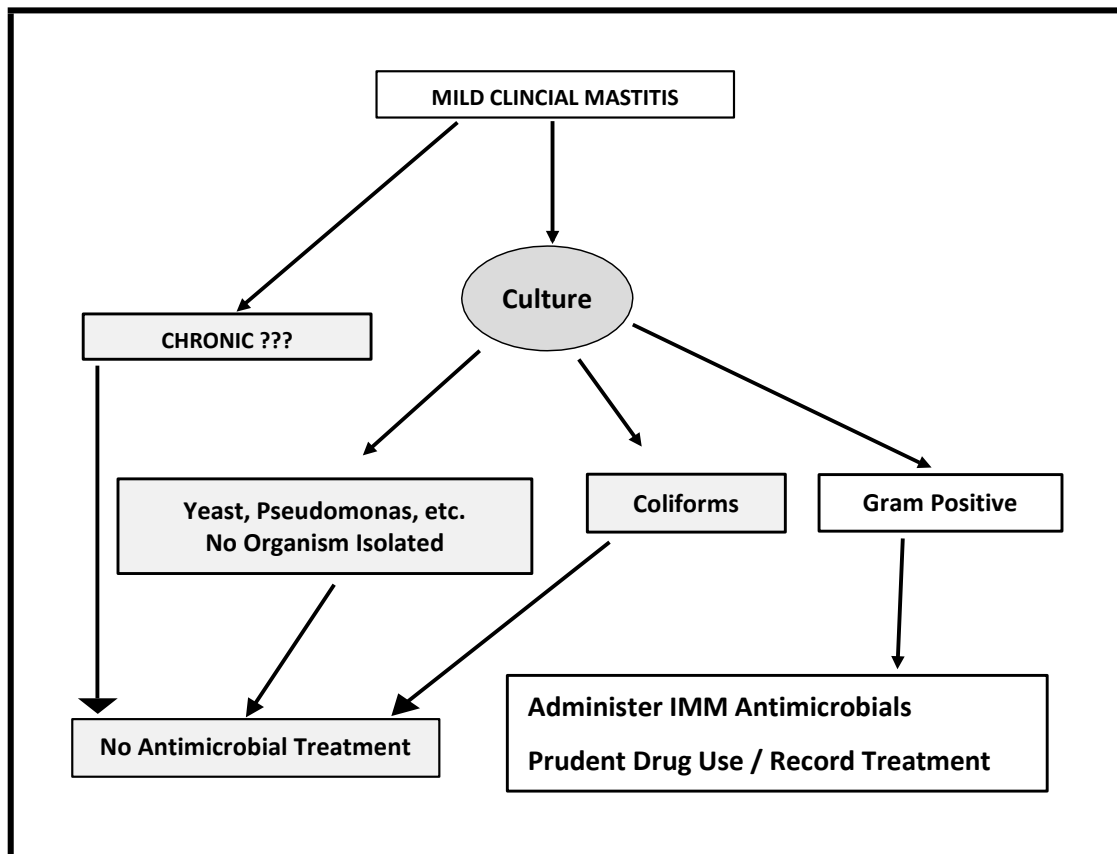
Figure 1. Flow chart for using culture to direct therapy for mild clinical mastitis

IMM = Intramammary (mastitis tubes)
Gram positive = Streptococci and Non-aureus staphylococci (coagulase-negative)
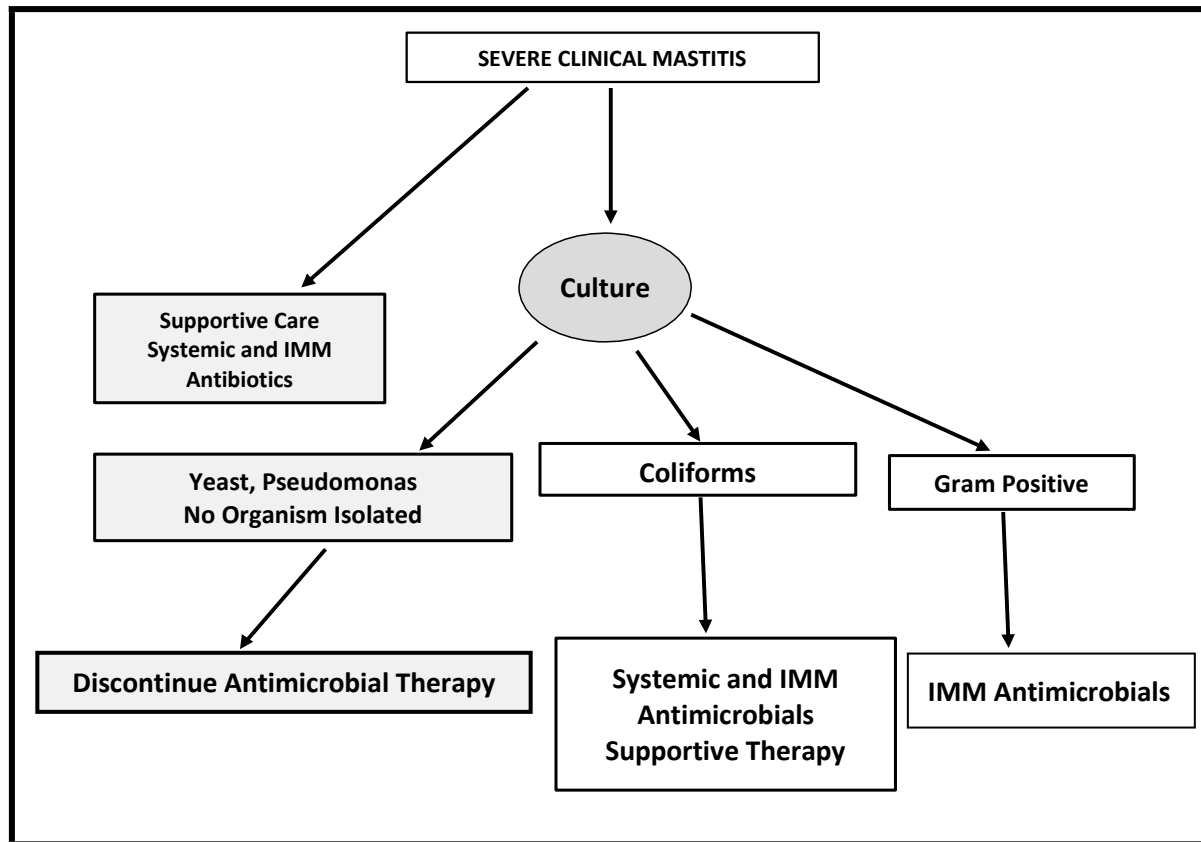
Figure 2. Flow chart for using culture to direct therapy for severe clinical mastitis

Don't Treat Repeat Offenders (Chronic Mastitis Cows)

A mastitis treatment protocol prevents any "cow-side" biases in treatment decisions. That is, the protocol should limit the treatment of repeat offenders, or identify those cows that are not likely to respond to therapy. Producers should stay on the game plan and not get creative with either repeat treatments or different drugs for that favourite cow. Most dairy producers report not having a written protocol, and extra-label drug use for the treatment of mastitis is common (Raymond et al., 2006; Oliveira and Ruegg, 2014).

The efficacy of mastitis treatment decreases for older cows, cows with high SCC, and cows with chronic infections (Pinzon-Sanchez and Ruegg, 2011). Particularly, chronic infections are likely to have poor therapeutic outcomes and treatment should be avoided. The best way to reduce the treatment of chronic mastitis is to maintain records of all cases that occur, including treated and untreated cases.

In the U.S., the majority of dairy herds only rarely culture milk samples from clinical mastitis cases (Schewe et al., 2015). A Wisconsin study found that over half of clinical mastitis cases treated with IMM antimicrobials were caused by *Escherichia coli* or cases where no organism was isolated (Oliveira and Ruegg, 2014). Also, the majority of U.S. dairy herds do not maintain treatment records, or maintain only partially complete records (Schewe et al., 2015). In Canada, treatment records were found to under report antimicrobial use when compared to inventories of discarded vials and tubes. Thus, as in the U.S., records were incomplete on many Canadian herds (Nobrega et al., 2017).

The majority of farms in the U.S. and Canada may not consistently follow the practices critical for prudent antimicrobial use for mastitis therapy. Farms were as likely to use oxytocin or organic or natural formulations (15–20%) for clinical mastitis as they were to use bacteriology in treatment decisions (Schewe et al., 2017).

The questions that should be answered before treating a cow with clinical mastitis are:

‣ How severe is the mastitis case? A cow with systemic signs (septic or toxic), such as off feed, fever, dehydrated, decreased milk production, will require immediate treatment that includes systemic antibiotic therapy, IMM therapy, supportive fluids and close monitoring compared with a cow with mild clinical signs that are limited to the udder and milk.

‣ Is this a new case of mastitis or a relapse? Repeated treatment of a recurrent case of mastitis is frequently unrewarding. If the recurring case of mastitis is to be treated, the therapeutic regimen might need to be more extended than previous treatment. However, no repeat case should be treated without the results of a milk culture from the affected quarter.

‣ How many quarters are affected? The expense and the likelihood of treatment failure increase as the number of affected quarters increases. A cow with three or four clinical quarters may have immune system deficiencies, teat canal problems, or an infection with an especially virulent or persistent bacterial strain—this is common for *Mycoplasma*, for example.

