Proceedings

2018 Cornell Nutrition Conference for Feed Manufacturers

80th Meeting

October 16 – 18, 2018

Doubletree Hotel

East Syracuse, New York

Cornell University

Department of Animal Science

College of Agriculture and Life Sciences

Ithaca, New York

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Proceedings and presentations will be available for download at ansci.cals.cornell.edu/CNC/.

Speaker Biographies

Dr. Dave Barbano
Cornell University
Dr. Dave Barbano is Professor of Food Science at Cornell University. Dave conducts an applied and basic research program on 1) natural cheeses and whey products, 2) improvement of methods of analysis of dairy foods, 3) raw milk and dairy food quality, 4) membrane filtration of milk and whey for protein separation and microbial removal. Recently, Dave has focused on developing new milk analysis measures of cow metabolic health for dairy herd management. Dave also delivers a technology transfer program to communicate research results to the dairy industry and teaches a dairy chemistry course. He has been very active in the analytical groups of International Dairy Federation and the Association of Official Analytical Chemists International for the past 30 years. He serves as Director of the Northeast Dairy Foods Research Center program that is funded jointly by national and regional milk promotion units, suppliers, and dairy product manufacturers.

Dr. Jack Britt
Britt Consulting
Jack Britt is a scientist, teacher, entrepreneur and consultant. He has served as a scientist and teacher at three Land Grant Universities, led programs at department, college, university and system levels and served as senior executive. He has worked with dairy farmers in 22 countries and is engaged as a futurist focused on the global dairy industry 50 years in the future. He has been a colleague and friend of Dr. Ron Butler since they both completed their graduate training in the early '70's.

Dr. Larry Chase
Cornell University
Dr. Larry Chase is a Professor Emeritus of Dairy Nutrition in the Department of Animal Science at Cornell University. Larry served as the General Chairman of the Cornell Nutrition Conference for 30 years. His current research activities are in the environmental impacts of dairy cattle rations and greenhouse gas emissions.

Dr. Heather Dann
Miner Institute
Heather Dann is a research scientist at the William H. Miner Agricultural Research Institute in Chazy, NY. She was raised on a dairy farm in the Finger Lakes region of New York. She received a B.S. degree from Cornell University, a M.S. degree from the Pennsylvania State University, and a Ph.D. degree from the University of Illinois. Her research at Miner Institute focuses on dairy cattle nutrition and management. She trains and mentors students and interns through a variety of experiential learning programs.

Mr. Michael Dineen
Cornell University
Mike was born in County Cork, Ireland where he grew up on a pasture based dairy enterprise. In 2014 he received his BS in Animal Science from University College Dublin. He also holds a Master of Philosophy from Queens University Belfast. Currently a 4th year PhD student in the lab of Michael Van Amburgh, Mike focuses on describing nutrient supply and animal requirements in pasture based systems along with a strong interest in aNDFom digestion. In 2017 Mike became a ruminant nutrition scientist with Teagasc Moorepark, who are an Irish government body involved in research, education and extension.

Dr. Rob Gilbert
Ross University
Dr. Robert Gilbert received his veterinary education in South Africa at the University of Pretoria. He spent time in private practice and corporate practice (artificial insemination) before returning to the University for post-graduate study. He later completed a residency in theriogenology at the University of Wisconsin-Madison before returning as a faculty member to the University of Pretoria (Onderstepoort). From 1988 through 2016 he served on the faculty of the College of Veterinary Medicine at Cornell University, where he also held various administrative positions. He joined RUSVM in 2016.

Dr. Rick Grant
Miner Institute
Rick Grant was raised on a dairy farm in northern New York State. He received a B.S. in Animal Science from Cornell University, a Ph.D. from Purdue University in ruminant nutrition, and held a post-doctoral position in forage research at the University of Wisconsin-Madison from 1989 to 1990. From 1990 to 2003, Rick was a professor and extension dairy specialist in the Department of Animal Science at the University of Nebraska in Lincoln. Since February of 2003, he has been President of the William H. Miner Agricultural Research Institute in Chazy, NY, a privately funded educational and research institute focused on dairy cattle, equine, and crop management. Rick's research interests focus on forages, dairy cattle nutrition, and cow behavior. He has been the recipient of the Pioneer Hi-Bred International Forage Award in 2010 and the Nutrition Professionals Applied Dairy Nutrition Award in 2015.

Dr. Sabrina Greenwood
University of Vermont
Sabrina is an Associate Professor at the University of Vermont. One of her central research focuses is the bovine milk proteome, including research to investigate nutritional, physiological, and management factors that affect the milk protein profile.

Ms. Allison Kerwin
Cornell University
Upon graduating with a Bachelor's degree from Cornell University, Allison Kerwin worked in the industry as a dairy nutritionist for a couple of years before returning to Cornell as a research technician under the direction of Dr. Overton. Allison proceeded to enroll in the employee degree program and is working on her PhD with a focus on transition cow nutrition.

Dr. Quirine Ketterings
Cornell University
Quirine Ketterings joined Cornell University in August 2000 to provide leadership for the field crop nutrient management extension and applied research program of the College of Agriculture and Life Sciences (CALS). Quirine received her MS from Wageningen Agricultural University and her PhD from The Ohio State University. She established and leads the CALS Nutrient Management Spear Program (NMSP), an applied research, teaching and extension program for field crop fertility management that aims to improve grower and agricultural industry management of crop nutrient needs (using manure, compost, rotations, cover crops, fertilizer). Research findings are extended to stakeholders through extension articles, software, factsheets, talks, and the NMSP website (http://nmsp.cals.cornell.edu).

Ms Niko Kochendoefer
Cornell University
Niko received her undergraduate degree in Animal Science and Agricultural Management at Anhalt University in Bernburg, Germany in 2012. She has experience managing large-scale sheep flocks in Germany and worked as whole farm consultant in the dairy industry. Currently she's a PhD student with Mike Thonney in the Cornell Graduate Field of Animal Science working on nutrition and management of milking sheep.

Dr. Xingen Lei
Cornell University
Xingen Lei received his B.S. and M.S. in China, and Ph.D. from Michigan State University. Since joining the Cornell faculty, he has pioneered developments of a new generation of bacterial phytases, feather-degrading enzymes, defatted microalgal feed proteins, and gene-knockout models to study nutritional genomics of selenium. He has been an active leader in applying new agricultural technologies in the global fight against human micronutrient deficiencies. Dr. Lei has 452 publications and has advised 98 graduate students and postdoctoral fellows.

Dr. Matt Lucy
University of Missouri
University of Missouri-Columbia. He received a B.S. from Cornell University, an M.S. from Kansas State University and a Ph.D. from the University of Florida. Dr. Lucy's current research program examines the physiological processes regulating fertility in dairy cows and explores practical methods that evolve from this research.

Dr. Joe McFadden
Cornell University
Dr. Joseph W. McFadden has a scientific interest to define the mechanisms of insulin resistance and fatty liver disease in dairy cattle. In 2003, he received a B.S. degree with Distinction in Research from the Department of Animal Science at Cornell University. He then completed an M.S. degree in Animal Science from the University of Illinois with a dairy cattle nutrition focus. In 2009, Dr. McFadden obtained a Ph.D. degree in Dairy Science from Virginia Tech. Following his Ph.D. training, Dr. McFadden gained experience in the field of mass spectrometry-based lipidomics as a postdoctoral fellow in the Department of Neuroscience and the Center for Metabolism and Obesity Research at Johns Hopkins University School of Medicine. In 2012, Dr. McFadden joined the faculty in the Division of Animal and Nutritional Sciences at West Virginia University as an assistant professor of biochemistry where he integrated hypothesis-driven lipidomics within the dairy sciences. Dr. McFadden recently joined the Cornell University Department of Animal Science as the Northeast Agribusiness and Feed Alliance Faculty Fellow in Dairy Cattle Biology. With federal and industry support, Dr. McFadden continues to employ lipidomics as a means to develop practical applications to improve hepatic health and lactation performance in cows.

Dr. Kristan Reed
Cornell University
Kristan received her PhD in Animal Science from UC Davis in 2016 after returning from 2 years of service in the Peace Corps in southern Africa. She moved from California to the midwest to work at the US Dairy Forage Research Center in Madison, WI studying whole farm nitrogen flows and simulation modeling. She began her position at Cornell University in August 2018 as an Assistant Professor of Dairy Cattle Nutrition. Her research continues to focus on whole farm

nutrient cycling and interactions between dairy cattle nutrition and environmental impacts of dairy farming.

Dr. Eduardo Rico-Navarrete
Cornell University
Dr. Rico is originally from Colombia, where he majored in animal sciences and focused primarily in nutritional science. He worked as a nutritionist in the feed industry for some years and then pursued his graduate studies in the US (Michigan State University and West Virginia University). He is currently a postdoctoral Research Associate at Cornell University where he is interested in investigating the potential of dietary manipulations to modulate metabolism, health and production performance. His recent work has focused on using mass spectrometry-based lipidomics to unveil the roles of sphingolipids in the development of insulin resistance in domestic animals, and identify metabolic fingerprints that define normal and pathological trajectories of lipid metabolism during the transition from gestation to lactation. To achieve this, he has utilized in vivo and in vitro approaches centered on lipid metabolism in key organs (e.g. liver, and adipose tissue) and their relation to insulin action. More recently, he has applied lipidomics to study the effects of nutritional interventions on markers of metabolic health in humans. Moving forward, Dr. Rico is interested in exploring the potential of nutritional manipulation to dairy cow diets to modulate milk composition and its nutraceutical potential.

Dr. Mart Ros
Cornell University
Mart Ros joined the Nutrient Management Spear Program (NSMP) at Cornell University in May 2017, after following BSc, MSc, and PhD programs at Wageningen University. His PhD research was on improving the utilization of soil phosphorus in managed grasslands. In his current job at the NMSP, he combines research and extension on nutrient management issues. Mart leads the project on whole-farm nutrient mass balances, which aims at improving the use efficiency of nitrogen, phosphorus, and potassium on dairy farms. Additionally, he coordinates regional efforts to compare and improve phosphorus indices in the Northeastern US. This project tries to mitigate phosphorus losses from agriculture to the environment. More information on these and other projects can be found on the NMSP website (http://nmsp.cals.cornell.edu).

Dr. Mike Van Amburgh
Cornell University
Mike Van Amburgh is a Professor in the Department of Animal Science and a Stephen H. Weiss Presidential Fellow at Cornell University. He teaches multiple courses and leads the Cornell Dairy Fellows Program, advises approximately 50 undergraduate students and is the advisor for the Cornell University Dairy Science Club.

For the last 20 years, a major focus of his research program has been to describe the nutrient requirements of dairy calves and heifers and aspects of endocrine control of developmental functions such as mammary development. This has evolved into describing and working to understand factors in neonatal life that establish lifetime productivity functions and outcomes.

Mike currently leads the development of the Cornell Net Carbohydrate and Protein System, a nutrition evaluation and formulation model used worldwide and through that effort is focused on enhancing the efficiency of nutrient use by ruminants to improve the environmental impact of animal food production. A significant focus of his current work is to understand whole animal and ruminal nitrogen metabolism and amino acid supply and requirements to enhance the development of the Cornell Net Carbohydrate and Protein System. Further, his group is active

in developing methods to better describe the interaction between forage and feed chemistry, rumen function and nutrient supply to compliment the model.

Pre-Conference Speakers

Dr. Barry Bradford
Kansas State University
Barry Bradford grew up on a cow/calf operation in Iowa and completed degrees at Iowa State and Michigan State. He has been on the faculty at Kansas State University since 2006, where serves as a Professor of Metabolic Physiology. Bradford and his team carry out research in the areas of nutritional immunology, metabolic inflammation of transition cows, and sustainability of dairy cattle diets. In addition, he teaches several courses in nutrition and endocrinology and currently serves on the board of directors of the American Dairy Science Association.

Dr. Tom Edrington
Diamond V
Prior to joining Diamond V, Dr. Tom Edrington spent 16 years with the USDA-ARS conducting research in pre-harvest food safety with an emphasis on Salmonella and E. coli O157:H7 in dairy and beef cattle. During his tenure at the USDA, Tom developed an internationally recognized program in pre-harvest food safety research involving collaborations with numerous partners in academia, industry, and government. He is author or co-author on over 150 peer-reviewed scientific journal articles, many of which examined Salmonella in dairy animals.

Dr. Guy Loneragan
Texas Tech University
Guy Loneragan is a veterinary epidemiologist. He received his BVSc from the University of Sydney and completed graduate training (MS and PhD) at Colorado State University. He now serves as a Professor of Food Safety and Public Health at Texas Tech University. Loneragan's research focuses societal challenges specifically related to the connection of animal, health and ecosystem health. His research portfolio includes exploration of approaches that control food-borne pathogens and antimicrobial drug resistance in agri-food systems. In his research, his approaches scale from the gene, to populations of animals, to policy advancement. Loneragan also works to characterize modifiable factors that influence animal health and welfare.

Mr. Steve Sands
Performance Feed Group
Steve Sands is Vice President of Protein for Performance Foodservice . He is a highly regarded foodservice industry veteran with over 35 years of experience in all phases of the meat business. In 1979, he became the fourth generation of his family to own and operate Standard Meat Co. During the 1980's Standard Meat became a leading supplier of steak and ground beef products to Sysco. The company focus expanded into export sales and branded beef programs during the 1990's and was sold in 2000 to Meyer Natural Angus. Over the next several years, Steve helped to build Meyer into the largest natural and organic beef company in the U.S. In 2003, he left Meyer to form Premium Protein Products, a beef and poultry slaughtering and processing company dedicated to high value specialty protein programs. PPP was sold in 2006 and after helping to transition the company to a new management team, Steve left in 2009 to join Performance Food Group as Vice President for Protein.

Dr. Bill Stone
Diamond V
Bill grew up on a beef and hog farm in southeastern Wisconsin. After obtaining a veterinary degree from the University of Wisconsin-Madison, he practiced veterinary medicine for three years in a dairy practice in Monroe, Wisconsin. Bill continued his education through graduate school at Cornell University. His program focused on applied aspects of dairy cattle nutrition and management. After completing his doctoral program he operated a dairy nutritional and management consulting business in central New York.

Bill worked in a veterinary herd health/nutrition position with the PRO-DAIRY program at Cornell University from 1998 to 2007 before joining Diamond V. His position with Diamond V enables him to help direct the company's dairy research and technical support programs, and to work with agribusiness and dairy producers throughout the United States and internationally. His primary areas of interest are dairy cattle nutrition and feeding management, forage management, and identification of bottlenecks on dairies.

Post-Conference Speakers

Dr. Mary Beth de Ondarza
Paradox Nutrition
Mary Beth grew up on an 80-cow registered Holstein dairy farm in southern New York. She received her B.S. at Delaware Valley College and her M.S. at Cornell University. In 1994, she received her Ph.D. from Michigan State University. She then worked as a nutritionist in the feed industry in the Northeast. Her primary responsibilities were to provide on-farm nutritional consultation, educate sales staff, and formulate and test new products. In 2003, Mary Beth became sole proprietor of a nutritional consultation business for the dairy feed industry, Paradox Nutrition. She now conducts studies on commercial dairy farms for feed ingredient supply companies. She also does computer modeling and literature review projects, and she continues to do some on-farm nutritional consultation.

Dr. Derek McIlmoyle
AB Vista
Dr. Derek McIlmoyle has been working in the agricultural industry since 2001 after graduating from Queen's University Belfast with an Honors degree in Agriculture followed by a Ph.D. in energy metabolism in dairy cows. Following completion of his Ph.D. Derek started his career in England and his previous positions include Technical Services Manager at a specialist feed & supplement premix company and prior to that as a Nutritionist for a large animal feed company based in the South West of England. With a strong in-depth knowledge on the ruminant market, Derek joined AB Vista in October 2007 and has been involved in both product sales and technical support at all levels within the agricultural sector in both the UK and Europe. With a blend of technical and commercial experience, Derek has been managing the Great Britain and Ireland Ruminant Business for AB Vista for the past 2 years.

Dr. Tim McAllister
Agriculture and Agri-Food Canada
Dr. Tim McAllister grew up on his parents' cow/calf farm in Innisfail, AB. He obtained a B.Sc. (Agr) and M.Sc. from the University of Alberta in Edmonton, and a Ph.D. (with distinction) in ruminant nutrition and microbiology from the University of Guelph, ON. He accepted an NSERC post-doctoral fellowship at the University of Calgary in 1991, and joined Agriculture and Agri-Food Canada in Lethbridge, AB in 1992. Dr. McAllister has been a research scientist in Rumen Microbiology, Feed and Nutrition since 1997. His research focuses on microbiology,

nutrition and beef production and on food and environmental safety issues related to livestock production, strategies for mitigation of Escherichia coli O157:H7, prion inactivation within the environment, antimicrobial resistance in bacteria in feedlots and investigating the discovery and characterization of fibrolytic enzymes from rumen microbes. He also has extensive research experience in GHG emissions within animals from manure and the impact of manure handling procedures, such as composting, on emissions. He is the author or co-author of over 660 peer-reviewed scientific papers and 60 reviews, as well as 1000 abstracts and conference proceedings, and over 100 final reports for collaborative research projects.

Dr. Tom Overton
Cornell University
Thomas R. Overton, Ph.D., is Professor of Dairy Management in the Department of Animal Science at Cornell University. Tom is recognized nationally and internationally for his research and extension efforts relating to metabolism, immune function, and nutritional physiology of the transition cow and his work on milk component production in cows. He serves as Director of the PRO-DAIRY program at Cornell, and as Associate Director of Cornell Cooperative Extension works with statewide and regional extension teams within New York to enhance the dairy and agricultural industries in New York State. He teaches the applied dairy cattle nutrition course for undergraduates and co-teaches a course in dairy nutrition for veterinary students.

Mr. Chris Piotrowski
AB Vista
Chris Piotrowski is a Director at Aunir, leading NIR calibration development company and a part of AB Vista. Chris leads the technical development of Near Infrared calibrations and applications at Aunir and has been instrumental in bringing novel on-farm NIR applications to the animal nutrition market. Chris is widely recognized globally for his contribution to the development of Near Infrared calibrations. Mr. Piotrowski has over 40 years' experience working in the field of Near Infrared analysis, across multiple industries.

Breakfast Speakers

Dr. Elliott Block
Arm & Hammer Animal and Food Production
Elliot was born and raised in Brooklyn, NY. After High School he received his B.S. from Cornell University in Animal Science and went to The Pennsylvania State University to complete his M.S. in Animal Nutrition (minor in Physiology) and his Ph.D. in Animal Nutrition (minor in Biochemistry). Upon his completion of his Ph.D. he moved to Montreal where we was hired as a professor of Animal Science with an appointment to the faculty of Medicine at McGill University. After 20 years at McGill he was offered a position as Research Manager at Arm & Hammer Animal Nutrition and was promoted to Research Fellow and Director. He has been at Arm & Hammer for the past 18 years. Elliot has authored and coauthored over 85 peer reviewed publications and numerous abstracts and popular publication articles. His major areas of interest has focused on Physiological Nutrition in Dairy Cattle but that has expanded over the past few years to include other livestock species and the areas of gut microbiome and its impact on health and productivity and into pre and post-harvest food safety.

Mr. Aidan Connolly
Alltech
Aidan Connolly is Alltech's chief innovation officer and vice president of corporate accounts. Responsible for the commercialization of Alltech's global research in addition to the company's corporate account strategy, his expertise is in branding, agriculture and international marketing.

NUTRITION 2.0:
THE INCREASINGLY COMPLEX WORLD OF HOW NUTRIENTS WORK

B. J. Bradford
Department of Animal Sciences and Industry
Kansas State University

INTRODUCTION

The first century of nutrition science focused heavily on the core nutrients required to sustain growth and basic health. Through these efforts, the 6 nutrient classes were defined and the most problematic nutritional diseases were understood. So why is it that, well into the 2nd century of nutrition science, we continue to carry out so much nutrition research? In this paper I will argue that we are watching "Nutrition 2.0" mature, and that this more complex view of nutrition has changed how we should approach diet formulation.

NUTRITION 1.0

The traditional view of nutrition classifies nutrients as fuels, building blocks (amino acids and some minerals), and enzyme cofactors (mostly vitamins and minerals). This paradigm (Fig. 1) has obviously been fruitful, allowing humanity to largely eliminate scourges such as scurvy and beriberi while developing relatively precise estimates of nutrient requirements to support animal productivity. Energy systems, though imperfect and subject to misuse, provide a practical means to incorporate multiple nutrients into a single requirement, reflecting the fuel flexibility of animals.

Nevertheless, this paradigm clearly fails to reflect the full impact of nutrients and non-nutritive feed components on animals (Bradford et al., 2016). For ruminant nutritionists, and increasingly monogastric nutritionists as well, one obvious gap in this simple view of nutrition is that it has no direct way of accounting for pre- or pro-biotic factors. Direct or synergistic effects of diet components can influence animals through enhanced digestibility or by altering physiology (i.e. gut health) to affect maintenance requirements. Consider how existing nutrition models incorporate well-established effects of feed additives; generally, these effects are "bolted on" to the model as a multiplier to some predicted nutrient supply, rather than being integrated into the model of the system. Beyond the impacts of feed additives, interactions of macronutrients within the gut lumen have drawn attention for years, with increasingly powerful analyses published recently (Weld and Armentano, 2017; de Souza et al., 2018).

One could defend the gaps above as being outside of the purview of the Nutrition 1.0 paradigm, as they cover pre-absorptive effects which primarily influence nutrients available to the animal (considering the gut lumen as external to the animal). However, absorbed nutrients also act as signals, and this is the key finding behind Nutrition 2.0.

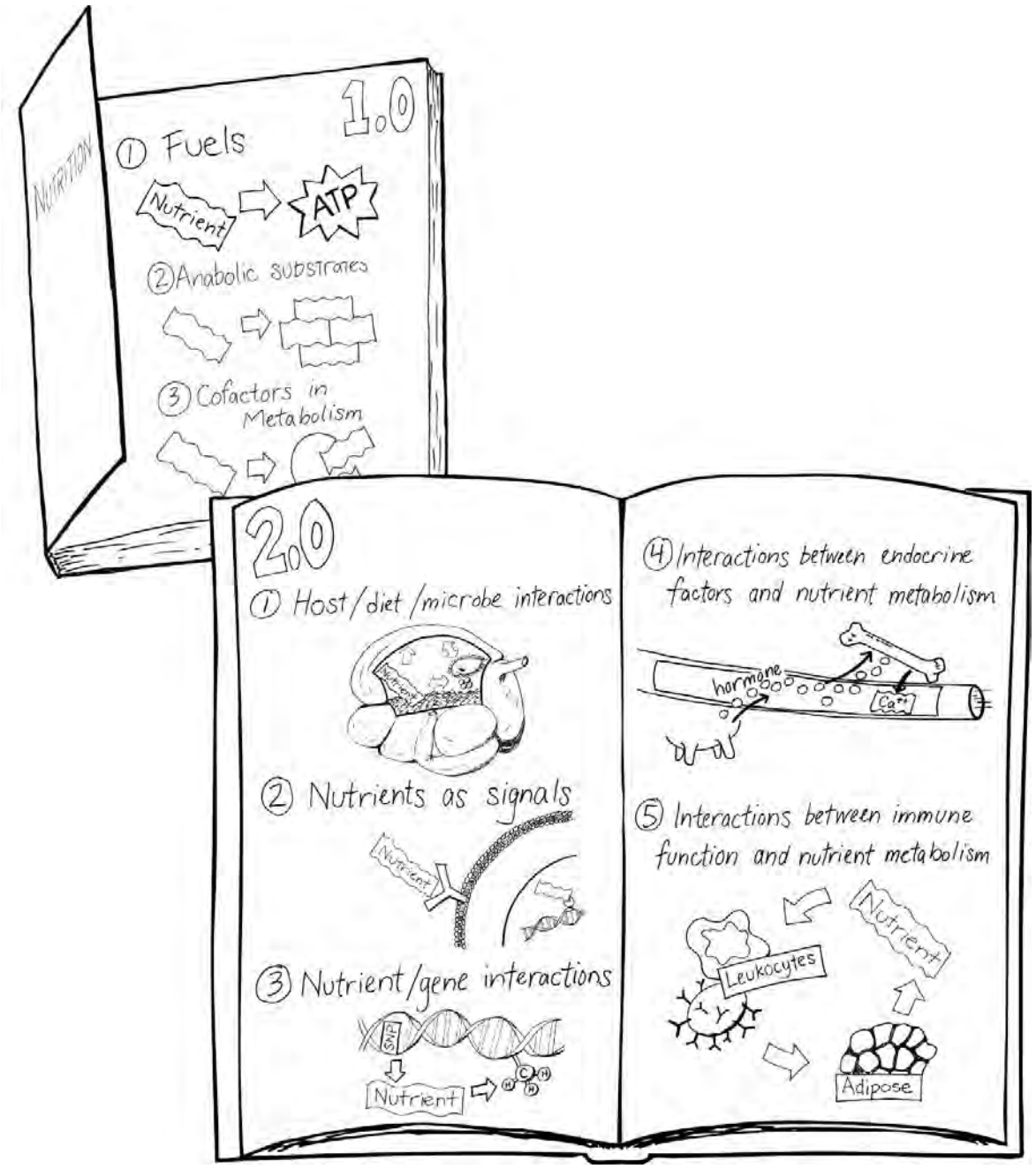

Figure 1. Early work in nutrition, continuing through the mid-20th century, revealed a variety of roles for nutrients that largely fit into 3 categories: fuels, anabolic substrates, and cofactors. This paradigm has been extremely useful for eradicating disease caused by gross nutritional deficiencies and for supplying the macronutrient needs of animals. However, discoveries in more recent decades have shown that Nutrition 1.0 was overly simplistic. These developments, collectively referred to as Nutrition 2.0, have revealed a variety of short- and long-term effects of nutrients, mediated by the gut microbiota, direct nutrient signaling, genotype-dependent interactions with the genome, interactions with an expanded list of metabolic hormones, and crosstalk with the immune system. These findings require a re-evaluation of how we think about diets. *Reprinted from Journal of Dairy Science, 99:4983 (2016), with permission from Elsevier and the American Dairy Science Association.*

NUTRIENTS AS SIGNALS

The most critical underlying biological phenomenon that upended Nutrition 1.0 is the discovery that nutrients can act as signals. This shift in thinking started with research into mechanisms underlying effects of polyunsaturated fatty acids on gene expression, which eventually revealed nuclear receptors that physically bind these fatty acids and then interact with DNA to influence transcription of target genes (Jump and Clarke, 1999). Prior to these discoveries, only signaling factors such as hormones, and drugs that mimic or disrupt these endogenous signals, were thought to work via such receptors.

For a while, fatty acids enjoyed some time in the spotlight as the only nutrients known to exert direct effects on cellular function. However, the release of the initial human genome sequence in 2001 led to the identification of nearly 1,000 genes encoding G protein-coupled receptors (GPR), most of them with unknown ligands and functions (Lagerström and Schiöth, 2008). In the decade after the publication of the human genome, a cottage industry of labs popped up to focus on characterizing the ligands and physiological roles of these “orphan” GPR. Somewhat surprisingly, a large number of these GPR were discovered to be sensors for nutrients (Efeyan et al., 2015). Cell-surface receptors have now been described for carbohydrates, amino acids, short- and long-chain fatty acids, purines, minerals, and vitamins. At this point, it’s clear that all nutrient classes have the ability to signal in an endocrine-like manner.

EPIGENETICS AND LONG-TERM NUTRIENT EFFECTS

Another startling shift in understanding has come from the discovery of epigenetics. Epigenetics refers to heritable changes in DNA beyond those encoded by the DNA sequence (Feeney et al., 2014). Several chemical alterations influence the 3-dimensional structure of chromatin (and thereby influence DNA’s accessibility for transcription), including DNA methylation and various histone modifications. Importantly, epigenetic marks are rewritten in specific tissues with changes in physiological state. For example, there are dramatic alterations in DNA methylation in the mammary gland at the start of lactation (dos Santos et al., 2015). Therefore, epigenetic mechanisms are potentially important as an unusual mechanism for inheritance but can also mediate long-term effects of events within an animal’s lifetime.

Nutrients are implicated in epigenetics in at least 2 ways. First, methylation is the primary chemical modification of DNA, and methyl donors therefore become very important in maintaining normal epigenetic status. For example, deficiency of folic acid, a required cofactor in methyl transfer reactions, led to altered sperm DNA and histone methylation in mouse sires and subsequent birth defects in their pups (Lambrot et al., 2013). There are other substrates for histone modification that could also potentially constrain epigenetic programming if they are limiting (Lempradl et la., 2015). Secondly, nutrients can regulate the activity of some enzymes that control histone and DNA modification, such as histone deacetylases. These mechanisms open up new

possibilities for long-term or even generational effects of nutrients on animal health and performance, as the fetal programming field has demonstrated.

NEW CONTROL MECHANISMS

Beyond these newly-defined roles for nutrients, the physiological systems that control them in the body have become much more complex, as well. For starters, deciphering the genome revealed unknown genes beyond the GPRs, and a surprising number have turned out to be hormones with some role in controlling metabolism. Furthermore, many of these hormones are not primarily produced by dedicated endocrine organs, but rather are released by organs with day jobs, including adipose tissue, liver, muscle, and even bone. The endocrine networks that regulate appetite, nutrient partitioning, and nutrient mobilization are far more complex than we imagined even in the 1990's.

In addition to the growing list of hormones exerting an influence on nutrient metabolism, we've also learned that immune cells resident in many tissues also regulate nutrient use (Iyer et al., 2015). For example, macrophages in adipose tissue provide negative feedback control of adipocyte lipolysis and alter the transition between white and brown adipose tissue. This is in addition to the broad systemic effects of systemic cytokine storms that occur during disease.

HOW DOES THIS INFLUENCE APPLIED ANIMAL NUTRITION?

Let's use a familiar nutrient to make the case for why these new discoveries alter the way we should think about diets and nutrition. Butyrate is a short-chain (volatile) fatty acid – the 3rd most abundant end-product of ruminal fermentation and also a major end-product of hind-gut fiber fermentation. According to Nutrition 1.0, butyrate can serve either as a fuel, generating ATP, or as a substrate for fatty acid and/or triacylglycerol synthesis. Fair enough – these are the primary fates of butyrate. But a lot of interesting things can happen along the way! We now know that intestinal butyrate serves as a ligand for several cell-surface GPR, resulting in alterations in gut peptide release, T cell development, epithelial cytokine signaling, and other physiological functions (Natarajan and Pluznick, 2014). Additionally, butyrate inhibits histone deacetylase activity, resulting in sustained impacts on chromatin structure. Ultimately, these effects combine to improve gut barrier function and enhance tolerance to commensal microbes, generating a loop whereby microbe-derive butyrate ends up permitting continued, unhindered colonization.

This single example neatly demonstrates how a humble fuel like butyrate – with a little assistance from gut hormones and immune cells – can have dramatic effects on tissue responses to the diet through its role as a signaling factor and epigenetic modifier. Suffice it to say that simply plugging in the ME value for a feed butyrate would not completely capture its potential function roles in the animal. Now, multiply this by all of the functional components of diets (and their metabolites), and you can see that Nutrition 2.0 will be providing plenty of questions for research in the coming years.

With all of this potential in mind, it is important to keep things in perspective. Although many functional impacts or “nutraceutical” applications are possible, our decades of experience in feeding livestock and conducting nutrition research has likely identified the most beneficial effects already. I would argue that the low-hanging fruit for taking advantage of these new discoveries is likely in feeding stressed animals. Whether it be heat stress, transitioning to lactation, or during an illness, there may well be functional effects of nutrients that could improve animal resilience, and it is less likely that we have discovered these tools already, because so little research has been done on nutrition during these traumatic times.

SUMMARY

The Nutrition 2.0 label is intended to point to the dramatic discoveries in the field over the past few decades that have fundamentally changed the way we think about nutrients. In addition to providing fuel, anabolic substrate, and enzyme cofactors, nutrients and other diet components serve as signals that activate specific receptors on many cell types, modulate epigenetic signatures on DNA to have long-term effects on animals and their offspring, and interact with a huge number of endocrine and immune regulators. Combined with our growing understanding of the microbiome, these discoveries complicate how we should think about diets, but also provide opportunities to improve animal health and performance, particularly during windows of stress.

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APPROACHES TO MITIGATING ON-FARM PATHOGEN BURDENS

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Diamond V

INTRODUCTION

Various pathogens can cause disease, reduced productivity, and mortality in livestock. All of these issues are a concern because they negatively influence animal welfare and farm profitability. However, a far greater concern is when any pathogens that originated from the dairy or feed yard cause sickness or death in people.

This paper is not meant to be all encompassing in pathogen management. A primary objective is to introduce, or re-introduce, the epidemiologic triad and to consider the importance of pathogen load in many of our disease control programs. The relationship between substrate supply and alterations in the microbiome of the digestive tract will be discussed, along with management and dietary approaches that can help stabilize the microbiome throughout the digestive tract.

THE EPIDEMIOLOGIC TRIAD

The epidemiologic triad depicts the fundamental relationship and interactions among the host (the cow), the agent (the pathogen), and the environment in which they reside (Figure 1). The “outcome” of these interactions, whether or not disease occurs and its severity, depends on the pathogen (infectious dose needed for disease, pathogen load and exposure), the host (innate and adaptive immune defenses), and how the environment is influencing both of these (stress events like overcrowding, high temperatures, poor cow comfort and bunk management; ventilation, humidity, management practices, etc.). Most conventional pathogen disease control programs primarily target the agent, but may also have recommendations for the host or the environment. For example, a control program for *Streptococcus agalactiae*, a contagious mastitis organism that only lives in the udder, will include milk culturing to identify infected cows. These animals will be treated with antibiotics to kill the pathogen, or culled, and recultured to ensure treatment success. A proper teat dipping program will be reviewed with milkers, again to reduce or eliminate the agent.

Johne’s control programs will also primarily focus on the agent, in this case *Mycobacterium paratuberculosis*. Cows will be fecal cultured or blood tested to identify infected animals. Positive animals may be culled or a separate calving area utilized. Colostrum won’t be pooled and may be pasteurized, or a colostrum replacer will be fed. Calves will quickly be removed from the calving area. Attempts will be made to keep manure out of feed, and not to feed lactating cow orts to youngstock. All of these approaches are designed to reduce exposure of the pathogen to the animal, especially the most susceptible calves and young heifers. The culling of positive animals, especially those that are heavily shedding the agent (*M. paratuberculosis*, in this case),

is designed to reduce the overall pathogen load throughout the environment and thus reduce the risk or degree of infection in other animals. Only rarely in this disease do we attempt to bolster the host's defenses against this specific pathogen with a vaccination.

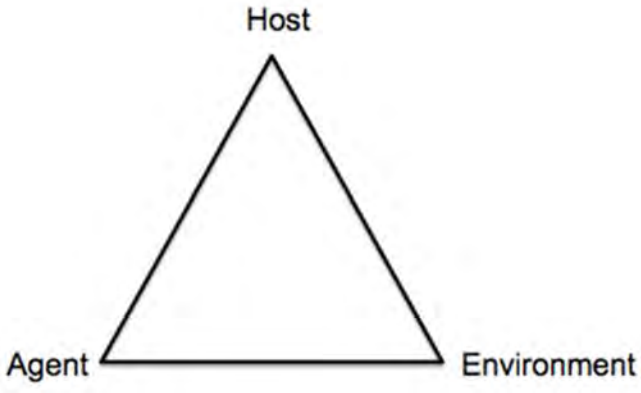

Figure 1. The Epidemiologic Triad

Pathogens from Feeds

A variety of pathogens can contaminate feedstuffs. Usually this contamination is related to manure coming into contact with the feedstuff. Manure contaminated irrigation water has been identified as the likely cause of *Salmonella* isolated from multiple feedstuffs on California dairies. Fortunately for dairies in the northeastern US, the acidic environment that occurs when forages go through a proper fermentation is toxic to many pathogens (*Escherichia coli, Salmonella, Listeria monocytogenes, Bacillus* and *Clostridium*) (Queiroz et al., 2018). There could still be problems, however, if silage does not go through a proper fermentation, or if there is excessive aerobic exposure during storage or feedout. There is also concern with the survival of *M. paratuberculosis*, the causative agent of Johne's disease, when manure is applied to alfalfa or grass fields following harvest. This is more of a concern with grass than alfalfa since regrowth of grass occurs from the cut blades, while alfalfa regrowth occurs from the crown. Fortunately, steps can be taken to reduce this risk. Spread manure on fields immediately after harvest and before regrowth begins; spread liquid manure as opposed to high solids manure to allow for the spreading of a relatively thin layer; limit applications to less than 5,000 gallons per acre per cutting; and finally try to avoid feeding silage and hay that did have manure spread on it following the prior cutting to heifers less than six months of age (Ev Thomas, Oak Point Agronomics, Ltd.).

Disruptions in the Microbiome

Our dairy industry contends with microbial agents that are commonly or always commensals in cattle, but can be extremely pathogenic when ingested by people. The microbes *E. coli* O157:H7, many types of *Salmonella*, and *Campylobacter* all fall into this category.

Diez-Gonzalez et al (1998) caused a great stir by indicating that fecal *E. coli* populations decreased 1,000 fold when cows were switched from a feedlot type of ration to one containing only grass hay. The authors theorized that starch eluding ruminal fermentation was being hydrolyzed in the hindgut, and the resulting sugars were then providing a medium for the growth of *E. coli.* The feeding of hay eliminated the hindgut starch source, and hence reduced the load of *E. coli*. In a review of studies evaluating the effects of forage feeding on *E. coli* populations, Callaway et al. (2003) concluded the following: "…abruptly switching cattle from a high grain ration to a high-quality hay-based diet has been shown to reduce generic *E. coli* and *E. coli* O157:H7 populations, but the magnitude of reductions has varied among studies. Switching all feedlot cattle in the United States from grain based diets to hay prior to slaughter is not currently feasible, in spite of the potential benefits." Reducing the *E. coli* O157:H7 fecal bacterial load (colony forming units per gram of manure) being shed would result in lower hide counts, thus reducing the risk of meat becoming contaminated.

Hemorrhagic bowel syndrome (HBS) leads to acute death in animals affected with the disorder. A proposed etiology for HBS is that an atypical amount of starch reaches the small intestine (Kirkpatrick et al., 2001), leading to an overgrowth of *Clostridium perfringes* type A, the release of toxins, reduced intestinal motility, cellular damage, hemorrhage, and finally the formation of a large blood clot in the lumen of the intestine. Sorting is likely a primary cause leading to the consumption of the atypical amount of starch.

These are both examples of the proliferation of a microbe that is part of the normal flora. The proliferation is thought to be caused by a change in diet, albeit in these examples one a chronic change and the other acute, which resulted in a change in the microbiome and either a large increase in bacterial shedding (the *E. coli* example) or disease (the HBS example).

Researchers have looked for ways to enhance the stability of the ruminant digestive system. One hypothesis is that a more stable microbiome throughout the digestive tract could enhance animal performance and, through this microbiome stabilization, reduce the overgrowth of undesirable microbes.

STABILIZING THE DIGESTIVE TRACT

Management Approaches

The technical support team at Diamond V, under the guidance of Dr. Tom Oelberg, developed the TMR Audit system. The results of thousands of audits have identified the ten most common reasons for variation in the TMR (adapted from Oelberg and Stone, 2014).

1. Equipment wear (augers, kicker plates, knives, etc.)
2. Mix time after loading the final ingredient
3. Load size

4. Levelness of mixer during mixing
5. Vertical mixer auger speeds
6. Liquid distribution
7. Restrictor plate settings in vertical mixers
8. Loading sequence
9. Loading position on the mixer box
10. Hay/straw quality and length prior to mixing

It follows that variation in the TMR could lead to variation or alterations in both nutrients the animal receives and the microbiome, which could adversely influence performance. Indeed, an improvement in production and components, along with a reduction in MUN variation, was observed in a case-study when TMR consistency was improved (Oelberg and Stone, 2014)

Dietary Approaches

Research was conducted to determine if *Saccharomyces cerevisiae* fermentation products (SCFP) could enhance ruminal stability; subsequent research would evaluate if this stability, along with subtle changes in the microbiome, was associated with changes in the shedding of fecal pathogens.

Lunguski et al. (2009) supplemented eight cows with a SCFP (Diamond V XP, 56 g) or the same amount of grain in a crossover design with 28 day periods. The starch source was abruptly switched from finely ground corn to highly fermentable high moisture shelled corn (HMSC) on the last 2 day of each period. When supplemented with SCFP, cows maintained milk fat levels and increased in FCM during the starch challenge, while fat percent and FCM decreased when they were not.

As a follow-up to this trial, Sun et al. (2016) supplemented six cows with a SCFP (Diamond V XPC, 14 g) or nothing in a crossover design with 28 day periods, and a 14 day washout between periods. Ruminal fluid was collected for *in vitro* fermentation during the last day of each period. The batch cultures were supplemented with a diet consisting of 55% alfalfa hay and 45% corn. The corn source was either corn meal or HMSC. Corn oil was sprayed on the hay at the rate of 2% of DM to provide a source of polyunsaturated fatty acids (PUFA). The fermentations were also run at two pH levels, 5.8 and 6.2. Rumen fluid obtained from cows during the periods supplemented with SCFP resulted in a 17% reduction in the level of trans-10, cis-12 CLA, one of the most potent fatty acids in causing MFD, compared to the periods without supplementation ($P < 0.001$). Additionally, the amount of linoleic acid that was fully biohydrogenated to stearic acid was increased from 38 to 41% ($P < 0.001$) when the rumen fluid was obtained from periods where SCFP was added to the diets. Both an altered rumen microbial system and the dietary inclusion of PUFA are necessary components in the formation of fatty acids like trans-10, cis-12 CLA (Bauman and Griinari, 2000). The results observed here, the reduction in trans-10, cis-12 CLA and the increase in stearic acid, indicate that a more “normal” fermentation occurred when SCFP was part of the diet.

Shen et al. (2018 and personal communication) top-dressed the TMR of five beef heifers (BW = 561 kg) with 90 g of a grain-molasses mix (Control = Cont), or the same grain mix with monensin (330 mg/d) and tylosin (110 mg/d) (ANT), or a SCFP (NaturSafe, 18 g/d) or two treatments with duodenal infusions of SCFP. The treatments were split and top-dressed twice daily. The heifers were fed a TMR averaging 87% dry rolled barley and 10% barley silage, with a composition of 29.7% NDF and 52.8% starch. The 5 x 5 Latin square design had 28 day periods, including 21 days for adaptation and 7 days for data collection. Ruminal pH was monitored continuously for 4 d from days 22 to 26 of each period. Focusing on the treatments that could be applied in the industry (top-dress as opposed to duodenal), mean ruminal pH did not differ between Cont, ANT, and SCFP, (5.77, 5.81, and 5.96, respectively), but the minimum pH was higher for SCFP than the other treatments (5.02^{c}, 5.13^{bc}, and 5.3^{a}, respectively). Additionally, the h/d that the pH was < 5.6 was significantly reduced in the SCFP periods as compared to the Cont and ANT periods (11.6^{a}, 10.4^{a}, and 5.6^{b} h/d for Cont, ANT, and SCFP, respectively; $P < 0.04$). Ruminal and total tract NDF digestibility followed the pH results, with total tract NDF digestibility of 56.2^{b}, 55.1^{b}, and 67.7^{a} for the Cont, ANT, and SCFP treatments, respectively.

Further evidence of a ruminal stabilizing effect of SCFP came from research done by Scott et al. (2017). In this trial, nearly 1,500 crossbred beef heifers averaging 359 kg were assigned to Cont (300 mg of monensin, 90 mg of tylosin, and 50 mg of the direct-fed microbial Bovamine Defend) or Treatment (Trt) diets (18 g of SCFP; NaturSafe, Diamond V). There were 10 pens per treatment, and pen was the experimental unit. Animals were on feed for 136 d before slaughter. There were no differences in final bodyweight (579.7 and 580.6 kg), DMI (10.3 and 10.4 kg/d), ADG (1.64 and 1.63 kg/d) or G:F (0.159 and 0.156) for Cont or Trt pens, respectively. There were no differences in yield grades. Finally, a numerically greater percentage (20.9%) of Cont animals developed liver abscesses (LA) as compared to Trt (16.3%) ($P = 0.27$).

In a recent review on liver abscesses (2015), Reinhardt and Hubbert noted that the mean prevalence of total liver abscesses in conventionally managed US feedlot cattle, which includes the feeding of tylosin and monensin, commonly ranges between 10 and 20%. Feeding tylosin reduces the prevalence of LA by 40 to 70%. *Fusobacterium necrophorum* is thought to be one of the primary culprits in causing LA, and tylosin reduces the ruminal concentration of *F. necrophorum* by 80 – 90%. Interestingly, *F. necrophorum* prefers lactate as a fermentative substrate as opposed to sugars. The pathogen is thought to exit the rumen through damage done to the epithelium from a low rumen pH. It is then transported to the liver, where bacterial growth commences followed by the subsequent liver abscess.

Based on the previous research we have reviewed, it is entirely possible that the lack of an increase in liver abscesses in the Trt animals described above, who were not receiving any tylosin, monensin, or a DFM, was due to a stabilization of the ruminal microbiome. This enhanced ruminal resiliency would have resulted in a higher ruminal pH, less lactate and thus less substrate for *F. necrophorum,* and a healthier ruminal

epithelium which would be less likely to allow for passage of *F. necrophorum* through the epithelium and to the liver.

Effects on Pathogen Shedding

Clearly, these feed additives can alter ruminal dynamics and help to stabilize the ruminal environment. Can this more stable environment have an effect on the shedding of pathogens?

In a blinded study, Brewer et al. (2014) fed forty 2 to 8 day old calves either SCFP in their milk replacer and as a bolus (Diamond V SmartCare and XPC; Trt, n = 20), or a bolus containing the same grain matrix as that used in the manufacture of XPC (Cont, n = 20) for two weeks before and three weeks after challenging them with *Salmonella enterica* serotype Typhimurium. Diarrhea scores, rectal temperatures, and fecal cultures were conducted throughout the trial. All calves were euthanized three weeks after the challenge, and samples of rumen and ileum were collected for histology and culture. Following the challenge with Salmonella, Trt calves had significantly lower rectal temperatures and diarrhea scores, and had negative Salmonella fecal cultures sooner than Cont calves. Additionally, there was a significant reduction in the *Salmonella* load in the ileum tissue collected at necropsy in the Trt calves, and their rumen papillae were significantly longer. Trt calves also grew significantly more than Cont calves post-*Salmonella* challenge.

Liou et al. (2009) randomly assigned eight yearling Charolais heifers to either a Control or Treatment diet. The diet of Cont animals was top-dressed daily with 200 g of ground barley, while the Trt heifers were top-dressed with the same grain mix, but where a SCFP (Diamond V XP, 56 g) was substituted for an equal amount of grain mix. A recto-anal junction mucosal swab (RAMS) was obtained approximately weekly from all heifers beginning at d –31 and cultured for *E. coli* O157:H7 to ensure that all heifers were negative for the pathogen; each animal was cultured and found to be negative six times before the challenge day. After 30 days on trial, all animals were challenged with a bolus dose containing 10^{10} CFU of *E. coli* O157:H7. The dose was placed directly into the rumen through the rumen cannula. Ruminal samples were collected 2, 6, 12, and 24 h post-inoculation, while they and RAMS were collected approximately every three days for the next 31 days and cultured for *E. coli* O157:H7. On the day of the challenge, *E. coli* O157:H7 was isolated from the rumen samples of all animals, and there were no significant differences in counts. However, by 24 h the Trt heifers had *E. coli* O157:H7 counts that were nearly one order of magnitude lower than Cont ($P = 0.04$). Only two of the Trt heifers were positive for the RAMS cultures from d 11 on, while all four of the Cont heifers were RAMS positive through d 25.

In a companion paper to Scott et al. (2017), Feye et al. (2016) swabbed the recto-anal junction of 200 carcasses per treatment at the abattoir. Reductions in Salmonella and *E. coli* O157:H7 were observed in animals fed the Trt (18 g of SCFP; NaturSafe, Diamond V) as compared to the Cont (tylosin, monensin, and DFM). Work is now being done to replicate this research in other feedlots and in commercial dairies.

SUMMARY

Pathogens are present on all farms. Some of these can be eliminated, while others can only be managed. A relatively stable environment throughout the ruminant gastrointestinal tract can allow the animal to perform normally and can influence the expression of the microbiome. Management techniques, ration design, and the use of researched feed additives can all assist in helping to stabilize the digestive ecosystem.

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PRECISON DAIRY FARMING IN THE FUTURE – CAN TECHNOLOGY REPLACE COW SENSE?

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OUTLOOK ON THE DAIRY INDUSTRY

It has been estimated that by 2067, the per capita consumption of dairy products is expected to increase from 87 kilograms (kg)/person to 119 kg (Koeleman, 2018). Most of us are well aware that the global population is set to increase by another 2-3 billion in the next 50 or so years. This means the dairy industry will need to produce 600 billion kilograms more milk (Koeleman). Today's dairy cow will either need to double her production, or we will need to dramatically increase the number of dairy cattle. We have seen some increase in productivity and over the last 25 years, have increased milk production by 61 percent (Carter, 2012) or about 2 percent per annum, but can we continue to grow sustainably?

The difference between a high and low performing cow can be considerable. We know that milk production is influenced by a myriad of factors including genetics and nutrition, consistency in mixing of feed, eating behaviors (such as sifting), social impact (other cows' bullying), water quality and environmental factors (heat). Trying to monitor all of this information would be quite a job and as many farms are growing in size or consolidating, the task itself becomes quite monumental. With fewer people interested in farming than has been the case in the past, managing for all of these circumstances and gathering data is nearly impossible. As a result, the information farmers are often basing their management decisions off of is determined by herd averages and not individual cows.

This has resulted in an obvious gap of data for producers. Farmers, particularly of larger dairy operations, are not aware of the details pertaining to each individual cow: how much is she eating, how much water is she drinking, what is her temperature, what is her stress level, etc., are all pieces of the puzzle that could help to determine what is affecting production, which often itself isn't documented consistently.

There is a way to bridge this gap that will enable farmers to manage their herds based on individual data, operating at a detailed level allowing them to not only determine the following factors, but also maintain accurate data to alleviate prosumer concerns regarding animal welfare and sustainability:

- Determine a cow's status in terms of comfort, illness, etc.
- Select the best animals for breeding and retaining
- Judge true profitability
- Increase milk production

The advent of new technologies in the agriculture world brings with it incredible opportunity. The “essential eight” as previously identified by PricewaterhouseCoopers (Huff Eckert) include artificial intelligence, augmented reality, blockchain, drones, the Internet of Things, robots, virtual reality and 3D printing. These eight technologies can be applied also to the agriculture industry, particularly if there is an adaptation to include sensors as a hardware, thereby moving IOT as a connecting software technology that enables the interconnectivity of the others (Figure 1).

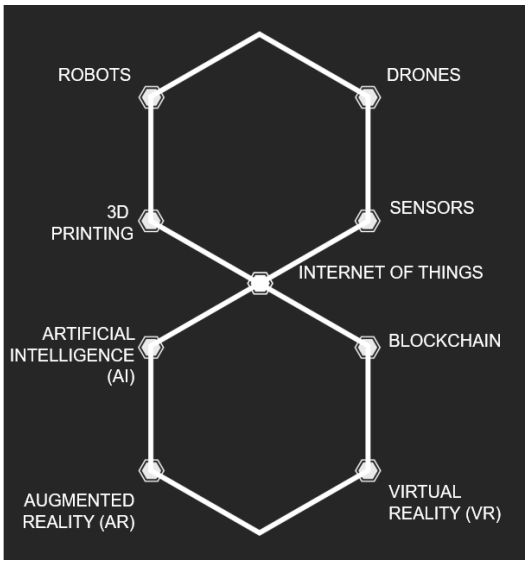

Figure 1. The eight technologies as they best apply to agriculture.

These eight technologies in agriculture allow for the greatest opportunity for the dairy industry to gather precise, real-time smart data. Some apply to the industry more obviously than others, but they all could, in some way, affect the farmers and producers in some manner that could assist in improving production and efficiency.

BRIDGING THE DATA GAP: THE EIGHT DIGITAL TECHNOLOGIEs

Hardware Technologies

These technologies are represented by the top portion of Figure 1 and include robots, drones, sensors and 3D printing. For many, the applications of these, particularly robots and sensors, is already fairly obvious. Other technologies, such as 3D printing require a little more understanding and imagination.

Robotic milking machines are probably the most well-known application for robots in the dairy industry. Their efficiency is well proven: One robot can milk 60 cows in a day (Mulvany, 2018). Automated milking systems (AMS) are often designed to allow cows to choose their own milking times, often procuring three or more milkings in one day (Dickrell, 2014). Less human handling generally causes less stress for cows as well. AMS find and clean the udders, aiding in proper hygiene techniques which is safer for the cow and better for production. These machines come in a variety of types and often are able

to assess vital information for daily documentation of metrics, allowing for individual analysis and better understanding of each cow's health and productivity.

Currently, the high cost of implementation, about $180-200K per machine, is the primary deterrent, but proper cost-benefit analysis would reveal in what timeframe these machines would begin to pay for themselves. Dickrell cites three variables as the primary evaluation metrics: labor savings, increases in milk production and milk prices (2014). The first is becoming increasingly easier to determine as labor costs are increasing and AMS machine costs are decreasing as the technology improves and the competition grows (Laine, 2018). Despite this, adoption will take time; thus far, only about 5% of US dairy farms have AMS (Mulvany 2018).

Robots are also used to clean and sanitize the barn, allowing for better biosecurity measures which will lead to healthier conditions for the cows, and also to feed cows, both of which can be performed completely autonomously on a scheduled basis that works in rotation with the rest of daily operations on the farm.

Drones are the aerial cousins of robots. Their primary purposes in the dairy industry would be that of inspection. In herd management, drones allow farmers to monitor the herd using thermal imaging (Black, 2017) to detect abnormal behavior often representing lameness, illness or calving. Drones, which could be flown by the farmer or autonomously, could also be used for inspecting the land, including fence lines for repair and to measure pasture growth using visual sensors. These visual sensors have also proven quite useful in surveying land and measuring pasture growth. There is also potential for drones to herd cattle as well. Drones may become more useful in these areas, particularly if battery life is prolonged and autonomous flying ability is improved.

The third hardware technology that offers benefit to dairy producers is 3D printing. Most likely the primary application would be for producing machine parts onsite. This may be of particular interest to rural farmers, saving valuable time both in travel and in downtime of the machine.

The last hardware technology offers the opportunity for the clearest cost/benefit analysis. That's because more than any other technological advancement, sensors can fill the data gap in dairy farming. Sensors allow farmers to monitor in real-time an individual cow's health, determining what "normal" is for each cow and allow for comparisons across time and with other members of the herd. With the information collected by sensors, producers are able to determine individual illness or lameness, substantially increasing the likelihood that quick corrective action will reduce the risk to other cows.

The most common sensors are wearables, which are similar to a Fitbit for humans and typically worn on the ears, neck, legs or tail. Depending on the brand, they monitor any (or all) of the following: temperature, heat detection, calving, locomotion or position in the field (GPS). Some more advanced sensors are designed to be implanted subcutaneously or as a bolus inside the rumen to monitor rumination and detect issues

such as rumen acidosis. Others are meant to detect symptoms of mastitis, a common ailment in dairy cows that can severely impact milk production. These sensors are designed to catch critical symptoms early on, thereby increasing chances of alleviating the issue before milk production is impacted.

Outside of the wearable market, sensors are also used to detect various other aspects of farm data. Sensors in grain bins can determine receding levels, grain content and mixture analysis. Sensors can be used to evaluate milk and determine milk fat, protein, somatic cell count, progesterone and antibiotic residues. Others monitor milk flow rate, quantity, temperature and then transfer the data to a management system for evaluation. Some use strictly sensors, others combine with NIR (near infrared) spectroscopy.

Software Technologies

All the information obtained by sensors is practically useless without analysis and interpretation. This is where artificial intelligence (AI) comes into play. Using machine learning and algorithms, computers are able to analyze data, predict outcomes and provide prognoses in order to help farmers make critical decisions for better production, improved efficiencies and to combat potential issues. In short, with the help of AI, big data has the potential to transform how a dairy farm operates.

Many of the sensor companies include this type of analysis in their programs. Often information can be accessed via tablet or smartphone, so farmers can see in real-time each cow's metrics and overall herd information.

Data can be collected for interpretation through means other than sensors, even using the computer itself. Some companies, such as Cainthus, use facial recognition software to monitor each cow's daily activity. Cameras installed in the ceilings of dairy barns check each cow and observe their habits including feed and water intake, where she likes to eat, if she's been bullied or if she's laying down too much. The software can alert farmers of early signs of lameness. Cargill has even taken a significant minority investment in Cainthus, indicating the approach as one of high interest and value.

Two similar software technologies include augmented reality (also known as mixed reality) and virtual reality. Augmented reality (AR) is the display of virtual images on a person's real field of vision. This can include visual interpretations of milk production, grain product or analysis, animal health monitoring or really any aspect of the farm operation that can be detected and measured would prove viable for use with artificial intelligence.

There is a phenomenal YouTube video (AgroTechICT, 2009) that perfectly displays this concept. Envision a farmer walking through his farm with a pair of glasses on. As he looks at his grain silo, it is displayed in his vision, the moisture content, mixture, capacity and any other relevant information he would be keen to know about that particular aspect of his farm. As he walks into his barn, and gazes at each cow, he is

informed immediately of her health statistics, milk production, 24-hour water consumption etc. AR truly has the ability to give farmers a completely different role in their day to day operations. Its immediate impact is probably small however, given that it would require significant investment on the part of other technologies, such as sensors and AI in order to implement.

Virtual reality (VR) also shows some promise, though it might be more welcome in the fields of learning and training rather than operations, particularly given its cost. VR is described as a digital environment which can be interacted with in a seemingly real way using electronic equipment. Its applications in the dairy industry vary from farm tours to veterinary training, with positive impacts on safety and efficiency. For example, in dairy farm training, VR can be used to allow trainees to navigate the farm, manufacturing facility or distribution site from a safe location and without interfering with the operations of the location. The headset itself replaces a human trainer and thus a person isn't required to walk the new employee through the facility, potentially saving costs in personnel as well as the previous positive benefits, safety and continued operations.

From a veterinary perspective, VR can be used to safely teach students about the reproductive tracts of the cow. Simulations allow students to navigate these internal structures without actually requiring a cow's presence and cooperation (Baille, 2016). Students can practice fertility examinations including pregnancy detection or evaluate reproductive concerns in a safe environment.

VR can also help to unite the producer and consumer. There is a disconnect between consumers and the agriculture industry, particularly their understanding of where their food comes from. Allowing consumers to virtually visit farms to better understand how operations work would be an advantageous aspect of virtual reality for the dairy industry and indeed, all of agriculture. Giving consumers an opportunity to experience firsthand how a dairy farm operates is an important component of influencing perception of the industry.

Along this same concept of consumer understanding would be where blockchain could come into play. This technology would help to instill traceability and trust to all members of the food supply chain. Particularly in the dairy industry, where the production chain is not as extensive as in other industries, such as beef, could blockchain help to alleviate some consumer concerns as well as general trust within the entire sector. The primary difficulty with adapting this technology is of course getting all members on board. This may take a few years to implement, but large corporations such as Walmart and IBM who are working together do help to drive interest and therefore increase the probability of this technology coming about more quickly.

These four hardware technologies and four software technologies are interconnected by the Internet of Things. This is how the information is transferred. It enables a sensor's data to be transmitted to a computer for interpretation; it enables that data to be analyzed and interpreted; and it enables the computer to then send prognosis

to the farmer for review. IoT and the other technologies can greatly influence the dairy industry most clearly in their ability to increase efficiencies, profitability and production.

Using the data

Previously farm management decisions were based on herd metrics or general dairy expectations and insight. With the advent of these technologies, farmers are able to use real-time, individual data to make decisions about each cow individually, but quickly and efficiently. New information is also available with sciences such as nutrigenomics and the understanding of a cow's nutrition and how it affects the genome is much more clearly understood. What nutrigenomics research has demonstrated is that specific nutrients and the inclusion of enzymes can greatly impact milk yield, develop healthier cows and thereby increase profitability significantly.

The future of dairy is nothing like what has been experienced in the past. So quickly now are the changes in agtech and the opportunities for new insight and understanding that farmers will be operating a connected farm most likely in the next 10 years. This opportunity to "farm" data, both new and historical, is what will enable farmers to bridge the data gap and improve dairy production through a new, digitized means of operation.

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RELATIONSHIPS BETWEEN UNDIGESTED AND PHYSICALLY EFFECTIVE FIBER IN LACTATING DAIRY COWS

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INTRODUCTION

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

Waldo et al. (1972) recognized that NDF needed to be fractionated into digestible and indigestible pools for calculation of digestion rates. The recognition that there is an indigestible portion of fiber led to research that improved our understanding of the digestibility of fiber in ruminant diets and the beginning of dynamic models of fiber digestion. 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) plus a fast- and 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 fiber particle are important for explaining ruminal fiber 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 physically effective NDF (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 among forage sources.

Recently, we have focused on the relationship between undigested and physically effective NDF at the Institute, and have conducted a study designed to assess the relationship between dietary uNDF240 and particle size measured as peNDF. The potential interaction between peNDF and uNDF240 is 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?

- Similarly, 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 fiber: forage or non-forage, since they differ dramatically in fiber digestion pools and particle size. Some nutritionists have even questioned how important particle size actually is as we better understand fiber fractions (i.e., fast, slow, and uNDF240) and their rates of digestion. This is a complicated question, but the short answer is – yes – particle size is important, although maybe for reasons we haven't always appreciated, such as its effect on eating behavior even more so than rumination.

MINER INSTITUTE STUDY: UNDIGESTED AND PHYSICALLY EFFECTIVE FIBER

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 versus ~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).

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 h 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).

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 fiber fractions. The lower uNDF240 diets contained about 47% forage and the higher uNDF240 diets contained about 60% forage on a DM basis (Table 1).

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). In Table 1 we see that this value ranged from 5.4% of DM for the lowUNDF240/low peNDF diet to 7.1% of DM for the high uNDF240/high peNDF diet. And by design, the two intermediate diets contained 5.9% of ration DM. A key 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. We are currently addressing that question in our Forage Research Laboratory at the Institute.

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 represent a range in particle size and indigestibility that would reasonably be observed in the field for these types of diets. And most importantly, we wondered if the two intermediate diets would elicit similar responses in DMI given their similar calculated peuNDF content.

In fact, 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/d. 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 versus lower uNDF240 diets. But, the important take-home result is the 0.45% of BW intake of uNDF240 for cows fed the high uNDF240 diet with hay that had been more finely chopped. The intake of peNDF was driven first by the uNDF240 content of the diet, and then by particle size within each level of uNDF240 (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/peNDF diets, respectively. And 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 fiber intake for cows fed diets differing in uNDF240 and peNDF.

	Low uNDF240[1]		High uNDF240		SE	*P*-value
Measure	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 h 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

A key question becomes: does lactation performance follow these observed responses in feed intake? Generally, 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/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/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.

	Low uNDF240[1]		High uNDF240		SE	*P*-value
Measure	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/peNDF diet had the lowest milk protein percentage, with cows fed the low/high uNDF240/peNDF diet being intermediate (Table 3). The 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 ruminating time (Table 4). This observation that dietary fiber characteristics may have a substantial effect on chewing during eating and time spent eating has been observed in multiple studies. A recent review found that higher forage content, greater NDF or peNDF content, and(or) lower NDF digestibility may all increase time spent eating for a wide range of forages (Grant and Ferraretto, 2018). The cows in our study spent up to 45 min/d more or less eating depending on the diet (Table 4). In fact, cows on the high/high uNDF240/peNDF diet spent 45 min/d longer eating and yet consumed nearly 3 kg/d less DM than cows fed the low/low uNDF240/peNDF diet. An important and practical management question is whether or not cows would have sufficient time to spend at the bunk eating with greater dietary uNDF240 that is too coarsely chopped? And if we consider an overcrowded feedbunk environment, the constraint on feeding time could be even more deleterious.

Cows fed the high/high peNDF/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 min/d 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 ($P \leq 0.05$).
[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 rumination time is related to the observation that cows tend to chew a bolus of feed to a relatively uniform particle size prior to 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 versus diet offered (% retained on sieve; DM basis).

	Sieve size, mm						Mean particle size, mm
Diet	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 h 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 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 peNDF in our study.

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/peNDF diet having the slowest ruminal turnover of fiber. 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 fiber digestibility is lower than desired, then a finer forage chop length will boost feed intake and lactational response. The enhanced lactational performance was associated with less eating time as well as more desirable ruminal fermentation and fiber turnover for cows fed the higher uNDF240 diet with lower peNDF.

Table 6. Ruminal fermentation and dynamics of fiber turnover.

	Low uNDF240[1]		High uNDF240		SE	*P*-value
Measure	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, m*M*	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 ($P \leq 0.05$).
[xy]Means within a row with unlike superscripts differ ($P \leq 0.10$).
[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 AND COW RESPONSES

We have combined data from three 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 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 ration DM (Miller et al., 2017).

Details of ration formulation may be found in the references for each study. Importantly, all of the diets fed in these three 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 three 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 (corn silage with hay and fibrous byproducts) because more research is required with varying forage types and sources of uNDF (forage versus 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- versus grass-based rations. In fact, research has shown that very 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 grasses (Palmonari et al., 2014; Cotanch et al., 2014).

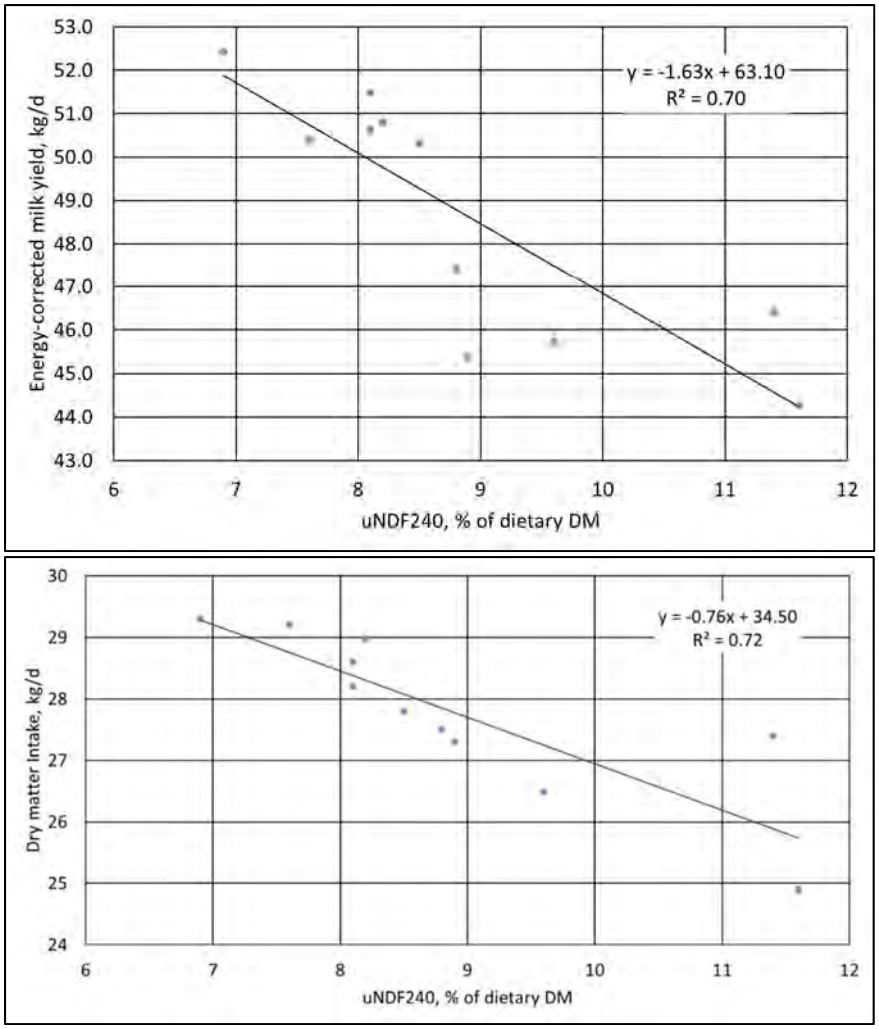

Figure 1. Relationship from three studies between dietary uNDF240 and DMI and ECM yield for cows fed diets based on corn silage, haycrop silage, and chopped wheat straw.

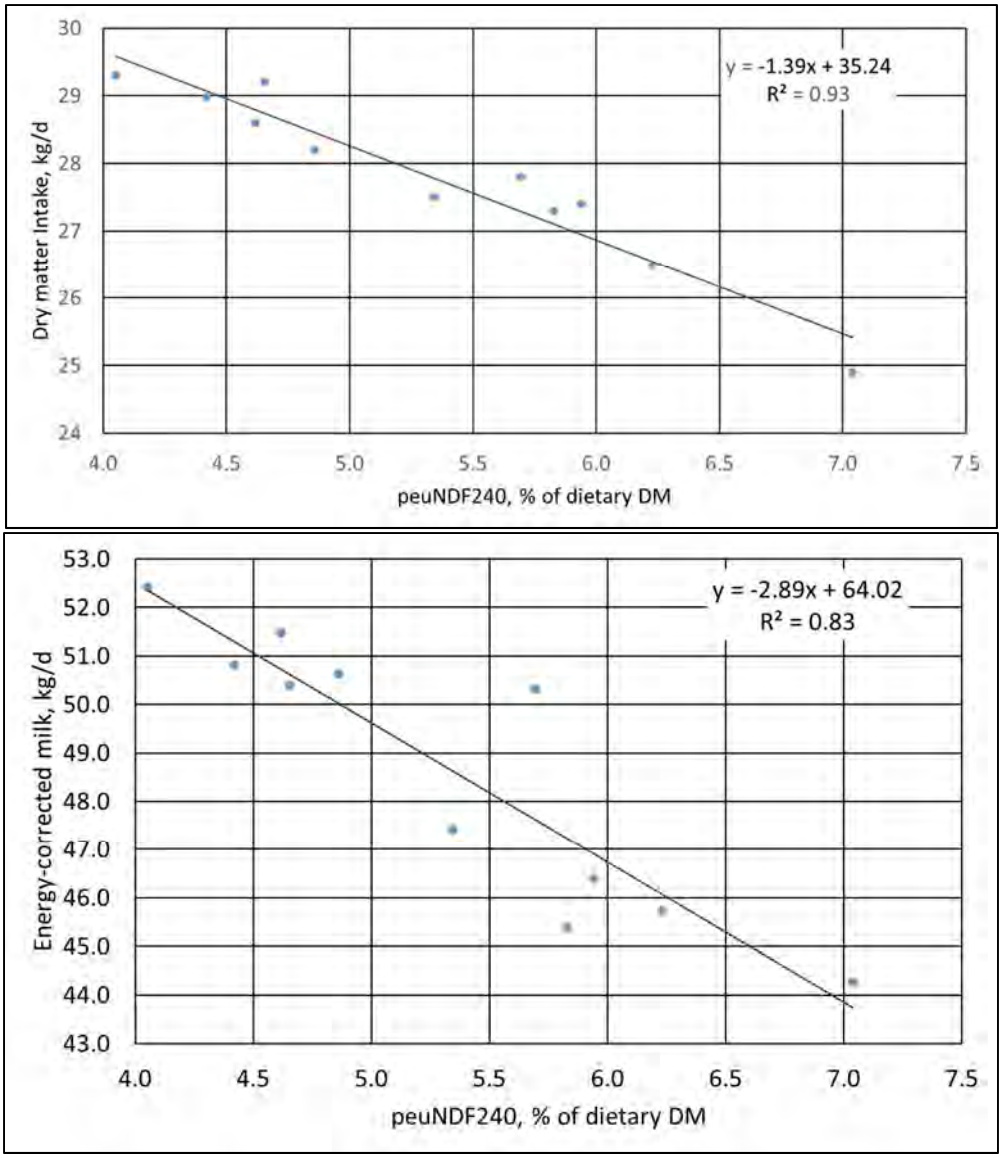

Figure 2. Relationship from three studies between dietary peuNDF240 and DMI and ECM yield for cows fed diets based on corn silage, haycrop silage, and chopped wheat straw (peuNDF240 = physically effective undigested NDF measured at 240 h of in vitro fermentation).

SUMMARY AND PERSPECTIVES: A TALE OF TWO FIBERS

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 coin 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 fiber digestibility is lower than desired, then 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 fiber.

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 fiber, it looks like we can have the best of times when we are able to integrate two measures of fiber – 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 US diet based primarily on corn silage.

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DETERMINATION OF FIRST LIMITING PHYSICAL FACTORS IN CORN SILAGE HYBRIDS: MODELING MULTIPLE POOLS OF RUMINAL aNDFom DIGESTION

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INTRODUCTION

The development of field usable models to describe nutrient supply and requirements has evolved and those models are now commonly used to evaluate most limiting nutrient supply on farms (NRC, 2001; Tylutki et al., 2008; Van Amburgh et al., 2015). To effectively use these models, characteristics of the forages, feeds, cattle and environment need to be inputted to establish the basis for the evaluation. Aside from the chemical composition of the forages and feeds being used, the other most relevant characteristics are the body weight (BW) and milk production of the cattle and their dry matter intake (DMI). From a modeling perspective, DMI is estimated by equations that are primarily developed using BW and energy corrected milk yield to drive demand (NRC, 2001; Tylutki et al., 2008). These equations account for approximately 60% of the variation in DMI and accordingly, other characteristics are used to describe intake such as particle size, feed availability, palatability, fermentation characteristics, cow time budgets, barn design, over-crowding and related functions that impact DMI (Grant and Albright, 2001; Allen et al., 2009; Gomez and Cook, 2010; De Vries et al. 2015).

Further, DMI can be affected by either physical or chemical fill mechanisms (Mertens, 1987; Allen, 2009) although few models have been able to fully describe these mechanisms. Among feeding systems, fiber has been described for its role in maintaining normal rumen health, chewing and rumination and milk composition (Mertens, 1997). Further, Mertens has indicated (Mertens, 1977; Mertens and Ely, 1979) that overall digestion is better predicted assuming that the potentially digestible NDF (pdNDF) fraction is the sum of two digestible fractions that can be described by two first order functions but with different rate constants. Following this, Ellis et al. (2005) demonstrated an improved fit of a two-pool pdNDF model that assumed two concurrently degrading sub-entities of pdNDF with different degradation rates. In addition, Huhtanen et al. (2008) has shown a marked improvement of a model when pdNDF was assumed to be comprised of rapidly and slowly degradable fractions.

The most recent version of the Cornell Net Carbohydrate and Protein System (CNCPS, v7) was developed based on these observations of multiple pools of aNDFom digestion. The model uses uNDF measured at 240 h in vitro digestibility (Raffrenato et al., 2018) and then partitions the aNDFom into two digestible pools, a “fast” and “slow” pool that are described relative to each other and digest concurrently with two distinctly different rates that most likely represent the degree of cross-linking between lignin and the carbohydrate fractions in the plant (Raffrenato et al., 2017). This approach, when

linked to the appropriate passage rate, appears to allow for a better description of rumen disappearance of aNDFom because the size of the pools can shift based on the agronomic effects on the growth of the plant and these shifts are not explained by a single pool, integrated rate of digestion or by the chemical composition of the plant. As the size of the fast pool increases, the rate of passage decreases, but the extent of digestion increases and in cattle fed high quality forages, a large percentage of the disappearance can be explained by microbial activity, independent of the chewing and rumination and particle size reduction.

Further, corn silage hybrids and varieties have been evaluated by many metrics that have been useful at selecting improved forages (e.g. Milk 2006; Schwab et al., 2003). Those approaches consider digestibility, starch content and also provide economic benefits for forage yield, which is an important variable, but do not consider factors that might impact DMI, such as characteristics that might affect physical fill. The approach they take for digestibility improves the prediction of energy intake by increasing DMI by 0.27 lb for every % change in 30 h NDFD. Given that digestibility can be different by growing season and this can impact both the total NDFD and the uNDF, it is likely that forages vary from year to year and these differences in digestibility are currently not accounted for with respect to what limits intake among seasons and this would be helpful as the development of diets would improve if these characteristics were accounted for.

MATERIALS AND METHODS

2017 NY and VT Corn Silage Hybrid Trials

In 2017 a total of 72 hybrids were evaluated in New York and Vermont from 16 suppliers (Table 1). Seed companies were invited to submit hybrids into either maturity group for a fee. The purpose of this trial was to provide independent, local data to aid in producer's decision making and consultation recommendations. Twenty-three hybrids were entered into the 80-95 day relative maturity group (Early-Mid) and were tested at two locations in NY (Hu-Lane Farm in Albion and the Willsboro Research Farm in Willsboro) and one location in VT (Borderview Farm in Alburgh). Forty-nine hybrids were entered into the 96-100 day relative maturity group (Mid-Late) and were tested at two locations in NY (Greenwood Farms in Madrid and the Musgrave Research Farm in Aurora) and one location in VT (Borderview Farm in Alburgh).

The average Growing Degree Days (GDD; 86-50°F system) from May through August for years 2005 to 2017 is 2031 GDD at Albion, 2025 GDD at Willsboro, 1971 GDD at Alburgh, 2071 GDD at Aurora, and 1939 GDD at Madrid (Table 2a and 2b).

All hybrids were planted using a two-row planter at 34,000 plants/acre. Each plot consisted of four 20' rows spaced 30 inches apart with harvest of the inner two rows. The early-mid hybrids were planted in Alburgh, VT on May 18th, in Albion, NY on May 17th, and in Willsboro on May 21st. The mid-late hybrids were planted in Alburgh, VT on May 17th, in Madrid, NY on May 18th, and in Aurora, NY on May 25th. Hybrids were planted in a randomized complete block design, with 3 replications. The Albion, NY site has a Hilton

loam soil type, was previously planted with soybeans and received 32 units N/acre at planting and an additional 132 units N/acre was applied as side-dress. The Willsboro, NY site has a Stafford fine sandy loam soil type, was previously planted with Fallow, received 15 units N/acre at planting and 90 units N/acre were applied as side-dress.

Table 1. Seed companies that participated in this hybrid evaluation process.

Hubner Seed	Dyna-Gro
Schlessman Hybrids	King Fisher
Growmark FS	Seedway
Dekalb	Augusta Seed
Pioneer	Wolf River Valley Seeds
Dairyland Seed	Doelber's
NK Syngenta	Channel
Masters Choice	Dyna-Gro

Both Alburgh, VT sites have a Covington silty clay loam soil type, were previously planted with corn and received 25 units N/acre at planting. Additionally, 125 units N/acre were applied as side-dress at both VT locations. The Aurora, NY site has a Honeoye silt loam soil type, was previously planted with winter wheat, and received 25 units N/acre at planting and an additional 107 units N/acre were applied as side-dress. The Madrid, NY location has a Stockholm loamy fine sand soil type, was previously planted with 4th year corn and received 94 units of manure N/acre prior to planting with an additional 32 units N/acre at planting. The Madrid site did not receive side-dress nitrogen.

The early-mid hybrids were harvested on Sept. 12th in Albion, Sept. 26th in Willsboro, and Sept. 20th in Alburgh. The mid-late hybrids were harvested on Sept. 20th in Aurora, Sept. 28th in Madrid, and Sept. 28th in Alburgh. From planting to harvest, the early-mid hybrids had 2004 GDD in Albion, 2131 GDD in Willsboro, and 1928 GDD in Alburgh (86-50 system). From planting to harvest, the mid-late hybrids had 1975 GDD in Aurora, 2087 GDD in Madrid, and 2077 GDD in Alburgh (86-50 system). The goal was to harvest all hybrids at about 65% (±3%) moisture at a target cutting height of 6 to 8 inches.

A sample, approximately 500 g, was taken in duplicate per plot replicate, resulting in 18 samples per entry across the three sites. Samples were sealed in a gallon-sized freezer bag and placed in a chest freezer with the addition of ice packs for transportation back to Cornell University or the University of Vermont where they were transferred to a -20°C freezer and/or shipped overnight for immediate analysis. One of the duplicate samples from each plot was kept as a retained sample while the other sample (9 samples/hybrid entry across the three sites) was submitted to Cumberland Valley Analytical Services (Waynesboro, PA) where NIR procedures were used to determine CP, starch, lignin, ash, total fatty acids (**TFA**), aNDFom, NDF digestibility (**NDFD**; 12, 30, 120, 240 h), undigested NDF (**uNDFom**; 30, 120, and 240 h) and 7-h starch digestibility. Several companies paid an additional fee for wet chemistry analysis on NDFD at 30 h.

Feed Chemistry Information and Management

All 648 replicates of the pre-ensiled material were submitted, as frozen samples, and a full suite of analysis was requested with aNDFom fermentation time point of 30h, 120h and 240h all by NIR analysis. Given the pre-ensiled nature of the forages, feed fractions such as soluble protein, ammonia, VFAs, organic acid, 7-h starch digestibility were discarded and replaced with the closest representative CNCPS feed library example of corn silage that matched the CP, starch content, aNDFom, ADF, lignin, ash, and the aNDFom digestibility. The corn silages were edited to reflect the chemistry and the digestibility of the trial forages.

Table 2a: The 2017 rainfall and growing degree days for the 80-95 RM corn silage varieties planted in New York and Vermont.

	Rainfall, inches			Growing Degree Days (GDD), 86/50		
	Alburgh, VT	Albion, NY	Willsboro, NY	Alburgh, VT	Albion, NY	Willsboro, NY
May	3.81	6.46	4.10	243	244	251
June	7.02	2.64	8.23	435	498	471
July	5.38	5.26	2.99	544	622	595
August	4.74	3.26	2.14	522	573	574
September	1.92	1.55	2.34	428	459	450
May-August	20.95	17.62	17.46	1743	1936	1890
May-September	22.87	19.17	19.80	2171	2395	2339
Average 2005-2017						
May-August	18.26	13.74	15.77	1971	2031	2025
May-September	22.30	16.90	18.77	2355	2443	2434

Table 2b: The 2017 rainfall and growing degree days for the 96-110 RM corn silage varieties planted in New York and Vermont.

	Rainfall, inches			Growing Degree Days (GDD), 86/50		
	Alburgh, VT	Aurora, NY	Madrid, NY	Alburgh, VT	Aurora, NY	Madrid, NY
May	3.81	4.54	6.88	243	253	249
June	7.02	4.14	5.84	435	467	453
July	5.38	6.99	6.76	544	595	555
August	4.74	1.56	3.81	522	529	514
September	1.92	2.29	2.05	428	424	407
May-August	20.95	17.23	23.29	1743	1843	1771
May-September	22.87	19.52	25.34	2171	2267	2177
Average 2005-2017						
May-August	18.26	14.28	16.97	1971	2071	1939
May-September	22.30	17.84	20.87	2355	2483	2320

In order to utilize the fermentation time points we calculated the rates and pool sizes for the aNDFom using the calculations of Raffrenato et al. (2018, accepted).

A large range in aNDFom and uNDF content and aNDFom digestibility among the corn forage samples was observed (Figure 1). This introduced complications as it was not feasible to evaluate all hybrids utilizing one standard diet at one initial intake level. Therefore, it was decided to split the data file into two distinct datasets, based on hybrid aNDFom content (roughly 30-40% and 40-50%). After assembling the feed analysis from each hybrid and a corresponding CNCPS corn forage template, a mass balance check was conducted for each hybrid to ensure accurate description of complete feed chemistry (i.e. CP + aNDFom + Sugar + Starch + Ether extract + Soluble fiber + Organic acid + Ash = 100). At this point, the corn forage feed chemistry data was in a compatible format that could be then imported into CNCPS version 7.0.

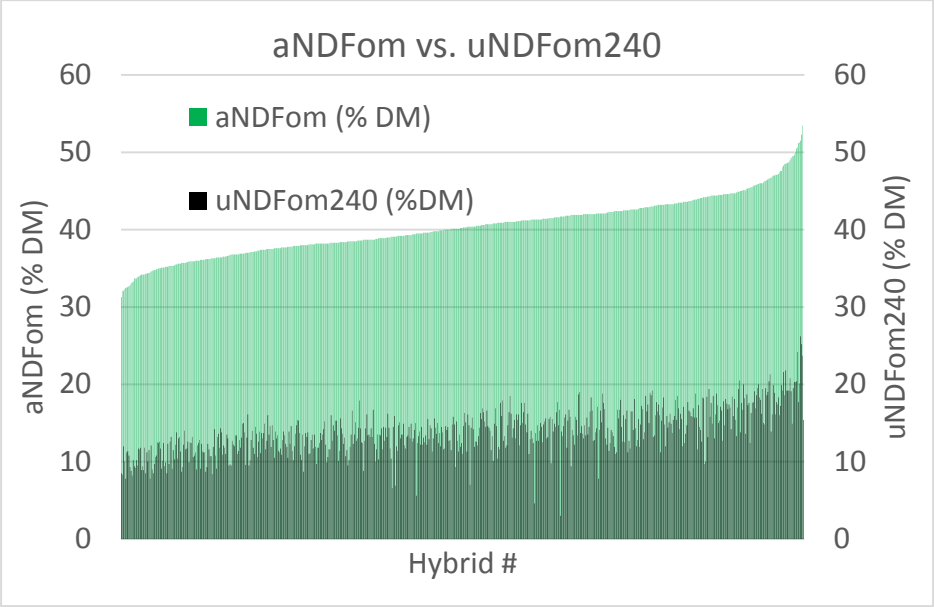

Figure 1. The aNDFom and uNDF of 648 replicates of 72 corn silages grown in three different locations in the Northeastern U.S. sorted on aNDFom from lowest to highest content.