‣ Has the cow had a history of high SCC? Cows with a history of high SCC for three months or more are more at risk to not respond to therapy, even if the clinical case is 'new'.

‣ Does the cow have other health problems? Other health problems such as metabolic stress (transition cow diseases), heat stress and milk fever (hypocalcemia) impair immune responses. The likelihood of successful mastitis therapy may be reduced in cows with concurrent illnesses.

▪ Barnyard Pharmacology—Top 7 Rules for More Effective Antimicrobial Therapy

1) Clean teat ends COMPLETELY before infusing mastitis tubes, otherwise you increase the risk for new infections.

2) Limit the volume of (intramuscular injections to 10 mL; larger volumes alter drug distribution in the body and increase the risks for residues.

3) Don't change the labeled route of administration—same reason as 2) above.

4) More is not better. For most antimicrobials used in dairy cows, increasing the labeled dose will NOT increase efficacy.

5) If the label says shake well before use, do it.

6) Drugs do NOT distribute to all parts of the body equally. This is especially true for systemic drug use for mild mastitis. Most antimicrobials drugs labeled for dairy cows do not reach effective concentrations in the udder.

7) Follow a written protocol in consultation with your veterinarian.

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POSTER

ABSTRACTS

The Effects of Neomycin in Milk Replacer on the Health and Performance of Dairy Calves

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The prophylactic use of oral antimicrobials is common practice in the veal and replacement heifer industry to prevent disease. However, the impacts on gut health are largely unknown. The objective of this study was to investigate the effects of oral antibiotic (neomycin) on calf health and growth. One hundred and fifty calves between 1 and 4 days of age were blocked by bodyweight (**BW**) and assigned to one of three dietary treatments (n = 50 per treatment): control (**CON**: non-medicated milk replacer**; MR**), short-term antibiotic (**ST**: neomycin mixed in MR from day 1-14), or long-term antibiotic (**LT**: neomycin in MR from day 1-28). BW was measured weekly for 49 days, weekly fecal samples for dry matter analysis were collected weekly, and daily fecal scores (**FS**) were collected for the first 28 days. A treatment effect (P = 0.005) was observed for FS, where CON calves experienced higher FS (0.640 ± 0.031) compared to ST (0.530 ± 0.034) and LT calves (0.409 ± 0.034). Significant differences were observed in scour days, where CON calves displayed longer bouts (P<0.002) of diarrhea (5.44 ± 0.35 days) compared to ST (3.78 ± 0.39 days) and LT calves (3.82 ± 0.38 days). At day 7, significant differences were observed in fecal dry matter (P = 0.003), where CON calves experienced lower fecal dry matter (0.130 ± 0.012%) than ST (0.200 ± 0.013%) or LT (0.170 ± 0.013%) calves. No significant differences in average daily gain (P=0.475; SEM=0.0546) were observed, suggesting that neomycin decreases the incidence and severity of diarrhea, but has no effect on growth performance.

Effect of dietary Selenium source on animal performance during immune challenge in lactating Holstein cows

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Selenium (Se) is an important dietary antioxidant required to mitigate free radical production, which can lead to oxidative stress and decreased animal health and performance. The objective of this experiment was to determine how source of dietary Se affects antioxidant status and animal performance during an intramammary endotoxin challenge. Twenty mid-lactation multiparous Holstein cows (591 ± 46 kg body weight, parity 2-4) were blocked by days in milk (157 ± 17 DIM) and randomly assigned to one of two treatments: 1) 0.30 ppm (100% NRC requirements) of dietary organic (selenized yeast) Se (dry matter basis) premix, or; 2) 0.30 ppm of dietary inorganic (sodium selenite) Se premix, top dressed and mixed into a basal ration daily. Dry matter intake (DMI) and milk production were recorded daily. Following a 75 day dietary adaptation period, cows were infused with 50 µg of lipopolysaccharide (LPS; *Escherichia coli* strain O111:B4) diluted in 10 ml of sterile saline in one front quarter. Rectal temperatures were recorded at -1.5, 0, 2, 4, 6, 8, 10, 12, 24 hr. During the adaptation period, average weekly DMI and milk production were not different (P > 0.05) between treatments. Results revealed no treatment differences in body temperature, daily milk production or DMI during the LPS challenge, although there was a time effect for all parameters (P < 0.001). There was a tendency (P = 0.07) for inorganic cows to have greater DMI in the 12 hours following LPS infusions. These results indicate that animal performance during immune challenge is minimally affected by source of dietary Se. Further analysis is required to determine antioxidant capacity and oxidative stress levels in cows supplemented with differing sources of Se.

Associations between herd management, barn design, lameness, and production in farms with robotic milking systems

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The objective of this study was to evaluate management, barn design, lameness, and productivity in farms using robotic milking systems. During visits to 76 dairy farms in Ontario, we surveyed producers regarding management practices and collected robotic milking system records. Farms averaged 99±73 lactating cows, 2.3±1.4 robot units, a milk yield of 36.7±4.7 kg/cow/d and 1607±343 kg/robot/d, a milking frequency of 3.0±0.4x/d, and a SCC of 215±88 (x1,000) cells/mL. Thirty cows/farm were gait scored (or 30% for herds >100 cows) using a numerical rating system (NRS; 1=sound to 5=lame) and were defined as clinically lame with NRS≥3 (28.6±11.7%) and severely lame with NRS≥4 (3.0±3.2%). Severe lameness prevalence increased by 0.15 percentage points (p.p.) when doubling the proportion of under-conditioned cows from the mean of 5.6 to 11.2% (P=0.04) and tended to be 0.35 p.p. greater for farms using a bedding product (P=0.06). Clinical lameness was 8.8 p.p less prevalent on farms using sand bedding (P=0.003), tended to be lesser with a greater daily frequency of raking lying stalls (P=0.07) and 5.7 p.p. lesser for farms that built new barns vs. retrofitting existing barns (P=0.07). Herd average milk yield/cow decreased with greater proportions of clinically (-0.1kg/d for 1 p.p. increase; P=0.01) and severely lame (-0.8kg/d with doubled prevalence from 3 to 6%; P=0.003) and over-conditioned cows (P<0.001). Milk yield per robot decreased with a greater prevalence of clinical lameness (-7.1kg/d for 1 p.p. increase; P=0.01). Milking frequency decreased with a greater proportion of over-conditioned cows (P=0.04). Milk SCC increased with a greater proportion of clinically lame (P<0.001) and under-conditioned cows (P=0.05). **Take Home Messages:** This study demonstrates that maintaining good hoof health, mobility, and body condition are key factors to optimize productivity and milk quality in robotic milking herds.

Mental health of dairy farmers using robotic milking systems

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Our objective was to survey dairy farmers using robotic milking systems to better understand their mental health. Of 76 farms in Ontario visited to survey management, cow health, and milk production, 34 farmers completed an online survey that included validated psychometric scales used to assess stress, anxiety, and depression. Associations between many variables were explored; results below indicate how those variables were associated, but they do not imply cause or effect. Because average milk yield per cow was negatively correlated with SCC and the proportion of lame and under-conditioned cows, only milk yield was kept for the multivariable models. Farmer stress was greater for females vs. males, and greater for those feeding manually vs. using an automated feeder (i.e. conveyer or automated delivery system); lesser stress was associated with greater herd average milk yield per cow and milk protein %. Depression was greatest for those working alone on the farm, those feeding manually, and for those with lesser average milk protein %. Anxiety was greater for females vs. males, those feeding manually, those working alone on the farm, and those with lesser average milk yield per cow and milk protein %. Greater resilience was surprisingly associated with lesser average milk yield per robot, greater herd average SCC, and for those using automated feeders vs. feeding manually. Comparing our results to a recent survey of all commodity groups across Canada, using similar methods, we demonstrated that dairy farmers using robotic milking systems may be experiencing less stress, anxiety, and depression than other farmers. Take Home Messages: The results highlight the potential benefits of automated milking and feeding systems for farmer mental health. We also identified a greater risk of poor mental health for farmers working alone, and we encourage everyone to reach out to their support networks and ask for help. Farmer mental health was also identified to be associated with milk yield, quality, and composition.

Herd-level management and housing of Canadian robotic milking herds

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The objective of this study was to describe housing and management practices of robotic milking herds across Canada. We visited 197 robot farms (Western Canada: n=50, Ontario: n=77, Quebec: n=59, Atlantic Canada: n=11) from April to September 2019 and collected data on barn design and farm management practices through an on-farm survey. Milk recording data for the 6 mo prior to farm visits were collected from Lactanet. Farms ranged from 38 to 1000 lactating cows (mean = 111±101), 1 to 17 robot units/farm (mean = 2.4±1.9), averaging 47.3±9.1 cows/robot, 36.6±4.9 kg/d of milk, 4.1±0.3% fat, 3.4±0.2% protein, and a herd level SCC of 200,882±94,276 cells/mL. Herd parity averaged 2.4±0.3 lactations, DIM averaged 174±20, and they were composed predominately Holstein (n=178; other breeds=19). Barns were predominately new builds (n=140), with the remainder being retrofits (n=57). The predominant barn design for lactating cows was free stall (n=183; pack: n=11; mixed n=3), with a free flow cow traffic system as the main design (n=173; guided=24). Tunnel ventilation was the main form of ventilation (n=61), followed by ceiling and natural (n=58), panel and natural (n=42), panel, ceiling and natural (n=19), and natural (n=16). Feed was delivered 2.8±3.6x/d (range = 1 to 14 x/d) and pushed up 12.8±8.4x/d (range = 1 to 41x/d). The primary method of feed push up was through the use of automated feed pushers (n=142; manual: n=37; other: n=18). Stalls were bedded 6.6±3.6 times per week, with the main bedding material being wood products (n=64; sand=52; straw=41; other=28). Stalls were raked 2.8±1.4x/d, alleys were cleaned 12.2±8.2x/d, and an alley scraper was the predominant cleaning method (n=173; autonomous=18; other=6). Overall, the results of this study demonstrate the current trends and benchmarks in management and housing practices across robotic milking farms in Canada.