Model Set Up

Two typical New York high corn silage-based diet (forage at ~60% of diet DM; corn silage ~70% of forage DM) were formulated in the CNCPS v7. Corn silage inclusion levels were maximized as much as possible as this was the variable of interest. Due to the higher levels of aNDFom and relatively high proportion of uNDFom of the corn silage analyzed, a digestible grass silage with low uNDFom was selected to complement this corn silage. Urea was also included as a rumen ammonia source to ensure adequate rumen ammonia levels so that fiber digestion by microbial action would not be impeded. The remaining ingredients are all typically included in Northeast diets and were included at levels so that diet formulation would match the standard animal requirements.

All diet ingredients were the same between both corn silage databases however, inclusion levels, in particular forage inclusions levels, were reduced to adjust for the moderate quality corn silage database. Ingredients included and nutrient composition of the standard diets are in Table 3. The two standard diets differed in the proportion of forage that comprised the diet and the total dry matter intake achieved. These two differences were largely due to the average quality/digestibility variation between the two corn silage databases. For each model simulation iteration, the corn silage hybrid and associated chemistry analysis was the only variable changing among runs. These diets were then fed to two standardized groups of cattle. The standardized cattle were developed based on typical Northeastern cattle parameters such as average body weight and performance capability. Dry matter intake was calculated to meet animal demands ensuring that intake (3-3.3 %BW) was realistic along with aNDFom intake (1% BW). The aNDFom intake levels were lower than typically achieved again due to the moderate quality of the corn silage being evaluated. The animals were 110 days in milk and were designed to be in a state where no tissue was being mobilized or deposited. Animal inputs of the two standard cows are described in Table 4.

Table 3. Base diet ingredients for CNCPS evaluations.	kg DM	% DM
Corn silage processed 35 DM 41 NDF medium	10.19	42
Grass silage 20 CP 48 NDF 5 LNDF	4.59	19
Corn grain ground fine	4.16	17
Canola meal solvent	0.99	4
Corn dist ethanol	0.38	2
Soybean hulls ground	0.50	2
Citrus pulp dry	0.50	2
Wheat midds	1.00	1
Extruded soybeans	0.89	4
Blood meal average	0.42	2
Energy Booster 100	0.17	1
MinVit +urea + rumen protected methionine	0.70	3.1
Total	24.49	100

Initially, all hybrids were simulated through the model at equal intakes. The simulated intakes were purposely lower than typically achieved (aNDFom intake <1.2% BW) as this year's corn silage had consistently lower digestibility than previous years. Typically, in this scenario, forage inclusion rate in the diet would decrease however as this was the variable of interest, corn silage inclusion was maintained as high as possible. Through utilization of the dynamic approaches of CNCPS v7, rumen aNDFom fill numbers were generated at steady state for each hybrid evaluated. Subsequently, the first limiting fill number was determined based on the quantity of aNDFom or uNDF fill in the rumen for each hybrid as every other ingredient in the diet was held constant.

Table 4. Animal characteristics and model inputs for the higher and lower intake evaluation groups. Two diets were developed because the upper and lower limits were not achievable using the entire database given the range in both aNDFom and uNDF240.

Animal inputs	Base High	Base Low
Dry matter intake, kg	24.50	22.84
Milk production, kg	41	36
Milk fat, %	3.7	3.7
Milk true protein, %	3.1	3.1
Milk lactose, %	4.78	4.78
Body condition score	3	3
Target body condition score	3	3
Age of first calving	22	22
Days in milk	110	110
Days pregnant	30	30
Calving interval	13	13
Calf birthweight, kg	44	44
Mature weight, kg	803	803
Age, months	39	39
Current weight, kg	750	750

There was a large range in predicted changes in DMI due to the range in aNDFom, uNDF and digestibility among the hybrids. The standard diets were formulated to achieve pre-determined rumen fill numbers based on rumen evacuation studies carried out at Cornell University and Miner Institute (Cotanch et al., 2014). These studies emptied the rumen contents on a number of animals and then tested a representative sample for aNDFom and uNDFom concentration. The maximum rumen uNDFom level in these studies was 0.66% BW. For our evaluation this resulted in a rumen uNDFom of 4.8 kg which corresponded to a total rumen aNDFom of 8.1 kg, which was ~1 % of body weight. These numbers were then established as the rumen fill set points. Hybrids that caused overfilling of the rumen by uNDF (i.e. poor digestibility), had to reduce their total diet DMI compared to those that under filled the rumen (i.e. high digestibility), where the calculated DMI was increased to achieve the set point. The set point was used to determine the difference in DMI from the standard diet as digestibility changed based on the rumen fill value from simulations at equal intake divided by standard diet rumen set point number (e.g. 5.24 lb/4.82 lb = 1.08). In this example, the rumen overfilled by 8%, which was determined infeasible based on rumen volume and therefore total DMI had to be reduced to achieve 100% of allowable fill (Figure 3). The fill adjustment factors were calculated for both total rumen aNDFom and uNDFom pool size and were applied on the basis of a first limiting physical fill approach. After the adjustment factors were applied to the total diet DMI, another model run was conducted, with the DMI adjusted to the first limiting levels based on the hybrid effect on rumen fill as described above. The total diet DMI was adjusted accordingly so that each hybrid met the first limiting allowable fill level. The resulting outcome due to altered DMI and corn silage digestibility, on allowable ME and MP milk production was recorded.

MODEL SIMULATION RESULTS

The model runs were conducted and examples are provided for two corn forages from the data set that are from each end of the range in digestibility to demonstrate the predictions for fill limitations. The potential to describe rumen fill based on aNDFom is in Figure 3. The base diet, with the average digestibility corn silage from the respective group, is the middle line on the curve and is highlighted by the rumen fill capacity line. The less digestible corn forage diet is described by the top line (Figure 3) and indicates at the given DMI, the rumen will overfill on aNDFom, so the DMI is infeasible with either the current inclusion level of corn silage or simply with the current diet formulation. The more highly digestible diet is described by the lower line and demonstrates that the digestion rate of the aNDFom is high enough to overcome an aNDFom fill limitation of that diet due to the difference in hybrid digestibility.

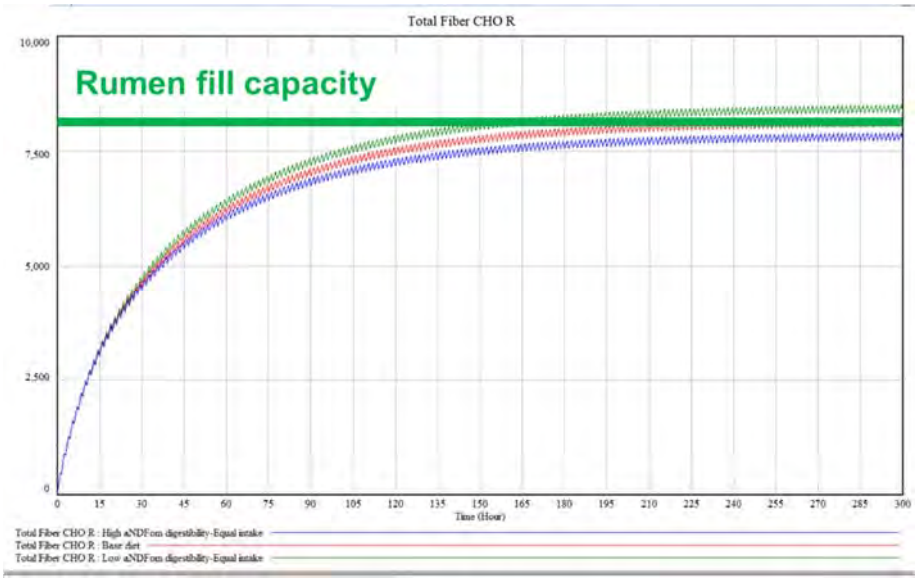

Figure 3. An example of an aNDFom rumen fill calculation from CNCPS v7 for diets a low, medium and high digestible corn silages.

A simple mathematical description of this behavior can be obtained by integrating the predicted passage rate and digestion rate of the respective pools of digestible aNDFom. In this example, we are using the passage rate for aNDFom incorporated in CNCPS v7 and with the current DMI, aNDFom level and BW, the passage rate is approximately 0.024/h. For one of the high digestibility forages, the fast pool is digesting at 0.012/h and is almost 70% of the total aNDFom, whereas for the low digestibility forage the fast pool is digesting at 0.07/h and is 53% of the aNDFom. Simple integration demonstrates that the fast pool of the higher digestibility corn silage is digesting 41% faster with an extent of digestion that is 11% greater than the lower digestibility corn silage. This difference creates more space in the rumen and potentially allows for greater intake because of the more rapid rumen disappearance (0.12/(0.12+0.024) =0.83 vs (0.07/(0.07+0.024) = 0.74. By integrating over time, in a dynamic model like CNCPS v7, this provides the capability of predicting first limiting physical fill characteristics, whether

it was total aNDFom or if the uNDF fraction is large enough to create a fill limitation (Figure 3).

The base diet was the reference diet and represented the average corn silage in the split data set (high and low digestibility and aNDFom). The comparisons were made on either an aNDFom or uNDF basis and this is demonstrated in Table 5 where a high and low digestibility corn silage were used to compare to the base diet. In this comparison, the first limiting factor for physical fill for the low digestibility corn silage was the uNDF240 where it was predicted to be 420 g over the base diet limit. Conversely, the high digestibility diet was not limited by uNDF240 and further, didn't meet the fill expectation of aNDFom by approximately 300 g, thus, cattle fed this diet would be expected to consume more total dry matter, assuming based on diet formulation that other nutrients were not first limiting.

Table 5. The predicted amount of aNDFom in the fast, slow and uNDF pools based on the digestibility of the corn silage hybrids represented in this comparison at a constant dry matter intake. The fill limits for each aNDFom pool are described in the standard diet.

	Low aNDFom digestibility diet	**Base aNDFom digestibility diet**	**High aNDFom digestibility diet**
aNDFom fast pool, g	1,588	1,632	1,698
aNDFom slow pool, g	1,588	1,655	1,715
uNDF240, g	5,239	4,819 (0.64% BW)	4,395
Total rumen NDF, g	8,415	8,106 (1.1% BW)	7,809
Formulated DMI, kg	24.5	24.5	24.5

The low digestibility diet was decreased in intake by the equivalent of 420g of uNDF and the adjusted calculations for pool sizes and dry matter intakes are found in Table 6. After adjustment for physical fill, the DMI for the low digestibility diet was 2 kg lower than the base diet, whereas the high digestibility diet was predicted to consume 900 g additional DMI to reach the aNDFom fill level, thus the ME and MP allowable milk predictions are described by those DMI differences.

Over the entire dataset, the predicted difference in milk yield per kg of DMI was 2.6 kg which is most likely high given the intake opportunity, and is being evaluated to understand why the model estimated such a feed efficiency (Figure 4). However, it was uniform among the forage comparisons, so we believe it represented the differences among forages. The prediction of milk yield represents the effect of increased DMI with changes in the digestibility of the forage that captures the energy of increased starch, sugar and fat intakes, and also represents the additional energy derived from the higher digestible forage itself.

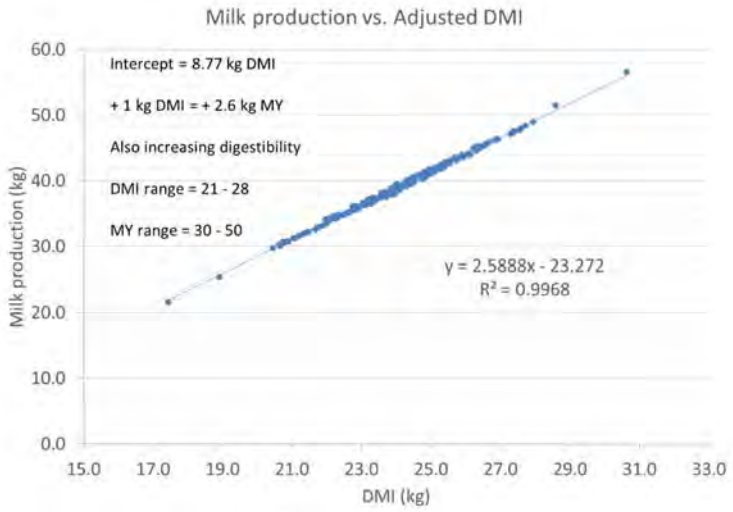

Figure 4. The prediction of dry matter intake and milk yield from the evaluation of the corn silage hybrids based on physical fill limitations of either aNDFom or uNDF240. The range in DMI among the data was 21 to 28 kg and the range in milk yield (MY) was 30 to 50 kg/d. The intercept of 8.77 kg represents the amount of intake needed to meet maintenance requirements.

Table 6. The predicted amount of aNDFom in each of the pools and total aNDFom in the rumen, and the overall dry matter intake and ME and MP allowable milk predictions after adjustment for rumen fill effects of either aNDFom or uNDF240. For the low digestibility diet, the fill was limited by uNDF240, whereas for the high digestibility diet, the fill was limited by total aNDFom which allowed for greater predicted dry matter intake on the high digestibility diet.

	Low aNDFom digestibility diet	**Base aNDFom digestibility diet**	**High aNDFom digestibility diet**
aNDFom fast pool, g	1,464	1,632	1,763
aNDFom slow pool, g	1,462	1,655	1,780
uNDF240, g	4,819	4,819	4,563
Total rumen NDF	7,745	8,106	8,106
Dry matter intake, kg	22.5	24.5	25.4
ME allowable milk, kg	35.8	40.9	43.3
MP allowable milk , kg	36.4	40.9	43.3

SUMMARY

The objective of this work was to develop an approach to rank the corn forage hybrids based on what we understand about rumen fill that integrated all of our current understanding of the behavior of aNDFom digestion in the rumen. The approach taken in this paper demonstrates the potential utility of having information and a procedure to evaluate the concepts of physical fill and DMI in conjunction with other components of the

diet. This approach requires refinement to better describe intake limits due to chemical characteristics and rumen fill effects. However, through utilization of the dynamic mechanistic structure of CNCPSv7 and basing our assumptions on rumen evacuation data, a more robust prediction of the potential impact of each hybrid on DMI and milk production can be achieved. Ultimately the goal is to help forward predict the productive capability of the corn silage hybrid or any forage and gain a greater understanding of how to proactively formulate/complement these forages.

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THE SYNERGY OF CHOLINE AND OMEGA-3 FATTY ACIDS FOR OPTIMIZED TRANSITION COW NUTRITION: A HYPOTHESIS

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INTRODUCTION

The development of postpartum fatty liver disease (**FLD**; i.e., hepatic steatosis or lipidosis) in dairy cattle continues to represent a nutrition and management challenge for the cow and producer. Of major concern, fatty liver is associated with decreased health status, fertility, and lactation performance (Wensing et al., 1997; Bobe et al., 2004). Hepatic lipid accrual, especially in the form of triacylglycerol (**TAG**), may limit gluconeogenesis and contribute to inflammation (Rukkwamsuk et al., 1999; Sordillo et al., 2009). Moreover, cows with fatty liver are at risk for acquiring other metabolic disorders including ketosis and displaced abomasum. For instance, fatty liver cows are predisposed to a heightened severity of infectious diseases including mastitis and metritis (Hill et al., 1985; Bobe et al., 2004). These outcomes may explain why cows with elevated hepatic lipid deposition are prone to infertility and compromised milk production later in lactation (Rajala-Schultz et al., 1999; Jorritsma et al., 2000). Herein, the current understanding of FLD mechanisms in dairy cows with a focus on very low density lipoprotein (**VLDL**) secretion is reviewed. Evidence is also provided to support the hypothesis that rumen-protected choline and docosahexaenoic acid (**DHA**) co-supplementation may represent a novel nutritional approach to minimize hepatic lipid accretion during the periparturient period.

MECHANISMS OF FATTY LIVER DISEASE

The advancement of negative energy balance during early lactation promotes adipose tissue lipolysis to enhance circulating concentrations of nonesterified fatty acids (**FA**). Dyslipidemia in turn elevates hepatic FA uptake where they are converted to fatty acyl-CoA by acyl-CoA synthetase. In the cow, the biochemical mechanisms of hepatic steatosis likely involve inadequate mitochondrial β-oxidation of fatty acyl-CoA, enhanced TAG esterification, and limited secretion of TAG within VLDL. First, the capacity to completely oxidize palmitoyl-CoA to CO_2 is not augmented during the transition from gestation to lactation (Litherland et al., 2011; McCarthy et al., 2015). In contrast, incomplete oxidation to acid-soluble products such as tricarboxylic acid cycle intermediates or ketones is maximum during peak lipolytic response (Dann et al., 2006; Litherland et al., 2011). In support, plasma lipidomic observations have also revealed marked elevations in circulating fatty acylcarnitines during the transition period (Rico et al., 2017b). As a consequence of increased hepatic FA uptake and inadequate oxidation, fatty acyl-CoA are partitioned towards TAG esterification which is mediated by various acyltransferases including glycerol-3-phosphate acyltransferase. In contrast to non-ruminant experimental models of non-alcoholic FLD in humans (Ferré and Foufelle,

2010), hepatic de novo lipogenesis of FA does not appear to contribute to TAG deposition in dairy cows with FLD (Pullen et al., 1990). Therefore, it is not surprising that observed postpartum elevations in liver palmitic (16:0) and oleic (*cis*-9 18:1) acids in dairy cows with FLD mimic observed elevations in plasma FA and the adipose tissue FA profile during the immediate postpartum period (Rukkwamsuk et al., 2000). The potential implications of these FA on VLDL synthesis and secretion are described below.

Although hepatic TAG esterification is upregulated during the transition period, concomitant elevations in VLDL-TAG secretion could limit the progression of FLD in periparturient dairy cows. In humans with simple steatosis and not the advanced inflammatory steatohepatitis form of non-alcoholic FLD, the secretion of TAG-containing VLDL increases linearly with TAG accrual (Donnelly et al., 2005; Choi and Ginsberg, 2011). However, the dairy cow has a reduced capacity to export TAG within VLDL from liver, relative to non-ruminants (Pullen et al., 1990). Although not a measure of VLDL secretion, our untargeted lipidomic approaches have confirmed dramatic reductions in circulating plasma TAG (e.g., TAG 60:1, 62:0, and 56:1) and TAG-rich lipoproteins in dairy cows transitioning from gestation to lactation (Davis et al., 2018; Saed Samii et al., 2018a). Assuming VLDL secretion is limited in postpartum dairy cows that develop FLD, relative to prepartum cows or clinically-healthy postpartum cows, then one possible explanation for limited VLDL export may be limited hepatic apolipoprotein (**Apo**) B_{100} concentrations. Apolipoprotein B_{100} is required for VLDL assembly and secretion (Sundaram and Yao, 2010); however, the mRNA expression of hepatic Apo B_{100} and circulating Apo B_{100} concentrations decrease as parturition approaches (Bernabucci et al., 2004; Bernabucci et al., 2009). Another possibility and focus of this review is a limited supply of hepatic phosphatidylcholine (**PC**), as proposed by Van den Top and coworkers (1995). Glycerophospholipids and some sphingolipids (i.e., choline-containing sphingomyelin) form a monolayer on the lipoprotein surface surrounding the TAG-rich hydrophobic core, and PC is the most abundant glycerophospholipid component of lipoprotein surface monolayers. Considerable evidence in biomedical non-ruminant experimental models demonstrate that reduced levels of hepatic PC impair the secretion of VLDL from liver (Yao and Vance, 1988; Fast and Vance, 1995). To the best of our knowledge, no additional evidence exists for any other glycerophospholipid or sphingolipid requirement for VLDL secretion including phosphatidylethanolamine (**PE**) or sphingomyelin, respectively.

PHOSPHATIDYLCHOLINE METABOLISM

Glycerophospholipid metabolism is complex. Two major types of glycerophospholipids include PC and PE. Of interest, the synthesis of PC involves the cytidine diphosphate (**CDP**)-choline pathway (i.e., Kennedy pathway) and the phosphatidylethanolamine *N*-methyltransferase (**PEMT**) pathway (Figure 1). Whereas the CDP-choline pathway utilizes choline as the key precursor, the PEMT pathway relies on the transmethylation cycle and the prerequisite methyl donor *S*-adenosylmethionine to methylate PE. It is generally recognized that PEMT is a liver-specific enzyme (Vance and Ridgway, 1988). Although choline may serve as a methyl donor for PE methylation, L-

methionine and betaine are other examples. Importantly, the CDP-choline and PEMT pathways appear to work in unison to synthesize PC in liver (Sundler and Akesson, 1975).

Phosphatidylcholine and choline-containing sphingomyelin are the predominant phospholipids in human VLDL with far less PE (Wiesner et al., 2009; Dashti et al., 2011). Indeed, PC comprises ~70% (mol %) of total phospholipids of rodent plasma VLDL (Agren et al., 2005). Earlier work by Fast and Vance (1995) demonstrated that the ratio of total PC to PE of nascent particles resembles hepatic organelle membrane phospholipid composition. Less clear is the specific types of PC (and maybe PE) that are preferentially required for VLDL assembly and secretion. Although attention has focused on the importance of choline within PC, we must be cognizant of the complete structure and potential functional importance of the two fatty acyl chains. Indeed, VLDL may contain hundreds of different types of PC, and early evidence suggests that PC 34:2 (number of carbons:number of double bonds; e.g., 16:0/18:2), 34:1 (e.g., 16:0/18:1), and 36:4 (e.g., 16:0/20:4 or 18:2/18:2) are prevalent on the surface of non-ruminant VLDL (Wiesner et al., 2009). The type of PC produced is likely governed by the preferentially utilization of specific fatty acids within the CDP-choline or PEMT pathways. For example, PC produced by the PEMT pathway are enriched in long-chain polyunsaturated FA (**PUFA**) including omega-6 arachidonic acid (20:4) and omega-3 DHA (DeLong et al., 1999; Pynn et al., 2011). Indeed, supplementing isolated rat hepatocytes with ethanolamine activates PEMT to promote the inclusion of DHA within PC (Samborski et al., 1993), and the quantitation of DHA-containing PC is utilized as surrogate measure for PEMT activity (da Costa et al., 2011). In contrast, PC synthesized by the CDP-choline pathway are enriched in saturated (e.g., 16:0 and 18:0) and unsaturated fatty acids with one or two double bonds including oleic and linoleic acids, respectively (DeLong et al., 1999; Pynn et al., 2011). It is also generally understood that saturated fatty acids are preferentially found at the *sn*-1 position of PC, whereas unsaturated fatty acids typically reside at the *sn*-2 position (MacDonald and Sprecher, 1991).

The synthesis and degradation of PC is further complicated by the involvement of other intermediary complex lipids including lysophosphatidylcholine (**LPC**), diacylglycerol, and ceramide. Liver LPC acyltransferase reacylates LPC to form PC, whereas PC is metabolized by the enzyme phospholipase A_2 releasing the fatty acid at the *sn*-2 position to form LPC. In circulation, LPC is a highly-abundant lyso-phospholipid, and LPC composition in plasma is a mixture of different species such as 16:0 (40%), 18:2 (20%), 18:1/18:0 (10–15%) and 20:4 (10%; Ojala et al., 2007). Diacylglycerol is utilized by choline phosphotransferase within the CDP-choline pathway, and sphingomyelin synthase transfers phosphocholine from PC to ceramide to form sphingomyelin. Although not the focus of this review, LPC, diacylglycerol, and ceramide are signaling lipids with diverse functions in inflammation, immunity, and insulin-mediated glucose utilization (Gräler and Goetzl, 2002; Summers, 2006; Erion and Shulman, 2010). Although the role of ceramide in the dairy cow has been extensively evaluated (Rico et al., 2016; Rico et al., 2017a; Rico et al., 2018b), the actions of LPC and diacylglycerol are less clear.

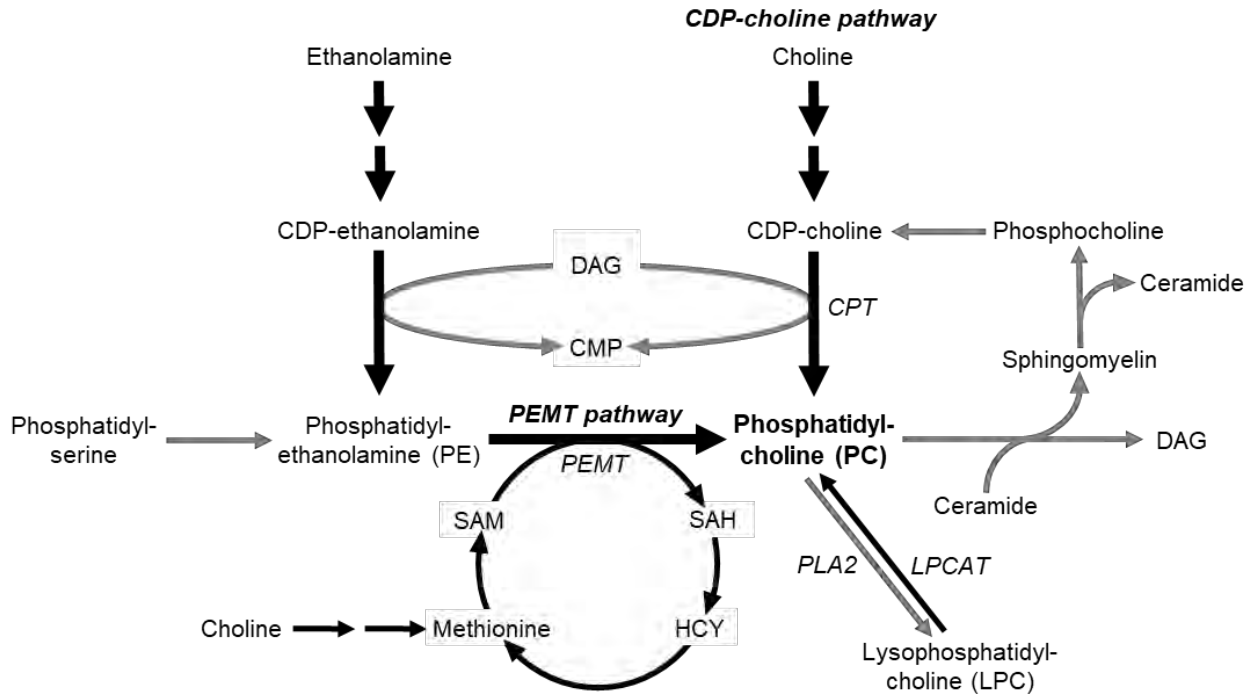

Figure 1. The complex synthesis and degradation of phosphatidylcholine. CDP, cytidine diphosphate; CMP, cytidine monophosphate; CPT, cholinephosphotransferase; DAG, diacylglycerol; HCY, homocysteine; LPCAT, LPC acyltransferase; PEMT, phosphatidylethanolamine *N*-methyltransferase; PLA2, phospholipase A_2; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

Phosphatidylcholine Biomarkers for Fatty Liver Disease in Dairy Cow

Early evaluation of the bovine plasma lipidome has identified candidate PC biomarkers for FLD in postpartum dairy cows (Saed Samii et al., 2017; Saed Samii et al., 2018a,b). Notably, we have identified multiple plasma PC that decrease during the transition from gestation to lactation, which are associated with the development of postpartum FLD (Figure 2; Bobe et al., 2004). These include PC 36:6, 32:3, 34:4, 34:6, and 37:6. Similar reductions in these PC species were observed in liver biopsied from postpartum cows, relative to prepartum (e.g., PC 36:6, Saed Samii et al., 2018a,b). Our lab has utilized these lipidomic features as initial targets for the development of nutritional therapies aimed at enhancing VLDL secretion in dairy cows. For instance, PC 36:6 may contain DHA; therefore, we consider the possibility that DHA supplementation may increase hepatic PC 36:6 synthesis and TAG export. These findings are supported by observed increases in palmitic acid concentrations within the cellular membrane phospholipid layer of hepatocytes with parallel decreases in eicosapentaenoic acid (**EPA**; 20:5) and DHA during the periparturient period in dairy cows (Douglas et al., 2007).

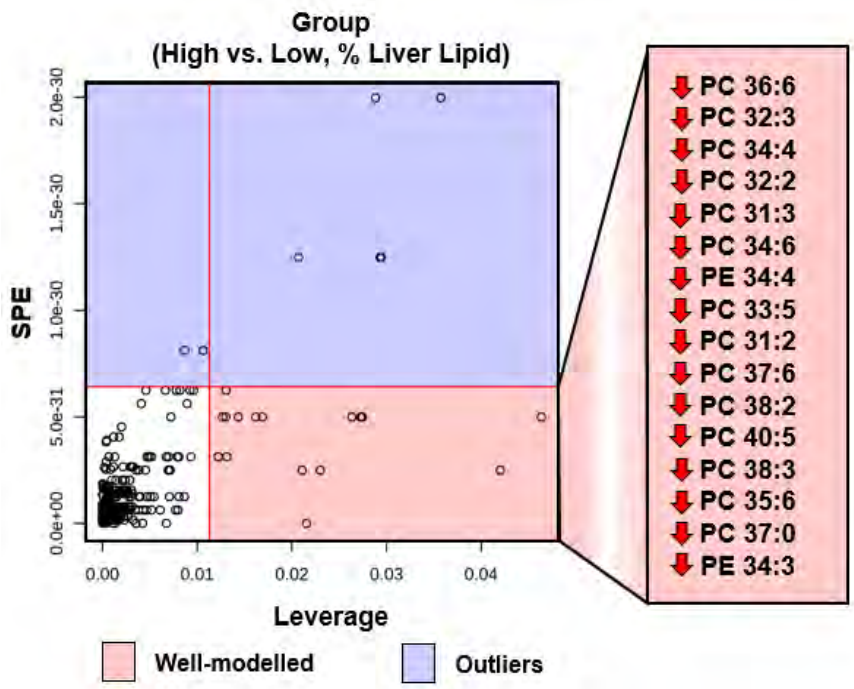

Figure 2. Suppressed plasma phosphatidylcholine (PC) levels are associated with fatty liver disease in periparturient Holstein dairy cows. Leverage/squared prediction error (SPE) plot of 301 complex lipids and their relationship with hepatic lipid accumulation. Normalized data represent plasma samples collected from periparturient Holstein dairy cows categorized into low (n = 7) or high (n = 7) mean (d 5 and 14 postpartum) liver lipid content (5 ± 1 vs. 12 ± 2 % of wet weight, respectively). Metabolites in lower right quadrant have high loadings and follow the expression pattern of the submodel (i.e., data demonstrate that out of 301 metabolites, the suppression of specific PC levels are most associated with fatty liver disease). Data were obtained using quadrupole time-of-flight mass spectrometry. PE = phosphatidylethanoline.

DIETARY CHOLINE AND OMEGA-3 FATTY ACID SUPPLEMENTATION

Choline Supplementation

Dietary choline supplementation has been considered a potential nutritional therapy to mitigate FLD in dairy cows and humans (Cooke et al., 2007; Guerrerio et al., 2012; Zenobi et al., 2018). For example, the supplementation of feed-restricted nonlactating multiparous Holstein cows during late gestation with rumen-protected choline ions (0 to 25.8 g/d) lowered liver TAG deposition in a dose dependent manner (Zenobi et al., 2018). To build on their findings, we applied fast protein liquid chromatography to demonstrate that this dietary approach increased circulating total TAG concentrations in TAG-rich and low density lipoproteins (**LDL**), and increased circulating total phospholipid levels in LDL (Table 1). The mechanisms of these outcomes may involve the ability of choline to stimulate hepatic PC synthesis, and thus provide key

glycerophospholipids for VLDL assembly. Recent findings obtained using bovine neonatal hepatocyte cultures suggests that choline chloride supplementation increases PC synthesis and VLDL secretion by predominantly activating the CDP-choline pathway (Chandler and White, 2017). Although choline may be transformed into betaine to support the transmethylation of PE, Chandler and White (2017) observed lower hepatocyte PEMT mRNA expression with choline chloride supplementation. Indeed, PC generated by the CDP-choline pathway was preferentially detected in VLDL fractions obtained from rodent hepatocyte cultures supplemented with radiolabeled choline (Vance and Vance, 1986). It is intriguing to postulate whether hepatocyte PEMT pathway activation can be enhanced during choline chloride supplementation while in the presence of preferential PE substrate containing very long chain PUFA such as DHA (DeLong et al., 1999; Pynn et al., 2011).

Table 1. Effect of increasing intake of ruminally protected choline ions on circulating concentrations of triacylglycerol (TAG) in plasma lipoprotein fractions collected using fast protein liquid chromatography.[1]

	TAG-rich lipoprotein fraction						**Low density lipoprotein fraction**					
	Intake of RPC, g/d				*P* =		Intake of RPC, g/d				*P* =	
Component	0	12.9	25.8	SEM	Linear	Quadratic	0	12.9	25.8	SEM	Linear	Quadratic
TAG, mg/dL	0.97	2.03	1.77	0.22	0.01	0.01	0.74	0.96	0.92	0.08	0.02	0.04
Cholesterol, mg/dL	0.65	1.04	1.11	0.13	0.01	0.21	34.0	38.5	41.2	3.10	0.09	0.80
Phospholipid, mg/dL	1.53	1.76	1.66	0.28	0.26	0.09	15.9	19.4	21.6	1.38	0.01	0.69

[1]Samples derived from lab of Dr. Charles Staples at the University of Florida following their evaluation of feeding increasing amounts of ruminally protected choline (RPC; ReaShure; Balchem Corporation) on hepatic health and lactation performance in nonlactating, pregnant Holstein dairy cows in negative energy balance (Zenobi et al., 2018). Lipoprotein fractions collected as described by Phipps et al. (2017).

Omega-3 PUFA Supplementation

Omega-3 long-chain PUFA include α-linolenic acid (**ALA**; 18:3), DHA, docosapentaenoic acid (**DPA**; 22:5), and EPA. Beneficial effects promoted by omega-3 PUFA are routinely observed in humans and various experimental models (Riediger et al., 2009; Kalupahana et al., 2011). These include reductions in circulating TAG in patients with hypertriglyceridemia (Mori et al., 2000; Schwellenbach et al., 2006), lower plasma levels of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β (Endres et al., 1989; Caughey et al., 1996), and the prevention of atherosclerosis and hypertension (Hino et al., 2004; Erkkilä et al., 2006). Although uncertain in humans (Poudyal et al., 2011), the dietary intake of fish oil rich in omega-3 PUFA has an insulin-sensitizing effect in adipose and liver tissues (González-Périz et al., 2009). In rats fed a 12% canola oil-based diet, improvements in estimated insulin sensitivity were observed with the supplementation of EPA and DHA, relative to ALA supplementation (Andersen et al., 2008). Improvements in insulin action with EPA and DHA supplementation may be due in part to reductions in lipogenesis and heightened FA oxidation (Kalupahana et al., 2011). Moreover, omega-3 EPA and DHA serve as

substrate for the synthesis of resolvins and protectins which may enhance insulin signaling by alleviating chronic inflammation (Serhan and Petasis, 2011). Collectively, these benefits associated with dietary DHA and EPA consumption have stimulated interest in promoting omega-3 PUFA intake for the prevention of metabolic diseases (Kinsella et al., 1990; Mostad et al., 2006).

Omega-3 FA supplementation is also being studied as means to alleviate hepatic steatosis in humans. Although not completely defined, the mechanisms of action appear to be multifaceted. Omega-3 PUFA are potent peroxisome proliferator-activated receptor-α activators which in turn stimulates FA oxidation (Pawar and Jump, 2003). Additionally, omega-3 PUFA inhibit lipogenesis by downregulating sterol regulatory element-binding protein (Xu et al., 1999). Improvements in hepatic oxidative balance and inflammatory status are also observed with EPA or DHA supplementation (Li et al., 2005; Ishii et al., 2009). As described earlier, activation of the hepatic PEMT pathway for PC production preferentially utilizes DHA (Samborski et al., 1993; DeLong et al., 1999; Watkins et al., 2003). Moreover, reduced PEMT activity confers susceptibility to FLD in humans (Song et al., 2005). Therefore, DHA supplementation may be a means to selectively activate PEMT to promote PC synthesis and thus support the secretion of TAG within VLDL. Such an approach may benefit humans (and potentially cows) experiencing reductions in total plasma and liver DHA/EPA levels during inflammatory FLD (Puri et al., 2009; Arendt et al., 2015). In support, randomized controlled trials have shown reductions in liver fat and circulating liver enzymes (i.e., alanine or aspartate aminotransferase) in children and adults with advanced non-alcoholic FLD (Nobili et al., 2013; He et al., 2016; Nogueira et al., 2016). Unfortunately, we cannot yet discern whether these beneficial outcomes are due to enhanced hepatic TAG secretion or because of other aforementioned beneficial effects associated with omega-3 FA consumption.

Omega-3 Fatty Acid Supplementation in Dairy Cows

In dairy cows, the effects of dietary supplementation of omega-3 FA including DHA on fertility and lactation performance has been extensively studied. Regarding reproduction, pregnancy losses are lower in cows fed rolled flaxseed which is rich in ALA, relative to cows fed rolled sunflower seed (Ambrose et al., 2006). Moreover, feeding dairy cows EPA and DHA may inhibit uterine prostaglandin $F_{2\alpha}$ synthesis, delay regression of the corpus luteum, and promote fertility by enhancing embryo survival (Burke et al., 1997; Staples et al., 1998). With regard to lactation, heavy emphasis has focused on the ability of omega-3 FA supplementation to enrich their concentrations in milk fat (Kitessa et al., 2004). Such a response is a perceived benefit for the production of functional foods that benefit human health (Lock and Bauman, 2004). Indeed, the consumption of omega-3 FA in milk may represent a preventive approach for the prevention of type 2 diabetes and coronary heart disease (Thorsdottir et al., 2004). Other research has focused on the ability of unprotected DHA supplementation to modulate the ruminal microflora and milk fat synthesis (Petit et al., 2002; Shingfield et al., 2003; Maia et al., 2007). Our understanding of the effects of DHA supplementation on metabolic health in cows is also developing. For instance, omega-3 FA may enhance insulin sensitivity in cows (Gingras et al., 2007), and elevate circulating insulin and insulin-like growth factor-I during early

lactation (Heravi Moussavi et al., 2007). Although the effects of omega-3 FA on TAG secretion in cows is uncertain, a recent evaluation of dietary rumen-protected DHA demonstrated accumulation of DHA in plasma phospholipids in dairy cows (Stamey et al., 2012).

Effects of Abomasal Infusion of Various Phosphatidylcholine Precursors on Lactation Performance, Metabolic Status, and the Hepatic Phosphatidylcholine Lipidome

An interest of our research group is to discover novel dietary approaches that maximize hepatic PC synthesis, TAG secretion, and health in dairy cows. In a recent proof-of-principle study performed at the Cornell Dairy Research Center, five multiparous late lactation Holstein dairy cows were enrolled in a 5 × 5 Latin Square design experiment. Cows were continuously abomasally infused for 6 d with emulsion preps containing palmitic acid (PA; 98% 16:0; BergaFat F-100 HP; Berg + Schmidt GmbH & Co.), PA + choline (50 g of choline ion delivered as choline chloride), PA + L-serine (170 g; 1X estimated duodenal flow), behenic acid (BA; 92% 22:0; Berg + Schmidt), or omega-3 FA (44% DHA, 0.7% DPA; algae-sourced life'sDHA; DSM Nutritional Products, Inc.). Although each cow was infused 301 g of total FA each day (12.54 g/h), infusions were balanced for the amount of palmitic acid and glycerol within the omega-3 oil (40 and 19 g, respectively). Moreover, each emulsion prep contained whey protein, polysorbate 80, ethoxyquin, and water. To justify our treatments (Figure 1), palmitic acid is a potent inducer of ceramide synthesis (Rico et al., 2016), detected at the *sn*-1 position of PC (MacDonald and Sprecher, 1991), and preferentially utilized by the CDP-choline pathway (Vance and Vance, 1986). Choline is the principal substrate for the CDP-choline pathway, but also believed to serve as a methyl donor for PEMT activation in a limited capacity. We also considered the role of choline in sphingomyelin synthesis and degradation. Although a non-essential amino acid, serine may be used for PC and ceramide synthesis (Rico et al., 2015a). Behenic acid is a very-long chain saturated FA chosen because of its preferential utilization for ceramide synthesis (Rico et al., 2015a; Rico et al., 2018a); however, the potential low digestibility of very-long chain FA is a concern. To the best of our knowledge our work represents the first intensive evaluation of behenic acid in dairy cows. Lastly, our omega-3 FA treatment rich in DHA and DPA was selected because of their potential to improve health and PC synthesis via the PEMT pathway (DeLong et al., 1999; Pynn et al., 2011; Siriwardhana et al., 2012). We are cognizant that our study evaluated clinically healthy late lactation cows in positive energy balance, and the observed effects may or may not be observed in early lactation cows at risk for FLD. Of relevance to our investigation, circulating PC concentrations increase, and plasma ceramide concentrations and nutrient partitioning decrease as lactation advances (Artegoitia et al., 2014; Rico et al., 2016).

Omega-3 infusion lowered dry matter intake, relative to PA treatment ($P < 0.01$). Milk yields recorded for cows abomasally infused with PA, PA co-infused with serine, or BA were comparable (Table 2). These data suggest that BA may also increase circulating very-long chain ceramides to support nutrient partitioning (Rico et al., 2015b; Rico et al., 2017a). In contrast to our observations in PA cows, omega-3 FA infusion significantly decreased ($P = 0.03$) and PA co-infused with choline tended to lower milk yield ($P = 0.08$).

Observed reductions in dry matter intake with DHA treatment were likely the cause considering feed efficiency was not modified. This may be due to the ability of omega-3 FA to promote satiety (Parra et al., 2008). Additionally, a working theory is that both DHA and choline lower ceramide levels and improve insulin sensitivity to reduce glucose partitioning to the mammary gland. Docosahexaenoic acid supplementation does improve bovine insulin sensitivity in vitro (Gingras et al., 2007), and a tendency for lower milk yield with high choline intake in mid-lactation cows has been observed (Sharma and Erdman, 1988). Such a response may explain the observed tendency for reductions in milk yield during late lactation but we argue that this effect would be highly dependent on stage of lactation. During early lactation, improvements in insulin sensitivity hold the potential to reduce lipolysis and minimize hepatic FA uptake. Indeed, lower levels of circulating FA have been observed with choline supplementation during the peripartum (Pinotti et al., 2003). Therefore, clear evidence exists for the improvement in hepatic health and milk production during early lactation choline supplementation (Pinotti et al., 2003; Zahra et al., 2006).

Table 2. Effect of abomasal infusion of palmitic acid (PA; 16:0), PA and choline chloride (PA+C), PA and L-serine (PA+S), behenic acid (BA; 22:0), or a mixed omega-3 oil containing docosahexaenoic and docosapentaenoic acids (DHA/DPA) on dry matter intake and milk production in late lactation Holstein dairy cows.[1]

	Treatment						
	PA	PA+C	PA+S	BA	DHA/ DPA	SEM	*P-value*
Dry matter intake, kg/d	24.4	23.7	22.8	22.9	21.3*	1.61	0.06
Milk yield, kg/d	29.2	26.2†	28.2	28.5	25.6*	1.72	0.09
Milk components, kg/d							
Fat	1.34	1.33	1.18	1.23	0.92	0.15	0.28
Protein	0.97	0.82	0.94	0.96	0.93	0.06	0.66
Lactose	1.32	1.25	1.30	1.31	1.20	0.08	0.44
Milk composition, %							
Fat	4.57	4.32	4.31	4.22*	3.62*	0.14	0.04
Protein	3.43	3.29	3.41	3.43	3.65	0.13	0.77
Lactose	4.49	4.64†	4.55	4.54	4.69*	0.06	0.09
Feed efficiency[2]	1.17	1.10	1.24	1.22	1.25	0.13	0.72

[1]In a 5 × 5 Latin Square design experiment, late lactation Holstein dairy cows were continuously abomasally infused for 6 d with emulsion preps containing PA (98% 16:0; BergaFat F-100 HP; Berg + Schmidt GmbH & Co.), BA (92% 22:0; Berg + Schmidt), PA + Choline (50 g of choline ion delivered as choline chloride), PA + Serine (170 g; 1X estimated duodenal flow), or omega-3 FA (44% DHA, 0.7% DPA; life'sDHA; DSM Nutritional Products, Inc.). Although each cow was infused 301 g of total FA each day, infusions were balanced for the amount of 16:0 and glycerol within the omega-3 oil (40 and 19 g, respectively). Data reflect LS Means ± SE for d 5 and 6 of infusion. Relative to PA: *, $P < 0.05$. †, $P < 0.10$.
[2]Feed efficiency = kg of milk/kg of dry matter intake.

Although milk fat yield was maintained with BA infusion, relative to PA cows, BA significantly lowered milk fat content ($P = 0.04$). Relative to palmitic acid, our data suggest that behenic acid digestibility and/or incorporation of behenic acid into milk fat TAG is lower. However, omega-3 FA infusion exhibited a far greater suppression in milk fat content and a numerical decrease in milk fat yield. These findings are especially intriguing

because we utilized an abomasal delivery approach; therefore, we consider the effects of omega-3 FA on biohydrogenation were negligible. The potent ability of omega-3 FA to suppress lipogenesis may be the cause (Xu et al., 1999). It is important to mention that DHA dietary supplementation at a much lower level (29 g/d) did not modify milk yield or milk fat composition in mid-lactation Holstein cows (Stamey et al., 2012).

The described treatments did not modify circulating glucose or total FA concentrations; however, circulating TAG and cholesterol concentrations were greatest with PA treatment, whereas the levels of these metabolites were lowest with omega-3 FA infusion (unpublished). Such outcomes were expected based on the previously described benefits linked with DHA consumption in non-ruminants. We also biochemically mapped the bovine hepatic glycerophospholipidome. Profound changes across hundreds of lipids were observed with treatment (Figure 3). For instance, DHA was markedly enriched in hepatic PC and LPC (e.g., biomarker PC 36:6). Elevations in DHA-containing PC may be indicative of enhanced PEMT activity (da Costa et al., 2011). In support, PEMT activation prefers PUFA-containing PE (DeLong et al., 1999). Indeed, DHA/DPA infusion robustly increased over 100 PC containing more than 3 double bonds, relative to PA cows. In contrast, PA infusion significantly increased a similar number of PC containing saturated FA or FA containing 3 or less double bonds. These PC may be preferentially synthesized by the CDP-choline pathway (Vance and Vance, 1986). We also observed modest increases in hepatic PC with choline chloride or L-serine infusion, relative to PA. Although some exceptions were observed, omega-3 infusion enhanced the incorporation of PUFA (>3 double bonds) into PC, whereas saturated FA feeding, choline, and serine elevated hepatic PC containing saturated FA, or FA with 1 to 3 double bonds.

CONCLUSION

Current evidence supports the ability of dietary rumen-protected choline supplementation to enhance TAG disposal in postpartum dairy cows at risk for metabolic disease. Our data suggest that the ability of choline to activate the CDP-choline pathway to promote PC synthesis may be the cause. However, potential exists to refine nutritional practices as a means to optimize liver PC production and VLDL secretion for enhanced liver health during the peripartum. Although research should continue to focus on whether methyl donor supplementation (e.g., choline and L-methionine) provides added benefit, dairy science advancements should determine which dietary FA inhibit or promote hepatic PC synthesis and TAG export. Moreover, the identification of methyl donors and fatty acids that work in unison to upregulate both the CDP-choline and PEMT pathways to maximize PC production and VLDL secretion for the prevention of fatty liver disease would represent a paradigm shift in transition cow nutrition.

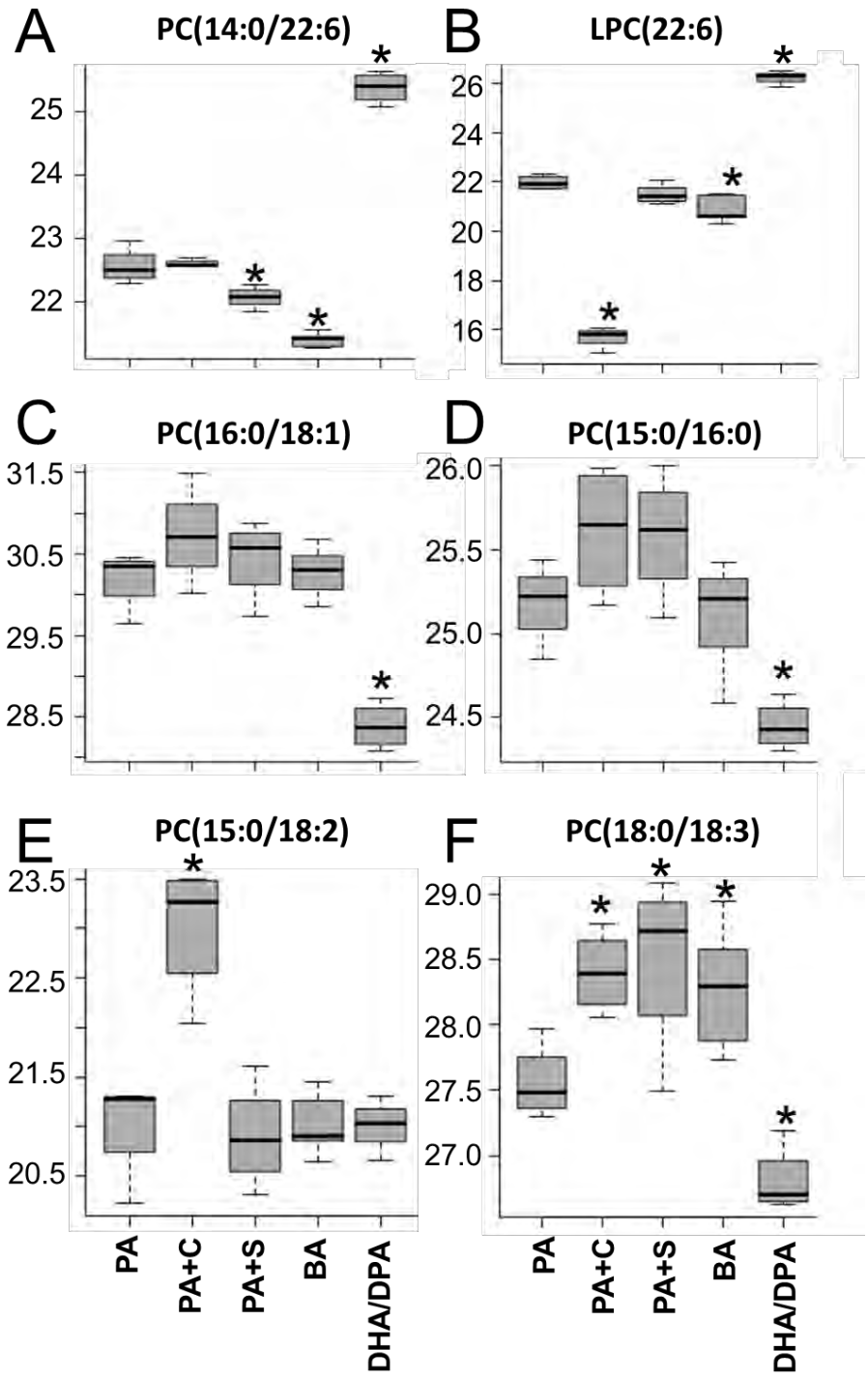

Figure 3. Effect of abomasal infusion of palmitic acid (PA; 16:0), PA and choline chloride (PA+C), PA and L-serine (PA+S), behenic acid (BA; 22:0), or a mixed omega-3 oil containing docosahexaenoic and docosapentaenoic acids (DHA/DPA) on select hepatic phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) levels in late lactation Holstein dairy cows. Generalized log-transformed intensities (Y-axis) derived from time-of-flight mass spectrometry. Samples reflect liver biopsied at the end of d 6 of each infusion.

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FERMENTABLE FIBER FOR YEAR-ROUND EWE MILK PRODUCTION

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INTRODUCTION

Seasonal production is a major constraint for ewe milk production in the US and Europe (Sitzia et al., 2015). Typical US dairy breeds (East Friesian and Lacaune) are seasonal polyestrous with low conception rates in off-season months, achieving one annual 160- to 180-day lactation. As a result, the US sheep dairy industry consists of a humble number of mainly small-scale family farms producing artisanal products in on-farm creameries for high-priced retail market segments. Yet, for many years the US has been the largest global import market for ewe milk cheeses, with annual imports of up to ~50% of the world's hard aged cheeses (FAO). China, with 12% of the annual global production, is currently the largest producer of ewe milk. Italy remains the largest exporter of hard aged cheeses (Balthazar et al., 2017). With 18% milk solids, the cheese yield from ewe milk is significantly higher than that from cow and goat milk, making ewe milk attractive milk for cheese production. However, seasonal availability of fresh ewe milk limits marketing opportunities and profitability of small-scale sheep farmers.

Opportunities to improve competitiveness are to increase the milk production of individual ewes to offset the challenge of seasonal production, to improve off-season conception rates and produce milk year-round, and to have a high lamb crop to tap into meat markets.

One option is to milk prolific traditional meat sheep breeds with high peak lactation yields. Enrolling meat breed ewes selected for accelerated lambing in a total dairy system utilizes their ability to breed out of season with high conception rates. A major consideration for the success of an intensely managed, dual purpose system is adequate nutrition throughout the production cycle. Fermentable dietary fiber is the focus of this nutritional research to use the abundance of idle forage acreage in the US Northeast and the advantage of grazing ewes during the gestational dry period. Successfully feeding ewes for an accelerated lambing, total dairy, multi-purpose system while maintaining optimal health and body condition for ewes is the main aim of this research.

MATERIAL AND METHODS

The hypotheses of this project were that the concentration of pfNDF in the diet would be positively correlated with feed intake and, thus, milk yield (Schotthofer et al., 2007) and that meat breed ewes managed in frequent and short lactations could achieve combined income from milk and lambs similar to that of long-lactation dairy breed ewes.

Between October 2016 and September 2018, 58 Finnsheep × Dorset crossbreed ewes were bred on the STAR accelerated lambing system and managed as a dairy flock. The ewes were divided into three management groups, with each group lambing three times during the experiment. Rebreeding was scheduled for day 73 of each lactation. Ewes within groups were milked in subsequent lactations year-round. This resulted in three 73-day lactation periods for each group with 219-day lambing intervals and nine 73-day lactations.

Table 1. Composition of experimental diets (% of DM). These diets were offered ad libitum with 350 to 500 g (varied with lactation) of hay per ewe per day.

Ingredient	30% pfNDF	35% pfNDF	40% pfNDF
Soy hulls	34.4	42.4	50.9
Wheat midds	20.1	20.1	20.1
Corn	31.5	24.1	16.2
Soybean meal	8.9	8.6	8.2
Molasses	1.7	1.7	1.7
Cornell sheep premix	1.06	1.06	1.06
Ammonium chloride	0.78	0.78	1.68
Calcium carbonate	1.34	1.12	0.89
Pellet binder	0.26	0.26	0.26
Estimated components			
DM (% of feed)	89.6	89.5	89.4
DDM	81.0	80.6	80.3
CP	17.0	17.0	17.1
NDF	36.1	41.1	46.5
pfNDF	30.5	35.1	40.1
INDF	5.6	6.0	6.4
NSCHO	38.9	34.0	28.7
EE	2.7	2.6	2.4
Ash	5.3	5.3	5.3

[1]Pelleted diets except for the first lactation of the first STAR group, which had no wheat midds, molasses, or pellet binder, but with 2.2% vegetable oil, more soy hulls, less calcium carbonate, more soybean meal, and 0.22% salt.

The experimental diets are presented in Table 1. Potentially fermentable fiber was defined by Thonney (2017) as the concentration of NDF in the diet minus the amount of indigestible NDF (INDF) at 1 X maintenance, with 1 X maintenance INDF being the concentration of indigestible dry matter at 1 X maintenance minus 10 to 15 percentage units of fecal DM as metabolic fecal losses (Van Soest, 1994). Soyhulls, an inexpensive by-product with a high proportion (~60%) of pfNDF, was the main source of fiber. Corn replaced soyhulls to achieve the desired pfNDF levels. The three levels of dietary pfNDF were formulated to be 30, 35, and 40% of the dietary dry matter. The diets were offered ad libitum with small amounts of hay to test their effect on intake and milk production.

At the beginning of the experiment, the ewes within each STAR group were randomly assigned to one of three pens. Three experimental diets rotated through these pens across the three lactations of each STAR group. Each pen was exposed to each experimental diet during the experiment.

Lambs were taken from each ewe within 12 hours of birth and reared artificially on cold milk. Beginning at DIM 1, milk yields for each ewe, and pen feed offered and refused were recorded twice daily. Weights were recorded weekly for each ewe and lamb. Milk, feed, and feed refusal samples were collected weekly. Ruminal fluid and ruminal fluid pH-values were collected twice during lactation 3 within each STAR group. Reproductive data were collected for each ewe.

The data were analyzed using the lmer procedure in R based upon the experimental design: a triply replicated (STAR group) Latin square with diet, pen within STAR group (rows), and lactation (columns) as fixed effects; ewe within pen as a random effect; and linear, quadratic, and cubic effects of days in milk (DIM) as possible continuous covariates to model lactation curves, with the possibility of different coefficients for each diet. Selected, preliminary results of the experiment are discussed below.

RESULTS AND DISCUSSION

The number of ewes per pen varied from 3 to 6 due to the breeding success prior to each lactation within STAR group. The data presented here include daily milk production for about 45 individual ewe lactations for each diet. As shown in Figure 1, ewes fed the 35% pfNDF diet produced the most milk ($P < 0.001$). Daily milk production averaged 1.3, 1.5, and 1.4 kg/day for ewes fed the 30, 35, and 40% pfNDF diets, respectively.

As portrayed in Figure 2, diet had a significant effect ($P < 0.001$) on DM intake. The DM intake of ewes fed the 35% diet correlates with higher milk production (Figure 1).

The small particle size of the pelleted diets did not have a negative effect on rumen health. Ruminal pH-values did not differ significantly among the diets (6.8, 7.0, and 6.8 for the 30, 35, and 40% pfNDF diets, respectively).

Feed efficiency was calculated by milk weight/DM intake (Figure 3). These values were not analyzed statistically but obtained by division; the modelled daily individual milk yield for each diet was divided by the modelled daily DM intake of each diet. Ewes fed the 35% pfNDF diet had higher feed efficiencies throughout lactation. Thus, ewes fed the 35% pfNDF diet were the most efficient and economical.

Rebreeding happened on day 73 of lactation. Weight loss in the beginning of lactation is not desirable. Ideally the ewes gain weight in early lactation up to breeding. This was achieved for each of the experimental diets. The weight gains showed an influence of diet, with the least weight gains for the ewes fed the diet containing 35% pfNDF. However, no significant difference due to diet was detected ($P = 0.884$).

There was no statistical influence of previous dietary level of pfNDF on conception rate (P = 0.273) or litter size (P = 0.856) in lactations 2 and 3. The ADG ranged between -0.189 g and 0.366 g for all ewes within all STAR groups and lactations did not show an effect on either conception rate (P = 0.812) or total number of lambs born (P = 0.361).

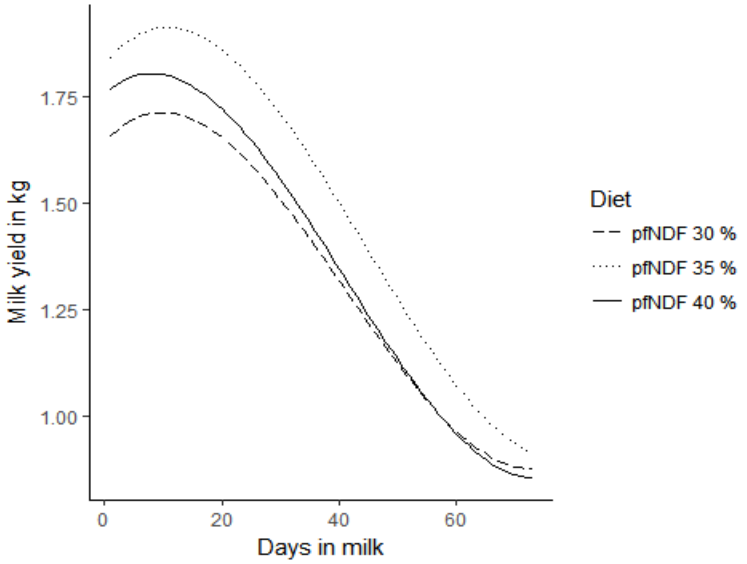

Figure 1. Lactation curves for ewes fed diets varying in concentrations of potentially-fermentable NDF.

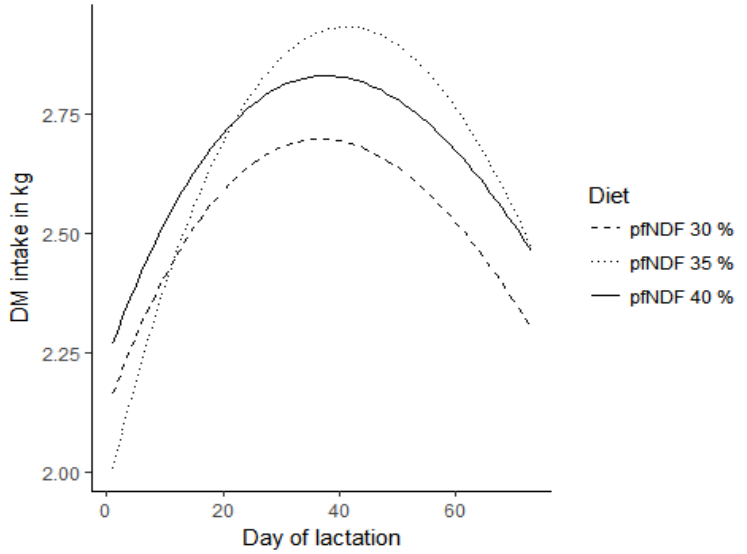

Figure 2. Daily dry matter intake.

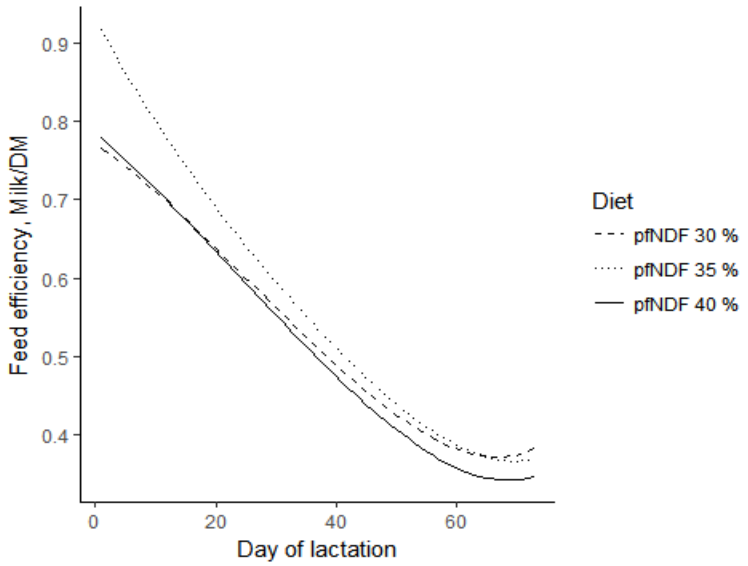

Figure 3. Feed efficiency.

The average weight of ewes increased from 68.7 kg in lactation 1 to 74.6 kg in lactation 3. Even though no statistical significance for lactation number was detected, the weight gains clearly had a positive impact on reproductive efficiency. Conception rates in the first reproductive cycle and the number of lambs born per lambing increased between lactations 1 and 3 (Table 2). No declines in fertility and fecundity, health, or body condition were detected in the 22-month experiment.

Table 2. Reproductive efficiency.

Item	Lactation 1	Lactation 2	Lactation 3
Conception rate, %	84	86	91
Conception in first cycle, %	85	79	93
Lambs born per lambing	2.3	2.2	2.7
Lambs raised per lambing	2.1	2.1	2.6
Lambs born per ewe per year	3.6	3.4	4.2
Lambs raised per ewe per year	3.3	3.3	4.1

Flushing strategies are advised to increase ewe weight and condition before and during breeding to increase the number of ovulated eggs and secure embryo survival, either with higher crude protein (Hoversland, 1958) or higher digestible carbohydrate (Habibizad et al., 2015) in the ration for various timespans prior and post introduction of the breeding ram. In this experiment, the ewes were fed relatively high protein and carbohydrate levels throughout lactation until breeding on day 73 of lactation. Six breeding groups (the second and third lactation of all groups) were taken off their concentrate ration immediately on day 73 of lactation to achieve dry-off. They were fed small amounts of low-quality hay (~ 9.3% protein, 72.1% aNDF, 50.3% ADF, 8.6% NFC) throughout the first two weeks of breeding to ensure optimal dry-off results. It seems the condition of the ewes during lactation prior to breeding had a higher impact on conception rate and fetal survival than digestible feed component level during breeding.

OUTLOOK

The achieved milk yields and lamb crop point toward success of accelerated dairy systems with Dorset and Finnsheep × Dorset meat ewes. The analysis of a variety of other measurements must be finalized for conclusive results. This includes blood NEFA, ruminal fluid VFA concentrations, and milk components.

Research has shown that East Friesian crossbred ewes can be manipulated to breed out of season in accelerated lambing systems (Peterson et al., 2005) and the advantage of lactational persistency of dairy breeds could be utilized by crossbreeding for a dairy setting. In a follow-up trial in Spring 2019, 30 yearling ewes from the Cornell flock with 25% East-Friesian dairy sheep genetics will be milked and rebred to study the impact of these genetics on lactation curve persistency, and ability to breed out of season in an accelerated system.

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MODELING THE NUTRITION-ENVIRONMENT NEXUS: COMPLETING THE CYCLE

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INTRODUCTION

The 1950s to the early 2000s saw dramatic change in the US dairy industry. Genetic improvement, advances in knowledge of dairy cattle nutrition, and other management improvements led to an increase in the national annual milk production of more than 50 million lbs from less than half the number of cows (~ 21 million cows in 1950 vs. ~ 9.2 million cows in 2000) (Blayney, 2004). With the increase in total milk production and productivity per cow came an intensification of dairy production due to decreases in the number of dairy farms and increases in the number of animals per farm (Blayney, 2004). The term Concentrated Animal Feeding Operations (CAFO) was introduced in the mid-1970s to begin regulation of large animal production facilities under the Clean Water Act, but it wasn't until the early 2000's that these regulations gained attention and required direct action of dairy producers to develop Nutrient Management Plans and apply for pollutant discharge permits (Hribar, 2010). Thus, although the field of dairy science continues to contribute to management improvements that increase milk production, industry objectives expanded around the turn of the century to include efforts to reduce the environmental impacts of dairy production and strengthen future sustainability of the industry. To support industry needs to increase production while reducing environmental consequences, dairy and agricultural science fields have and will continue to elucidate connections between management practices, production, and downstream environmental impacts. A key component of efforts to reduce environmental impact is the ability to quantify each impact under a variety of management practices. Because measurement of things like farm emissions, runoff, and leaching is impractical or infeasible, prediction and simulation models are necessary for evaluating dairy production's contributions to these environmental pollutants (Kohn, 2015).

FROM FEED TO THE ENVIRONMENT

Obvious connections between dairy production and the environment are the pathways from dairy feed digestion to the environment. These include enteric methane emissions, manure methane, ammonia and nitrous oxide emissions, and manure N and P runoff and leaching. As a potent Greenhouse Gas (GHG), reducing enteric and manure methane emissions could reduce contributions of dairy production to global climate change. Depending on storage practices, application rates, and nutrient concentrations, dairy manure can produce GHG emissions, cause build-up of nutrients in the soil, and contaminate water and air resources. Growing awareness of and popular press attention to dairy and animal agriculture contributions to carbon emissions, water contamination, and algal blooms (e.g. Gardiner, 2015), have made these important areas of focus. Although increased productivity itself has been demonstrated to reduce the GHG production per unit of milk (Capper and Bauman, 2012), nutritional efforts to improve dairy

sustainability over the past 20 years have also focused on reducing enteric methane and manure nutrient concentrations. Development of models to predict and describe these processes have increased so that we can quantify and compare environmental impacts of competing dairy management practices within the larger context of food production.

Methods to reduce enteric methane through nutritional supplements and feeding strategies have been widely studied with variable success (Knapp et al., 2014). Models that can predict methane production under a range of animal and dietary conditions using varying degrees of empiricism and mechanism (e.g. Hristov et al., 2017; van Lingen et al., 2018; Rotz, 2017) are available and have proved useful in quantifying this source of GHG. Similarly, the connections between dairy cattle nutrition and manure N and P excretion have been extensively studied with models following suit (e.g. Reed et al., 2015; van Lingen et al., 2018; Powell and Broderick, 2011; Satter et al., 2005; NRC, 2001). This kind of information has been useful for more precisely and efficiently meeting nutrient requirements which can reduce feed costs while improving environmental metrics as well as for national GHG and non-point source pollutant inventories (Cerosaletti et al., 2004; Thoma et al., 2013).

However, nutritional interventions are only part of the dairy system and have a limitations in their scope to improve sustainability before they are constrained by economic or biological feasibility (e.g. Moraes et al., 2015). Manure management is, therefore, the next logical piece of the dairy nutrition-environment puzzle and one that has also received a lot of attention in the past decades. Continuing innovation in manure management includes anaerobic digestion, liquid-solid separation, and improved application recommendations, among others (Leytem et al., 2018). Similar to nutritional strategies, manure management innovation has been accompanied by models to quantify benefits of implementing these practices (Leytem et al., 2018) but have physical and economic limitations in the scope of their impact. Anaerobic digestion, for example, requires a large investment in equipment, and, without supplemental substrate for digestion, may not produce enough energy from manure alone to be practical (Innovation, 2014). Also, improvements in manure N storage and application methods have small opportunities for improvement in relation to whole farm N efficiency and may shift more N to the soil and increase potential losses from the field (Reed et al., 2017).

Combining models of dairy cattle nutrition and excretion with those of manure management and field scale crop and soil models, one can trace nutrients and quantify their environmental impact from the feed all the way to their air or water loss pathways. However, this is the point in the dairy nutrient cycle where most investigations have stopped. Although it is well known that mineral N fertilization influences nutritional quality of forages in mostly predictable ways (i.e. increasing CP and fiber content) (Coblentz et al., 2017), manure fertilization has less predictable quality outcomes, especially for warm season grasses (Peyraud and Astigarraga, 1998; Coblentz et al., 2017), which makes it difficult to connect nutrition in Year 1 to forage quality and subsequent excretion and environmental loss in Year 2. Closing this nutrient cycle is an important area of investigation for dairy scientists and will support holistic investigations into long term

sustainable management practices to prevent shifting impacts from one location to another or from one year to the next.

CLOSING THE CYCLE

In order to evaluate dairy sustainability in a holistic, rigorous way, we need whole farm models that represent the latest state of knowledge and complete dairy nutrient cycles such that the downstream impacts of management choices can be evaluated. Few whole-farm dairy models exist compared to individual farm component models, such as stand-alone crop production, animal feeding, or soil and environmental quality models. Furthermore, existing whole-farm models all have limitations in their ability to completely represent the complexities of modern dairy farms and their technologies. IFSM, arguably the most comprehensive of existing dairy farm models, often uses algorithms developed from science of the 1980s and 1990s and so does not capture the most recent improvements in nutrition and manure management. Further, IFSM simulates one year at a time, which prevents evaluation of long-term nutrient cycling and use efficiency and carry-over effects of management practices between years.

For example, choice of fertilizer and crop rotation have been shown to have variable long-term impacts on things like soil organic carbon and N mineralization that impact soil fertility and thus crop production (Oberlitz et al., 2018; Poffenbarger et al., 2018; Zavattaro et al., 2017; Triberti et al., 2016). There is also evidence that manure N from dietary alfalfa N is more readily available to oats and corn than manure N from dietary corn N resulting in higher DM yields (Powell and Broderick, 2011; Powell et al., 2017). Incorporation of the relationships between diet ingredients, manure composition, and subsequent year crop quality will improve whole farm simulation models and allow investigations into longer term consequences of management choices like:

- How does herd nutrition management affect nutrient fate in crop uptake and crop quality?
- Does reduction in enteric methane shift emissions to manure or soil based on the amount of volatile solids excreted?
- What are the impacts of different manure wastes streams and application quantities and methods on crop feed quality and nutrient losses to the environment?

Further, since management decisions begin long before ration formulation or manure application, closing the gaps in dairy nutrient models will also help inform larger scale management decisions by answering questions such as:

- How do crop system choices (e.g., increasing perennial legume use) affect fertilizer purchase requirements, animal diet formulation, animal productivity, and post-animal nutrient availability for future crop and feed production?
- How does dairy nutrition affect changes in soil health parameters (erosion, soil carbon) and subsequent crop growth and farm productivity?

These are just a few of the systems questions that a modern, whole-farm dairy simulation model can help to answer. To continue to improve the sustainability of the dairy

industry we must build on the progress of the past decades using modeling to integrate knowledge of all parts of the dairy system and think holistically about both short and long term impacts of management choices.

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SOLUBLE LIGNIN AND ITS RELATION TO KLASON LIGNIN, ACID-DETERGENT LIGNIN AND DIGESTIBILITY OF NDF

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INTRODUCTION

Measurements of lignin are among the most used to evaluate forages. The most popular is acid detergent lignin with sulfuric acid (ADL)(Van Soest, 1963) and is currently used in the Cornell Net Carbohydrate and Protein System (CNCPS) (Tedeschi and Fox, 2016), followed by Klason lignin (KL)(Theander et al., 1995). These methods produce disparate values (Hatfield et al., 1994). The ADL values are lower than KL, and are criticized for low recovery of lignin (McSweeney et al., 1994, Lowry et al., 2002). Soluble lignin (∆L) is the difference between KL and ADL, called dispersible lignin in the Australian papers. The difference between values obtained by these methods is substantial, and has been ignored as a biological variable. NDFD48 is a measure of in vitro digestibility of NDF at a time point of 48 hours, expressed as percent of NDF. This paper investigates the ∆L and its relation to KL, ADL, and NDFD48.

Soluble lignin was found in the rumen fluid of a cow fed Australian Spear grass (Gaillard and Richards, 1975), and has also been observed from the action of fungal cellulases upon cell walls of grasses, (Grabber et al., 2002, Mc Sweeney et al., 1994). The soluble lignin found in the rumen by acid precipitation at pH 3 from clarified rumen fluid is indigestible (Neilson and Richards, 1978), but is not known to have negative effects on fiber digestion (untested). The rumen residue also contains small amounts of protein and hemicellulosic carbohydrates (Gaillard and Richards, 1975).

DATA SETS AND ANALYSIS

The results presented here are based on two data sets: one by J.B. Robertson in the Cornell Laboratory consisting of 7 grass forages, 13 maize plants, 4 corn silages and 15 alfalfa hays. The second set is from Jung et al. 1997, table 1 in their paper, and digestibilities of NDF (NDFD48) from their figures, decoded by Van Soest (2015). The data set of Jung et al. consists of 16 C_3 grasses, 8 C_4 grasses, and 12 legumes, including 6 alfalfas and 6 other legume species and was conducted with the purpose of discrediting ADL. The NDFD48 of one C_3 grass could not be read from their figures. Forages were analyzed for in vitro digestibility (NDFD48), acid-detergent lignin (ADL) (Van Soest 2015) and Klason Lignin (KL) by the method of Theander et al. (1995). Soluble lignin (∆L) was calculated as the arithmetic difference between KL and ADL, as percent of dry matter, and also as a percent of Klason Lignin (∆L/KL).

RESULTS AND DISCUSSION

Correlations of ΔL with NDFD48, KL and ADL are shown in table 1. Correlations of NDFD48 with ΔL on a dry matter bases are insignificant, and vary from the negative to the positive. This result is surprising in view of the high negative correlations of ADL and KL with digestibility (Jung et al. 1997). Correlations with KL are variable and positive, as expected, because ΔL is a part of KL. Correlations with ADL are low and mostly insignificant.

Table 1: Correlations of ΔL on a dry matter basis with NDFD48, KL and ADL.

Component	N	NDFD48	KL		ADL	
Cornell Data						
Grasses	7	-0.27	0.57		0.15	
Maize Plants[a]	17	-0.34	0.85	**	0.27	
Alfalfas	15	-0.22	0.93	**	0.62	*
Jung et al. (1997)						
C_3 Grasses	16	0.12	0.71	**	0.00	
C_4 Grasses	8	0.39	0.89	**	0.17	
Legumes	12	0.29	0.41		-0.45	

[a] includes silages;
* P < 0.05; **P < 0.01

The data are shown in a different way in table 2, as mean values of concentrations of dry matter for ADL and KL. The ranges of ΔL are presented as percentages of KL. These amounts are large and variable and average about 50 percent of the KL. Values of ΔL/KL vary to over 80 percent of KL (table 2), probably in the most immature grasses of lowest ADL. The Ranges of ΔL observed by Gaillard and Richards (1974) and McSweeney et al., (1994) are similar. Values of ΔL on a dry matter basis are obtained by subtracting column 3 from column 2 in table 2. The associated decline in ΔL/KL with increasing ADL is seen in all groups (table 3). Correlations of ΔL/KL in table 3 are negative with ADL and positive with NDFD48. The decline in ΔL/KL with increasing ADL suggests the conversion of ΔL to ADL as forages mature, a matter of need for further study. Unfortunately, the dates of cutting and description of forage stages are lacking.

The peculiar nature of the ADL-KL-ΔL relationships is shown in in figure 1. Soluble lignin and KL are plotted against ADL on a dry matter basis. The ΔL relationship with ADL is flat and statistically insignificant and relatively constant as a proportion of dry matter. The KL rises with a unity slope relative to ADL (1.14-0.14). The intercepts of the two regressions are the same at 6.3 percent of dry matter. The rise in values of KL against almost constant ΔL leads to a decline in proportion of ΔL as a part of KL, (ΔL/KL). This is a large statistical interaction due to increasing ADL, and contributes to negative associations between ΔL/KL and ADL. The declining relations of ΔL/KL with ADL are shown in in figures 2 and 3. The variability is greater in mature forages (Figure 3) than in growing forages (Figure 2). However, considerable

association occurs in mature forages, like maize and other C_4 plants grown for their seed. The decline in ΔL/KL with increasing ADL, gives a strong statistical interaction with positive correlations with NDFD48, which occur in all groups (Table 3 and Figure 4). The positive relations between ΔL/KL and NDFD48 indicate that ΔL has no negative effect on NDFD48, and refutes claim that KL represents the main negative factor affecting NDFD48 and forage quality.

Table 2: Mean values of Klason, acid detergent lignin, ΔL/KL and their ranges.

Component	**N**	**KL**	**ADL**	**ΔL/KL (%)**	**Range ΔL/KL**
Cornell Data					
Grasses	7	13.8	6.4	55	42 – 73
Maize Plants	13	6.4	2.8	56	47 – 70
Maize Silage	4	7.7	3.2	59	53 – 65
Alfalfa	15	11.3	6.6	41	25 – 52
Jung et al. (1997)					
C_3 Grasses	16	9.9	3.3	67	49 – 83
C_4 Grasses	8	8.5	3.4	59	46 – 70
Alfalfas	6	12.0	8.4	30	22 – 36
Other Legumes	6	12.2	7.1	43	28 - 54

KL – Klason lignin
ADL – Acid detergent lignin
ΔL/KL – Acid detergent soluble lignin divided by Klason lignin (%)

Table 3: Correlation between acid detergent soluble lignin expressed as percentage of Klason Lignin (ΔL/KL) with acid detergent lignin (ADL) on a dry matter basis and with in-vitro digestibility of neutral detergent fiber (NDFD48).

Forage Class	**N**	**ΔL/KL * ADL**	**ΔL/KL * NDFD48**	**ADL * NDFD48**
Cornell				
Grasses	7	-0.85 *	0.76 *	-0.95 **
Maize Plants	17	-0.64 **	0.44	-0.78 **
Alfalfas	15	0.07	0.12	-0.55 *
Jung				
C_3 Grasses	16	-0.90 **	0.83 **	-0.79 **
C_4 Grasses	8	-0.41	0.73 *	-0.59
Legumes	12	-0.81 **	0.58 *	-0.69 *
All Legumes Combined	27	-0.48 *	0.34	-0.53 **

* $P < 0.05$; **$P < 0.01$

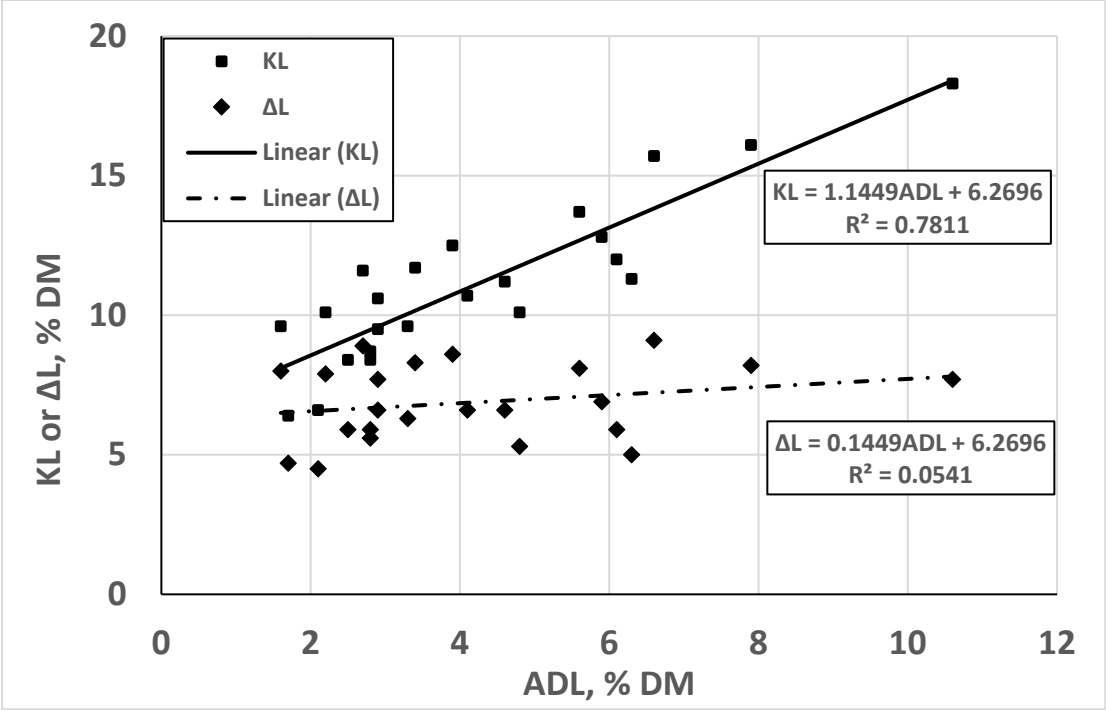

Figure 1: Relationships of KL and ∆L with ADL for C_3 forages from Van Soest and Robertson and Jung et al. (1997).

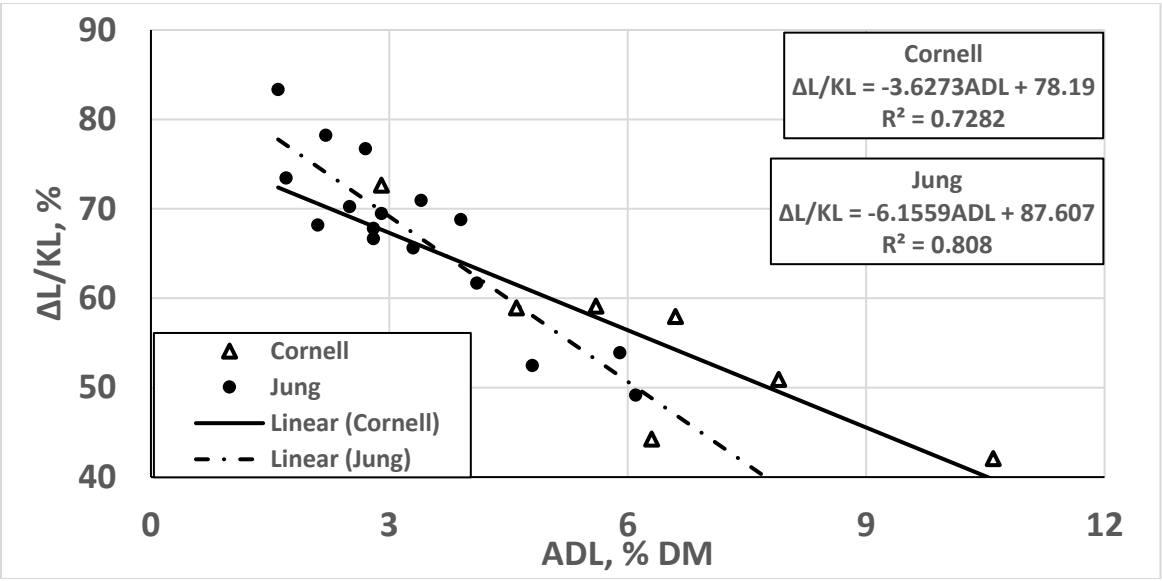

Figure 2: Relation of acid-detergent soluble lignin as a percent of Klason lignin with acid-detergent lignin as a percent of dry matter. Combined data for forage grasses from Robertson (Cornell) and Jung et al 1997.