Associations of milk production and quality with management and housing of Canadian robotic milking farms

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The objective of this study was to identify factors associated with milk production and quality on Canadian robotic milking farms. We visited 197 robot farms (Western Canada: n=50, Ontario: n=77, Quebec: n=59, Atlantic Canada: n=11) from April to September 2019 and collected details of barn design and herd management practices through an on-farm survey. Milk recording data for the 6 mo prior to farm visits were collected from Lactanet. Farms averaged 111±101 lactating cows, 2.4±1.9 robot units/farm, 47.3±9.1 cows/robot, 36.6±4.9 kg/d of milk, and a herd-level SCC of 200,882±94,276 cells/mL. Cow traffic system was associated with milk production; on farms with guided systems (n=24) cows tended to produce 1.5 kg/d less (P=0.08) milk than in free cow traffic farms (n=173). A greater number of cows per robot was associated with lesser milk yield; every 10 additional cows was associated with 0.77 kg/d lesser production per cow (P=0.01). The predominant bedding type used was wood products (n=64; sand=52; straw=41; Other=28); farms with sand bedding tended (P=0.07) to have a 1.3 kg/cow/d greater milk production compared farms with wood products. SCC was positively associated (P=0.05) with cows per robot; for every 10 additional cows per robot, SCC increased by 9,932 cells/mL. Additionally, every 10-d increase in herd average DIM was associated with a 10,060 cells/mL increase in herd average SCC (P<0.001). The results of this study demonstrate that greater milk production and milk quality are being achieved on Canadian robotic milking farms by using free flow cow traffic systems, keeping the stocking density of cows per robot lower, and using sand as the predominant bedding type for their free stalls.

Heifers with short ano-genital distance conceive sooner and require fewer inseminations

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Raising replacement heifers is the second largest expense for a dairy farm, where every extra day to first calving increases the cost of rearing a heifer. Selecting heifers for superior fertility, therefore, could yield significant economic benefits. Ano-genital distance (AGD, the distance from the anus to the clitoris in cattle) is a phenotype believed to reflect androgen exposure during a critical time in a heifer's fetal development. A previous study in lactating Holstein cows found that cows with long-AGD (≥127 mm) are less fertile than cows with short-AGD (<127 mm), demonstrating its potential as a new fertility trait. This study was to determine if the same negative relationship exists between AGD and fertility measures in maiden Holstein heifers. AGD was measured by one individual in 671 heifers (11 Alberta & BC dairies); it followed a normal distribution pattern with a mean (± SD) of 114 ± 11.5 mm (range, 81 to 148 mm). Using the mean AGD as the cut-point, heifers were categorized into short (<114 mm) and long (≥114 mm) AGD groups, and associations with fertility were determined. Heifers with a short-AGD were younger at conception (444 ± 8.5 vs. 457 ± 8.4 days; $P < 0.01$) and required fewer inseminations (1.6 ± 0.13 vs. 1.8 ± 0.13 times bred; $P = 0.02$) than those with long-AGD. Moreover, pregnancy to first AI in short-AGD heifers was greater than that in long-AGD heifers (62.2 ± 5.7 vs. 52.5 ± 5.8 %; $P = 0.01$). In summary, a negative association between AGD and fertility measures, i.e., age at conception, times bred and pregnancy to first AI, was evident. Therefore, AGD may be a useful reproductive phenotype for genetic selection, although studies on a larger scale are necessary to confirm these findings. **Take Home Messages**: (1) Maiden heifers with short AGD are more fertile than those with long AGD (2) These are preliminary findings which remain to be confirmed.

How does Low Blood Calcium close to Calving Relate to Health, Production and Reproduction in Dairy Cows?

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Blood samples were taken between 2 and 7 days in milk (DIM) in 398 Holstein cows from 11 dairy herds in Alberta. Blood serum was analyzed for macro minerals, including calcium (Ca), metabolites, liver enzymes, hormones and haptoglobin. Cows were categorized into either Low- (≤ 2.10 mmol/L; n = 136) or High- (> 2.10 mmol/L; n = 262) Ca groups. Cow health was monitored daily and diseases were diagnosed by farm staff or blood information. Production measurements included milk yield at 25 and 90 DIM, as well as 305-day mature equivalent milk yield. Reproduction outcomes were cyclicity by 35 DIM, pregnancy to first AI and pregnancy by 150 DIM. Low-Ca cows had lower concentrations of Mg, Na, K, albumin, globulin, total protein and cholesterol, and greater concentrations of urea, aspartate aminotransferase, β-hydroxybutyrate, non-esterified fatty acids and haptoglobin than High-Ca cows. Low-Ca cows had greater incidence of retained fetal membranes (18 vs. 4%), metritis (33 vs. 11%) and ketosis (27 vs. 9%) compared with High-Ca cows. In addition, culling (11 vs. 2%) and death rate (6 vs. 3%) were greater for Low-Ca cows. Low-Ca cows had lower milk yield only by 25 DIM compared with High-Ca cows (714 vs. 794 kg).The proportion of cows cyclic at 35 DIM (56 vs. 67%) and pregnant to first AI (31 vs. 43%) tended to be lesser for Low-Ca cows compared with High-Ca cows, with no difference in the proportion of cows pregnant by 150 DIM (66 vs. 68%). **Take Home Message:** Cows categorized as Low-Ca had lower serum concentrations of macro minerals, greater concentrations of markers for fat mobilization and inflammation, greater incidence of postpartum diseases and poorer reproductive outcomes compared with those categorized as High-Ca. Ensuring adequate blood Ca concentrations and dry matter intake after calving is essential for a smooth transition period.

How does the eSense Ear Tag Activity Monitor Perform in Dairy Heifers?

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In total, 281 dairy heifers from a commercial farm near Edmonton, AB were fitted with an ear tag activity monitor (SCR eSense, Allflex) once they were eligible for breeding (>13.5 months of age). Heifers were synchronized using prostaglandin, given 2 weeks apart, and were also given an estrus detection patch (Estrotect). Heifers were bred based on activity alert from the system or if the estrus patch had 50% colour change. All heifers received sex-sorted semen for the first AI and conventional semen for subsequent breedings. Pregnancy diagnosis was performed at 30 days post AI and heifers had 4 opportunities to become pregnant to AI. All heat events were confirmed using estrus patches and/or transrectal ultrasonography (lack of a CL > 20 mm; presence of uterine fluid and tone). The system recorded a heat index, maximum activity change, minimum rumination change and duration of heat. The pregnancy to first AI was 66.5% and total proportion of heifers pregnant after 4 breedings was 97.9%. Out of 469 heat events, the sensitivity (ability to correctly identify heat; true positive) was 91%, with 8% false positives (the system incorrectly identified a heat) and 8% false negatives (the system missed a heat). When comparing true to false positive heats, there was a significant increase in heat index (84 vs. 35), heat length (12 vs. 3 h), activity change (68 vs. 33) and rumination change (-52 vs. -12). There was also an increase in activity (68 vs. 17) and rumination change (-52 vs. -35) when comparing true to false negative heats. Interestingly, 85% of false positive heats had a heat index <45 and a rumination change < -20, while 82% of false negative heats had a rumination change ≥ -20 combined with a positive activity change. **Take Home Message:** The eSense SCR system showed a high sensitivity to identify heifers in estrus, which resulted in a high pregnancy rate. Our data would indicate that producers may be able to further identity true heats using a combination of heat index, activity and rumination information. Thank you to the Agriculture Funding Consortium for financial support, and to Breevliet Ltd and SCR by Allflex.

Optimizing the Performance of the SCR eSense Activity Monitor in Heifers with Timing of AI

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In total, 281 dairy heifers from a commercial farm near Edmonton, AB were fitted with an ear tag activity monitor (SCR eSense, Allflex) once they were eligible for breeding (>13.5 months of age). Heifers were synchronized using prostaglandin, given 2 weeks apart, and were also given estrus detection patches (Estrotect). Heifers were bred at random times based on activity alert from the system or if the estrus patch had 50% colour change. All heifers received sex-sorted semen for the first AI and conventional semen for subsequent breedings. Pregnancy diagnosis was performed at 30 days post AI and heifers had 4 opportunities to become pregnant to AI. Data from six non-pregnant heifers were not used for statistical analyses. In a subset of heifers (n = 150), ovaries were scanned every 12 h starting at the time of AI until ovulation. The system recorded a heat index, maximum activity change, maximum rumination change and duration of heat. The average (range) interval between onset of activity alert and ovulation, and peak activity and ovulation was 28 h (16 to 46 h) and 22 h (10 to 40 h), respectively. Overall, pregnancy risk using conventional (72.5%) and sexed semen (72.0%) were not associated with any heat characteristics measured by the system. For conventional semen, each hour increase in interval from onset of activity or peak activity to AI reduced the predicated probability of pregnancy (PPP) by 3.8 and 4.2%, respectively. For sex-sorted semen, the greatest PPP was observed 12 to 16 h and 0 to 2 h after the onset of activity and peak of activity, respectively. **Take Home Message:** When using conventional semen, the sooner you breed heifers after estrus alert, the better. When using sex-sorted semen, delaying breeding to as close to peak activity as possible will result in the best pregnancy outcome. Thank you to the Agriculture Funding Consortium for financial support, and to Breevliet Ltd and SCR by Allflex.