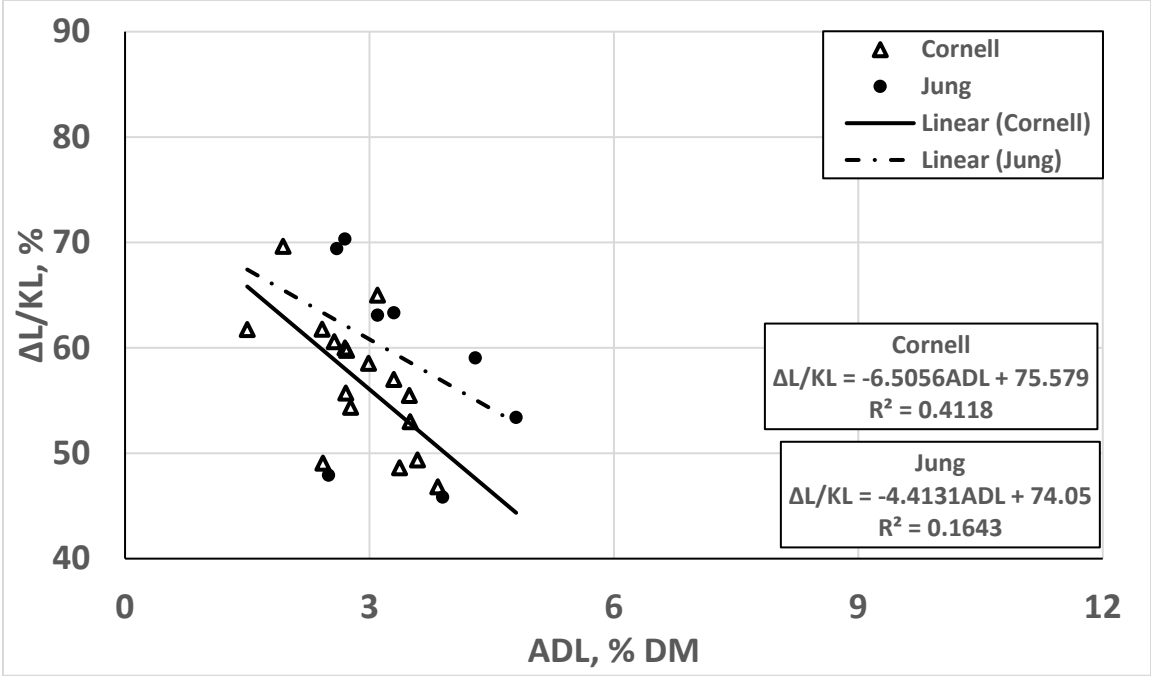

Figure 3: Relation of acid-detergent soluble lignin as a percent of Klason lignin with acid-detergent lignin as a percent of dry matter for C_4 mature plants. Combined data from Robertson (Cornell) and Jung et al 1997 (n=25).

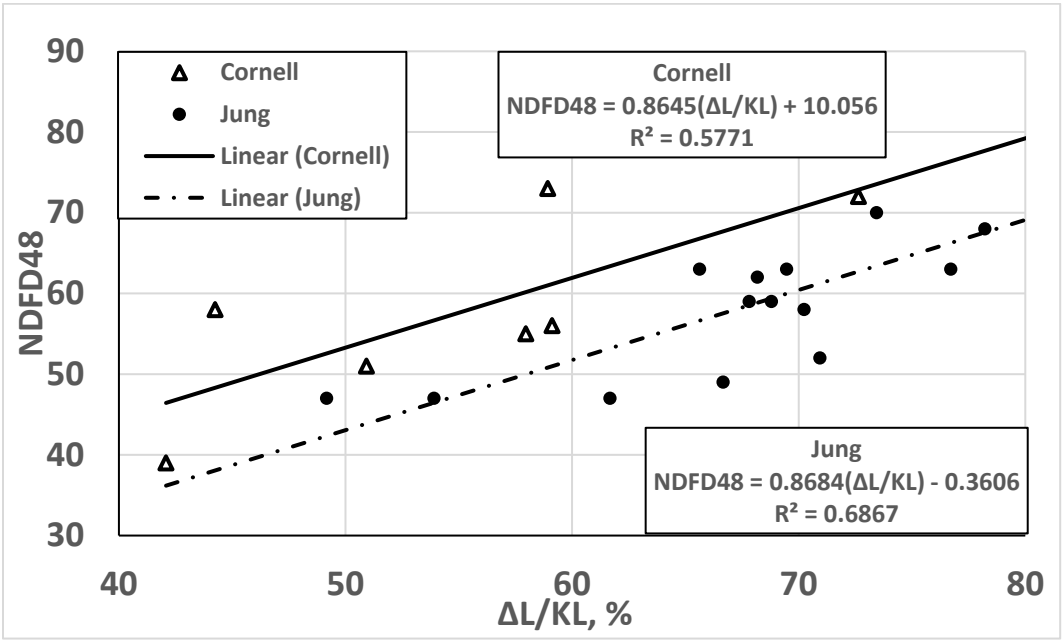

Figure 4: Relation between the in vitro digestibility of NDF (NDFD48) with acid-detergent soluble lignin as a percent of Klason lignin (ΔL/KL). Combined data for forage grasses (n=23).

The variances of ∆L and ADL are the same on a KL basis, because the sum of the two are a constant 100 percent. Thus a correlation of positive sign for ∆L (Figure 4) has the opposite negative value with ADL. Correlations of ∆L and ADL on a KL basis with NDFD48 are in Table 3. The relation of NDFD48 with KL is more variable, because the KL is a composite of the two differing fractions. The high variance of KL was noted by Jung et al., 1997. The KL does not appear to be a uniform substance in a nutritional sense (Van Soest 1994, chapter 22).

The data of Jung et al (1997) included here and reanalyzed as an independent set supports our conclusions. The paper of Jung et al was conducted to discredit ADL and was unsuccessful because their statistical analyses failed to show any advantage of KL, which also has the disadvantage of being a more laborious and time consuming procedure. Jung et al and related literature was used to justify KL, and overlooked the biological role of lignin as a major plant protection factor. Lignin is energetically very expensive (Jung et al, 1999), and is irretrievable in times of plant stress, such as dry conditions and cold (Van Soest and Hall 1998). Corn plants reduce lignification in favor of seed production in unfavorable conditions, as a matter for survival.

Historically at the time of the original publications of the ADL (Van Soest 1963; Van Soest, 1973), it was thought that the precipitate formed on prolonged boiling in acid represented non-lignin matter (Moon and Abou-Raya 1952). This precipitate is however the main component of ∆L. Since that time developing lignin chemistry changed view to regard KL as total lignin. This paper shows the divergent characteristics of ∆L and ADL (Figures 2, 3, 4 and Table 3). The ADL is unanalyzable and cannot be resolved into identifiable phenolics (McSweeney et al 1994), although such phenolics are found in ∆L (Raffrenato 2011).

CONCLUSIONS

Current views of lignin have criticized ADL for a lack of recovery of ∆L, overlooked as an independent anti-quality factor. Soluble lignin differs from KL in its statistical associations, and is variably related to KL, of which it is a part. Klason lignin appears as a heterogeneous entity. However, when ∆L is expressed as a proportion of KL, it has a strong negative association with ADL. This interaction yields positive correlations with NDFD48. The negative association of ∆L/KL with ADL occurs in all forage classes. However, ADL is consistently related to indigestibility of NDF and its use in the CNCPS is validated.

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MILK ANALYSIS FOR DAIRY HERD MANAGEMENT: TODAY AND IN THE FUTURE

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INTRODUCTION

In 2014 (Barbano et al., 2014), we introduced the application of mid-infrared (**MIR**) for rapid milk fatty acid (**FA**) analysis and reported positive correlations of bulk tank milk fat test with a higher proportion and concentration of de novo FA in bulk tank milk. The analytical aspects of reference milk FA analysis and model development and validation were reported by Wojciechowski and Barbano (2016) and Woolpert et al. (2016). The form of the FA data from the MIR was structured to provide information on the relative proportions of de novo (C4 to C15), mixed origin (C16:0, C16:1, C17:0), and preformed (C18:0 and longer) FA in milk, the mean FA chain length (carbon number) and degree of unsaturation (double bonds/fatty acid). With experience in the field testing of bulk tank milk from individual on farms we found that providing this FA information in units of grams per 100 grams of milk was more useful. Since that time, we have continued to collect data on milk FA variation in bulk tank milk and it's relationship to feeding and farm management.

Woolpert et al. (2016, 2017) have reported the results of two studies to determine feeding and farm management factors influencing milk FA composition and their relationship to bulk tank milk fat and protein test and production per cow per day of fat and protein. In the first study (Woolpert et al., 2016) 44 commercial dairies were identified as either predominantly Holstein or Jersey in northern Vermont and New York. The yields of milk fat, true protein, and de novo FA per cow per day were higher for high de novo (**HDN**) versus low de novo (**LDN**) farms. Woolpert et al. (2016, 2017) estimated the impact of differences in de novo fatty acid concentration in milk among farms on bulk tank fat and protein, and estimated the impact of those differences e on farm income per 100 cows per year. Higher milk de novo fatty concentration drove higher milk fat, milk protein, and grew revenues from milk.

Based on data from these studies the following graphs (Figures 1 thru 4) for Holstein farms were developed to help farms understand the relationships between bulk tank milk FA composition and bulk tank fat and protein tests. Work is currently in progress to produce similar graphs of these relationships for milk produced by Jersey cows. These relationships of milk fatty acid composition to bulk tank milk fat and protein concentration are the basis of use of milk fatty acid composition in combination with herd management information to aid in making decisions to adjust dairy cattle ration composition or management to improve the production of milk fat, protein, and milk volume per cow per day. It has been shown that seasonal varation of fat and protein conentratroin in bulk tank milks in the northeastern US is related to seasonal varation in de novo fatty acid concentration and production in grams per cow per day (Barbano et al., 2017).

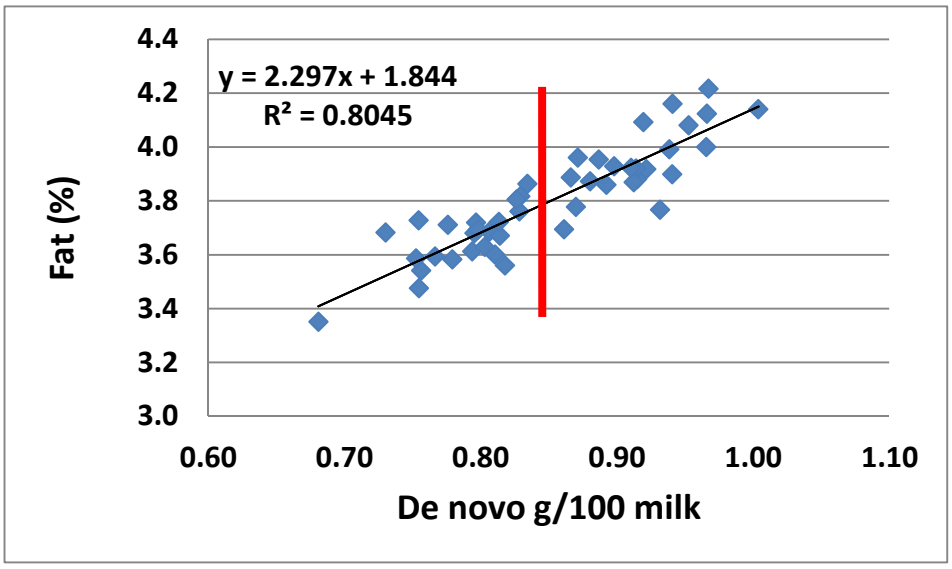

Figure 1. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of de novo FA in milk. In general, a farm needs to have a concentration of de novo FA higher than 0.85 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

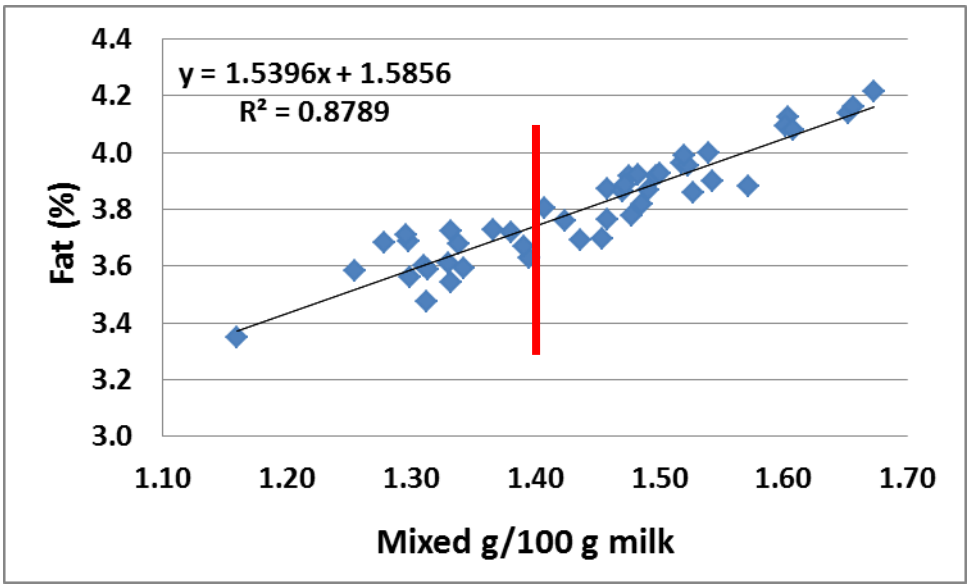

Figure 2. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of mixed origin FA in milk. In general, a farm needs to have a concentration of mixed origin FA higher than 1.40 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

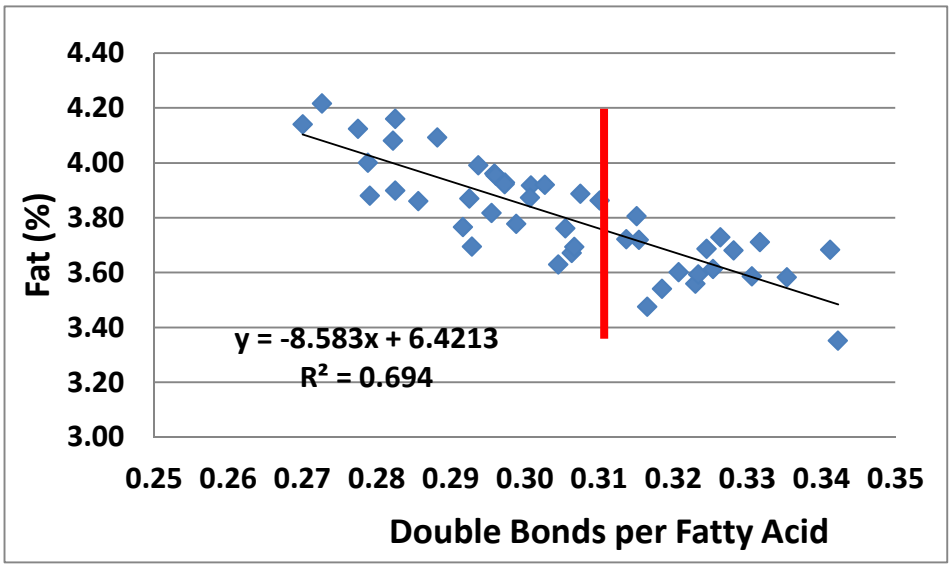

Figure 3. Relationship of bulk tank milk fatty acid unsaturation with bulk tank milk protein test. As double bonds per fatty acid increases the bulk tank milk fat test decreases. To achieve a 3.75 % fat test a farm needs to have a double bond per fatty acid of less than 0.31.

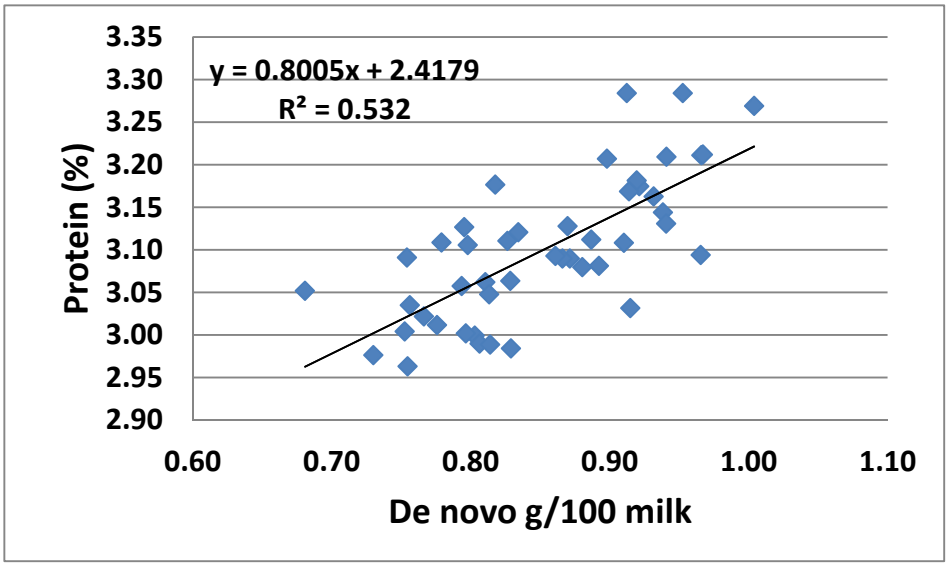

Figure 4. Relationship of bulk tank milk protein concentration with change in de novo milk fatty acid concentration. As de novo milk fatty acid concentration increases milk protein increases.

MILK ANALYSIS BY MIR

Measurement of Milk Fat, Protein, and Lactose

Mid-infrared spectrophotomers have been used extensively in the dairy industry for measurement of the concentration of fat, protein, and lactose in milk since the late 1970's. The first instruments used physical optical wavelength selection filters mounted in a rotating filter mounting wheel and each filter (up to 8) was moved in and out of the infrared light beam that passed through the same in the instrument cuvette. The filters were designed in pairs of a sample and reference wavelength. There were 4 filter pairs called the fat A, fat B, protein, and lactose filter pairs (Kaylegian et al., 2009a). Because these optical filters were layers of minerals that were selected to block most MIR wavelengths from passing through the filter, the beginning and ending wavelength varied from filter to filter and total energy passage though each filter was slightly different (Smith et al., 1995). Details of precalibration instrument adjustment and calibration adjustments have been reviewed (Lynch et al., 2006). With the advent of interferometers and less expensive computing power, it became feasible to use Fourier transform optical system with an interferometer to quickly calculate a full infrared spectra of each milk sample instead of using optical filters. This increased the sample analysis speed of the MIR milk analyzers and today the fastest instrument can test 500 to 600 mlk samples per hour.

The availability of the full spectra for each milk sample opened up the possibility of development of partial least squares (**PLS**) statistical models to predict the concentration of fat, protein, and lactose in each milk sample, instead of the traditional basic filter approach. The availability of the full spectra also provide an opportunity to refine the basic traditional filter model approach because "virtual" filters could be digitally cut from each spectra to use the wavelengths that were formerly used as optical filters. Kaylegian et al. (2009a) conducted a systematic study designed to optimize the center wavelength and wavelength band width that could be selected from each spectra for the fat A, fat B, protein, and lactose filter wave lengths. Those wave lengths were published (2009a) and can be used uniformily in all MIR milk analyzers, regardless of equipment manufacturer to provide a traceable and well defined method of measurement for fat, protein, and lactose content of milk. Total solids, solids not fat, and other solids can be calculated from those main components. Once the filter wavelengths have been set in an instrument, then the primary slope of the uncorrected signals can be set to achieve a 1 to 1 relationship with milk chemistry and intercorrection factors can be calculated (Lynch et al., 2006). The advent of modified milk calibration samples for MIR analyzers (Kaylegian et al., 2006) can also be used to calculate the fixed intercorrection factors for each MIR. The USDA Federal Milk Markets currently use optimized traditional filter models (70/30 fatB/fat A, protein and lactose) because this approach is not proprietary, it is well defined, traceable, and yields accurate test results for fat, protein and lactose. Extensive evaluation of PLS models for measurement of milk fat, protein, and lactose have been performed in the USDA FMMA labs and PLS were not found to provide better accuracy than the properly set up traditional filter models for measuring fat, protein, and lactose content of milk.

Measurement of Minor Components in Milk

The first minor milk component of milk to be measured routinely using infrared milk analysis was milk urea nitrogen. The combination of either low concentration of a minor milk component or the fact that the milk component does not have a unique signature wavelength related to its structure, causes the developer of MIR analytical methods to use chemometric statistical approaches to predict the concentration of that milk component. The most common chemometric method used in MIR milk analysis is PLS. A PLS model is a mathematical equation that uses a weighting factor for the light absorbed at each wavelength to calculate a predicted concentration of the milk component. The actual number of individual wavelengths used in the model calculation will be a function of the range(s) of wavelength selected by the researcher doing the PLS modeling and the spectral resolution (i.e., inverse centimeter frequency of data points) of the optical system used for the measurements. Different optical systems from different manufacturers may differ in inverse centimeter resolution. The developer of the PLS model has to collect a population of milk samples and then uses the spectra of each sample and the chemical reference value for the component being measured to develop the PLS equation. Each new PLS model developed to measure a milk component is unique and its predictions may differ in sensitivity to external factors such as preservative concentration (both active and inert ingredients), preservative type, and homogenizer performance. Thus, every new prediction model or new version of a PLS model to predict the same parameter needs to be tested for it ruggedness with respect to external factors.

The reference chemistry, the PLS modeling procedures to produce PLS models to predict the concentration of milk de novo, mixed origin, and preformed fatty acids and fatty acid mean chain length and double bonds per fatty acid, and the modeling performance statistics, were reported by Woolpert et al. (2016) and Wojciechowski and Barbano (2016), respectively. The details of post GLC calculation of milk fatty acid composition for herd management are very specific and laboratories that run GLC analysis typically do not follow these procedures. To obain comparible results for herd management specific data post GLC data handing procedures are required and have been described (Kaylegian et al., 2009b). In brief, only a specific standard group of fatty acids are included in the calculation, data for unidentified peaks are never included, methylation recovery factors for short chaing fatty acids may be needed, and all data are normalized to a 100% basis before providing final milk fatty acid reference values.

After PLS modeling, the reporting of model performance statistics (i.e., SECV, r-squared and RPD) and external model validation statistics (standard deviation of the difference between instrument predictions and reference values) for an independent set of milks that were not used in the modeling should be reported to provide users with an estimate of the expected performance of each PLS model. This is true not only for milk fatty acids but also for any other milk component estimated using PLS models for MIR milk spectra. The raw prediction of a milk component concentration or structural parameter metric by a PLS model is an uncalibrated value and a set of milks with an adequate range of concentration and good distribution/dispersion of concentrations within that range is need to periodically adjust the calibration slope and intercept of the output of the prection model.

Interpretation of Milk Analysis Data for Feeding and Health Management

Milk analysis data should not be used in isolation. The key milk analysis data are fat, protein, lactose, SCC, MUN, de novo, mixed, and performed fatty acids, fatty acid chain length, and double bonds per fatty acid plus milk weight per cow per day when looking at bulk tank milk composition data for a herd of dairy cows that averages between 150 to 200 days in milk. High frequency information (i.e., daily or multiple loads per day) is critical to follow the status of the herd and quickly identify when something is changing. The best opportunity to get this stream of data is as part of the routine milk payment testing of individual producer milks. In the Northeast US, we have developed this condition at the St Albans Cooperative Creamery, AgriMark Cooperative, and Cayuga Milk Ingredients through their milk payment testing. Producers and their nutrtionists receive these data soon after milk testing is completed, because the data are posted on a secure web site. The cooperatives do not provide any interpretation of the data, they simply make it available in a timely fashion. At this point the producer/dairy nutritionist has the opportunity to bring the milk data together with the feeding intake data, environmental, and ration formulation data.

Examples of Herd Managment Data Behavior and Interpretation

Interpretation of data is always attempting to find a single solution to a multifactorial problem. Often, one issue is we don't stand back far enough to see the multifactorial nature of issues. This is particularly true in relation to the intersection of animal health, nutrition and feed, and management issues. There are examples of expected changes in milk fat, protein and lactose composition and production per cow per day, as a result of specific single conditions that are know to impact milk compositon and production. While these may be useful examples in learning how to use and interpret milk composition, it is possible that multiple factors will be influencing milk composition at the same time and this need to be considered for the interpretation of real time herd data. An example is when periods of extreme heat stress are super-imposed on any of the conditions described below. Heat may also produce changes in the release of fat from the feed and those changes in feed characteristics may persist in the feed after the heat stress conditions have been removed and may continue to have an impact.

Trans Fatty Acid Induced Milk Fat Depression

Biohydrogenation of unsaturated milk fatty acids in the rumen of dairy cows has been documented to cause milk fat depression (Harvatine and Bauman, 2011). Rumen produced trans fatty acids are carried through the cow's blood stream to the mammary cells where they are taken into the mammary cells and inhibit the chain elongation mechanism in de novo synthesis of milk fatty acids produced within the mammary cells. Conditions in the rumen that set up cows for milk fat depression are a diet too high in rumen unsaturated fatty acid load (RUFAL), high levels of unsaturated fat in the dietary forages, and oil seed supplements that is released too fast in the rumen from the feed structure, or management factors (e.g., over crowding) that cause slug feeding resulting in low rumen pH. Low rumen pH favors a population of rumen microflora that convert dietary cis unsaturated fatty acids to trans fatty acids in the rumen. Different cows at different stage of lactation and production levels in

different feeding groups will go into milk fat depression at different rates. Some cows in a herd may be clearly in milk fat depression and others may not. It should be remembered that the bulk tank milk test and changes in milk fatty acid composition represent the average of the dairy herd. As a higher percentage of the cows in the herd are in milk fat depression there will be a gradual change in milk composition with time.

When the fat content of milk decreases there can be multiple causes. New milk fatty acid analysis using rapid MIR milk analysis can be used to try to understand the cause of lower milk fat test or milk fat production in a dairy herd and allow appropriate corrective farm management measures to be taken. Table 1 contains an example of how milk composition and milk production may change as a result of milk fatty acid induced milk fat depression. This data can be obtained on bulk tank milk from a milk payment testing laboratory that is properly set up with a MIR milk analyzer capable of doing milk fatty acid testing. The test is done on the same milk sample used for milk payment testing, so there is not extra milk sample collection and it is done with the same instrument that is doing the measurement of milk fat and protein test for payment testing.

The typical sequence of changes in milk composition and milk fatty acid are shown in Table 1. It is useful to integrate data on the milk production per cow along with the milk composition data to obtain a better understanding of how, on average, the dairy herd is interacting with feed, environmental conditions, and managmenet practices.

First, the nutritionist must keep in mind where a specific herd performance is with respect to established correlations among concentration of milk fatty acids in grams per 100 grams of milk and bulk tank fat and protein concentration, as shown in Figures 1, 2, 3 and 4. If the herd is a normal well-managed herd that is producing very well, then their data should be somewhere near the regression lines in Figures 1, 2, 3, and 4. The first change in bulk tank milk composition that can be noticed when a herd is moving into trans fatty acid induced milk fat depression is that the mixed origin fatty acids expressed as grams per 100 grams of milk decreases. Palmitic acid (C16:0) is the longest fatty acid produced in the chain elongation process of de novo fatty acid synthesis and it is the major fatty acid of the mixed orgin fatty acid group. Palmitic acid is the first de novo fatty acid to decrease in production when trans fatty acid induced milk fat depression begins. As you can see from Table 1, the change in mixed origin fatty acid leads the change in fatty acid composition from week to week. As that occurs, the concentration of de novo fatty acids also begins to decreases. The higher level of trans fatty acids produced in the rumen begin to have a negative impact on rumen microbe growth and reduce microbial biomass. This reduces total availability of rumen fermentation produced essential amino acids from the rumen microbial biomass and milk true protein concentation and production per day decrease. Average milk fatty acid chain length will gradually incease and double bonds per milk fatty acid will also increase. For TMR fed high producing Holstein herds, when the double bonds per fatty acid exceed a value of 0.31 double bonds per fatty acid in the bulk tank, the probability of trans fatty acid milk fat depression increases.

Once the milk fat depression gets more severe and extensive in a dairy herd, it becomes apparent that the de novo fatty acids are also decreasing in g/100 g of milk. Generally, if this is the only problem in the herd, both bulk tank milk fat percentage will decrease but milk output per cow will probably not change if there is adequate available glucose for milk lactose synthesis (i.e., no other cow health issues). The grams per day output of de novo and mixed origin fatty acids decrease and preformed fatty acid output per day stays about the same. On a relative fatty basis, the de novo and mixed orgin fatty acids as percentage of total milk fatty acids decrease and the preformed fatty acids as a percentage of total fatty acids increase but the output of performed fatty acids per day (g/cow/day) is not increasing and the per day output (g/cow/day) of both de novo and mixed origin fatty acids are decreasing.

Table 1. Example of expected changes in milk production and composition for bulk tank milk on corn based total mixed ration fed Holstein cows that are progressively moving into rumen produced trans fatty acid induced milk fat depression.

	A Holstein Farm Transitioning into Trans Fatty Acid Induced Milk Fat Depression										
		X1000	fatty acids per 100 g milk				fatty acids per 100 g milk			carbon #	DB/FA
week	lbs	SCC	Fat	Lactose	Protein	MUN	Denovo	Mixed	Preformed	FA CL	FA Unsat
1	92.0	147	3.88	4.61	3.25	9.7	0.91	1.41	1.34	14.54	0.28
2	91.8	155	3.80	4.63	3.25	9.9	0.90	1.35	1.34	14.60	0.30
3	91.6	162	3.71	4.62	3.17	10.3	0.85	1.30	1.36	14.68	0.31
4	91.4	170	3.63	4.61	3.14	10.7	0.80	1.25	1.38	14.78	0.33
5	91.3	158	3.42	4.61	3.10	11.2	0.72	1.15	1.36	14.90	0.34

		fatty acids								
	Milk kg	Denovo	Mixed	Preformed	Lactose	Fat	Protein	fatty acids per 100 g fatty acids		
week	per day	g/day	g/day	g/day	g/day	g/day	g/day	Denovo	Mixed	Preformed
1	41.8	380	589	560	1927	1621	1358	24.86	38.50	36.64
2	41.7	375	563	559	1930	1584	1355	25.06	37.59	37.36
3	41.6	353	541	566	1921	1543	1318	24.22	37.04	38.75
4	41.5	332	519	573	1915	1506	1303	23.32	36.44	40.23
5	41.5	300	477	564	1911	1418	1284	22.40	35.55	42.05

Immune System Activation and Response to an Immune Challenge

Activation and response of the immune system in a dairy cow requires the use of energy from the diet, body reserve, or both to provide energy for the immune system response. In general, the form of energy used to support an immune response to a health challenge is blood glucose. The use of blood glucose in support of an immune system response can be quite high. Blood glucose is also required for lactose synthesis in the mammary gland. The synthesis and output of milk lactose in grams per cow per day is highly correlated (r-squared > 0.99) with milk output per day.

An example of expected changes in milk fatty composition and milk production per cow in response to a hind gut immune system response is shown in Table 2. The primary observed sign for this condition from a milk production is the extreme decrease in milk yield per cow per day. However, it should be remembered that the decrease in milk yield is tightly correlated with a decrease in lactose output per cow per day due to the fact that there is lack of sufficient blood glucose to maintain milk lactose output. In this case milk output has to decrease. If milk output per cow per day decreased substantially and synthesis of milk fat and protein remained the same, then it would be expected that there was a large increase in concentration of fat and protein concentration (g/100 g milk). While there may be some small inceases in milk fat and protein concentration, very large increases are not usually observed and therefore when synthesis of lactose for milk is reduced, in general g/cow/day synthesis of both fat and protein decrease. This is observed in the data in Table 2.

If the nutritionist only looks at the concentration of milk fat, protein, and lactose in milk (g/100 g milk), then the milk looks relatively normal. In addition, when looking at the relative proportion of de novo, mixed, and preformed fatty acids (Table 2), it looks like little has changed. This would indicate that feed utilization and rumen fermentation is likely functioning well and has not been impacted by the immune system response of the dairy cow outside of the rumen. Often it is said by nutritionists and producers, the milk composition looks good but the cows just are not milking. This may be puzzling for a nutritionist (when the impact of heath is not considered) and this is why the dairy industry needs to looking at more complete picture of health and nutrition together to correctly interpret and understand changes in milk component concentration and output per cow per day to make management decisions to address real time issues that influence milk production and profitability.

Table 2. Example of expected changes in milk production and composition for bulk tank milk on corn based total mixed ration fed Holstein cows that are progressively experiencing a hind gut immune system challenge (e.g., leaky gut, virus infection, etc.). The key change is the progressive and decrease in grams of lactose per cow per day, while concentration of lactose in milk remains unchanged.

A Holstein Farm that is developing a hind gut problem causing an immune system activation.											
		X1000	fatty acids per 100 g milk				fatty acids per 100 g milk			carbon #	DB/FA
week	lbs	SCC	Fat	Lactose	Protein	MUN	Denovo	Mixed	Preformed	FA CL	FA Unsat
1	92.0	147	3.89	4.61	3.25	9.9	0.91	1.40	1.34	14.54	0.29
2	87.0	150	3.92	4.63	3.20	9.6	0.93	1.43	1.34	14.60	0.31
3	84.0	160	3.87	4.64	3.22	10.1	0.87	1.40	1.38	14.62	0.30
4	81.0	169	3.85	4.65	3.18	9.6	0.86	1.39	1.40	14.58	0.31
5	78.0	149	3.95	4.61	3.22	10.1	0.90	1.39	1.42	14.60	0.29

		fatty acids									
	Milk kg	Denovo	Mixed	Preformed	Lactose	Fat	Protein	fatty acids per 100 g fatty acids			
week	per day	g/day	g/day	g/day	g/day	g/day	g/day	Denovo	Mixed	Preformed	
1	41.8	380	587	560	1927	1626	1358	24.90	38.41	36.69	
2	39.5	367	565	529	1829	1548	1264	25.14	38.65	36.22	
3	38.1	332	534	526	1770	1476	1228	23.84	38.36	37.81	
4	36.8	316	511	515	1710	1416	1169	23.56	38.08	38.36	
5	35.4	319	492	503	1633	1399	1140	24.26	37.47	38.27	

Immune System Activation and Response to Mammary Immune Challenge

Any health challenge that causes an increase in immune system activity in the dairy cow will increase the demand for blood glucose need to support the immune system response. Thus, availability of glucose to support lactose synthesis in reduced and milk and milk component production per cow per day will decrease. In the case of increasingly severe mastitis (Table 3) there may also be an associcated decrease in milk lactose concentration with increasing milk SCC. Milk fat and protein concentration (g/100 g milk) may stay relatively unchanged but the quality (i.e., enzymatic damage) to the milk fat and protein increase. Output per cow per day of fat and protein will decrease in proportion to milk yield, but concentration of milk fat, protein, and milk fatty acids may remain relatively constant.

The increase in milk somatic cell count and the localized activation of the immune system in the mammary tissue causes increased proteolytic and lipolytic activity in the mammary tissue and milk with damages milk protein and fat and reduces milk quality (Murphy et al., 1989). This is the basis for use of milk SCC in the milk payment system to reduce the value of milk as milk SCC increases due to expected decreases in cheese yield (Barbano et al., 1991; Klei et al., 1998).

Table 3. Example of expected changes in milk production and composition for bulk tank milk on corn based total mixed ration fed Holstein cows that are progressively experiencing a mammary infection immune system challenge that is characterized by an increase in milk SCC. The key change is the progressive and decrease in grams of lactose per cow per day, while concentration of lactose in milk decreases slight and milk and milk component output per cow per day decreases, while the milk fatty acid composition remains relatively stable.

	A Holstein Farm an immune system challenge due to increasing milk SCC.										
		X1000	fatty acids per 100 g milk				fatty acids per 100 g milk			carbon #	DB/FA
week	lbs	SCC	Fat	Lactose	Protein	MUN	Denovo	Mixed	Preformed	FA CL	FA Unsat
1	92.0	150	3.89	4.65	3.25	9.7	0.91	1.40	1.36	14.54	0.29
2	90.0	237	3.88	4.61	3.24	9.9	0.90	1.38	1.38	14.60	0.30
3	88.0	324	3.88	4.57	3.23	10.3	0.90	1.39	1.38	14.61	0.31
4	86.0	411	3.89	4.54	3.25	10.7	0.90	1.38	1.40	14.58	0.30
5	84.0	500	3.90	4.52	3.26	11.2	0.90	1.39	1.39	14.60	0.31

		fatty acids								
	Milk kg	Denovo	Mixed	Preformed	Lactose	Fat	Protein	fatty acids per 100 g fatty acids		
week	per day	g/day	g/day	g/day	g/day	g/day	g/day	Denovo	Mixed	Preformed
1	41.8	380	587	568	1942	1626	1358	24.78	38.22	37.00
2	40.9	368	564	564	1884	1585	1324	24.59	37.70	37.70
3	40.0	360	555	551	1826	1550	1290	24.52	37.87	37.60
4	39.0	351	539	547	1773	1519	1269	24.46	37.50	38.04
5	38.1	343	530	530	1724	1487	1243	24.46	37.77	37.77

An Example of an Error in TMR Formulation/Reformulation

As availability and cost of various feed resources change, dairy cattle rations need to be reformulated. When dairy cattle rations are reformulated to use new or different batches of feed the same ingredients, representative sampling and analytical testing of the feed ingredient are needed to calculate the reformulation of the dairy cattle ration to maintain optimum milk production performance and dairy cow condition. In the process of feed sampling there is always the possibility of error. Milk analysis prior to and after a TMR reformulation can be an indicator of whether the diet formulation was properly adjusted for the composition and quality of the new TMR ingredients. The milk composition response to changes in feed is normally relatively rapid (days instead of weeks) compared to overall herd health changes or changes in rumen fermentation.

When energy density of the ration is decreased, a response is seen almost immediately. The first and most sensitive response is in the milk fatty acids. Typically, if dietary energy input is suddenly decreased, the cow begins to mobilize body fat, no matter where she is with respect to stage of lactation. Periods of lack of access to feed will also cause a similar and rapid response in milk compositon. The two most responsive indicators are the relative % of de novo, mixed, and preformed milk fatty acids, and MUN. They will begin to change before there is any noticeable change in milk per cow per day and total milk fat and protein concentration. MUN will decrease and the relative proportion of preformed fatty acids will increase and mixed orgin fatty acids will decrease. The relative proportion of mixed and preformed fatty acids may invert over a period of 3 or 4 days, compared with baseline levels established before the ration change. Ultimately, if the ration formulation is not corrected, milk output, fat and protein concentration and output will decrease in the following week as the cows and rumen microflora adjust to the new dietary input.

Table 4. Example of expected changes in milk production and composition for bulk tank milk on corn based total mixed ration fed Holstein cows due to a TMR reformulation where an error in sampling or feed analysis cause the energy density of the new TMR to be lower than the old TMR. The time line in this table is days instead of weeks that was in previous tables.

A Holstein Farm with a ration formulation error that unintentionaly decreased the energy density of the ration.

		X1000	fatty acids per 100 g milk				fatty acids per 100 g milk			carbon #	DB/FA
Day	lbs	SCC	Fat	Lactose	Protein	MUN	Denovo	Mixed	Preformed	FA CL	FA Unsat
1	92.0	147	3.89	4.61	3.25	9.7	0.91	1.40	1.36	14.54	0.29
2	92.0	155	3.88	4.64	3.24	9.9	0.90	1.38	1.38	14.60	0.30
3	91.8	162	3.85	4.61	3.20	9.0	0.88	1.34	1.42	14.68	0.31
4	91.4	170	3.79	4.62	3.18	8.7	0.85	1.32	1.42	14.72	0.30
5	90.1	158	3.70	4.61	3.17	7.9	0.80	1.26	1.44	14.75	0.31

		fatty acids								
	Milk kg	Denovo	Mixed	Preformed	Lactose	Fat	Protein	fatty acids per 100 g fatty acids		
Day	per day	g/day	g/day	g/day	g/day	g/day	g/day	Denovo	Mixed	Preformed
1	41.8	380	587	568	1927	1626	1358	24.78	38.22	37.00
2	41.8	376	576	576	1938	1621	1353	24.59	37.70	37.70
3	41.7	367	558	592	1923	1605	1334	24.18	36.81	39.01
4	41.5	353	548	589	1917	1573	1320	23.68	36.77	39.55
5	40.9	327	515	589	1886	1513	1297	22.86	36.00	41.14

Future of Real-time Milk Analysis

Historically, milk analysis data have been used for calculation of milk payment and for DHIA dairy production record keeping that provides data in support of genetic selection of dairy cows. Recently, as dairy herd size has increased greatly in the US, there is need for higher frequency milk composition data that can be used for a more proactive management of both nutrition and health of dairy cows. The need for information in support of dairy farm management is becoming more encompassing. Currently, milk analysis is evolving rapidly and there may be important new milk analysis metrics for dairy herd management that emerge in the next few years. Going forward, merging mik composition data produced by a larboratory (or in real-time on the farm) and management information (e.g., activity monitor, health measures, etc.) collected at the farm will be needed to optimize both productivity and animal health and well being. Ultimately, in the longer run future for dairy farm management, the technology to measure milk composition parameters should be integrated into the milking system at the farm. This will require the development of new in line milk analysis sensor technology that is different than technology that is currently available today.

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MID-INFRARED MILK TESTING FOR EVALUATION OF HEALTH STATUS IN DAIRY COWS

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INTRODUCTION

Feeding and managing dairy cows for optimal health, productivity, and fertility is critical for our dairy industry. One of the challenges we face is early detection and treatment of health issues, especially for our early lactation (i.e., fresh) cows. Milk composition is recognized as being a relatively good source of health and metabolic information given that there is interaction between circulating blood and milk synthesis outcome (Gengler et al., 2016).

Mid-infrared (MIR) spectroscopy is used for routine payment testing of milk and provides information about the major components (i.e., fat, protein, and lactose) found in milk. Traditionally, information such as the fat to protein ratio, has been used to make feeding and management decisions. There is tremendous interest in moving beyond using MIR spectroscopy of milk for only major components. It has the potential to identify minor (fine) milk components and health issues in dairy cows (De Marchi et al., 2014; Gengler et al., 2016; Barbano et al., 2018). Also, changes in milk detected through the MIR spectra allows blood biomarkers not found in milk to be predicted. Blood testing is invasive, requires labor, and is costly. Milk is readily available with harvest occurring two or three times daily in most herds. Research needs to focus on automated and cost-effective methods for biomarker quantification and use of MIR spectroscopy is one possible approach.