Business Analysis of Visual Observation, IRT and Ovsynch as Reproduction Strategies in Alberta Dairies

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Estrus detection (ER) is an important component in decision-making in Alberta dairies. The objective of this project was to compare the business analysis on traditional breeding strategies (Visual Observation; VO, and Ovsynch; OVS), and infrared thermography (IRT) at different ER and pregnancy rates (PR). The current study used the average costs of dairy production activities, inventories, capital purchases, milk sales and feed purchases from the *Economics of Milk Production in Alberta 2017 - 2009*. The costs associated with Breeding, Feeding per AI service, Operation costs, Return to equity and Culling risk (%) per ER (30 – 100%) and PR (PR per parity group; 1-2, 3-4, and >4) determined the financial benefit of the breeding strategies. Breeding cost results (CAD/cow) showed a higher cost in OVS ($138.99) compared to VO ($115.78), and IRT ($118.28). Pregnancy rate costs were affected by Breeding cost, however, the ER had significant effect on PR expense for each breeding strategy, VO (ER 30%; 205.93, ER 60%; 167.36, & ER 100%; 129.39 CAD/cow), OVS (ER 30%; 247.21, ER 60%; 200.90, & ER 100%; 155.33 CAD/cow), and IRT (ER 30%; 210.38, ER 60%; 170.97, & ER 100%; 132.19 CAD/cow). The Total production cost and Return to equity varied depending on Conception per AI service (1st - 4th), VO (1st; 2439.13, 2nd; 2672.17, 3rd; 3129, & 4th; 3129.13 CAD/cow), OVS (1st; 2439.13, 2nd; 2621.91, 3rd; 2804.69, & 4th; 2987.47 CAD/cow), and IRT (1st; 2439.13, 2nd; 2672.17, 3rd; 3129, & 4th; 3129.13 CAD/cow). Culling risk cutoff per ER varied depending Parity, ER 50% for Parity 1-2 (3.51%), 60 % for Parity 3 – 4 (0.67%) and 80% for Parity > 4 (4.22%) despite breeding strategy. **Take Home Messages:** Understanding the financial impact of estrus detection rates using different breeding strategies provides dairy farmers with decision-making information.

Ano-genital distance is not affected by the estrous cycle stages in dairy cows

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Genetic selection for novel reproductive phenotypes could be a strategy in the sustainable improvement of fertility in dairy cows. Anogenital distance (AGD), one such phenotype, is the distance from the center of the anus to the base of the clitoris in dairy cows. Recent studies found that cows with short AGD (≤127 mm) are more fertile than cows with long AGD (>127 mm) and that AGD has moderate heritability (0.37), making it a worthy candidate for further evaluation because most reproductive phenotypes have low heritability. In addition to heritability, a phenotype should be highly repeatable (the closeness of measurement taken at different time points by the same person). The estrous cycle is regulated by hormones such as estrogen and progesterone. We hypothesized that an increase in estrogen during proestrus may cause swelling of the vulva, altering the AGD. The objective was to know if AGD is affected by the estrous cycle stages in dairy cows. Accordingly, twenty lactating Holsteins were subjected to estrous synchronization. Ovaries were scanned (transrectal-ultrasonography), blood samples collected (to quantify progesterone), and AGD measured in triplicate (mean value used), every other day through an entire estrous cycle, i.e., from one ovulation to the next. The stages of the estrous cycle were classified as metestrus, diestrus, proestrus, and estrus based on ovarian structures and progesterone concentrations. The AGD measurements taken at different stages of the estrous cycle within the same animal did not differ (P = 0.99) and were highly correlated (r = 0.98; P <0.001). In summary, AGD measurements at different stages of the estrous cycle were highly repeatable, indicating that AGD does not change through the estrous cycle. **Take home messages:** (1) AGD was not affected by different stages of the estrous cycle (2) Because of its high repeatability, AGD can be measured at any stage of the estrous cycle (3) This finding will be valuable if AGD becomes established as a fertility trait.

Higher embryo quality and viability in heifers with short anogenital distance

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Recently, the genetic selection of dairy cows for fertility with superior reproductive phenotypes is of interest. The potential use of anogenital distance (AGD; the distance from the center of the anus to the base of the clitoris in dairy cows) as a reproductive phenotype is strengthened by previous findings that cows with short AGD (<127 mm) had a greater fertility than those with long AGD, and AGD is a moderately heritable trait. Moreover, a recent preliminary study suggested that primiparous cows with short AGD have greater embryo yield and viability than those with long AGD. The objective of this study was, therefore, to determine if embryo yield and quality varied between heifers of different AGD categories. AGD was measured using digital calipers in heifers (n = 13) that were previously superovulated and flushed (average 2.4 flushes/animal) for embryos. The embryo data set (n = 30) from these heifers were collected. Heifers were categorized as either short (<114 mm) or long (≥114 mm) AGD based on the mean (114 mm) obtained from a population of 671 heifers in a different study. The proportion of viable (58% *vs.* 42%; $P = 0.02$) and grade 1 (58% *vs.* 42%; $P = 0.05$) embryos were significantly higher and the proportion of fertilized ova (55% *vs* 45%; $P = 0.07$) tended to be greater in heifers with short AGD compared to those with long AGD. The proportion of viable embryos out of total structures recovered (67% *vs.* 56%; $P = 0.08$) within short and long AGD categories tended to be greater for heifers with short AGD than those with long AGD. In summary, although the yield of embryos did not differ, the embryo quality was greater for heifers with short AGD than heifers with long AGD. **Take Home Messages:** (1) Fertilization rate and embryo viability are important for a successful pregnancy. (2) Our findings that heifers with short AGD have greater viable embryos than those with long AGD, therefore strengthening the potential for AGD as a novel reproductive phenotype.

The Diversity of Bovine Digital Dermatitis Bacteria: How to Work Around the Complexity

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Digital dermatitis (DD) is common cause of lameness as it is a painful and chronic skin lesion of the foot. DD is polybacterial in nature, meaning that multiple types of bacteria are involved in the disease. Because of this, a thorough understanding of the most important bacteria remains a major gap in our ability to develop more effective DD prevention strategies. Our study aims to provide a better understanding of a 'core group' of bacteria consistently identified across DD lesions. We track bacterial populations and how dynamic they are throughout different stages of diseases as an effective strategy to investigate their potential roles in disease development. By using a deep sequencing methodology, it allows us to identify all bacteria present in DD lesions and quantify their abundance at all stages in the disease. We use this data to compare across cattle breeds and production systems, enabling us to observe a consistent group of potentially pathogenic bacteria in DD-positive cows. Historically, *Treponema* bacteria have been most commonly associated with a DD-causing role. In addition to *Treponema*, we identify an additional novel group of bacteria that appear consistently in DD lesions while absent in healthy skin. Through deep sequencing and culturing techniques, we observe the presence of multiple bacterial species that are potentially involved DD lesion development: *Porphyromonas levii*, *Fusobacterium* spp., and *Bacteroides pyogenes*. Implications: Establishing the 'core group' of DD-relevant bacteria is a significant step in progressing towards a confirmation of what causes DD and how. Current treatments may be ineffective at completely eliminating these pathogens, therefore future intervention strategies specifically for these bacteria may be beneficial in order to 1) completely heal lesions by clearing pathogens and returning to normal skin; and 2) reduce the reoccurrence of the disease.

Mastitis-related antimicrobial use: Current practices on Canadian dairy farms

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Prevention and treatment of mastitis accounts for the majority of antimicrobial use (AMU) on dairy farms. Stewardship programs aimed at reducing AMU in the dairy sector have emerged over the last decade. In Canada, the 'Canadian Dairy Network of Antimicrobial Stewardship and Resistance' (CaDNetASR) encompasses 150 dairy farms in five regions and monitors antimicrobial resistance (AMR) and AMU patterns. To identify areas for reduction of mastitis treatments, structured questionnaires captured current on-farm AMU practices on a subset of 110 farms in British Columbia, Alberta, Ontario and Nova Scotia. Of these farms, 35% practiced selective dry cow therapy (DCT). Multifactorial methods were used to select cows for antimicrobial treatment: SCC (cut-off: 150,000 cells/mL), timepoint of previous clinical mastitis (CM) case and the number of CM events in the current lactation. For CM treatments, 54% of farmers used inclusion criteria to select cows for treatment: severity of symptoms, confirmed or suspected presence of bacteria and SCC (cut-off: 300,000 cells/mL). These outcomes will inform intervention studies in British Columbia and Alberta that measure the effect of selective DCT and selective CM treatment on AMU and AMR through the CaDNetASR program. Take home messages: There is an opportunity for reducing AMU related to DCT and treatment of CM on dairy farms in Canada.

Communication between veterinarians and dairy farmers: Effect of communication training on communication skills and mental wellbeing in veterinarians, farmer satisfaction and herd health outcomes

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Diseases reduce dairy cattle health and welfare, causing huge losses in the Canadian dairy sector. Veterinarians are a key information source for dairy farmers regarding disease prevention and treatment. Well-developed communication skills increase farmer satisfaction and increase adherence to veterinary advice, thereby enhancing herd health. Additionally, concerns about veterinarians' mental wellbeing are rising, and improving client-centered communication might benefit their mental health. To date, no longitudinal studies have assessed efficiency of communication training on communication competencies, mental health and farm consultancy. Key objectives of my PhD project are to determine: 1) communication characteristics specific to veterinarian-dairy farmer interactions; and 2) effects of communication training on communication skills and mental wellbeing in veterinarians, farmer satisfaction and herd health outcomes. First, I will conduct interviews on participants' current knowledge/attitudes about communication during farm visits and assess herd health status by examining farm data. To examine communication patterns, I will record and analyze veterinarian-farmer interactions during these visits. Secondly, veterinarians will participate in a novel communication training. Finally, I will determine effects of training on communication skills and mental wellbeing in veterinarians, farmer satisfaction and herd health outcomes by repeating initial measurements. **Implications**. Communication training will benefit the Canadian dairy sector by enhancing farmer satisfaction with herd health consultancy, increasing adherence to veterinary advice and improving livestock health and productivity. Additionally, results of this study are expected to enhance mental health of veterinarians and inform development of teaching communication skills in veterinary students and graduate veterinarians.

Developing and testing a live attenuated Johne's disease vaccine as a JD control measure

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Alberta has a high prevalence of Johne's disease, caused by *Mycobacterium avium Subsp. paratuberculosis.* This highlights the need for better JD control to benefit the Alberta dairy industry. A live attenuated vaccine against JD is most promising to protect calves against MAP infections. In this project, we aim to make mutants and fine-tune the attenuation of mutant strains, so that it stimulates protective immune responses without being harmful, persistent or infectious. For this purpose, we will create single and double mutants in MAP genes that were previously identified as being essential for the survival of MAP in the intestinal tissues of dairy calves. We have already created 2 different KO mutants and the reagents (engineered phages) are available to produce any combination of mutations in a single strain. In previous studies we tested these mutants ex vivo in macrophages to measure macrophage viability, MAP viability and cytokines production. These mutants have been also tested in vivo in mice infection model to study histology and size of infected organs. Previous studies showed that mutation in essential genes might result in different pro-inflammatory and anti-inflammatory responses. In the current study these candidate vaccine strains will be tested in our validated calf infection model. We will characterize the protective responses by immune cell and cytokine profiling. This will inform our selection of the best vaccine strain to test in a vaccination/challenge experiment in dairy calves. Implications: As part of an interdisciplinary project, we will contribute to significant new insights on the entirety of JD to the dairy and beef industry and identify control practices that can reduce the massive burden that JD has put on the dairy industry for years.

The voice of dairy farmers: Implementation of ethnographic field methods to address antibiotic use

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Antibiotic resistance, or the ability of a microbe to withstand treatment with antibiotics, is an emerging health issue that has been largely attributed to the inappropriate use of antibiotic treatments. The aim of this research is to understand dairy farmers' perceptions of antibiotic use (AMU), resistance (AMR), and regulation in Central Alberta and interpret them in light of the broader context. Current approaches aimed at addressing AMR have been focused on behavioral change interventions as a means to achieve absolute reduction in use. This project offers a new approach to tackling this issue by working to understand the underlying contextual drivers of antibiotic use, including the belief systems and values of the farmers. Ethnography was employed to address the above aim. Ethnography is an immersive field method traditionally used in cultural anthropology. By immersive, we mean that data collection is done in natural settings and involves direct interaction or close involvement with the people or animals of interest for extended periods of time. To apply this method, the researcher resided in Ponoka, a dairy farming community in Central Alberta for approximately three months. During that time, participant and direct observation were used to gather information on the everyday life of the community and to absorb their mode of reasoning. The implementation of these methods will shed light on which values and beliefs are most important to this community. Semi-structured interviews were also conducted with members of the dairy farming community on topics related to antimicrobial use, resistance, and regulation. **Implications.** 1) This research will allow the farmers to make their voices heard with regard to the development of an antibiotic stewardship initiative. 2) Through this project, we will gain a more in-depth understanding of the sociocultural context in which decisions concerning antibiotics are made. 3) This will provide policymakers with context-specific information to develop locally-appropriate and sustainable strategies to improve stewardship in the dairy sector in Central Alberta.

Development of an alternative therapeutic method for the control of Bovine mastitis in dairy cows.

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Mastitis is one of the most frequently occurring diseases affecting dairy cattle. Cattle with mastitis suffer from painful inflammation of the udder, which results in low milk production, culling, discarded milk and sometimes death, factors that have a great economic impact on the dairy industry. Current treatments of bovine mastitis include antibiotics, which increase the burden of antimicrobial resistance in livestock and as a result also in humans and the environment. In this project, we aim to develop alternative therapies for bovine mastitis by understanding the causative and beneficial organisms in the commensal microbiota of the mammary gland. We propose the use of state-of-the-art genomics and metagenomics approaches to identify influential members of the udder microbiota with central roles in maintaining udder health and modulating susceptibility to mastitis. Metagenomics is the use of genomic technologies and bioinformatics to help study the entire microbial community. Milk samples will be collected from commercial farms in Alberta at different time points of lactation for each quarter. An innovative approach will be used for targeted isolation and characterization of these beneficial "foundation species" from the native microbial community of the udder. The goal of this research is to develop a combination of these foundation species that will be used for the development of a synthetic mini-microbial community, the antimicrobial activities of which will be tested against major mastitis pathogens under laboratory conditions.

Communication is the Key – Mitigating Lameness is Only Possible When Working Together

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Even though Canadian dairy producers identified lameness as the most important dairy cattle health issue and lameness has an undeniable negative influence on the economics of a dairy farm and animal welfare, cow-level lameness prevalence in Alberta has maintained at 20% over the last decade. In light of an acceptable lameness prevalence of 10% (Dairy Code of Practice), it is time for action. Lameness is a unique health issue in terms of the variety of causes and their prevention and control, along with an indirect economic benefit. Despite knowledge on lameness, there is little knowledge of dairy producers' perception of lameness. Because each dairy farm has its unique facilities and management, we must embrace the difference among farms and find an individual solution tailored to each specific farm and include communication in our path towards lameness reduction. The first step was done in the summer of 2019. In-depth interviews were conducted with nine dairy producers in an effort to garner an understanding of their perspectives and perception of lameness as well as the day–to–day challenges that they face. Interviews were transcribed and methods used to identify a range of themes that came from conversations with producers. These interviews revealed a variety of challenges and a range of attitudes, values, beliefs, concerns and feelings regarding the significance of lameness. Combining the knowledge of the producer perceptions with knowledge-based lameness control programs will provide a more effective approach to the reduction of lameness occurrence. Take home message: Communication is the key to work on solutions to mitigate lameness together. Taking the team approach has the potential to reduce the negative economic and welfare aspect by reducing the lameness prevalence.

Developing Novel Therapeutic Alternatives for Bovine Digital Dermatitis

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Digital dermatitis (DD) is an ulcerative and painful skin disease of the foot in cattle, mostly associated with Treponemes bacteria, where skin healing is severely impaired in DD and oxytetracycline is the most effective treatment to date. DD is common in dairy cattle (affecting up to 15% of cows and 94% of herds in Alberta), resulting in economic and major welfare concerns. Using oxytetracycline in treating DD increases the burden of antimicrobial resistance in dairy cattle in Alberta, leading to antibiotic residuals in the food chain. Therefore, developing a novel therapeutic for DD is indispensable to produce healthy dairy products. Our research has established that cows with active DD lesions display accumulation of neutrophils. Timely removal of neutrophils by distinct phenotypes of macrophages is necessary for the skin wound healing process to begin. Neutrophils contain matrix metalloproteinases (MMPs) that regulate tissue remodeling during skin inflammation. Increased neutrophil infiltration and dysregulated protease or MMP activity exacerbate acute and chronic skin wounds in inflammatory skin disorders. Our research objective is to understand why foot skin does not heal in DD and how impaired healing function can be reinstated. Our first aim is to determine the influence of pro-inflammatory and anti-inflammatory macrophages in skin inflammation during active stage (M2) of DD. Our second aim is to perform MMP activity analysis in DD lesions obtained from DD cattle hooves at both acute and chronic stages of DD to find out whether upregulation of MMP activity is associated with tissue destruction in DD. Reducing inflammation and promoting wound healing without the use of antibiotics represent a novel therapeutic approach which aim to heal refractory DD ulcers and chronicity in order to reduce lameness in dairy cattle with less reliance on antibiotics, facilitating welfare and economic benefits for the cattle industry.