USE OF MILK MID-INFRARED SPECTRA TO DETECT HEALTH ISSUES IN DAIRY COWS

Metabolic Status

In early lactation, dairy cows experience negative energy balance that can affect their health, productivity, and fertility. Studies have been conducted to determine the feasibility of using MIR spectra of milk to indicate the energy status of cows by predicting energy intake and balance, dry matter intake, and residual feed intake (McParland et al., 2011, 2012, and 2014; Shetty et al., 2017). The models of McParland et al. (2011, 2012, and 2014) in general were moderately accurate during cross-validation but were not accurate during external validation because of the limited variability in the dataset population (McParland and Berry, 2016). Shetty et al. (2017) had better success in predicting dry matter intake with confinement housed dairy cows, especially when milk yield and body weight were modeled with the MIR spectral data. Interestingly, the milk fat region of the spectra was responsible for the majority of variation in dry matter intake.

A more viable option to understand the metabolic status of cows, especially during early lactation, is the use of biomarkers related to negative energy balance and ketosis. Many biomarkers require a sample to be collected invasively (e.g. drawing blood) and are expensive and time consuming to analyze in a laboratory. Cow-side tests for biomarkers in urine, milk, or blood are available, but can be labor intensive for dairy producers. The use of MIR spectrometry may allow some biomarkers to be measured easily, quickly, and cost effectively. Milk beta-hydroxybutryate (BHB) and milk acetone are biomarkers that have been most extensively researched and implemented to date.

Hyperketonemia (i.e., ketosis) is determined by measuring blood BHB. Given the correlation between blood BHB and milk BHB, researchers (de Roos et al., 2007) developed a prediction model for milk BHB and ketosis monitoring. Although the relationship between the blood-based method and the MIR predicted method for BHB was moderate ($r = 0.79$), they suggest the MIR prediction of milk BHB could be part of a useful screen tool for subclinical ketosis. The MIR predictions of milk BHB and acetone were better (80% sensitivity) at predicting hyperketonemia than the traditionally used milk fat to protein ratio (66% sensitivity; van Knegsel et al., 2010). The use of a milk BHB threshold of ≥ 0.20 mmol/L had excellent accuracy to detect hyperketonemia in dairy cows and its use was proposed in herd-level surveillance programs (Denis-Robichaud et al., 2014).

Santschi et al. (2016) found that testing milk for BHB with models developed by de Roos et al. (2007) and Denis-Robichaud et al. (2014) was a fast and inexpensive approach to determining herd-level prevalence and that prevalence was affected by parity of the cow and season of calving. They noted that a major limitation of the testing was the frequency of sampling with monthly DHI milk samples. The use of the de Roos et al. (2007) milk BHB and acetone models was found to be not suitable for individual detection of cows with ketosis due to the length of the test-day interval and the poor predictive performance (van der Drift et al., 2012). Inclusion of cow test-day information improved marginally the model performance (van der Drift et al., 2012). Similarly, Chandler et al. (2018) found that including cow test-day milk and performance variables in logistic and multiple linear regression models improved the ability to predict hyperketonemia at a herd-level. Grelet et al. (2016) took a novel approach to modeling milk BHB and acetone by using cows of different breeds, countries, and management systems along with milk spectral data from different analytical equipment. They found R^2 of cross-validation of 0.71 for BHB and 0.73 for acetone which is considered moderately accurate. Thus, it appears that milk BHB can be determined by MIR analysis as part of a Dairy Herd Improvement testing program thereby providing a fast and inexpensive alternative to blood BHB analysis.

The possibility of using MIR spectra from milk to predict blood biomarkers (i.e., metabolites and hormones) related to metabolic status is gaining interest. This is a rather novel approach as the concentration of a biomarker in a fluid (i.e., blood) is determined by the spectroscopic analysis of another fluid (i.e., milk) that may not even contain the biomarker of interest. Pralle et al. (2018) predicted blood BHB using milk MIR spectra,

milk composition, and producer-reported variables. The models were very good (0.83 ≤ AUC ≤ 0.90) but not excellent at determining hyperketonemia. The researchers noted that none of the models achieved the sensitivity and specificity of cow-side blood tests that are available currently. Before the Pralle et al. (2018) study, the prediction of blood BHB in dairy cows from milk MIR spectra was poor with several models evaluated and all yielding R^2 in the range 0.21 to 0.38 on cross-validation (Belay et al., 2017).

Grelet et al. (2018) used MIR spectra from milk to predict blood components such as glucose, insulin-like growth factor 1 (IGF-1), nonesterified fatty acids (NEFA), and BHB. Unfortunately, the quantitative models were weak for glucose, IGF-1, NEFA, and BHB with a R^2 of cross-validation of 0.44, 0.61, 0.39, and 0.70, respectively. Therefore, quantitative values could not be predicted accurately. However, Grelet et al. (2018) suggested that the models allowed for screening of individual milk samples for high or low values that represented a healthy, a moderately impacted, and an imbalanced metabolic status. This approach was more promising with discriminant models correctly identifying up to 74% of the samples. Luke et al. (2018) also found it difficult to use milk MIR analysis to predict biomarkers of metabolic health and nutritional status of early lactation cows quantitatively. They found a good estimation of blood urea nitrogen (R^2 = 0.89), but moderate estimations of serum NEFA (R^2 = 0.46) and BHB (R^2 = 0.49), and poor estimations of serum albumin (R^2 = 0.26), globulin (R^2 = 0.20), calcium (R^2 = 0.18), and magnesium (R^2 = 0.17). Interestingly, the researchers noted the importance of including data from as many herds as possible in the calibration data set to improve performance with the validation data sets.

We (Barbano et al., 2015) developed a model that predicted blood NEFA directly from the milk MIR spectra. This was a unique approach since it was not by calculation from milk fatty acid data as others have proposed with gas chromatography (Jorjong et al., 2014). The milk-predicted blood NEFA model worked well for Holstein cows during the first 3 wk of lactation. This model is being used at Miner Institute to evaluate fresh cows at a frequency of 1 milking/d. Figure 1A shows as expected the decrease in NEFA concentration as days in milk (DIM) increase and the difference between primiparous and multiparous cows. Milk-predicted blood NEFA concentration is monitored over time (Figure 1B) and can be used to make nutritional and management decisions. The milk-predicted blood NEFA model is being applied to milk collected inline from pens of fresh cows across the US and analyzed at Miner Institute. This information is helping nutritionists identify nutritional and management opportunities for their dairy producers. We find that monitoring groups of milk fatty acids determined by MIR analysis (Wojciechowski and Barbano, 2016; Woolpert et al., 2016; Figure 2), such as de novo fatty acids and preformed fatty acids, is beneficial for individual fresh cows (Barbano et al., 2018) or pens of fresh cows. These metrics are related to body weight change and health of the cows.

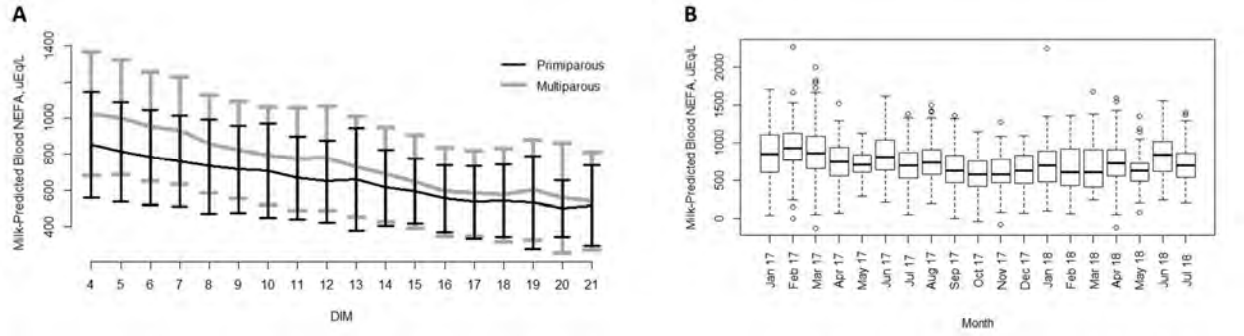

Figure 1. Concentration of milk-predicted blood nonesterified fatty acids (NEFA) for Holstein cows fed a total mixed ration during the first 21 days in milk (DIM) summarized by DIM and parity (A) or monthly average (B).

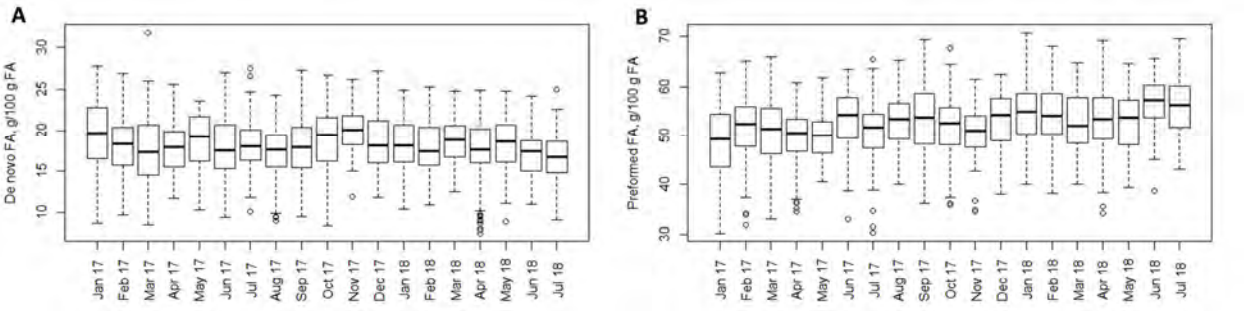

Figure 2. Concentrations of de novo fatty acids (A) and preformed fatty acids (B) for Holstein cows fed a total mixed ration during the first 21 days in milk.

One area in which milk composition is potentially useful is in providing warning of impending health events before clinical signs are visible. We (Pape et al., 2018) examined the relationship between changes in milk composition based on MIR analysis and the onset of ketosis or displaced abomasum (DA) in fresh cows. The approach used was to test the extent to which machine learning models could differentiate between milk samples from cows that went on to experience either ketosis or a DA and milk samples from cows that did not at approximately the same date and DIM (Figures 3 and 4). Milk-predicted blood NEFA, preformed fatty acids, and the fat/protein ratio were all elevated leading up to DA and ketosis, while de novo fatty acids was depressed. For both DA and ketosis, milk-estimated blood NEFA, de novo fatty acids, preformed fatty acids, and the fat/protein ratio all provided strong predictive power. Milk BHB did not. Our models yielded an area under the curve of ~0.8 from 5 to 0 d in advance of the event, with predictive power generally increasing up to an area under the curve of ~0.89 on the day of the health event. Overall, this suggests that there is a great deal of potential for improving early warning of health events like ketosis and DA and enabling better preventative treatment. The next step is to implement an early warning system in real-time. So far, we have developed a pilot version of such a system for the Miner Institute herd, providing general-purpose alerts for health issues in fresh cows to the farm staff. We are evaluating its usefulness currently.

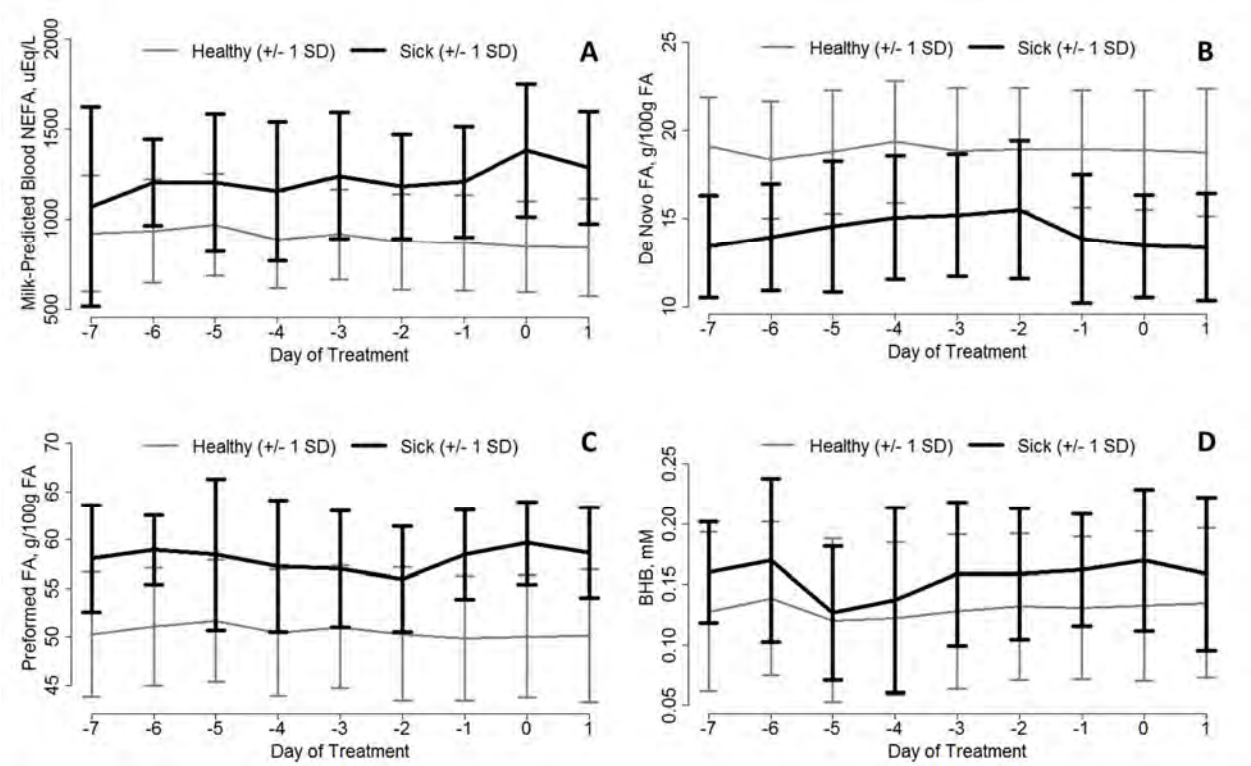

Figure 3. Changes in milk-predicted blood nonesterified fatty acids (NEFA; A), milk de novo fatty acids (FA; B), milk preformed FA (C), and milk BHB (D) for lactating cows that had ketosis or were healthy and matched by date and days in milk to cows with ketosis.

Immunological Biomarkers and Inflammation

German researchers (Zoldan et al., 2017) used healthy and diseased (e.g mastitis or systemic) Holstein cows to identify and validate immunogenic biomarkers in milk that indicated inflammatory diseases outside of the mammary gland. They demonstrated with receiver operator characteristic analysis (with a 94% specificity) that haptoglobin (82% sensitivity), secretory component (59% sensitivity), lactoferrin (55% sensitivity), and vascular endothelial growth factor (67% sensitivity) showed the highest discriminatory capability for healthy and diseased cows. Haptoglobin was the best single-use biomarker based on multinomial logistic regression. This is not surprising as acute phase proteins are generally considered appropriate biomarkers for inflammation. In milk, haptoglobin, serum amyloid A, and lactoferrin are often identified as biomarkers for mastitis.

We have evaluated several regression models to predict inflammation based on blood acute phase protein from milk MIR spectra from dairy cows. We matched serum haptoglobin and serum amyloid A data with milk composition and spectral data for 420 Holstein cows that were either healthy, clinically ill, or possibly experiencing a subclinical health issue. The coefficient of determination from cross-validation of the models was poor for serum haptoglobin (<0.4) and serum amyloid A (<0.3). Some of the issue with predicting blood acute phase proteins from milk are related to a lack of validated methods

that accurately measure the biomarkers of interest. Given that a dairy producer is probably not interested in the actual value of acute phase proteins, but is interested to identify if a cow is has having an inflammatory response or not, we used classification models to categorize cows into low (10th, 25th, and 33rd) and high percentiles (90th, 75th, and 66th) for each acute phase protein. The receiver operating characteristic area under the curve for the different models ranged from 0.81 to 0.90 for serum haptoglobin and 0.85 to 0.95 for serum amyloid A. Using another approach, the cows were classified into low and high serum haptoglobin categories based on a percentile threshold. This approach resulted in receiver operator characteristic area under the curve values between 0.7 and 0.9 when thresholds of 50 to 90 percentile were used. These models indicate to us that cows that are experiencing systemic inflammation can be identified through milk sample analysis.

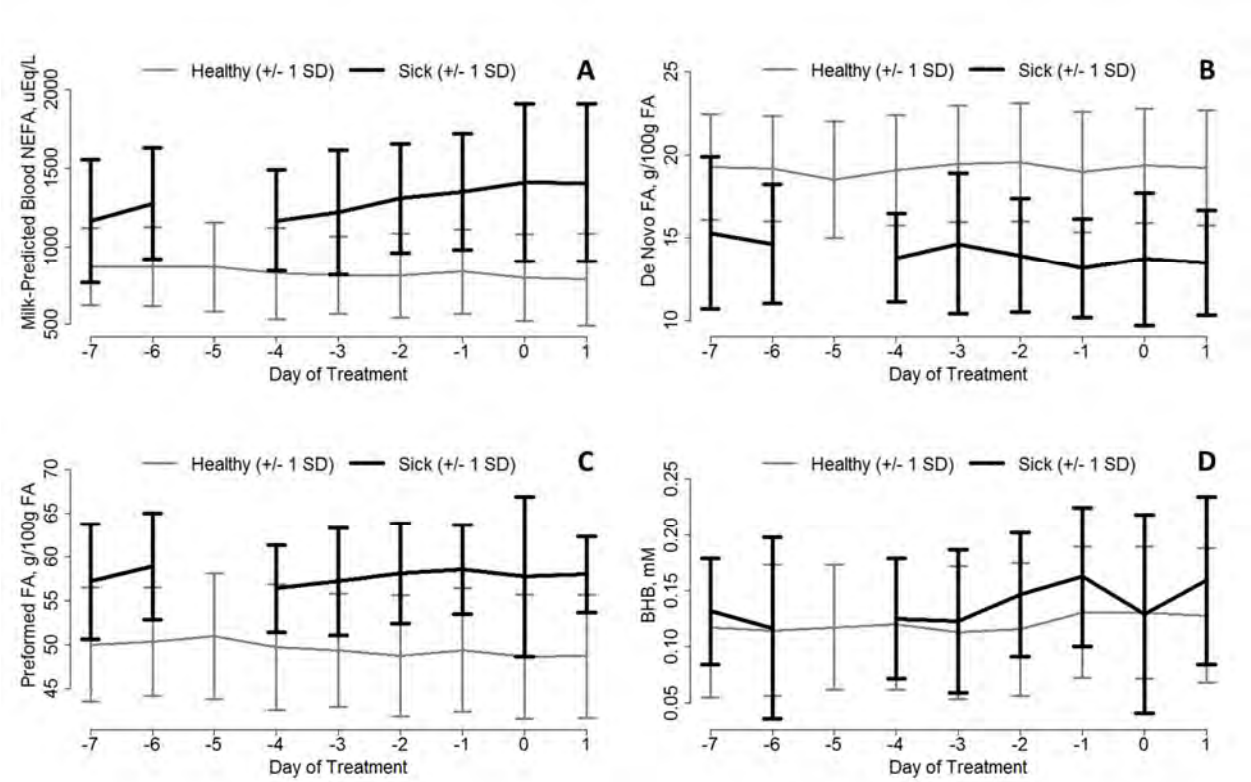

Figure 4. Changes in milk-predicted blood nonesterified fatty acids (NEFA; A), milk de novo fatty acids (FA; B), milk preformed FA (C), and milk BHB (D) for cows that had displaced abomasum or were healthy and matched by date and days in milk to cows with a displaced abomasum.

Subacute Ruminal Acidosis (SARA)

The ratio of milk fat to protein was found to not be specific enough to detect low ruminal pH values in early and mid-lactation dairy cows (Guegan et al., 2015). This is not surprising given that 1) ruminal samples were collected by an oro-ruminal probe method that can have saliva contamination, and 2) the relationship between SARA and milk fat content is inconsistent in several studies. Many factors can affect fat content such as

breed, season, and days in milk. Interestingly, the daily ruminal pH mean and variation have been shown to be related to specific milk fatty acid changes (measured by gas chromatography) in Holstein-Friesian cows (Colman et al., 2012). The susceptibility of dairy cows to SARA was shown to be reflected in milk fatty acid proportions measured by gas chromatography with C18:1 trans-10 as primary and C15:0 and C18:1 trans 11 as secondary indicators (Jing et al., 2018).

In general, a decrease in ruminal pH will result in changes in ruminal biohydrogenation, C18 biohydrogenation intermediates, and microbial populations (source of odd and branched chain fatty acids) that alter the milk fatty acid profile. Thus, Giger-Reverdin et al. (2018) viewed milk fatty acid composition as a good candidate to detect SARA in dairy goats. They used two methods to determine milk fatty acid composition: gas chromatography (expensive and time-consuming method) and MIR spectroscopy analysis (inexpensive and quick method). An index of short and medium chain fatty acids (≤ C13) to long chain fatty acids based on gas chromatography was positively related (r = 0.60) to ruminal pH. However, the index calculated from MIR analysis was not correlated as the MIR method overestimated the short and medium chain fatty acids and underestimated the long chain fatty acids. They concluded that MIR analysis was not a viable method for prediction of SARA until the inaccuracy in the prediction of fatty acids is addressed.

Recently, Luke et al. (2018) suggested that a number of fatty acids have been proposed as potential biomarkers of SARA and that MIR analysis can predict some fatty acids with good accuracy so MIR spectra may be able to be used to predict ruminal pH and SARA. They evaluated several pH metrics: mean pH, time below pH 6, area under the pH curve between milkings and pH at the time of milk sample collection along with milk spectra from ruminally cannulated dairy cows. The partial least squares models to predict pH metrics from milk spectra had poor to moderate accuracy with R^2 values between 0.22 and 0.59. Discriminant analysis models categorized cows as either having or not having SARA with a sensitivity of 81% and a specificity of 72%. The researchers concluded that the MIR analysis of milk shows promise as a useful tool for monitoring ruminal pH in lactating dairy cows.

Mastitis

It is well-documented (review by Brandt et al., 2010) that milk composition changes with subclinical and clinical mastitis. Laine et al. (2014) suggested that use of MIR analysis of milk might provide indicators of mammary health that could allow for earlier detection of mastitis and identification of cows with chronic infections while being quick and cost-effective. Traditionally, somatic cell count determined by flow cytometry has been used to monitor mammary health. Laine et al. (2014) found that milk composition described by MIR predicted components or the MIR spectrum was different before and during mastitis. They identified lactoferrin, urea, sodium, and titratable acidity as early indicators of mastitis. However, they did not investigate the sensitivity and specificity of those indicators to predict mastitis.

Lameness

Early detection and treatment of lameness is important to support animal welfare. Lameness is associated with physiological changes such as inflammation and modified feeding behavior that potentially alter the milk composition. Mineur et al. (2017) found that categorizing all types of lameness together was not useful in predicting lameness from milk MIR spectra. The sensitivity and specificity were generally in the 60 to 70% range indicating too many cows were not being identified as lame. However, sensitivity and specificity increased generally to the 80 to 90% range when lameness issues like heelhorn erosion or whiteline disease were grouped separately from other types of lameness and modeled. The researchers indicated that future work requires the use of data from varied cows and herds and validation steps before implementing this technology on a large scale.

CHALLENGES WITH MODEL DEVELOPMENT AND IMPLEMENTATION

The use of milk MIR spectra to detect health issues in dairy cows is promising. However, there are some challenges to wider implementation. Currently, application of MIR spectra to detect health issues is limited primarily to milk BHB for the determination of prevalence of hyperketonemia at the herd-level. Typically, the testing is conducted as part of the monthly DHI milk-testing program. The frequency of testing needs to be at a much higher frequency for the detection and treatment of individual cows (Barbano et al., 2018). In the future, development of on-farm MIR milk analyzers in needed to allow high frequency sampling and the generation of real-time actionable alerts.

The accuracy of a prediction model based on milk MIR spectra depends on the precision of measurement of the validated method of the biomarker of interest. This may be a larger issue for blood-based biomarkers compared with traditional milk components. Laboratories analyzing biomarkers need to have methods validated and ideally participate in an external quality assurance program (i.e., interlaboratory proficiency tests) to justify the effort in developing milk prediction models for commercial use. Model development is not about the quantity of milk spectra one uses, but is about the quality of milk spectra and balancing the variation of the biomarker of interest and designing the variation into the background chemistry of the modeling dataset.

Models will need to be validated and then implemented by the milk analyzer equipment manufacturers. Implementation may be very time consuming. Calibration samples may be needed by the laboratories. Laboratories will need to determine how to process and report the information while dairy producers and their consultants will need to determine how to use/interpret the information.

SUMMARY

Mid-infrared spectroscopy of milk is a tool to predict health issues in dairy cows. It has the advantage over other methodologies in that it is noninvasive, it is lower-cost, and the sample (i.e., milk) is available multiple times per day. Several studies have

demonstrated the usefulness of milk BHB and acetone in predicting herd-level hyperketonemia (i.e., ketosis). Other studies have identified its use for the prediction of energy balance and metabolic status, inflammation, and digestive issues. The real advantage of the tool will be realized when it can be used to provide real-time information to dairy producers regarding the health and physiological status of their cows.

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RON BUTLER'S RANGE OF SCIENTIFIC INQUIRY

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INTRODUCTION

Dr. W. R. (Ron) Butler began his career studying testicular function in the rat in the late 1960's. From that time, he has traversed from the hypothalamus to the ovary to the uterus in a diverse array of species. His work has covered the span from basic to applied studies. Regardless of the species or reproductive organ studied, a recurrent theme in his work is the timeliness and importance of the question asked. This has led to a career of "firsts" where important findings arose from hypothesis-driven research. Ron Butler has trained under, collaborated with, and trained some of the best-known animal reproductive biologists of his era. His resume includes over 100 refereed journal publications with over 8000 citations. His h-index (Scopus) is an impressive 45.

EARLY YEARS

Ron began his academic career at Ohio State working with W. R. "Reg" Gomes on testicular function in the rat (Butler et al., 1968). He later moved to Purdue University training under Dr. Paul Malven and collaborating with Dr. Doug Bolt from USDA Beltsville. It appears that his love for luteinizing hormone (LH) began at Purdue with the studies of pituitary LH release in the ovariectomized ewe (Butler et al., 1972). This work, published in the journal *Endocrinology* not only included detailed profiles of LH but also included the development and validation of a radioimmunoassay for ovine prolactin. Radioimmunoassay was a new technology at the time and the development of the prolactin assay to replace cumbersome bioassays was an important scientific achievement. Following the completion of his work at Purdue, Ron moved to the laboratory of Ernst Knobil; one of the most iconic scientists in all of reproductive biology and a graduate of Cornell Animal Science. Knobil was Chairman of the Department of Physiology at the University of Pittsburgh School of Medicine where he studied the regulation of the menstrual cycle in rhesus monkeys. The classic work from the Knobil laboratory created the basis for much of our understanding of how LH controls reproductive cycles in mammals. To this day, reproductive biology students are taught the "Knobil model" for LH control that includes elements of Ron's work. Fellow lab mates from this era included Fred Karsch who later moved to an illustrious career at the University of Michigan where he continued to work on LH secretion and developed surgical procedures for cannulation of the portal vein in sheep. Ron was an NIH fellow within the Knobil laboratory and published 15 papers with Knobil. His publication record with Knobil was second only to that of Rob Gilbert of the College of Veterinary Medicine at Cornell (co-author of 16 publications). His two first-author publications in *Endocrinology* included work on the control of thyroxin secretion (Butler et al., 1975b) and work on the luteolytic mechanisms in the rhesus monkey (Butler et al., 1975a).

GETTING STARTED AT CORNELL

Ron moved to Cornell in 1975 and immediately established his endocrine laboratory. Early collaborations were with Bill Hansel from the Department of Physiology of Cornell where they studied the endocrinology of dogs (Concannon et al., 1978). Ron's first publication in the *Journal of Dairy Science* was a description of a radioimmunoassay that could be used to measure cortisol in milk (Butler and Des Bordes, 1980). He followed this work with several studies in which milk cortisol was examined as a possible indicator of stress in dairy cows (Fox et al., 1981; Termeulen et al., 1981).

Ron's work in the Knobil laboratory studying the reproductive cycles of monkeys clearly stimulated an interest in understanding the mechanisms that control the interval to first ovulation in dairy cows. Cows that fail to ovulate postpartum are infertile. First ovulation, therefore, was then and continues to be a keen interest of reproductive biologists. His earliest publication on this topic was a collaboration with Cornell dairy geneticist Bob Everett and fellow Cornelian C. E. Coppock (Butler et al., 1981a). The abstract of this paper concluded, "energy balance during the first 20 days of lactation is important in determining the onset of ovarian activity following parturition". Also included in this publication was the idea that first ovulation occurred approximately 10 days after the nadir in energy balance. These two concepts that energy balance controlled the interval to first ovulation in dairy cows and that the first ovulation occurs 10 days after the energy balance nadir are widely held to this day. Authors have cited this paper over 225 times.

THE 1980'S

The 1980's was a period of intense scientific discovery within the Animal Science Department at Cornell. Ron's laboratory turned to puberty and seasonality in the ewe and investigated the effects of photoperiod, nutrition, and ram exposure (Butler et al., 1981b, 1987; Fitzgerald and Butler, 1982, 1988; Fitzgerald et al., 1982). In addition to these studies, he collaborated with those working actively on animal growth including Dr. Dean Boyd working with pigs (Boyd et al., 1985) and Dr. Don Beerman working with cimaterol in sheep (Beerman et al., 1987; O'Connor et al., 1991b; a). His work in reproductive biology included three studies in swine in which ovulation rate, pregnancy, and the use of relaxin to synchronize farrowing were studied (Butler and Boyd, 1983; Pope et al., 1986; Fu et al., 1990). Ron's colleague, Dean Boyd, considers the work on relaxin to have had a major impact on modern farrowing management in the swine industry. Ron also completed studies on the control of gonadotropin secretion with fellow Cornell reproductive biologist Bob Foote (Butler et al., 1983).

BACK TO LH

It was the mid to late 1980's when Ron returned to the topic of energy balance and postpartum cyclicity in dairy cows with graduate student Rick Canfield. Their work during this period clearly established the model for LH pulsatility and the resumption of

postpartum reproductive function in dairy cows that continues to be used today (Canfield and Butler, 1990, 1991). It was at about this time that Ron published his most highly cited paper “Interrelationships between energy balance and postpartum reproductive function in dairy cattle” with fellow Cornelian R. D. Smith (Butler and Smith, 1989). This paper documented the steady decline of reproductive performance of dairy cattle over time and detailed physiological mechanism that could potentially explain the decline. This paper was the first in a series of highly cited review papers authored by Ron on energy balance and reproductive function in dairy cows (Butler and Smith, 1989; Beam and Butler, 1999; Butler, 2000). Collectively, authors have cited these review papers nearly 1500 times.

PROTEIN NUTRITION AND FERTILITY

In addition to their work on LH, Canfield and Butler published groundbreaking work on protein nutrition and fertility with Cornell dairy nutritionist Charlie Sniffen (Canfield et al., 1990). At the time, farmers were overfeeding protein to maximize milk production. Canfield reported that feeding a 19% compared with 16% crude protein in the diet increased plasma urea concentrations and reduced first service conception rate by 17 percentage points. Canfield’s initial work was followed soon thereafter by graduate student Charlie Elrod working in Butler’s laboratory. Elrod was able to show that overfeeding soluble protein and increasing plasma urea caused a decrease in uterine pH (Elrod and Butler, 1993; Elrod et al., 1993) that is apparently detrimental to the embryo. Elrod’s work was followed by a field trial conducted by Butler and graduate student Steve Beam showing a 20-percentage point reduction in conception rate for cows with a plasma or milk urea concentration of greater than 19 mg/dL (Butler et al., 1996). The collective work from his laboratory led to one of the most highly-cited review papers on protein nutrition and reproduction in the *Journal of Dairy Science* entitled “Effect of protein nutrition on ovarian and uterine physiology in dairy cattle” (Butler, 1998) (302 citations). In later work, Butler graduate student Michelle Rhoads performed mechanistic work and showed urea infusion would decrease uterine pH compared with a saline-infused control (Rhoads et al., 2004). Furthermore, she demonstrated that elevated plasma urea nitrogen decreased the viability of the early embryo (Rhoads et al., 2006).

INSULIN-LIKE GROWTH FACTORS COME INTO FOCUS

The groundbreaking work from the Butler lab clearly implicated LH pulsatility as a major driver of postpartum ovulation of the dairy cow. It became clear, however, that the concentration of systemic growth factors could modulate the LH response. One of the best studied of these was insulin-like growth factor I (IGF1); a major hormonal product of the liver that was known to respond to the energy balance of the cow. Butler teamed up with Cornell graduate student Steve Beam in a series of studies to establish the link between energy balance, circulating IGF1, and ovarian follicular growth measured by ultrasound. Their first paper published in the *Biology of Reproduction* established the important concept that there are three fates of the first wave dominant follicle postpartum; namely ovulation, failed ovulation (turnover) or cyst formation (Beam and

Butler, 1997). These fates were tied to circulating estradiol concentration (arising from the follicle) and circulating IGF1 (favorable outcomes associated with greater IGF1 in the circulation). The important link between energy balance, IGF1, follicular function and ovulation early postpartum was further explored and confirmed in a second paper published in the *Journal of Dairy Science* (Beam and Butler, 1998). Their collective work was presented at the prestigious Fifth International Symposium on Reproduction in Domestic Ruminants held in Colorado Springs Colorado in August 1998. The review paper that arose from that presentation presented a clear model for how IGF1 and LH work collectively to increase circulating estradiol and trigger the LH surge leading to first ovulation (Beam and Butler, 1999). The working model is widely accepted today. The paper is Ron's third most-highly cited publication (343 citations).

LINKING INSULIN TO IGF1

The systemic IGF1 that Beam and Butler studied arises primarily from the liver and is under metabolic control arising from the nutrition and energy balance of the cow. The somatotropic axis is "uncoupled" for cows in negative energy balance. The mechanism underlying the uncoupling was an important question of the time. Postpartum cows have low circulating glucose and insulin concentrations. Ron explored this question with graduate student Stephen Butler. In a landmark study, Butler demonstrated that insulin infusion recoupled the somatotropic axis and increased IGF1 through a mechanism that involved growth hormone receptor (GHR) 1A (Butler et al., 2003). Stephen Butler went further to demonstrate that positive effects of insulin and IGF1 on the follicle could occur independent from changes in LH pulse frequency (Butler et al., 2004). Butler later went on to test propylene glycol drench as a practical method to improve hormonal and metabolic status and stimulate follicular development postpartum (Butler et al., 2006). Although the treatment failed to improve reproductive outcomes, the publication reestablished the importance of negative energy balance and introduced the relatively new concept that the low dry matter intake and energy balance in non-ovulatory cows could be detected *before* calving. This study was one of the first that linked postpartum reproduction to the metabolic profile of the dry cow.

PRACTICAL SOLUTIONS FOR THE POSTPARTUM COW

Ron always had an interest in solving problems that face dairy producers. His laboratory published a series of papers that examined the utility of feeding different formulations of fat including protected fats (Beam and Butler, 1997, 1998) and fish oils (Moussavi et al., 2007a; b). He collaborated with Dale Bauman's lab in their work on conjugated linoleic acid (CLA) (Chouinard et al., 2001; Castañeda-Gutiérrez et al., 2005, 2007; Corl et al., 2006). Collaborating with Michael De Veth, Butler demonstrated that feeding CLA early postpartum could improve reproductive outcomes (de Veth et al., 2009). The potential to use CLA to improve postpartum reproduction was confirmed in subsequent studies (Csillik et al., 2017).

In addition to feeding, Butler explored practical ways to increase progesterone and stimulate cyclicity in postpartum cows. This included work in postpartum cows (Larson et al., 2007). Butler's lab also participated in the original trials that let to FDA approval or progesterone-releasing CIDR devices that are widely used today (Lucy et al., 2001). In one of Ron's last collaborations with Dr. William Hansen before Hansel's retirement from Cornell, Rod worked on a process called "inembryonation" where estrus was synchronized using a CIDR device and $PGF_{2\alpha}$ and a "one-step" frozen embryo was transferred on day 7.

LATER YEARS

During the later years of his career, Ron continued his research with his long-time collaborator Yves Boisclair from the Cornell Department of Animal Science. Yves and Ron shared an interest in metabolism and potential effects on fertility in the dairy cow. Several important concepts arose from their work on the adipose tissue hormone leptin. They showed that 1) leptin decreased in response to negative energy balance (Block et al., 2001); 2) hypoinsulinemia postpartum caused an increase in leptin receptor in liver (Thorn et al., 2008) and; 3) leptin is an important signal leading to the conservation of glucose in early postpartum cows (Ehrhardt et al., 2016). Their latest work includes one of the few publications on adiponectin in dairy cows (Krumm et al., 2017).

Ron co-published more papers with Rob Gilbert (Cornell University College of Veterinary Medicine) than any other author. Ron and Rob's final period of collaborative work focused on uterine disease and its effect on fertility in the postpartum cow as well as basic biology of the ovarian follicle postpartum (Galvão et al., 2009, 2010; Vieira-Neto et al., 2014; Cheong et al., 2016, 2017). The lead authors on their collaborative publications included two Ph.D. students who are now highly regarded faculty members in Colleges of Veterinary Medicine (Klibs Galvão now at the University of Florida and Soon Hon Cheong at Cornell).

SUMMARY

Ron Butler's career spanned several decades and contributed greatly to the field of animal reproductive biology. His career embodied that of a creative and productive faculty member who remained active and relevant from early to later years. He collaborated with many individuals both within and outside the Department of Animal Science at Cornell. He trained many great scientists who work in both industry and academia. Their work will continue his scientific legacy into the future. Not included in these pages is a list of the many students who benefited from Ron's knowledge both inside and outside the classroom. These students may not have completed and published research projects under his guidance but nonetheless benefited from his knowledge and inspiration.

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RON'S IMPACT ON THE WORLD DAIRY INDUSTRY

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Professor Ron Butler is a renowned animal scientist! His scholarly publications reflect research conducted with 12 different species. His graduate and post-graduate training was completed in three top laboratories in the USA, and his early research on disconnection of the pituitary from the hypothalamus in rhesus monkeys plowed new ground in neuroendocrinology.

OUR CONNECTIONS

Ron and I traveled parallel paths. We graduated with B.S. degrees from different universities in 1966 and pursued graduate training in reproductive physiology and endocrinology. I first became acquainted with Ron when I visited Purdue University in 1969 to learn how to assay progesterone in blood from dairy cows. I was taught to do assays by Allen Garverick, who was a PhD student in the laboratory of Professor Ralph Erb. Nearby was the laboratory of Professor Paul Malven, where Ron was pursuing a PhD degree.

In 1993, Ron and I had a once-in-a-lifetime happenstance. We were invited to participate in a national symposium on nutrition and reproduction in Zeist, The Netherlands. My Delta Airlines Boeing 747 flight from Atlanta to Amsterdam was delayed 4 hr, because a catering truck backed into our plane just before we pushed back from the gate. We had to disembark and get another plane. When I arrived in Amsterdam, conference hosts asked if I would speak ahead of Ron, because his flight had been delayed. When Ron arrived, we learned that his TWA Boeing 747 flight from JFK to Amsterdam was taxiing to the runway when an unmanned catering truck rolled down the ramp into his plane. When we calculated the probability of this happening to both of us, the zeros after the decimal ran for several pages.

Ron's family and mine connected more closely in the early 1990's when my daughter Stephanie and Ron's daughter Stacy became classmates at Cornell's Hotel School. In visits to Ithaca, I saw Ron regularly.

Beyond these connections, Ron and I both pursued studies related to dairy cattle fertility and impact of nutrition and energy balance on reproduction in livestock. Our projects turned out to be complementary and we often cited each other's work. His work on energy balance and protein nutrition in high-producing dairy cows set the stage for many others to pursue studies and develop methods and technologies to deal with these two important issues.

IMPACT ON THE DAIRY INDUSTRY

Measuring impact is not simple, but I doubt there is a dairy farmer or dairy nutritionist or dairy cattle veterinarian in the developed world that does not deal with negative energy balance or excess blood or milk urea nitrogen on a regular basis. This is where the practical application of research findings from Ron's team has made its global mark.

Important research findings move quickly from the laboratory to adoption within an industry. Publication of research findings in credible journals and presentation of these findings at national or international meetings accelerates adoption of better husbandry practices. To assess Ron's impact from a scholarly standpoint, it is informative to look at how often his publications have been cited. According to Google Scholar, his top 10 papers had been cited 6,348 times as of 29 August 2018 (Appendix Table 1). Notice that these papers deal primarily with two topics: energy balance in the periparturient cow and impact of protein nutrition on fertility.

ENERGY BALANCE

Butler and Smith. The most cited paper with W R Butler as an author was published in 1999 in the Journal of Dairy Science by Ron Butler and David Smith. It was from an American Dairy Science Association symposium on *Interactions of Nutrition and Reproduction*. This paper provides an excellent summary of relationships between energy balance and reproduction in the dairy cow.

Nevertheless, my perception is that Ron's earliest and most significant contribution in this area was from his 1983 paper with Bob Everett and Carl Coppock.

Butler, Everett and Coppock. In this paper, Ron, Bob and Carl utilized data from a previous study that focused on utilization of urea in diets of 13 Holstein cows in early lactation. The original study was published by Kwan et al. (1977). Cows in this study produced an average of 7,334 kg (16,135 lbs.) in 305-day lactations.

The Butler team measured hormones in blood from these cows to estimate interval to first ovulation and used feed intakes and milk production to estimate energy balance. Then they looked at relationships among energy balance and several reproductive traits. Their most significant finding was that cows ovulated and started estrous cycles while they were still in daily negative energy balance.

There are several important lessons from this paper:

- Milk yield is related inversely to energy balance – cows that produce more milk generally have a more negative energy balance (Figure 1).
- Days to first ovulation is related inversely to energy balance – cows with more negative energy balances take longer to ovulate after parturition (Figure 2)
- Energy balance reached its nadir (most negative point) near the time of peak milk yield

- First ovulation occurred just after the energy balance nadir, before cows reached positive energy balance.
- Finally, there is valuable information in existing data sets that may not have been included in the original published paper.

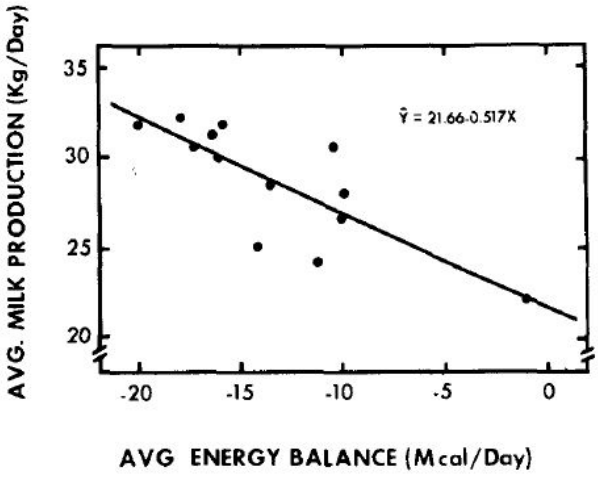

Figure 1. Relationship between milk yield daily energy balance.

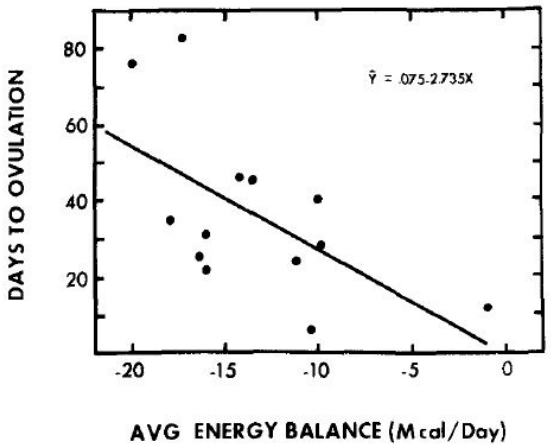

Figure 2. Relationship between days to first ovulation and daily energy balance.

Ron's team was among the first to demonstrate a positive effect of feeding supplemental fat on development of ovulatory follicles about 2 wk postpartum in high-producing Holstein cows (Beam and Butler,1997). They fed three levels of tallow (3.3, 5.2 and 7.1% of ration DM) and noted that cows fed the two higher levels had more ovulatory follicles at 2 wk postpartum. This focus on dietary fat and postpartum follicle development laid groundwork for development of fat-based dietary supplements that are used broadly in the dairy industry.

Ron's work caused many reproductive biologists and nutritionists to become more aware of antagonistic relationships between milk yield and energy balance, between negative energy balance and postpartum anestrus and between negative energy balance and fertility after AI. This led to numerous studies focused on feeding during the dry period and transition period to reduce amount of weight loss, and it refocused attention on how body condition at calving can impact subsequent weight loss. It also led to increased attention on what happens physiologically with rapid weight loss, particularly mobilization of fatty acids and changes in blood glucose, insulin, IGF and somatotropin. There was also interest in whether negative energy balance exerted longer term effects on germ cells and uterine function.

Britt (1992) proposed that developing follicles and oocytes could be affected adversely if their prolonged development occurred during a period of severe negative energy balance (Britt Hypothesis; Figure 3). Substantial data support this hypothesis and that the adverse effects may be mediated through epigenetic mechanisms. For example, Carvalho et al. (2014) reported that conception rate at timed AI at 80 $\pm$ 3 days postpartum was 25%, 38% and 84% for Holstein cows that lost, maintained or gained body condition score during 3 wk postpartum. Additionally, they showed that oocytes from cows that lost most body condition were fertilized, but then died at a high rate during the first 7 d of development, a classical epigenetic-type effect.

In the future as we mine dairy data from the "cloud" we will be looking for temporal relationships between environmental factors like energy balance and subsequent responses, some of which may occur months or years later. For example, we know that feeding calves to gain more in their first 2 months of life is reflected in their yield in first lactation. What is the long-term impact of severe negative energy balance in a cow's life? Ron would encourage us to ask that type of question.

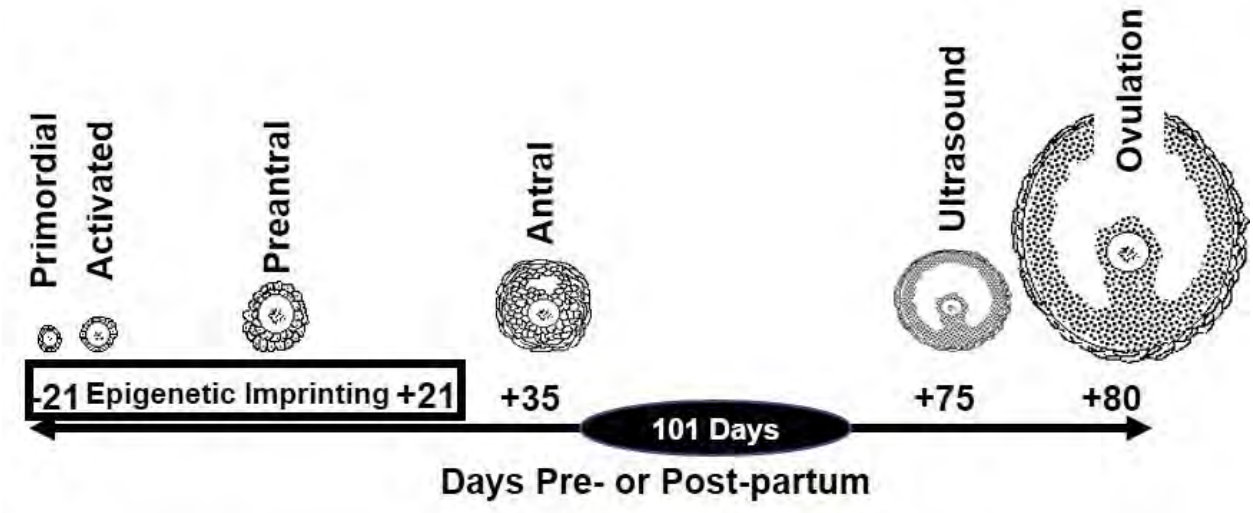

Figure 3. Illustration of a potential mechanism whereby negative energy balance during the transition period can affect the developing oocyte and result in failure of conception 11 to 12 weeks postpartum. Adopted from Britt et al. (2018).

Ron's work has had lasting impacts. Over the last 3 decades, dairy scientists, nutritionists, veterinarians and farmers responded in many ways to address adverse impacts of negative energy balance in the postpartum cow. For example:

- Body condition scoring became a routine practice
- Body condition scores at calving were nudged downward
- Rations were reformulated to improve digestibility and intake for fresh cows
- Transition management focused on 3 weeks before to 3 weeks after calving
- Dietary lipids were altered to address fat's potential impact on intake
- Dietary fatty acids were developed to that have beneficial effects on fertility
- Micronutrients, yeasts and other ingredients were boosted in fresh cow TMRs

There is a move to determine whether we can develop dairy cows that do not experience severe negative energy balance and yet still produce equivalent amounts of milk in a complete lactation (Zachut and Moallem, 2017). Analyses of data from 92 cows selected randomly from an Israeli research herd (Volcani Center; Rishon LeZion, Israel) demonstrated that cows differ consistently in postpartum weight loss over 4 consecutive lactations (Figure 4).

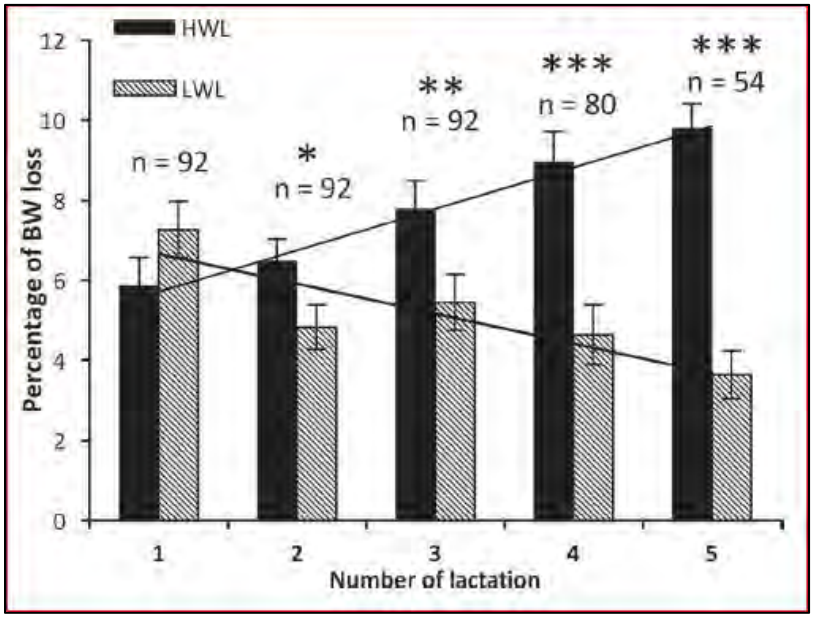

Figure 4. Differences in weight loss during early lactation among two groups of Holstein cows identified to have different patterns in weight loss. Adapted from Zachut and Moallem, 2017.

Application of genomic selection is likely to accelerate progress in identifying bulls that sire cows which produce the same amount of milk, are healthier and do not experience swings in energy balance that we see in today's modern dairy cow. Recent studies of daughters of Brown Swiss bulls revealed that some bulls sire daughters that

are clearly more robust and healthier and produce similar amounts of milk and components to daughters of bulls that do not transmit this robustness (Ha et al., 2017)

Our efforts to understand impacts of negative energy balance on many traits in dairy cattle trace their roots back to Ron's work in the postpartum cow. But he also led us to look at another nutritional-based issue – what are the impacts on fertility of over-feeding protein.

EXCESS SOLUBLE PROTEIN AND FERTILITY

If a little is good, let's add some more. That may have been our attitude for a long period about how much protein and what form of protein to include in rations of dairy cows. We knew we had to feed the organisms in the rumen, and we could do that easily with products like urea. Gradually crude protein levels in rations crept upward, reaching more than 20% in many cases.

On the other hand, fertility was declining, and inquisitive folks started looking at what was happening. Ron's work with energy balance and his nature to let graduate students seek a relevant problem to pursue led to a series of papers that illustrated how too much soluble protein could lead to lower fertility in dairy cows.

His research team showed that too much soluble protein could change the pH of the uterine lumen at a critical time and this led to substantial reductions in fertility. To avoid complications with energy and protein, they did their insightful work with Holstein heifers that were not in negative energy balance and not producing milk.

This was challenging work. How does one measure pH in the lumen of the uterus repeatedly over several days? How does one maintain as much similarity among rations for experimental groups and still vary the amount of crude protein significantly? Through ingenuity, they conducted experiments that provided a lot of insight. They kept rations similar by simply adding a bit more urea to one and tweaking a few ingredients in the other. It worked!

Higher soluble protein levels led to significantly lower conception rates in fertile heifers! This was associated with a drop in pH in the uterine lumen at a critical time of early embryonic development. They measured milk or blood urea nitrogen to estimate the excess amount of protein. Subsequent work demonstrated that the change in uterine pH in response to excess protein was due to changes in the transport of ions across the endometrium. It is now accepted wisdom among dairy nutritionists and veterinarians that excessive dietary soluble protein is not only expensive, but also impairs fertility through these alterations in the uterine environment.

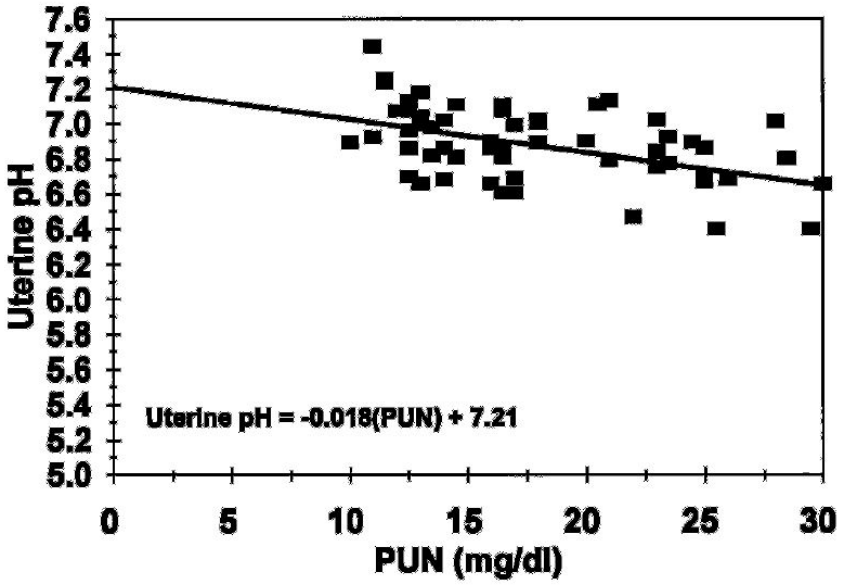

Figure 5. Uterine pH in heifers with various concentrations of plasma urea nitrogen (PUN) in blood on day 7 after estrus. Adapted from Elrod and Butler (1993).

The response to these dietary protein studies was quick. Every milk analyses laboratory added MUN to their stable of assays. Nutritionists and veterinarians started monitoring MUN, BUN and PUN and asking whether it was getting into the critical range. At the same time, our nutritional knowledge and models became more sophisticated in matching available carbohydrate and protein sources to achieve more efficient fermentation in the rumen and less wastage of nutrients.

All of this made our industry much more efficient in using protein in our rations. Protein percentages started creeping down and we were putting less nitrogen into soils and waterways. It is having a long-term benefit on our ecosystems.

Few scientists have had multiple long-term impacts on practices that have benefited the dairy industry worldwide. Ron's research on mechanisms through which nutrition affect postpartum reproduction and fertility led to improvements in feeding and management that are now hallmarks of best management practices. Well done Ron!

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Appendix Table 1. Top 10 cited scientific papers authored by W. R. Butler*

Title and Authors	Citations
Interrelationships between **energy balance** and postpartum reproductive function in dairy cattle; 1999; WR Butler, RD Smith; Journal of Dairy Science 72 (3), 767-783.	1,402
Nutritional interactions with reproductive performance in dairy cattle; 2000; WR Butler; Animal Reproduction Science 60, 449-457.	833
Effects of **energy balance** on follicular development and first ovulation in postpartum dairy cows; SW Beam, 1999; WR Butler; Journal of Reproduction and Fertility Supplement-, 411-424.	629
Effect of **protein nutrition** on ovarian and uterine physiology in dairy cattle; 1998; WR Butler; Journal of Dairy Science 81 (9), 2533-2539.	623
Plasma and **milk urea nitrogen** in relation to pregnancy rate in lactating dairy cattle; 1996; WR Butler, JJ Calaman, SW Beam; Journal of Animal Science 74 (4), 858-865.	558
Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows; 2003; WR Butler; Livestock Production Science 83 (2-3), 211-218.	548
Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat; 1997; SW Beam, WR Butler; Biology of Reproduction 56 (1), 133-142.	528
Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally **degradable protein**.; 1993; CC Elrod, WR Butler; Journal of Animal Science 71 (3), 694-701.	458
The relationships between **energy balance**, milk production and ovulation in postpartum Holstein cows; 1981; WR Butler, RW Everett, CE Coppock; Journal of Animal Science 53 (3), 742-748.	414
Decreased concentration of plasma leptin in periparturient dairy cows is caused by **negative energy balance**; 2001; SS Block, WR Butler, RA Ehrhardt, AW Bell, ME Van Amburgh, YS Boisclair. Journal of Endocrinology 171 (2), 339-348.	355

*(Google Scholar, https://scholar.google.com/citations?user=__d-NQoAAAAJ&hl=en&oi=sra Accessed 29 August 2018)

THE MANY FACETS OF RON'S INFLUENCE AT CORNELL
RON BUTLER: GENEROUS COLLABORATOR, SELFLESS ADMINISTRATOR, DEDICATED TEACHER

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Ross University

In academia I have encountered two types of effective leaders. Some are very focused, energetic and aggressive in pursuing the interest of their own "team" - be that a section, department, college or university. They argue that if their counterparts are equally aggressive, it is up to the next level of administration to make good decisions. A smaller number of academic leaders takes a wider view, carefully considering the overall good of their own unit and the greater whole, sometimes endorsing a path that may not be in the short term interest of their own unit but is likely to be best overall. In my opinion, Ron Butler belongs to the latter group. This is part of the reason that I am proud to call him a friend. Ron Butler was a team player at Cornell for 42 years, and Chaired the Department of Animal Science for 10 or 11 of those years. He has many, many accomplishments in that time but I want to highlight just a few.

A trait that characterized Ron Butler throughout his career, and especially during his formal leadership term, was collaboration and collegiality. For example, there is tension in many universities between colleges of veterinary medicine and departments of animal science where both exist. This is not so at Cornell. Of course, the good relationship between the College of Veterinary Medicine and the Department of Animal Science is long lasting, and not an achievement of Ron's, but he actively cultivated and developed this collaboration. Ron went out of his way to develop collegial relationships with faculty of the veterinary college. He knew what was going on at the vet school. He had an interest in the research activities of the veterinary faculty. He encouraged and participated in research and teaching collaborations and encouraged others to do the same. There are numerous examples, and I am the beneficiary of Ron's generous reaching over the boundary between the schools.

One example of Ron's willingness to work for the greater good of all is his term as Chair of the Institutional Animal Use and Care Committee (IACUC). Ron took over the leadership of this crucial committee during a particularly fraught period politically speaking. Very strong personalities in the animal care and use and research communities had led to severe conflict, alienation and bitterness with research and teaching colleagues. Chairing the IACUC was not an inviting option, but it was a role that Ron accepted. He may not have been the only person who could have achieved what he did, but we will never know that. What we do know, is that Ron brought about a period of collaboration between faculty who used animals in teaching or research, and those charged with the oversight of animal care and use. IACUC and the animal care and use staff came to be seen as facilitators of responsible use of animals, rather than combatants raising objections and complications at every step. Instead of the previous adversarial relationship, a period of collaboration was ushered in. It was clear everyone was on the

same team. People worked actively to solve problems. I believe that the IACUC under Ron's leadership achieved what must surely be the goal of every such committee: an environment in which everyone knew that responsible animal use, and impeccable animal welfare were shared ideals and cooperated to achieve those ends. The framework and philosophy brought about under Ron's leadership endure still, and are a model for other institutions to follow. He deserves real credit for this.

Ron's tenure as Chair of the Department of Animal Science coincided with an economic downturn and stringent financial circumstances. Nevertheless, it was a period during which the Department maintained a positive and productive mood. This alone is testament to Ron's skills. An achievement that deserves special mention is the rejuvenation of the Teaching and Research farm at Harford. By the time Ron became Chair, it was clear that the farm's facilities had outlasted their usefulness. The dairy had grown in a haphazard way, facilities were aging, cow welfare was compromised and it was becoming increasingly difficult to carry out research that was directly relevant to modern production systems. An initial effort to define the scope and costs of refurbishing the T&R had indicated that the project would cost multiples of the funds that could realistically be made available. It would have been easy to conclude that a good faith effort had been made and that nothing could be done. Ron did not do that. He worked constantly with the leadership of the College of Agriculture and Life Sciences to seek innovative solutions. He advocated tirelessly for the teachers and researchers who needed the facility and for the animals whose welfare depended on it.

During this time, there was an additional sensitive wrinkle. The College of Veterinary Medicine had had its own small dairy herd, right on campus, since the mid 1950s. This too had become antiquated, and it was displaced by the construction of the New Diagnostic Laboratory. The Veterinary College had plans and funding to replace the unit on the outskirts of the Ithaca campus. The site chosen for this dairy was too small to accommodate the needs of the Department of Animal Science. However, the establishment of a dairy herd right on campus had obvious negative implications for the effort to renew T&R in a way that was consistent with 21st century standards and the needs of the Department of Animal Science. The simple position would have been for Ron (and the rest of the College leadership) to oppose the building of the Vet College Dairy Herd. Instead, Ron supported the Vet College initiative but continued to argue forcefully for the needed initiative in Harford.

Ultimately, he and the College leadership achieved both. The veterinary college project was successfully completed, and a novel, innovative solution was found for the Harford farm in the form of the Cornell University Ruminant Center. Today, this solution, painstakingly put together and achieved, is regarded as a model for other universities. It is a wonderful facility with happy cows (always important to Ron), and a great place to do research. It assures Cornell's positon in driving leadership and innovation in the modern dairy industry, to the benefit of New York and the country. I know this project occupied a great deal of Ron's attention and effort, and he deserves a considerable portion of the credit for the commendable outcome.

The most enduring thing a Department Chair does in many cases is appoint new faculty. This sets the direction of the department, potentially for decades, and appointment of capable, high achieving colleagues sets the standard for the department going forward. In this realm, Ron Butler achieved great success. Remember that Ron's term was marked by fiscal restraint and more effort was expended in saving positons than in filling new ones. Nevertheless, Ron had some marked success. Vimal Selvaraj is now well-established as a professor of great intellect and energy who is generating novel research findings across a broad range of topics of basic and applied relevance. Similarly, Julio Giordano has enjoyed breathtaking success in a short time. Both promise to carry the torch of Animal Science at Cornell for a long time. Similarly, the appointment of Samantha Brooks was met with great excitement, and it was a pity that she felt her interests began to diverge from the necessarily narrower focus of the Department.

Ron's collegiality, ability to see the big picture and predisposition to work across boundaries was also reflected in his teaching career. For his 42 years at Cornell, Ron taught every year. Undergraduate students meant a lot to Ron and he took pride in the number of people he influenced as undergraduate majors in Animal Science. Many he guided through undergraduate research projects and in some cases into more formal research careers, or futures in aspects of animal science or veterinary medicine. Ron has been recognized for his teaching prowess. His influence and efforts included a devotion to extension, where again he was unusually successful. In 1991, Ron Butler and Joanne Fortune initiated a graduate course in reproduction that has been a great success and continues. More recently, Ron introduced a new course in comparative animal reproduction, in which he sought to highlight similarities and differences in mammalian reproductive strategies, a popular course in which I enjoyed participating on a couple of occasions.

I hope these few examples serve to give a glimpse of a man who was a leading scholar and teacher, but who is marked by his personal humility and devotion to a greater good. This has been evident in his career in general, and especially in those occasions where he has been called upon to play a leadership role. He is proof that you can be a distinguished professor, an effective leader, and a fine person, all at the same time. Ron's achievements are entirely consistent with the person he is every single day, and are evidence of his hallmark trait of always being dedicated to something bigger than himself. Cornell University, and particularly Animal Science at Cornell are better off for having had Ron Butler there for 42 years. I am very grateful to the organizers of this conference for giving me the opportunity to describe ways in which this very distinguished professor, but humble person, has made a huge difference, mainly by being himself. I am proud to have worked with Ron as a colleague and to have him as a friend. Congratulations, Ron, on a truly exceptional career.

OF COWS AND MEN: REVIEWING THE LINK BETWEEN MILK FAT AND HUMAN HEALTH

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[2]CRSAD; [3]Department of Animal Science, Université Laval

INTRODUCTION

Milk is a unique, nutrient-dense, food. Key to early development of mammals, milk constitutes a major source of energy, high-quality protein, and vitamins and minerals such as vitamin D, calcium, and potassium (Smilowitz et al., 2005; Gaucheron, 2005). Beyond its key role in neonatal nutrition, milk and milk-derived products are also major sources of nutrients for adults. The ability of humans to carry on drinking milk through adulthood has developed gradually over the past eight millennia, in association with agriculture and dairying (Curry., 2013), and it is due to the persistence of the enzyme lactase past early childhood. The rise of distinct genetic mutations for lactase persistence developed in at least four different geographic regions on the planet (i.e., lactase hotspots), and, arguably, provided a major selective advantage (Bersaglieri, et al., 2004). Today, one-third of humans produce lactase during adulthood, with prevalence being greater in places like northern Europe, where over 90% of people can drink milk, but also in West Africa, the middle east, and south Asia (Curry., 2013; Liebert et al., 2017).

Despite the arguably obvious benefits that milk can provide to human populations as a source of nutrients, its place as a component of a healthy diet has been questioned. Indeed, over the past five decades, changes in consumer perception, dietary guidelines, public health messages, and policy, have all resulted in a shift in the patterns of consumption of milk and dairy products, particularly those of higher fat content. The start of these changes can be traced to the emergence of the “diet-heart hypothesis” in the 1950’s. Based on limited epidemiological data, its fundamental premise was that fat consumption could cause cardiovascular disease (**CVD**) in humans. In spite of being criticized as “invalid” and being based on a “tenuous association” from the onset (Yerushalmy and Hilleboe, 1957), the diet-heart hypothesis became a central piece of dietary advice in the following decades, in particular, to reduce CVD risk. A generalized “fear of fats” has spread since then and shaped consumer’s choices, who nowadays tend to look for reduced-fat foods as healthier alternatives to full-fat options, in hopes of reducing intake of fat and ‘calories’, and the risk of heart problems and obesity. Simultaneously, milk avoidance has become more prevalent (Figure 1), and new beverages of plant origin with low contents of saturated fats have become available, replacing cow’s milk in the diet (e.g., the so –called soy and almond milks). In contrast to current perceptions and dietary advice, an important body of literature has emerged over the past decade that challenges the contemporary views associating saturated animal fat consumption to human disease. Such studies suggest that dairy in general, as well as full-fat dairy, may decrease the risk for CVD. Furthermore, recent studies suggest that full fat dairy may actually protect from obesity and associated chronic diseases. This review

will summarize available evidence from recent meta-analyses, and observational and randomized controlled trials (**RCTs**), on the effects of dairy consumption on human health. The public health role of dairy products in relation to their potential to prevent or ameliorate the onset of chronic diseases will be discussed.

FAT CONSUMPTION AND HUMAN DISEASE

The Diet-heart Hypothesis - Background and Classic Studies

The now prevailing concept that saturated fats of animal origin are detrimental to human health can be traced to some suggestive pieces of evidence originated in the 20th century. First, the ground-breaking studies of Ignatowski in 1908 and Anichkov in 1913 demonstrated the ability of animal fats and, specifically, cholesterol, to cause atherosclerotic lesions, raise plasma cholesterol, and cause death in rabbit models of atherosclerosis (Konstantinov et al., 2006). Second, during the 1950's, Ancel Keys produced epidemiological data that seemed to identify dietary fat as a major cause of heart disease. In two commonly known studies, Keys and collaborators showed some seemingly strong associations between national death rates for middle-aged men from arteriosclerotic and degenerative heart disease and the proportion of fat-calories available in their national diets (Keys, 1953; Keys et al., 1966). His data relating availability of energy from fat and cardiovascular-related death led him to conclude that "dietary fat somehow is associated with cardiac disease mortality, at least in middle age". These studies propelled the extensively known diet-heart hypothesis that related dietary factors to the incidence of cardiovascular disorders. The validity of this hypothesis was quickly challenged by Yerushalmy and Hilleboe (1957), and its acceptance has remained far from unanimous ever since it was first proposed. In their methodological note, the authors attempted to evaluate whether the proposed hypothesis could actually reflect "known or ascertainable facts" which may allow for the generalization of this premise. The authors pointed at several limitations, including 1) small sample size (i.e., only 6 countries were used in Key's original study); 2) the possible effects of unaccounted confounders (e.g., underreporting in countries with lower economic status who cannot afford meat and dairy); 3) the fact that no actual data on fat consumption was used (i.e., availability was used); 4) the lack of specificity of the relationship (i.e., protein consumption also related to death); 5) variation in deaths from cardiac disease are largely variable across countries at any given fat availability category. Some interesting points emerging from their methodological analysis include: first, the strength of the association is greatly reduced when more countries are included (n= 22); second, the relationship is not specific to fat; third, "almost no association" was found when correlating fat or protein with all causes of death. This last point merits attention because CVD is the leading cause of death in industrial countries, and consequently, the relationship in Keys' studies would be expected to also hold for all-cause mortality. More recently, others (Willet, 2012) have pointed out that the countries chosen by Keys to represent low fat intake and low incidence of CVD were in fact less industrialized and showed differences in smoking habits, physical activity and obesity, thus complicating the generalization of the diet-heart hypothesis.

Dietary Lipids and CVD Risk

The importance of cholesterol in the development of CVD in humans was first suggested by data extracted from the Framingham study, which enrolled 5127 men and women aged 30–59 years in Massachusetts, starting between 1948 and 1950. Following six years of longitudinal evaluation, cholesterol was identified as one of three risk factors for CVD (Kannel et al., 1961)[1]. Several other studies between the 1950's and 1980's found positive associations between serum (total) cholesterol and risk of CVD. With the advent of techniques to identify and measure circulating lipoproteins, it was further shown that cholesterol contained in very low-density lipoproteins (**VLDL-C**), as well as low-density lipoproteins (**LDL-C**; i.e., 'bad cholesterol'), correlates positively with CVD risk, while that found in high density lipoproteins (**HDL-C**; i.e., 'good cholesterol), correlated negatively (see review by Parodi, 2009). In fact, LDL-C became the lead marker for atherogenicity and CVD risk, and thus, the main target and factor guiding CVD treatments in the last few decades (Stone et al., 2014). However, the role of LDL-C recently has been put into question as it is considered a very poor predictor of CVD (Sachdeva et al., 2009; Ravnskov et al., 2018). On the other hand, HDL-C (an indicator of cholesterol efflux) levels are used as a marker of reduced CVD risk (Rohatgi et al., 2014; Monette et al., 2016), particularly when used as a ratio of total cholesterol to HDL-C (Castelli, 1988). In addition, increased triglyceride to HDL-C ratio is a more powerful predictor of coronary heart disease (Luz et al., 2008).

While much of the focus has been historically placed on cholesterol, other factors seem to be important to predict CVD risk. Some of these include obesity, serum triglycerides, inactivity, hypertension, cigarette smoking and diabetes. Results from the Framingham cohort illustrate the importance of these cofactors, as, for example, accounting for glucose intolerance, high systolic blood pressure, smoking, and left ventricular hypertrophy increased CVD risk to 60.2, compared to only 3.9 when cholesterol alone was used (Kannel et al., 1979).

Saturated Fats and Blood Lipids

The ability of saturated fatty acids (**SFA**) to raise blood LDL-C is consistent across the literature, and it is well documented (Micha and Mozaffarian, 2008). Interest in this relationship stems from hypothesis that LDL particles may increase cholesterol accumulation in arterial walls, facilitating the formation of atheromatous plaque, and therefore increasing CVD risk (Kruth et al., 2001). Consequently, efforts have been made to determine the atherogenic potential of individual SFA as a marker of CVD. For example, Ulbricht and Southgate (1991) proposed the atherogenic index, which is calculated by dividing the sum the SFA lauric (12:0), myristic (14:0) and palmitic (16:0), by the sum of omega 3 and 6, 18:1c9, and other monounsaturated fatty acids. Based on Hegsted's work (1965), each factor is multiplied by an empirical constant according to its

[1] Indeed, interest in cholesterol seemed justified, as atheromatous lesions are rich in free and esterified cholesterol, relative to normal arterial walls (Windaus, 1910). Moreover, cholesterol alone can cause atheromatous lesions in the vascular wall (Anitschkow and Chalatow, 1913).

capacity to raise or decrease cholesterol, using a value of 1 for all fatty acids and a value of 4 for 14:0. Because these presumably atherogenic fatty acids (12:0, 14:0, 16:0) represent 30-40% of cow's milk triglycerides (Jensen, 2002; O'Donnell-Megaro et al., 2011), some have concluded that dairy fat is a potential cholesterol-raising food, and, consequently, consumers have reduced the consumption of full-fat dairy (Wang and Li, 2008). Simultaneously, official advice has focused on reducing the consumption of fat and saturated fat specifically. The dietary fat guidelines introduced in 1977 (US) and 1983 (UK) (Harcombe et al., 2015) recommend to 1) reduce overall fat consumption to 30% of total energy intake and 2) reduce saturated fat consumption to 10% of total energy intake. It is important to note that, with these guidelines, the ability of SFA to raise blood HDL-C, and to reduce CVD risk, is implicitly ignored (Parodi, 2009). In fact, a meta-analysis of 60 controlled trials showed that SFA have no effect on the ratio of total cholesterol to HDL-C (lower is better) when SFA replace dietary carbohydrates (Mensink, et al., 2003). Furthermore, the allegedly "atherogenic" lauric acid reduces the ratio, mostly by increasing HDL-C. These observations challenge the notion that SFA are indeed atherogenic and that SFA sources like milk fat may have health-adverse effects.

The disconnect between the expected effects of SFA-containing foods on CVD risk markers and the actual outcomes is well exemplified by the work of Lefevre et al., 2005. In this double-blind, randomized cross-over study, the authors report the effects of milk fat on health markers in males aged 22-64. Dietary supply of fat and SFA were adjusted by substituting low fat or non-fat dairy products for their higher fat equivalents. Energy from milk fat was progressively reduced to achieve fat and SFA energy consumption equivalent to an average American diet (**AAD**; 37% fat and 14% SFA; above current recommendations), and two diets with reduced fat and SFA content (**Step I diet**, 28% fat and 8.8% SFA; **Step II diet**, 24% fat and 6.2% SFA). The last two diets met current recommendations for energy intake from fat and SFA (Table 1).

Table 1. Reductions in dairy fat consumption result in higher blood triglycerides and Total:HDL-Cholesterol (Lefevre et al., 2005)

Effect of diets on lipid and lipoprotein concentrations[1]

	AAD 14% SFA	Step I diet 8.8% SFA	Step II diet 6.2% SFA
Total Cholesterol, mmol/L	4.82±0.69	4.59±0.6[2]	4.39±0.66[2,3]
Triacylglycerol, mmol/L	1.06±0.65	1.20±0.76[2]	1.22±0.80[2]
LDL Cholesterol, mmol/L	3.25±0.58	3.03±0.56[2]	2.87±0.52[2,3]
HDL Cholesterol, mmol/L	1.07±0.23	0.99±0.22[2]	0.95±0.22[2,3]
Apolipoprotein A-I, g/L	1.23±0.14	1.17±0.13[2]	1.15±0.12[2,3]
Apolipoprotein B, g/L	0.97±0.19	0.93±0.20[2]	0.9±0.18[2,3]
Total:HDL cholesterol	4.7±1.08	4.84±1.18[2]	4.85±1.26[2]

[1] All values are mean ± SD; n = 86. AAD, average American diet.
[2] Significantly different from AAD, $P < 0.05$ (ANOVA with Bonferroni corrections).
[3] Significantly different from Step I diet, $P < 0.05$ (ANOVA with Bonferroni corrections).

As expected, the reduction of dairy SFA consumption resulted in reduced circulating LDL-C. However, reductions of similar magnitude were observed for HDL-C. Furthermore, low fat diets increased circulating triglycerides and the ratio of total cholesterol to HDL-C (**Total:HDL-C**; i.e., the atherogenic ratio), both proxies for increased CVD risk. In this way, elevations observed by reducing dairy fat intake cast some concerns about the efficacy of low-fat diets to reduce CVD risk. Arguably, increased CVD risk may actually result from following this type of low-fat approach. Although factors like adiposity and insulin resistance of subjects may have influenced the responses to low-fat diets in this study, it is evident that the effects of such diets on the commonly used biomarkers (e.g., LDL-C, HDL-C, Total:HDL-C, and triglycerides) were certainly not what many may expect in terms of alleviation of CVD risk. Moreover, the validity of CVD biomarkers like LDL-C has dwindled recently, and the role of lipoproteins on CVD has shifted focus into their size (i.e., small and dense LDL profile is worse), number (i.e. Apolipoprotein B as a marker of atherogenic particles), and oxidation propensity, rather than their cholesterol content (Krauss, 2005; Parodi, 2009). An example comes from the guidelines of the American Heart Association, who, based on a recent review of available data, reported being unable to find evidence to support continued use of specific LDL–C and/or non-HDL–C treatment targets. In this way, the “bad cholesterol” is no longer the main factor guiding treatment (Stone et al., 2014).

Even leaving CVD lipid biomarkers aside, under the diet-heart hypothesis, the effects of SFA on actual clinical outcomes should reflect harmful consequences on cardiovascular endpoints. Contrary to this expectation, a meta-analysis of prospective cohort studies that followed 347,747 subjects for 5 to 23 years found no association between saturated fat intake and CVD, both fatal and non-fatal (Siri-Tarino et al., 2010). Similarly, in a systematic review and meta-analysis of prospective cohort studies and RCTs (n = 78) with 649,812 participants, Chowdhury et al. (2014) reported no increase of relative risk of coronary outcomes associated with dietary or circulating SFA. Moreover, the authors reported an inverse association between circulating margaric acid (17:0, a marker of dairy fat intake) and coronary disease. Taken together, available evidence from prospective epidemiologic studies and RCT does not support guidelines encouraging reduced saturated fat consumption, particularly those from dairy. Whether this evidence can be considered sufficient to totally vindicate SFA and dairy, is still a matter for discussion; however, currently available data suggest the heavy focus on saturated fats may be not only unnecessary, but perhaps also detrimental. This is particularly true when considering that dairy fats may have been replaced with industrial *trans* fats of plant origin (e.g. margarines with high *trans* fat content), as well as refined sugars (i.e., fructose). Some trends exemplifying these dietary substitutions can be seen in Figure 1. Full fat milk has been partially substituted with lower-fat versions, while soft drinks consumption and fructose has risen in linearly. Importantly, there are cogent reasons to believe that the simultaneous reduction in consumption of some dairy products and the increase industrial *trans* fats and sugars could be detrimental. For example, non-ruminant (i.e., industrial) *trans* fats are nowadays recognized as harmful (Micha and Mozaffarian, 2008) and they relate strongly to heart disease and all-cause mortality (Oomen et al., 2001; de Souza et al., 2015). Similarly, a growing body of evidence indicates that most US adults currently consume excess added sugar (a source of fructose), and this is significantly associated

with obesity, metabolic syndrome, and CVD mortality (Johnson et al., 2009; Lustig et al., 2010; Yang et al., 2014). In fact, the American Heart Association has recommended the reduction of dietary sugar intake by more than half (Johnson et al., 2009).

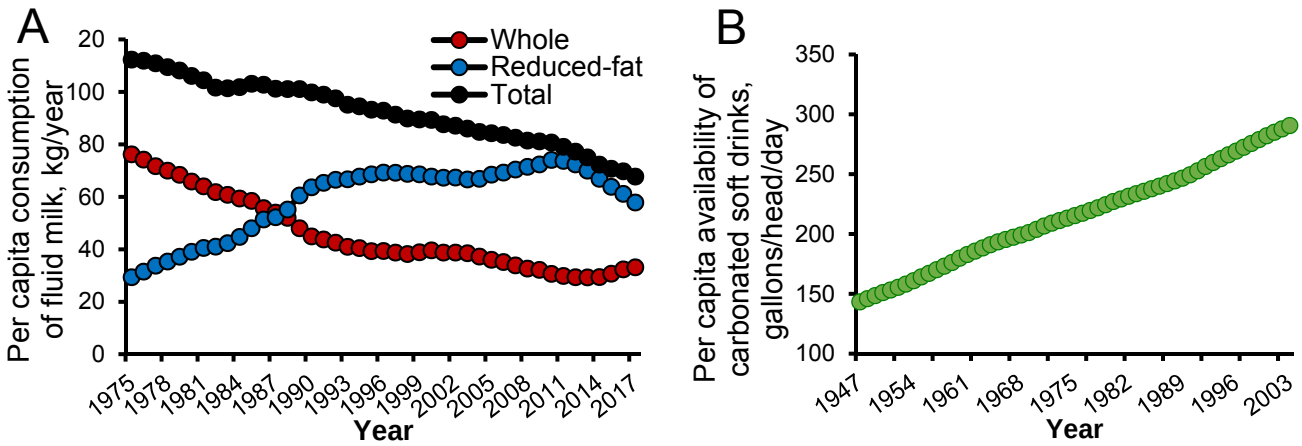

Figure 1. (A) Trends of consumption of fluid milk as reduced-fat milks and whole milk from 1975 to 2017 in the U.S. (B) Per capita availability (gallons/head/day) of carbonated soft drinks, 1947 - 2003 Source: USDA, Economic Research Service. 2012; 2018.

DAIRY CONSUMPTION AND HUMAN HEALTH OUTCOMES

A Note on the Assessment of Evidence

Evidence for claims related to lipid intake and human health come from a range of studies with varying degrees of causal strength, going from observational studies to controlled randomized clinical trials. Young and Karr (2011) give some perspective on the frequency at which observational claims fail to replicate, at an alarming rate of 80%. Further, in about 10% of the cases, when the claims from observational studies were tested in clinical trials they moved significantly in the opposite direction (Young and Karr; 2011). These authors suggested that ‘‘any claim coming from an observational study is most likely to be wrong – wrong in the sense that it will not replicate if tested rigorously’’. Therefore, assessing the observational evidence that relates dietary intakes to common disease outcomes is, at the very least, problematic (Prentice, 2014). For instance, the validity of observational studies can be compromised by the necessary reliance placed on self-reported food intakes and can further be complicated by the effects of confounders, such as lifestyle factors that may impact the association. This highlights some of the problems with claims originated from ecological studies, such as the diet-heart hypothesis, which now seems to lose validity in the face of stronger pieces of evidence, such as RCTs and prospective cohort studies, which contradict its original presuppositions (e.g., Micha and Mozaffarian, 2008; Ramsden et al., 2016). While bearing these limitations in mind, the interpretation of observational studies relating dairy intake and health outcomes can be in many instances a useful starting point for

investigation and validation. Regardless, causation may only be derived from properly controlled experimentation.

Obesity and Type 2 Diabetes

One arguably important reason for the current trends of dairy fat avoidance (Figure 1) is related to the interest in reducing excess energy intake. The common presumption is that dairy fat can be stored as body fat and thus contribute to weight gain, obesity, and cardiometabolic risk. This has driven dietary guidelines to recommend the consumption of low-fat dairy (Jensen et al., 2014). In contrast to guidelines and prevailing public sentiment, available evidence indicates dairy fat consumption is not related with the risk of weight gain. The comprehensive review of Kratz et al. (2013), which used a combination of observational and controlled studies, indicates that dairy fat consumption, both recorded or assessed via odd-chain fatty acid content in blood (e.g., 15:0 and 17:0), was inversely related with obesity risk. Similar findings were reported in a cross-sectional evaluation of full-fat milk consumption in three-year-old children (Beck et al., 2017). The multivariate analysis included potential demographic and nutritional confounders. The authors reported reduced odds for severe obesity in association with higher milk fat consumption, suggesting a protective effect of dairy fat against obesity in three-year-olds. Similarly, in a prospective cohort study of 18,438 healthy middle-aged women followed during 11 years and belonging to the Women's Health Study, greater consumption of total dairy products reduced the risk of becoming overweight or obese. Furthermore, the lowest risk was observed at the highest quintile of high-fat dairy product intake (Rautiainen et al., 2016). Finally, in a meta-analysis of 29 RCTs, Chen et al. (2012) reported dairy consumption does not increase body weight gain or body fat gain. Moreover, dairy consumption results in modest beneficial effects on weight loss in short-term and energy-restricted RCTs.