Effective and economic Johne's disease control using new early disease detection assays

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Johne's disease (JD) is an infectious chronic enteritis characterized by diarrhea, loss of body condition, and lower productivity. A potential association between *Mycobacterium avium ssp.* paratuberculosis (MAP) and Crohn's disease, a chronic enteritis of humans, is also reported. New experiments demonstrated that young calves also shed MAP, on average, one MAP-infected calf infects, ~3 non-infected pen mates. However, there is no research that evaluated the economic effects of a Johne's disease control program based on the transmission of MAP in calves. We aim to: i) design an improved JD control program, including prevention of calf-to-calf transmission; ii) determine accuracy of new technologies for early detection of MAP-infected dairy cows; and iii) model economic impacts of the new control program. The new JD control program will be implemented on 10 Alberta dairy farms based on a previous risk assessment to determine difficulties in JD control. Blood samples to detect the interferon-gamma (IF-gamma) response and fecal samples for PCR analysis will be collected every 2 mo from all female young stock (≤12 mo) and every 6 mo from all dairy cows to determine accuracy of biomarkers. Environmental sample analysis and IF-gamma response will be linked to management data to generate interventions. Analysis of actual MAP prevalence will enable us to validate the predictive value of actual infection status and to identify, separate and remove infected animals early in disease development. Results should be widely applicable as average herd size and housing system in Canada are similar to other countries. Take home messages: The new JD control program will prevent MAP infections and, as a consequence, reduce incidence and prevalence of other transmitted infections and improve health of young stock, as well as reduce economic losses due to MAP infections.

Cattle Health Surveillance System (CHeSS): Monitoring major infectious diseases and antimicrobial resistance in the Western provinces

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Significant investments have been made to understand endemic infectious disease control in Canadian dairy. However, most projects have focused on the control of one specific disease over a limited time period. These projects have demonstrated that the prevalence of important diseases varies widely among herds. To introduce a comprehensive approach, project objectives are: 1) determine the prevalence of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma bovis*, leukosis, leptospirosis, neosporosis, Johne's disease and *Salmonella* dublin on Western Canadian (BC, AB, SK, MB) dairy farms; 2) develop control measures for each infectious disease on a subset of infected farms, increasing the understanding of farm-specific risk factors for infectious diseases; 3) determine how to improve producer motivation for disease control and understand drivers and barriers towards adopting biosecurity measures; 4) economically evaluate each disease control program; and 5) determine antimicrobial resistance (AMR) prevalence in AB and BC dairy and its relationship to antimicrobial use. By focusing on the surveillance and control of multiple important infectious diseases and AMR, this study will provide practical and performance-based standard operating protocols that could be adapted to the industry as a whole. CHeSS will have major emphasis on knowledge transfer to the dairy industry by utilizing written reports, annual producer meetings and demonstration farms to facilitate peer-to-peer learning opportunities. Take home messages: CHeSS will result in understanding the prevalence and relevant risk factors for infectious diseases on Western Canadian dairy farms, and identify best practices related to AMR. This will improve dairy farm profitability and animal welfare, improved animal and public health, and higher consumer confidence in Canadian milk and dairy products.

Rapid detection of antibodies against bovine leukemia virus by bacterial surface complementation assay

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Enzootic Bovine Leukosis (EBL) is a cancerous disease caused by Bovine leukemia virus (BLV). It accounts for considerable economic losses to the dairy industry.The most common and reliable method to diagnose BLV infection is ELISA. However, a major limitation of ELISA is the requirement of manual handling of samples and the long processing time which makes it unsuitable as an on-farm diagnostic test. Hence, there is a need for an alternative test with rapid detection and reduced manual labour. In this regard, antigen-specific antibodies can be detected with a novel biosensor, based on a novel split trehalase (TreA) enzyme, developed in Dr. De Buck's lab. In the current study, the existing biosensor platform was modified to detect BLV antibodies in clinical serum by using bacterial surface complementation of fusion proteins expressed on the outer membrane of *E.coli*. The integration of bacterial surface display system in biosensor format is cost-effective and enhances the speed and sensitivity of the assay. The cells expressing these fusion proteins when incubated along with diluted bovine clinical serum and trehalose (acting as enzymatic substrate) results in production of glucose which can be measured by a commercial glucometer or a visual assay. To validate the assay, a panel of 17 characterized serum samples obtained from BLV positive and negative cattle were tested in comparison with ELISA results. Evaluation of this panel resulted in positive detection of all BLV positive serum. However, 6 false positives were observed among the healthy samples due to the presence of anti-*E. coli* antibodies in the serum samples. Our results indicate that, using this assay, it is possible to detect anti-BLV antibodies in clinical serum and distinguish between healthy and BLV positive samples. In conclusion, the BLV assay is a potential tool for simple and rapid diagnosis of BLV infection which is compatible with both lab-based and a more user-friendly on-farm format.

Deep learning improves mastitis detection in automated milking systems

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Automated milking systems (AMS) are used in 12% of Canadian dairy farms and >20% of dairy farms in Western Canada, with indications of increasing adoption. Therefore, a need exists for accurate, early automated disease detection. Although many AMS models incorporate chemical sensors to assess milk, including mastitis detection, udder health often declines in the year after transitioning. AMS generate much more data than milk characteristics, and animal behavior data may offer novel indicators for disease onset, with great potential to improve mastitis detection when analyzed with state-of-the-art machine learning methods. Initial models were developed using data from 13 farms in Ontario, of which 3 were used to validate model performance, including 400 animals followed for the first 50 DIM. The models were used to predict daily probability of an animal being diagnosed with clinical mastitis. Deep learning models use a series of connected neurons to identify relationships between variables, and recurrent networks capture time-dependent relationships and base predictions on individual animal patterns. Using a prediction window of 3 days, accuracy was 86% (13% false-positive and false-negative). Furthermore, a combination of milk characteristics and behavioral traits resulted in prediction accuracy of 85%, 9% higher than when using milk characteristics alone. Additional data have been collected from 64 farms: 3 in BC, 2 in Manitoba, 2 in New Brunswick, 55 in Ontario, 15 in Quebec, and 6 in Saskatchewan. These data include cows followed between January and August 2019 in all stages of lactation. Updated results including these farms will be presented at the seminar, increasing the robustness and external validity of the models. **Impact.** AMS data bring novel opportunities. Earlier detection of mastitis would decrease long-term udder health impacts, reduce antimicrobial use and improve milk production, quality, and profitability.

Underlying genetic architecture of mastitis: A systematic review, meta and gene prioritization analysis of GWAS results

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Genome-wide association studies (GWAS) have been widely used to identify single nucleotide polymorphisms (SNPs) and genes associated with mastitis. However, no systematic review and gene prioritization analysis has been conducted to date. Hence, the objective was to perform a systematic review, meta and gene prioritization analysis of GWAS studies to identify key genetic markers and genes associated with mastitis-related traits in dairy cattle. Four electronic databases and 8 conference proceedings were searched. The systematic review was guided using the PPO framework (population: dairy cattle; prognostic tool: GWAS; outcome: mastitis/SCS). Identified articles were screened and data were extracted. Meta-analysis of the reported gene effects will be performed using a random effects model and will be followed by a gene prioritization analysis. Initially, 3,396 articles were identified. After implementation of inclusion and exclusion criteria, 89 studies remained. Preliminary analysis on 5 studies reporting key candidate genes associated with mastitis identified 6 genes shared by >2 studies. Preliminary results indicated that a clearer understanding of genetic architecture of mastitis could be achieved. Moreover, the functional and gene prioritization analyses will allow for pinpointing genomic regions associated with mastitis and their corresponding best functional candidate genes. **Implication:** This systematic review will summarize and confirm GWAS findings to better understand the genetic architecture of mastitis in dairy cattle.

Investigating virulence factors of *Treponema* spp. with newly developed molecular tools

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Digital Dermatitis (DD) is the best known infectious claw disorder affecting dairy herds worldwide. DD is described as an ulcerative foot lesion, characterized by a complex pathology. DD results in cattle lameness and impairs animal welfare, this leads to significant economic losses worldwide. The suspected causative agent of DD is *Treponema* spp. They are anaerobic microbes that are difficult to study due to their fastidious nature and the lack of molecular genetic tools. This lack of knowledge hampers the understanding of disease pathogenesis and further limits effective, evidence-based treatment. In order to better understand the pathogenesis, we seek to understand the bacterial virulence factors underlying DD through genetic manipulation followed by animal infection experiments. To do this, we will create mutants of *Treponema* spp. and determine which mutants don't survive in the host by comparing to the original inoculum (sample containing microbes for infection). By identifying non-viable mutants, we can elucidate essential genes. With this genetic insight we will generate new mutants and compare their survival and pathogenicity to wildtype strains. This study will provide a wealth of knowledge about the genetic content of *Treponema* spp. and reveal details as to how those genes contribute to the establishment and survival of *Treponema* spp. in DD lesions. This information will serve as the foundation for the future development of novel drug targets and diagnostic tools for the prevention of DD. Take-Home Message: Through the identification of virulence genes, our approach to combating digital dermatitis becomes more direct and specific to limiting *Treponema* spp. survivability in lesions. Ultimately this will reduce economic loss and improve animal welfare.

Bovine Leukemia Virus control in dairy cows: Effect of selective removal of high-risk animals on herd prevalence

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Bovine leukosis is a highly transmissible viral disease affecting a large percentage of Canadian dairy cows. The causative retrovirus, bovine leukemia virus (BLV) incorporates its genomic material (RNA) as DNA into that of the host's DNA of the B-lymphocyte cells. This so-called provirus or proviral DNA enables the virus to establish a lifelong infection. BLV-infected animals can have different amounts of this proviral DNA and can be categorized as either high proviral load (HPL) or low proviral load (LPL) BLV-infected animals. HPL animals are more likely to develop leukocytosis, tumors. Moreover, these HPL cows are considered high-risk animals as they appear to more readily transmit the virus, probably due to a high percentage of infected B-lymphocytes. We propose a strategy whereby we will work with the dairy herds across Alberta to identify and remove these HPL animals from the herd. This intervention was proven effective in a recent study in three medium-sized (70-200 cows) dairy herds in the USA. We hypothesize that this intervention significantly reduces BLV transmission to non-infected cows. As the standard methods of proviral load quantification (qPCR) are expensive, we are evaluating alternative, more affordable ways of HPL detection such as antibody titers, white blood cells and B-lymphocytes counts. Removing all infected animals from a high BLV prevalence farm is not practical. We propose a system that identifies high-risk animals, and advise only to remove this subset of BLV-infected animals from the herd. This selective culling of HPL animals is expected to significantly speed up the reduction of BLV prevalence in a dairy herd and be a major step towards eradication of BLV. We are recruiting dairy farms in Alberta that want to participate in our effort to eradicate BLV from dairy herds. If you are interested to know more about the project please contact sulav.shrestha1@ucalgary.ca / (403) 708-2342.

Motives and barriers to providing outdoor access for dairy cows

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Canadian citizens view outdoor access as an important element for dairy cow welfare. Dairy cows are highly motived to access pasture, especially at night. A recent study on alternative types of outdoor access showed that dairy cows also prefer to access an outdoor bedded pack at night. However, approximately 35% of the lactating and 65% of the dry cows in Canada are provided access to pasture; numbers on the use of alternative outdoor areas are unknown. We aim to understand the motives and barriers of dairy farmers to provide lactating dairy cows access to pasture and to other forms of outdoor areas. We will recruit approximately 50 Alberta dairy producers to participate in semi-structured interviews, including those who 1) do not provide outdoor access, 2) do provide outdoor access, 3) do not provide outdoor access now but did so within the past 10 years, and 4) do provide outdoor access now but did not do so within the past 10 years. Interview questions will be designed to assess perceived barriers and motives to providing different forms of outdoor access. **Take home messages:** Only a minority of lactating dairy cows are provided outdoor access in Canada, despite outdoor access being highly valued by cows and by the Canadian public at large. We aim to understand the perceived barriers and motives for implementing outdoor access by producers.

Accumulating bacteriocin genes in a non-aureus Staphylococccus strain to make it protective against bovine mastitis pathogens

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Background: Bovine mastitis is the most common and economically important disease affecting the dairy industry. Intramammary infection with *Staphylococcus aureus* is one of the leading causes of mastitis. Although antibiotics have been the main method of treating and preventing bovine mastitis, alternative treatments are required due to the emergence of antibiotic-resistant bacteria. A promising alternative is the use of bacteriocins - antimicrobial proteins produced by bacteria. Bacteriocins function through different modes of action, therefore, creating a probiotic strain which produces a cocktail of different bacteriocins, will reduce the risk of resistance against bacteriocins. Hypothesis: A super-probiotic non-aureus staphylococcus (NAS) strain by introducing several unique bacteriocin gene clusters will allow the inhibition of *S. aureus* intramammary infections. Methods: We have a large library of bovine NAS samples including some already with inhibitory effects against *S. aureus* as recipient strains for additional bacteriocin gene clusters. The recipient strains will be selected based on its ability to: persist during infection in bovine mammary glands; not cause significant increases in somatic cell count; and ability to take up external genes. After identifying a recipient NAS strain, bacteriocin gene clusters will be transferred into the recipient strain's genome. We have five donor NAS strains each with a single bacteriocin gene cluster that inhibits *S. aureus* growth, with each gene cluster belonging to a unique bacteriocin category. The modified recipient strain will be tested in a mouse mastitis model and then a heifer model to evaluate its efficacy against *S. aureus*. Impact: Creating a super-probiotic strain will reduce the reliance on antibiotics which are losing their efficacy as the primary treatment of mastitis. Take home message: With the creation of this super probiotic-strain, it will be used as an alternative for antibiotics and will reduce the heavy reliance on antibiotics, the rise of antimicrobial resistance and the current economic impact of mastitis.

Marketing of Male Dairy Calves – Findings and Consensus of an Expert Consultation

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A group of experts – including dairy farmers, calf growers, veterinarians, auction operators and regulators – was convened for a focused 2-day discussion of potential health and welfare problems associated with the marketing of male dairy calves. Written notes and audio-recording were used to summarize the information provided, and content analysis was used to develop a consensus statement on concerns, possible solutions, and recommendations. The group noted that calves across all Canadian regions are commonly transported at 3-7 days of age and undergo transport up to 48 hours depending on the location of their dairy farm of origin. Some regions use auctions almost exclusively for marketing, while others rely more on direct sales. Better criteria are needed for calf fitness for transport, maintaining farm biosecurity when calves are collected, reducing the use of antimicrobial therapy in calf production, and improving education for farmers and veterinarians on the importance of neonatal calf care for male dairy calves before transportation. Experts noted that major changes in male dairy calf marketing will be required to comply with new regulations on animal transportation in Canada; collaboration between the industry and regulators will be needed to develop an implementation plan that safeguards animal health and welfare as the transition is made. Take home message: Male dairy calf marketing in Canada can involve transportation of 3-7 day old calves for up to 48 hours. New regulations limit the transport of unweaned calves to 12 hours which may improve animal health and welfare but will require significant procedural changes. A collaborative effort is needed to safeguard calf health and welfare as the dairy and calf-rearing industries transition to comply with the new regulations.

Effects of lipopolysaccharide on the metabolic function of ruminal epithelial cells

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Ruminal acidosis has been reported to induce inflammation and increase risk for non-desired molecules, such as lipopolysaccharide (**LPS**), to cross rumen wall and enter circulation. Such inflammation has been reported to increase glucose requirements and decrease milk yield, but it is not clear whether cells within the rumen wall (rumen epithelial cells) are implicated in this response. The objective of this study was to investigate the effects of LPS on rumen epithelial cell metabolism. Ruminal papillae from nine yearling beef heifers were used to isolate and culture REC. Cells were grown for 12 d before use and then exposed to normal media or media containing SCFA (2 mM butyrate and 5 mM propionate) with (50,000 EU/mL) or without LPS for 24 h. Incubation media samples were collected for analysis of glucose and SCFA use. Cells were also collected to measure expression of genes associated with inflammation (tumor necrosis factor [*TNF*] and interleukin 1β [*IL1B*]), nutrient transport (*GLUT1* and monocarboxylate transporters [*MCT1*, *MCT4*]), and nutrient metabolism (*ACAT1*, *MCU*, *IGFBP3* and *IGFBP5*). Protein expression of ketogenic enzymes (BDH1 and HMGCS1) were also analyzed using flow cytometry. Cells tended to consume more glucose when exposed to LPS compared to without (31.8 vs 28.7 ± 2.7 %; P = 0.072). Expression of inflammatory genes (*TNF* and *IL1B*) were upregulated when exposed to LPS (P < 0.001). In cells exposed to LPS, there was a downregulation of ketogenic genes (*ACAT* and *IGFBP5*) and an upregulation of genes involved in nutrient transport (*GLUT1*, *MCT4*) and nutrient metabolism (*MCU* and *IGFBP3*) (P < 0.05). Media with SCFA led to greater expression of *MCU. MCT1* was upregulated in cells grown with SCFA compared to REG and those exposed to LPS (P = 0.001). Ketogenic enzyme expression was not affected, however BDH1 tended to be less in cells exposed to LPS (P = 0.076). **Take Home Messages:** Exposure of the rumen epithelial cells to LPS increases glucose uptake and metabolism suggesting that the rumen epithelium may contribute to the greater glucose requirement due to inflammation.

Nutritional management practices in Manitoba and Saskatchewan farms with automatic milking systems

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There has been considerable debate regarding the quantity of concentrates that should be provided in automatic milking systems (AMS). Previous studies have looked at how much producers are currently providing; however, these studies did not include any information regarding the partial mixed ration (PMR). Twenty-two farms with AMS in Saskatchewan and Manitoba participated in a study looking at benchmarking the quantity of concentrates provided in the AMS in relation to the stage of lactation and milk yield, as well as the feed composition and chemical composition of both the partial mixed ration (PMR) and the AMS concentrates. An in-person survey was conducted at each farm, seven months of individual milking and feeding records were collected and, one year of milk yield and milking component records were obtained from dairy herd improvement (DHI). In addition, samples from the PMR, and the AMS concentrates were taken. The milking and feeding results were separated based on days in milk (DIM) into five categories early (1-45 DIM), peak (46-99 DIM), mid (100-199 DIM), late (200-305 DIM) and over (>305 DIM). They were also separated into milk yield categories low (< 30kg/d), medium ($\geq 30 \leq 50$ kg/d), and high (> 50 kg/d). The resulting data was summarized with descriptive statistics which provide a summary of the common feeding practices. The common practices include feeding pellets in the AMS. The quantity of pellets was programmed based on a table which used DIM and milk yield to set each individual animal's amount. The average concentrates provided ranged between 3.73 and 5.86 kg/cow/d in the AMS. The highest amounts observed were provided to high yielding cows with a mean of 7.01 kg/cow/d. Milking frequency was highest in higher producing cows with an average of 3.77 milkings/d. **Implications.** The results from the study provide an idea of the current practices being implemented in the industry, and will provide valuable information for future studies.

Effects of Steam Pressure on the Chemical and Rumen Degradation Characteristics of Faba Bean in Dairy Cattle

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The aim of this study was to identify the effects of processing times on the nutritional value of Faba bean (*Vicia faba*) seeds for dairy cattle. A total of 15 samples were used (1 variety x 3 yrs. harvest x 5 treatments). Seeds were autoclaved at 121 °C for 30, 60, 90, and 120 min, while untreated seeds were used as control treatment. Mixed model procedure and RCBD experimental design ($Y_{ij} = \mu + T_i + \beta_j + e_{ij}$) were used for statistical analyses with SAS 9.4 (SAS Institute, Inc., Cary, NC, US). Significance was declared at P < 0.05. The average DM, CP, ST, and NDF content was (90.65 %, 29.16, 36.49, and 36.03 %DM, respectively). Soluble crude protein (%DM) was significantly higher ($P <0.0001$) in the control than the averaged heat treatments (23.90 vs 5.19 %). The total digestible nutrients (TDN_{1x}) and energy values were significantly different among treatments ($P <0.001$) by decreasing gradually as heating time increased (TDN: - 2.78, - 3.87, - 6.44, and - 7.71 %DM at 30, 60, 90, and 120 min, respectively). Rumen undegradable crude protein (RUP-NRC, 2001) was significantly different ($P <0.0001$), increasing its content with increasing heating times (+ 65.33, + 114.80, + 131.24, and + 143.97 g/kg DM at 30, 60, 90, and 120 min, respectively). The effective degradability of NDF in the rumen was significantly different ($P= 0.001$), increasing with increasing heating times (+ 97.44, + 148.73, + 150.93, and + 220.77 g/kg DM at 30, 60, 90, and 120 min, respectively). The potential of steam pressure to decrease protein rumen degradability of Faba bean (*Vicia faba*) for cattle rations has proven effective. Further studies to determine the benefits of an optimal steam pressure time on the metabolism and animal performance of dairy cattle are still needed.

The effect of intestinal Ca-gluconate and Ca-butyrate on ruminal short-chain fatty acid (SCFA) absorption and SCFA concentrations in the gastrointestinal tract of heifers

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The objective of this study was to evaluate ruminal short-chain fatty acid (SCFA) absorption and short-chain fatty acid concentrations within the gastrointestinal tract (**GIT**) of heifers infused either with Ca-gluconate or Ca-butyrate. Thirty-two ruminally cannulated heifers, fed a common diet, were provided a ruminal infusion of water (**CON**), ruminal infusion of Ca-gluconate embedded in a fat matrix (0.0192% BW; **RGluc**), an abomasal infusion of Ca-gluconate (0.0077% BW; **AGluc**), or an abomasal infusion of Ca-butyrate (0.0029% BW; **ABut**). Treatments were designed to provide the same amount of butyrate to the small intestine. DMI was restricted to 95% of voluntary intake and the temporarily isolated and washed reticulum-rumen (WRR) technique was used to measure SCFA absorption. Samples of the rumen, jejunum, and colon were collected. Data were analyzed to determine the effect of the CON vs. ABut, CON vs. treatments with Ca-gluconate, and for ABut vs. treatments with Ca-gluconate. DMI was not affected by treatment averaging 7.7 kg/d ($P = 0.77$). Ruminal concentrations of major SCFA were not affected. The concentration of SCFA in the colon was greater for ABut than for treatments with Ca-gluconate (29.5 $vs.$ 22.6 mM; $P = 0.026$) and the molar proportion of acetate was greater for CON than ABut (76.2 $vs.$ 73.5%; $P = 0.036$) and CON than Ca-gluconate (73.0%; $P = 0.046$). The molar proportion of colonic propionate was greater for ABut than CON (17.8 $vs.$ 16.6%; $P = 0.049$) and greater for treatments with Ca-gluconate than CON (17.9%; $P = 0.020$). The rate of ruminal SCFA disappearance was greater for ABut compared to CON (678 mmol/h $vs.$ 486 mmol/h; $P = 0.014$), and greater for ABut than Ca-gluconate treatments (535.31 mmol/h; $P = 0.039$). **Take Home Message**: These data indicate that supplying Ca-butyrate or Ca-gluconate to the intestine affect SCFA in the colon and stimulate SCFA absorption across the rumen.

Association between Protein Molecular Spectral Profiles and Metabolizable Protein Supply, Protein Rumen Degradation Characteristics and Estimated Intestinal Protein Digestion to Dairy Cattle Before and After Rumen Incubation of Faba Bean Partitions and Faba Bean Silage

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The primary objectives of this study were to: (1) compare faba bean samples among whole crop, stem, whole pods, leaf, and whole crop faba silage in terms of predicted metabolizable protein supply; protein rumen degradation characteristics, and estimated intestinal protein digestion; (2) determine how protein spectral features of faba bean samples are changed during rumen incubation using ATR-FTIR molecular spectroscopic technique and (3) test the relationship between protein related spectral profiles before and after microbial digestion and protein digestibility and availability. Faba bean samples were firstly determined for chemical compositions and then incubated in the rumen for the determination of CP degradation kinetics and protein intestinal digestibility. Protein related molecular spectral features before and after rumen incubation were also obtained. Results showed that the function of microbial digestion to the change of protein related molecular structure of faba bean samples could be detected by both univariate and multivariate molecular spectral analyses. In addition, the spectral features of rumen incubation residues of faba bean had a strong correlation with the NDICP content, while the relationship between spectral profiles and other nutritional compositions and availability was diminishing during rumen incubation. In conclusion, the protein availability and digestion characteristic are mainly associated with its spectral profiles in original samples; the change of spectral profiles may have minimal effect on facilitating protein enzymatic hydrolysis. Overall, when evaluating nutritional value of feed to dairy cattle using ATR-FTIR spectroscopy, spectral features of original samples should be first considered.

Association between Carbohydrate Related Molecular Structure Spectral Profiles and Chemical Profiles, Energy Profiles, CNCPS Profiles and Rumen Degradation Parameters to Dairy Cattle Before and After Rumen Incubation of Faba Bean Partitions and Faba Bean Silage

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The primary objectives of this study were to: (1) compare chemical profiles, energy profiles, CNCPS fraction profiles and rumen degradation parameters among faba bean samples including whole crop, stem, whole pods, leaf and whole crop faba silage; (2) compare carbohydrate related spectral parameters of faba bean samples before and after rumen incubation; (3) test the relationship between carbohydrate related molecular spectral profiles before and after rumen incubation and chemical profiles, energy values, CNCPS carbohydrate fractions and NDF rumen degradation parameters. Faba bean samples were determined for chemical compositions, energy values and CNCPS carbohydrate fractions. Faba bean samples were also incubated in the rumen for estimation of NDF degradation kinetics. In addition, carbohydrate related spectral features before and after incubation were obtained using ATR-FTIR spectroscopy. The results showed that spectral features were altered during rumen incubation and a significant interaction between faba bean samples and incubation time was observed. Furthermore, spectral features of incubation residue were found to have a stronger correlation with less degradable carbohydrate fractions (NDF, ADF, ADL). Based on the results of multivariate analysis, the spectral profiles of original samples could be separated from the incubation residues, however spectral profiles of 12 and 24 h incubation residues could not be separated. In conclusion, carbohydrate related spectral features were altered during first 12 h of rumen incubation, and the change of spectral features were associated with its nutritional characteristics. Overall, carbohydrate related spectral features could be used as indicators for faba bean nutritional evaluation in dairy cattle.

Determining the optimal dosage of an innovative fibrolytic enzyme on NDF and DM degradability and kinetics of whole crop faba bean silage in western Canada

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The main objective of this study was to determine the optimal dosage level of fibrolytic enzyme solution (derived from *Trichoderma reesei*; a mixture of xylanase and cellulase; AB Vista, Marlborough, UK) when applied to faba bean silage (*Vicia faba* L.). The faba bean silage was dried at 55°C for 48 hours and ground through a 1-mm screens using a Wiley mill. Seven doses of the enzyme: 0, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mL of enzyme/ kg DM of silage were applied directly onto the substrate for both *in situ* and *in vitro* analysis for 0, 6, 24, and 48 hours of incubation time. Four cannulated lactating dairy cows at Rayner Dairy Research and Teaching Facility (RDRTF, University of Saskatchewan) were used for the *in situ* study. Two ANKOM Daisy[II] incubators (ANKOM Technology, Macedon, New York, USA) were used for the *in vitro* study. The *in situ* results showed that there was a cubic (P<0.05) effect of enzyme dose levels on DM degradability (DMD) and a quadratic (P<0.01) effect on the neutral detergent fiber degradability (NDFD) of faba bean silage. There was no effect of the enzyme dosage level (P>0.05) on DM degradation rate (Kd), however there was a significant linear effect on Kd of NDFD. Similar results were observed in the in vitro study, with a quadratic (P<0.01) effect on in vitro DMD and a cubic (P<0.01) effect on in vitro NDFD of the faba bean silage. **Implications:** This study demonstrated that the new generation of fibrolytic enzyme solution has a potentially positive effect on DMD, NDFD and kinetics of faba bean silage. Further study is required to examine the effect of the fibrolytic enzyme on the lactational performance, and nutrient digestibility in high producing dairy cows fed with a faba bean silage based diet.

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