Type 2 diabetes (**T2D**) is rapidly rising worldwide, paralleling the epidemic increase in obesity. Because of its high content of calcium, magnesium, vitamin D, and whey proteins, which could reduce insulin resistance, dairy products could be hypothesized to protect against T2D (Rice et al., 2011). The meta-analysis of Aune et al. (2013) evaluated the association between intake of dairy products and the risk of T2D from prospective cohort and nested case-control studies (n=17). Non-linear, inverse associations were found between the risk of T2D and intakes of dairy products, low-fat dairy, yogurt, and cheese, the latter being the highest in fat content. The risk responses to dairy intake were dose-dependent, and flattened at higher intakes. Interestingly, high fat dairy did not alter the T2D risk in this study, although a meta-analysis focused specifically on butter consumption (Pimpin et al., 2016; 11 country-specific cohorts and 201,628 participants) reported butter intake was associated with a reduction of T2D risk. This discrepancy may suggest that the effects of dairy fat may be food-specific (e.g. cheese different from butter), a concept that merits further investigation. Other recent meta-analyses add support to the protective effects of dairy consumption against T2D (Forouhi et al., 2014; de Souza et al., 2015; Yakoob et al., 2016). For example, the prospective associations between circulating fatty acids in phospholipids and T2D were reported in individuals from the EPIC-InterAct case-cohort study (17,928 T2D subjects and 16,835 participants in a

random subcohort; Forouhi et al., 2014). By design, this study combines the temporal sequence and power advantages of a larger prospective cohort, with the measurement efficiency of a case-control. Forouhi et al., 2014 reported reduced hazard ratios for incident T2D in association with the odd chain SFA 15:0 and 17:0, both of which are mostly derived from dairy products. Similarly, using two prospective cohorts with 3333 adults aged 30 to 75 years, free of T2D at baseline, and followed during 15 years, Yakoob et al. (2016) found that individuals at the highest quartile of plasma 15:0, 17:0, and *t*-16:1n-7 had reduced risk of incident diabetes mellitus (-44%, -43%, and -53%, respectively; Figure 2). This last finding is of particular interest given that other studies have showed that circulating *trans*-palmitoleic acid (*t*-16:1n-7) is associated with lower insulin resistance, atherogenic dyslipidemia, and incident diabetes (Mozaffarian et al., 2010; de Souza et al., 2015). Importantly, whole-fat dairy consumption is most associated with elevated plasma concentrations of *trans*-palmitoleic acid (Mozaffarian et al., 2010). Whether the apparently beneficial effects of dairy on T2D risk are mediated by *trans*-palmitoleic acid or other components of dairy, remains to be experimentally elucidated; regardless, this possibility constitutes an exciting new direction for fatty acid research.

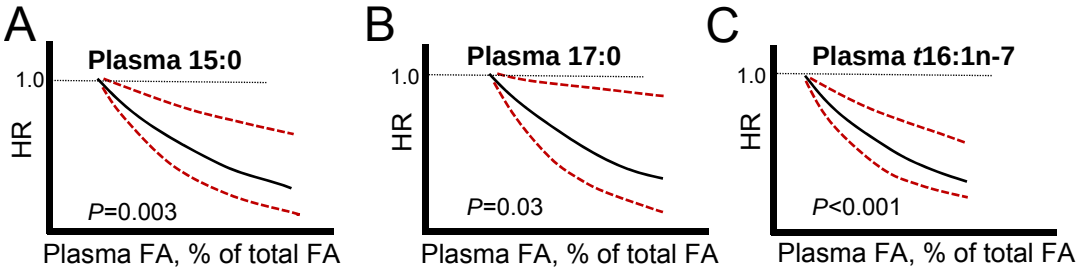

Figure 2. Circulating biomarkers of dairy fat intake and risk of incident diabetes mellitus in two large prospective cohorts using 3,333 adults in a 15-year follow-up (Adapted from Yakoob et al., 2016). Solid-black and dashed-red lines represent hazard ratios (HR) and their 95% confidence intervals, respectively, for plasma A)15:0, B)17:0, and C) *t*-16:1n-7.

Metabolic Syndrome

Metabolic syndrome (**MetS**) consists of a cluster of cardiovascular risk factors that include central obesity, hyperglycemia, hypertriglyceridemia, low HDL-C, and hypertension (Alberti et al., 2009). Moreover, MetS is closely associated with CVD risk, T2D, all-cause mortality and cancer (Saely et al., 2007; Wu et al., 2010; Esposito et al., 2012). Despite the recognition of the potential of dairy products to prevent or alleviate CVD, T2D and blood pressure in adults (e.g., 2010 Dietary guidelines for Americans; USDA/USDHHS, 2010), data on the relationship between dairy consumption and MetS is very limited. Chen et al. (2015) evaluated currently available data from cross-sectional/case control studies (n=16) and prospective cohort studies (n=7) in two different meta-analyses. Comparing high vs. low dairy products intake, both meta-analyses showed a reduction in MetS risk with high dairy consumption, an observation that was maintained when the studies were evaluated by stratified subgroups (e.g. geographic region, sex, type of dairy, and follow-up duration). Finally, a dose response analysis of

prospective cohorts in the same study showed an inverse relation between MetS risk and dairy consumption (Chen et al., 2015). Interestingly, the reduction in risk became evident when dairy intake was higher than 2 servings per day, and behaved linearly thereafter. This would suggest a minimum amount of dairy may be needed to impact MetS risk in a beneficial manner.

CVD Risk and CVD Mortality

As discussed previously, the relationship between SFA consumption and CVD is not straightforward, and it was historically derived from the diet-heart hypothesis, with two important premises: 1) SFA can influence circulating cholesterol (i.e., increase LDL-C), and 2) cholesterol is a risk factor for CVD. The resulting assumption was, therefore, that SFA consumption can cause CVD. Given the disconnect between SFA consumption and the anticipated clinical CVD outcomes (e.g., Siri Tarino et al., 2010; Chowdhury et al., 2014), the strength of the diet-heart hypothesis has been questioned. Considering that dairy products may have protective effects against obesity, T2D, and metabolic syndrome, it is important to elucidate whether this may also be true for CVD, which remains a major cause of death in the United States (Mozaffarian et al., 2015). When looking at the effects of dairy consumption on CVD risk factors, the most salient finding is that, contrary to expectations, reducing SFA intake from dairy increases CVD risk, as determined by commonly used markers (Lefevre et al., 2005; Table 1). This sobering observation seems to receive further support from other studies that report significant associations between milk-derived fatty acids and a more favorable LDL particle size distribution (i.e., reduction in small dense LDL particles; Sjogren et al., 2004). Furthermore, some SFA found in milk fat, such as lauric acid, are actually associated with a reduction of CVD risk (Micha and Mozaffarian., 2010). Importantly, these pieces of evidence align with the solid, general observation, that SFA are neutral or even beneficial in terms of CVD risk.

Focusing on evidence from prospective data, Elwood et al., (2008) conducted a meta-analysis of 15 prospective cohort studies reporting the association between milk and dairy consumption and the incidence of vascular diseases in the UK. The relative risk (RR) of stroke and/or heart disease was significantly reduced in subjects with high milk or dairy consumption (RR =0.84 and 0.79, respectively), compared with the risk in those with low consumption. These findings highlight once more, the disconnect between the hypothesized effects of SFA-containing foods like dairy, and the actual clinical outcomes of interest. Similarly, a systematic review of the available literature indicates that most studies do not support the expected effects of dairy fat on CVD, and that discrepancies may be associated to country-specific effects (Kratz et al., 2012). Specifically, the Nurses' Health study (from the US) found a consistent positive association between dairy fat intake and CVD, while 11 other studies across Europe, Costa Rica, and Australia, showed either no association or an inverse relationship between CVD and dairy fat intake. Only one of these 11 studies reported a discrepancy, as it found an inverse association in men, but a positive one in women (Kratz et al., 2012). The authors suggested that residual confounding from lifestyle factors associated with dairy intake, as well as differences in food sources of dairy fat, may help explain the discrepancy between US and non-US data.

Relevant to this point, the recently published results from the Prospective Urban Rural Epidemiology (**PURE**) study evaluated the effects of dairy consumption on death and major CVD events across 21 countries and 5 continents in an 9-year follow-up (Dehghan et al., 2018). Dietary intakes of dairy products for 136,384 individuals were recorded using country-specific validated food frequency questionnaires. Dairy foods evaluated included milk, yoghurt, and cheese, and these were grouped into whole-fat and low-fat dairy. Dairy intake above 2 servings per day reduced the risk of total mortality, CVD mortality, major CVD, and stroke, relative to no intake. Similarly, whole-fat dairy (> 2 servings per day) was inversely associated with total mortality and major CVD. Interestingly, the CVD response to whole-fat dairy appeared to be dose-responsive, as it increased progressively from <0.5 to 0.5-1, 1-2, and >2 servings per day. Cheese consumption (>1 serving per day) was associated with reduced mortality and major CVD, while the effect of butter was neutral (i.e., no increase in risk). The PURE study suggests that dairy intake, especially whole-fat dairy, might be beneficial for preventing deaths and major cardiovascular diseases. Moreover, there seems to be no disadvantage associated with the consumption of full-fat dairy, compared with the low-fat counterparts. The authors conclude that consumption of dairy products should not be discouraged and perhaps should even be encouraged, particularly in low-income and middle-income countries where dairy consumption is low.

The mechanistic modes of action by which milk and dairy products may be protective against CVD and other health outcomes is likely complex and still requires investigation. It is important to bear in mind that bovine milk contains an outstanding number of bioactive components (Park, 2009), which may interact additively, synergistically, or antagonistically. The heavy focus on single nutrients during the past decades (e.g., saturated fats should be avoided) has proven narrow in scope and of limited ability to predict health outcomes. As proposed by others (Mozaffarian, 2014), food-based guidelines that reduce confusion for consumers and are based on prospective evidence for effects on clinical endpoints are needed.

CONCLUSION

We may be in the midst of a paradigm shift in human nutrition. The revaluation of the validity of classic literature and the emerging plethora of evidence over the past decade, strongly contradict the long-held idea that dietary saturated fats cause adverse effects on health. Moreover, as shown in this review, current evidence indicates that dairy products, including full-fat dairy, may exert protective effects on metabolic health, reducing the incidence of obesity, T2D, MetS, CVD, and mortality. In light of this evidence, a general call to revise the guidelines on dairy consumption seems strongly justified and necessary, particularly as dairy products may help combat the spread of chronic diseases. Moreover, the historic focus on individual nutrients (e.g., fat, calories) has proven limited in terms of predicting clinical outcomes. In this sense, a whole-food approach to studying the effects of the ensemble of nutrients contained in dairy foods on human health outcomes seems warranted. Lastly, policy changes should be guided by a more nuanced interpretation of observational studies, and reflect the value of repeatable, randomized controlled studies, as the latter may provide insights on causality and the role of dairy on public health.

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POTENTIAL ROLE OF METHIONINE IN IMPROVING PERFORMANCE OF BROILER CHICKS UNDER HEAT STRESS

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INTRODUCTION

Approximately 9 billion broiler chicks were raised within the U.S. alone in 2017, which provided 55.6 billion pounds of broiler products and generated a market value of 30.2 billion dollars (USDA, 2018). The majority of the poultry production is concentrated within southern States where summer temperature is high (National Chicken Council, 2016). Thus, broilers raised in those States are likely to experience heat stress during the summer. Modern broiler houses are built to raise high density of chicks for efficient use of space. High stocking density is known as another stress factor for these animals. Both factors impair growth performance, immune responses, and health status of chicks (Dozier et al., 2005; Grashorn & Kutritz, 1991; Lara & Rostagno, 2013; Shanawany, 1988; Quinteiro-Filho et al., 2010).

Oxidative stress is the main consequence induced by heat and stocking density stresses (Altan et al., 2003; Lin et al., 2006). Reactive oxygen species (ROS) are oxygen-containing free radicals that are generated during oxidative stress and cause oxidative damage to protein, lipids, and nucleic acids in living cells. Antioxidants remove ROS by either sequestering them or degrading them. These substances include vitamin E, carotenoids, and the sulfur containing amino acids methionine and cysteine (Holst & Williamson, 2008; Oroian & Escriche, 2015).

Methionine and cysteine are considered to be the most limiting amino acids in poultry diets, aside from lysine. Requirements for digestible methionine + cysteine and digestible methionine range from 0.88% to 0.74%, and 0.45%, to 0.39% in starter and finisher diets, respectively (National Research Council, 1994). As chicks have the capacity to convert methionine into cysteine, it is considered to be more essential than cysteine (Bhagavan, & Ha, 2011). Corn and soybean provide the majority of crude protein and amino acids in poultry diets. Additional sources of methionine: synthetic DL-methionine (DL-Met) and methionine hydroxyl-analogue calcium salt (MHA), have been shown to improve performance of broilers (Elvert et al., 2008). At the same time, supplementing synthetic amino acids in the diets can reduce the use of crude protein, which can lower the nutrient excretion especially nitrogen in the manure and help prevent pollution to the environment(Han and Lee, 2000).

Methionine is necessary for tissue growth, and is also a key antioxidant with the ability to prevent lipid peroxidation and protein oxidation in the brain (Butterfield and Lauderback, 2002; Butterfield et al., 2010), liver (Singal et al, 2011; Zhu et al., 2012) and muscle (Wang et al., 2009; Willemsen et al., 2011). It has been shown that methionine residues in proteins have potent antioxidant function as they are readily oxidized prior to other amino acids. Oxidizing the surface exposed non-functional methionine (Met) into methionine sulfoxide (MetO) protects other critical residues in the protein to maintain its integrity and function (Atmaca, 2004; Levine et al., 2000; Levine et al., 1996). A metabolite of methionine, S-adenosyl-L-methionine (SAM), is involved in antioxidation as it is necessary for synthesis of glutathione (GSH), a tripeptide necessary for cellular ROS removal (Brown et al.,2012). Growing broilers fed diets containing concentrations of methionine exceeding the NRC requirement showed improved growth performance, antioxidant status, and immune function (Chen et al., 2013; Rao et al., 2003; Swain & Johri, 2000). Another major function of methionine is that it serves as a dietary source of methyl groups and at the cellular level methionine is utilized as the initiating amino acid in the protein synthesis (Cavuoto & Fenech, 2012; Niculescu & Zeisel, 2002).

Methionine plays a key role in the protein synthesis and catabolism in the immune system and development of the immune organs (Al-Mayah, 2006; Mirzaaghatabar et al., 2011). Several researchers have found that methionine supplementation in the diets can increase the weight of bursa of Fabricius and spleen in chicken (Wu et al., 2012; Wu et al., 2012), while methionine deficiency can cause immune organs dysplasia (Zhang and Guo, 2008). Methionine can also promote the T lymphocyte proliferation and differentiation to enhance the cellular immune function (Kinscherf et al., 1994; Shen and Xie, 2004).

Fast growing chicks may face metabolic complications under heat stress and high stocking density. Dietary supplemental methionine may alleviate these stresses through improving the anti-oxidant status, inflammatory response, and immunity of the broilers to ultimately increase performance and animal wellbeing.

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PREPARTUM DIETARY MANAGEMENT OF HYPOCALCEMIA THROUGH THE USE OF A SYNTHETIC ZEOLITE A

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INTRODUCTION

Around the time of parturition, it is common for dairy cows to experience decreased blood Ca due to a decrease in dry matter intake (**DMI**) and an increase in Ca demand for colostrogenesis and subsequent milk production. Hypocalcemia ensues when blood Ca drops below normal ranges, and is associated with an increased risk of developing other health disorders (Kimura et al., 2006; Chapinal et al., 2011; Martinez et al., 2012), decreased milk production (Chapinal et al., 2012), and reduced reproductive performance (Chapinal et al., 2012; Martinez et al., 2012; Caixeta et al., 2017).

One strategy for preventing hypocalcemia is to feed a Ca-deficient (< 20 g/d available Ca) prepartum diet, which will cause a slight decrease in blood Ca and stimulate parathyroid hormone (**PTH**) secretion (Goings et al., 1974; Kichura et al., 1982). Parathyroid hormone mobilizes skeletal Ca when blood Ca concentrations drop and increases Ca absorption efficiency in the small intestine and Ca reabsorption in the kidney (Goff, 2006). Due to Ca concentrations found in common feedstuffs, it is difficult to formulate a prepartum diet sufficiently low in Ca to result in homeostatic changes (Horst et al., 2005). However, the effects of a low Ca dietary approach can be actualized by decreasing the bioavailability of dietary Ca through the use of a Ca binding agent. In an *in vitro* study, Thilsing et al. (2006) showed that synthetic zeolite A, a sodium aluminum silicate, has the capacity to bind to Ca, P and Mg in rumen fluid at varying pH levels. However, the binding capacity of zeolite A to microminerals that may play an important role in combating oxidative stress has not been investigated. Previous studies have shown that feeding zeolite A during the prepartum period results in improved Ca status during the periparturient period and is hypothesized to be a result of actualizing a low Ca prepartum diet (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Pallesen et al., 2008). Only one study has observed the effects of feeding zeolite A for more than 2 weeks. Despite observing improved Ca status on the day after calving in this study, cow numbers were limited as only 9 Jersey cows were fed zeolite A (Thilsing-Hansen and Jørgensen, 2001). Subsequent studies limited zeolite A supplementation to the 2 weeks prior to expected calving (Thilsing et al., 2007; Pallesen et al., 2008; Grabherr et al., 2009). It is common for North American commercial dairy farms to utilize a 2-group dry cow strategy such that animals move to a close-up pen 21 to 28 d prior to expected calving. Despite demonstrating improved blood Ca status, further research is needed to investigate the effects of feeding zeolite A on intake and performance as well as the potential effects on

immune function and reproductive performance that may result from altering blood mineral status.

The objective of this study was to determine the effects of feeding zeolite A for 3 weeks prior to expected calving to multiparous Holstein cows on serum mineral status, plasma oxidant status, DMI, milk production, and reproductive performance. We hypothesized that zeolite-fed cows would have improved Ca status during the postpartum period, resulting in improved postpartum outcomes.

EXPERIMENTAL DESIGN

All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee prior to the onset of the experiment. Cows (n = 55) were enrolled in a completely randomized design study, with randomization restricted to balance for parity group (entering 2nd vs. 3rd and greater lactation) and previous 305-d mature equivalent milk production. Cows were fed a control diet beginning at 28 d prior to expected parturition for a covariate (pretreatment) period of 7 d. Animals were housed in individual tie-stalls with individual feed bins. At 21 d prior to expected parturition, cows were assigned to 1 of 2 treatment groups, Control (**CON**) or Experimental (**EXP**). Diets were identical except for the addition of synthetic zeolite A (X-Zelit, Protekta Inc., Lucknow, Ontario, Canada/Vilofoss, Graasten, Denmark) in the EXP diet. After parturition, all cows were fed the same fresh cow diet and managed similarly until 28 DIM.

Diets were formulated using the Cornell Net Carbohydrate and Protein System (**CNCPS**, version 6.55, Cornell University, Ithaca, NY). Ration ingredient composition and analyzed diet composition are presented in Table 1. In the EXP diet, zeolite A was fed at 3.3% of DM, targeting 500 g/d as fed. Cows were individually fed and the weight of feed delivered and refused was recorded to calculate daily feed intake. Weekly TMR samples and feed ingredient samples were collected for determination of DM and the as-fed inclusion rate of the ration ingredients were adjusted weekly based on the DM values. Forages and TMR samples were dried and ground; TMR samples were composited at 4-wk intervals and feed ingredients were composited over the course of the study. Composited samples were submitted to a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA) for wet chemistry analysis.

Body weight was measured and a BCS was assigned weekly for each cow from enrollment through 28 DIM. Rumination data were collected with HR tags (SCR Dairy, Netanya, Israel) in 2-h intervals from enrollment through 28 DIM. Colostrum was harvested within 2 h of calving, weighed, and sampled for determination of IgG concentration (Cornell University Animal Health Diagnostic Center, Ithaca, NY). All cows were milked 3×/d and daily milk weights were recorded. Milk samples were taken weekly at 3 consecutive milkings for wk 1 through 4 of lactation and analyzed for milk composition in the Barbano lab at Cornell University using Fourier transform mid-infrared (FTIR) techniques (Barbano et al., 2014).

Table 1. Formulated and analyzed (mean ± SD) diet composition of the prepartum diets and the common postpartum diet.

	Prepartum		
Item	**CON**	**EXP**	**Postpartum**
Ingredient, % of diet DM			
Corn silage	40.00	38.60	40.25
Alfalfa hay	—	—	3.58
Wheat straw	33.33	32.17	4.48
Alfalfa silage	—	—	10.74
Canola meal	8.33	8.03	5.37
Ground shelled corn	—	—	3.58
Steam flaked corn	—	—	6.27
Citrus pulp	3.33	3.24	2.02
Blood meal	1.67	1.62	—
Amino Plus[1]	6.67	6.47	6.14
Smartamine M[2]	0.03	0.05	—
LysAAmet[3]	—	—	1.77
Soybean hulls	5.00	4.85	3.74
Dried molasses	—	—	4.08
X-Zelit[4]	—	3.40	—
Urea	—	—	0.41
Other	1.6	1.56	7.53
Analyses, % of diet DM			
CP	13.6 ± 1.0	13.5 ± 0.7	16.4 ± 0.4
NDF	46.4 ± 1.4	46.0 ± 1.7	30.6 ± 2.8
Starch	16.8 ± 1.7	16.3 ± 0.3	26.1 ± 1.6
Sugar	3.2 ± 0.8	3.3 ± 0.4	3.3 ± 0.5
Fat	2.24 ± 0.13	2.25 ± 0.30	2.64 ± 0.24
Ash	6.12 ± 0.53	7.99 ± 0.36	9.14 ± 0.49
Ca	0.68 ± 0.05	0.65 ± 0.03	1.00 ± 0.07
P	0.39 ± 0.03	0.38 ± 0.02	0.38 ± 0.01
Mg	0.42 ± 0.05	0.42 ± 0.03	0.51 ± 0.04
DCAD, mEq/100 g of DM	11.03 ± 2.06	26.87 ± 1.71	40.75 ± 2.54
MP, g/kg of DM[5]	87.24	85.41	123.04

[1] Ag Processing Inc., Omaha, NE.
[2] Adisseo, Antony, France.
[3] Perdue AgSolutions LLC, Salisbury, MD.
[4] Protekta, Inc., ON, Canada/Vilofoss, Graasten, Denmark.
[5] Metabolizable protein as predicted by the Cornell Net Carbohydrate and Protein System, based on analyzed forage composition.

Blood samples were collected 1×/wk from enrollment until 7 d prior to expected calving, then daily through 7 DIM, with 2 samples collected within 24 h of parturition, and 3×/wk from 8 to 28 DIM. A subset of serum samples were analyzed for Ca, P, and Mg concentrations at the Cornell University Animal Health Diagnostic Center (Ithaca, NY). Samples were classified as normal Ca (Ca ≥ 2.12 mmol/L) or low Ca (Ca < 2.12 mmol/L) to determine subclinical hypocalcemia (**SCH**) prevalence (Goff, 2008). The 4 serum

samples analyzed between calving and 3 DIM were evaluated to determine overall Ca status of the cow. Similar to Caixeta et al. (2017), cows were also retrospectively classified as having eucalcemia (**no SCH**; 0 samples with Ca < 2.12 mmol/L), SCH (1 to 3 samples with Ca < 2.12 mmol/L), or chronic SCH (**cSCH**; all 4 samples with Ca < 2.12 mmol/L) between calving and 3 DIM.

A subset of plasma samples were analyzed for oxidant status according to Abuelo et al. (2016). In order to assess oxidative stress during the transition period, the oxidant status index [**OSi**; OSi = (reactive nitrogen and oxygen species; **RONS**) / (antioxidant potential; **AOP**)] was evaluated as it reflects the changes in redox balance (Abuelo et al., 2013, 2016).

Prepartum and postpartum data were analyzed separately. All statistical analyses were conducted with SAS (version 9.4, SAS Institute Inc., Cary, NC). The prevalence of hypocalcemia between treatments by day was tested with a Fisher's exact test using PROC FREQ. Continuous measures not repeated over time were subjected to ANOVA using the MIXED procedure with fixed effects of treatment, parity group and the interaction between treatment and parity group. Data analyzed over time were subjected to repeated measures ANOVA using the MIXED procedure and the repeated statement for time. Fixed effects included in the model were treatment, time, parity group, and all two-way interactions with the random effect of cow within treatment. Pretreatment measures were included in the model when available as covariates. The effect of treatment and Ca status, both with the fixed effect of parity, on time to pregnancy by 150 DIM were analyzed by a Cox Proportional Hazards model using the PHREG procedure. Cows that were removed from the herd before becoming pregnant or were not pregnant by 150 DIM were right censored.

RESULTS

Prepartum and postpartum serum mineral concentrations and plasma OSi are presented in Table 2 and treatment by day effects are presented in Figure 1 for serum Ca, P, and Mg. Cows fed EXP during the prepartum period had higher serum Ca concentrations, most notably as parturition approached and in the immediate postpartum period. Serum P concentrations in the EXP-fed cows were approximately half the concentration of the CON-fed cows during the entire prepartum period. Serum P levels in EXP-fed cows were significantly lower than CON-fed cows during the immediate postpartum period yet increased through 14 DIM such that EXP-fed cows had significantly higher P concentrations at d 14. Magnesium concentrations steadily decreased during the prepartum period for EXP-fed cows and on average, were lower than the CON-fed cows. Serum Mg was markedly lower for EXP-fed cows within the first DIM but we did not observe treatment by day interactions thereafter. There were no significant treatment differences in the OSi during the prepartum or postpartum periods.

Table 2. Least squares means and SEM for prepartum and postpartum serum mineral concentrations and geometric means with back transformed 95% confidence intervals for oxidant status index (OSi).

	Treatment			*P*-value	
Variable	**CON**	**EXP**	**SEM**	**Trt**	**Trt × Day**
Prepartum					
Ca (mmol/L)	2.31	2.47	0.02	<0.001	0.06
P (mmol/L)	2.03	1.03	0.04	<0.001	0.04
Mg (mmol/L)	0.92	0.85	0.01	<0.01	<0.01
OSi[4]	1.33 (1.24–1.44)	1.34 (1.24–1.45)	—	0.93	0.60
Postpartum					
Ca (mmol/L)	2.15	2.33	0.02	<0.001	<0.001
P (mmol/L)	1.39	1.19	0.04	<0.001	<0.001
Mg (mmol/L)	0.92	0.91	0.01	0.51	<0.001
OSi	0.97 (0.90–1.05)	0.99 (0.91–1.07)	—	0.77	0.33

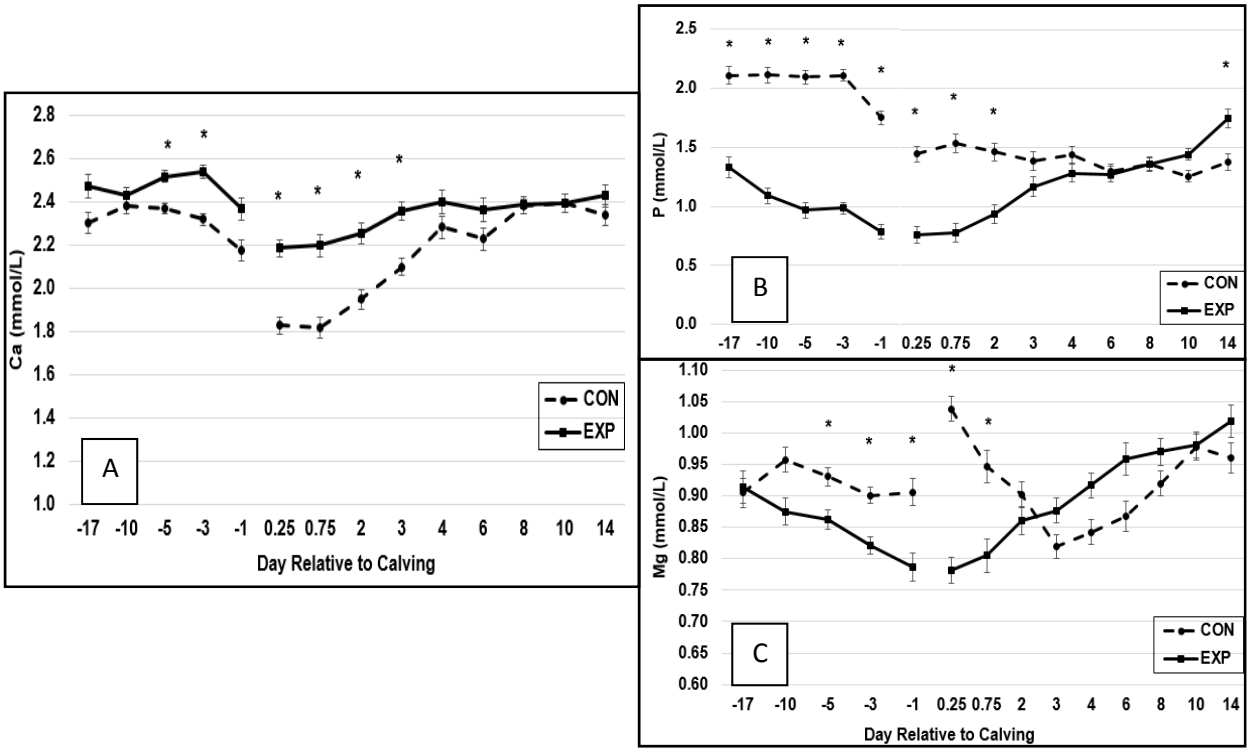

Figure 1. Serum concentrations of Ca (A), P (B), Mg (C), by day relative to calving with significant differences indicated by an asterisk (*; $P \leq 0.05$).

Subclinical hypocalcemia (**SCH**) prevalence for the two treatment groups at different time points are presented in Figure 2A. There were 3× as many CON-fed cows that had serum Ca concentration < 2.12 mmol/L within the first DIM compared to the EXP-fed cows. The highest prevalence of SCH occurred within d 1 postpartum for both treatment groups. The CON-fed cows had a greater prevalence of SCH from 3 days prior to calving through 3 DIM. Fourteen out of 55 cows were categorized as not having SCH from calving through 3 DIM; only one of these cows were in the CON-fed group. Fifty

percent (n = 13) of EXP-fed cows and 62.1% (n = 18) of CON-fed cows were categorized as having SCH. We did not observe any EXP-fed cows with cSCH while 34.5% (n = 10) of CON-fed cows were categorized as having cSCH (Figure 2B).

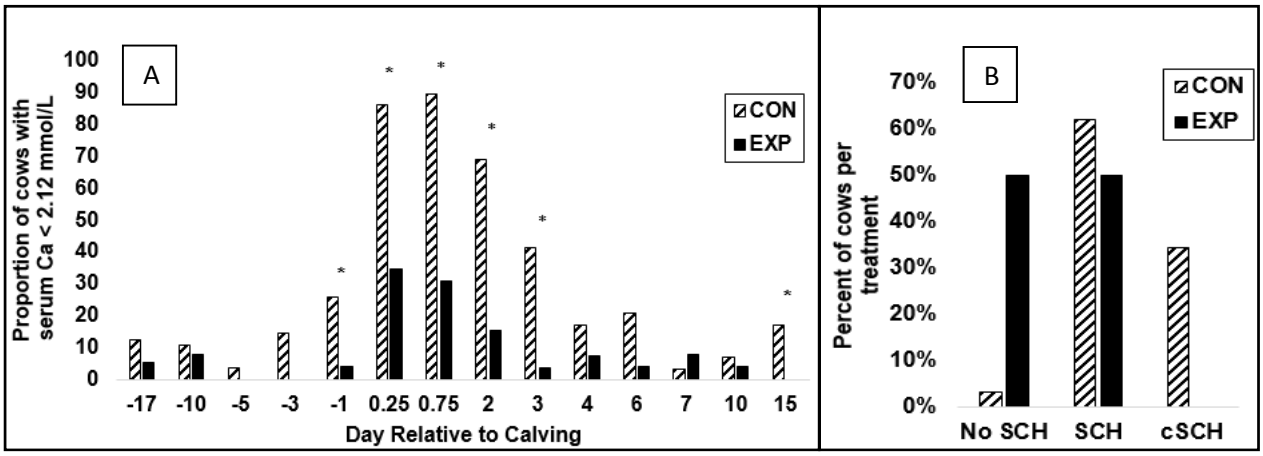

Figure 2. A) Prevalence of subclinical hypocalcemia (Ca < 2.12 mmol/L) by day relative to calving. Days at which treatments significantly differed are indicated with asterisks (*; $P \leq 0.05$). B) The proportion of cows classified based on the severity of hypocalcemia from calving through 3 DIM. Cows were eucalcemic (no SCH) if 0 samples were < 2.12 mmol/L, subclinically hypocalcemic (SCH) if 1 to 3 samples were < 2.12 mmol/L, and chronically subclinically hypocalcemic (cSCH) if all 4 samples were < 2.12 mmol/L.

Prepartum and postpartum DMI, EBAL, rumination, BW, change in BW, and BCS are presented in Table 3. We observed a treatment by week effect such that EXP-fed cows had similar DMI at 3 weeks prior to calving but had a greater reduction in DMI compared to CON-fed cows as parturition approached. There were no effects of treatment or interactions between treatment and week on postpartum DMI. While not significantly different at individual weeks, numerically DMI was 1 kg/d lower in the week before parturition and 1 kg/d higher in the first week postpartum for cows fed EXP compared to CON-fed cows. Cows fed the EXP diet ruminated for less time per day compared to CON-fed cows during the prepartum period with no effects of treatment during the postpartum period. Cows fed EXP gained less weight as calving approached compared to CON-fed cows. Prepartum BCS was lower in EXP-fed cows however, the absolute differences are small. We observed a treatment by week effect for BCS such that EXP-fed cows had a numerically lower BCS during wk 1 and 3 and was not different at wk 2 and 4.

Colostrum measurements, milk yield, and milk composition results are presented in Table 3. Despite improved Ca status during the periparturient period in cows fed the EXP diet, these cows did not have improved IgG concentration or IgG yield compared to cows fed the CON diet. We found no differences in milk yield between treatments nor did we observe a difference in ECM. Fat and protein yield also did not differ between treatments however, we observed a tendency for EXP-fed cows to have a higher protein and total solids concentration over wk 1 through 4.

Table 3. Least squares means and SEM for prepartum and postpartum DMI, energy balance (EBAL), rumination, BW, BCS, colostrum measurements, and milk composition over the first 4 wk of lactation.

	Treatment			*P*-value	
Variable	**CON**	**EXP**	**SEM**	**Trt**	**Trt × Time**
Prepartum					
DMI (kg/d)	14.6	14.0	0.2	0.07	0.04
DMI (% of BW)	1.82	1.79	0.03	0.44	0.10
EBAL (Mcal/d)	5.4	4.4	0.4	0.05	0.11
Rumination (min/d)	521	500	7	0.03	0.77
BW (kg)	808	790	4	<0.001	0.02
BW change[1] (kg)	17	6	4	0.04	—
BCS	3.37	3.29	0.02	0.008	0.32
Postpartum					
DMI (kg/d)	21.7	22.2	0.4	0.51	0.16
DMI (% of BW)	3.09	3.16	0.06	0.36	0.20
EBAL (Mcal/d)	-11.8	-11.9	0.7	0.91	0.66
Rumination (min/d)	512	523	11	0.61	0.34
BW (kg)	713	708	5	0.42	0.82
BW change[1] (kg)	-36	-36	7	0.96	—
BCS	3.14	3.11	0.03	0.34	0.01
Colostrum weight (kg)	7.3	5.8	0.7	0.16	—
Colostrum IgG (mg/dL)	7628	8342	469	0.29	—
Colostrum IgG yield (g)	494	441	40	0.35	—
Milk yield (kg/d)	48.0	47.5	0.7	0.58	0.99
Fat (%)	4.17	4.32	0.08	0.17	0.05
Fat (kg/d)	1.98	2.03	0.04	0.35	0.26
3.5% FCM[2] (kg/d)	52.9	53.6	0.9	0.59	0.45
Protein (%)	3.19	3.30	0.05	0.09	0.24
Protein (kg/d)	1.51	1.55	0.03	0.33	0.88
Lactose (%)	4.58	4.59	0.02	0.78	0.59
Lactose (kg/d)	2.22	2.20	0.04	0.67	0.54
TS (%)	13.03	13.32	0.11	0.07	0.18
TS (kg/d)	6.23	6.30	0.10	0.65	0.66
ECM[3] (kg/d)	53.0	53.8	0.9	0.50	0.57
ECM/DMI	2.47	2.47	0.05	0.95	0.47
MUN (mg/dL)	12.46	10.90	0.43	0.01	0.87
SCS	1.07	1.08	0.25	0.98	0.72

[1] Change in BW from wk -3 to -1 (prepartum) and wk 1 to 4 (postpartum), relative to calving
[2] 3.5% FCM = (0.432 × kg of weekly average milk yield) + (16.216 × kg of fat).
[3] ECM = (0.327 × kg of weekly average milk yield) + (12.95 × kg of fat) + (7.65 × kg of true protein).

For the reproductive analysis, 11 out of 52 animals (CON = 8 cows, EXP = 3 cows) were right censored due to being culled before becoming pregnant (n = 1) or not becoming pregnant during the first 150 DIM (n = 10).The median time to pregnancy during the first 150 days postpartum for animals fed the EXP diet was 70 DIM while the CON-fed cows had a median time of 89 DIM (Figure 3A). Treatment [hazard ratio (95% CI) for

EXP vs. CON = 1.76 (0.93 – 3.33); *P* = 0.08] tended to be associated with time to pregnancy such that EXP-fed cows had a higher hazard of becoming pregnant by 150 DIM.

In relation to Ca status, 0/12 eucalcemic cows, 5/30 SCH cows, and 6/10 cSCH cows were right censored. The median time to pregnancy during the first 150 days for eucalcemic cows was 69 DIM compared to 73 DIM for cows categorized as having SCH. Only 40% of cows with cSCH became pregnant by 150 DIM (Figure 3B). Calcium status was found to be associated with time to pregnancy (*P* = 0.01). Compared to eucalcemic cows cSCH cows had a significantly lower hazard [0.18 (95% CI = 0.06 – 0.57); *P* < 0.01] of becoming pregnant within 150 DIM while cows with SCH did not differ from eucalcemic cows (*P* = 0.29).

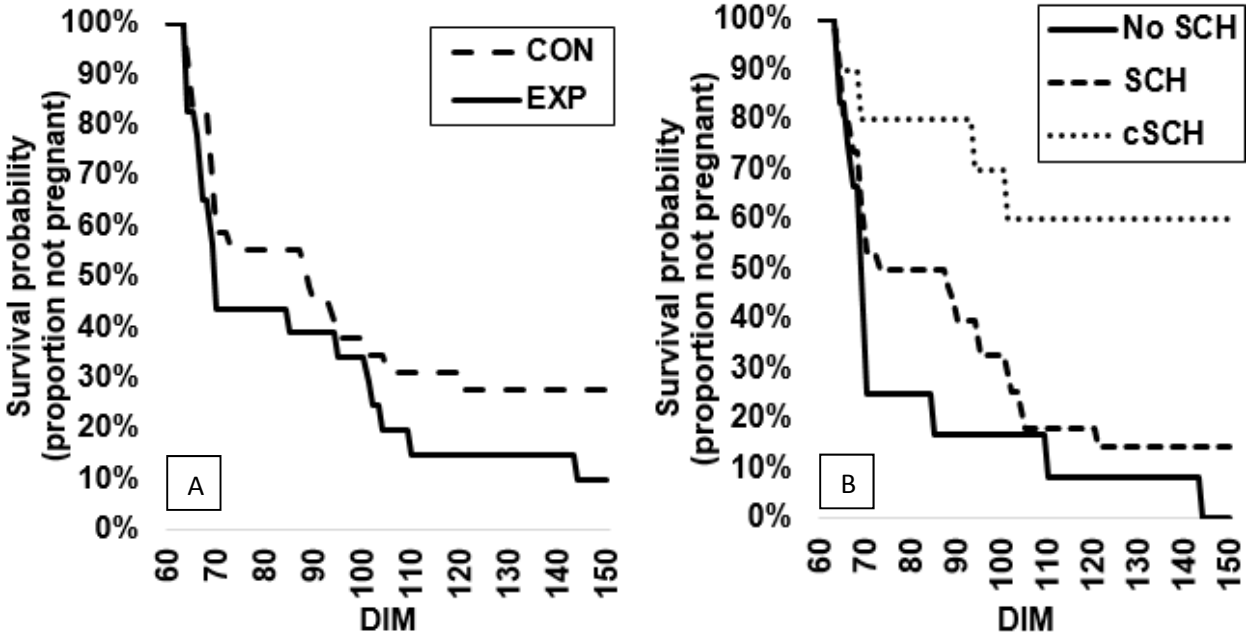

Figure 3. A) Survival curves estimating the time to pregnancy by treatment group B) Survival curves estimating the time to pregnancy for cows categorized as being eucalcemic (**no SCH**; 0 samples with Ca < 2.12 mmol/L), subclinical hypocalcemic (**SCH**; 1 to 3 samples with Ca < 2.12 mmol/L), or chronic subclinical hypocalcemic (**cSCH**; all 4 samples with Ca < 2.12 mmol/L) between calving and 3 DIM.

To our knowledge, this is the first study evaluating the effect of synthetic zeolite A on reproductive measures. Evaluating reproductive performance was not a primary objective of this study and the study was not powered to evaluate differences between treatments. These results should be interpreted as preliminary findings in need of further research involving more cows to validate the improved time to pregnancy findings of this study.

CONCLUSIONS AND IMPLICATIONS

Supplementing cows with synthetic zeolite A for three weeks before parturition improved Ca status during the periparturient period however, postpartum performance was similar between the two treatment groups. Numerically, cows fed zeolite A had improved time to pregnancy, though further work needs to be done to validate these findings. We also did not observe a treatment effect on oxidative metabolism as reflected in the lack of change of OSi.

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THE BOVINE MILK PROTEOME: WHAT'S IN IT AND HOW CAN IT BE MANIPULATED?

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INTRODUCTION

Thousands of proteins have been identified in bovine milk to date, and the dairy industry has an opportunity to benefit from identification and characterization of this proteome on both the cow and consumer side. Pre-weaned calves are responsive to the quality of colostrum and mature milk, which could be in part due to the protein profile and could impact their immune system development and growth. Consumer health is also impacted by the protein profile of bovine milk, with some proteins in milk being heat resistant and persisting as intact bioactive compounds even in pasteurized milk products sold at the supermarket. Beyond the interest in the enhancement of bioactive proteins within milk, the possible use of proteins within milk as biomarkers for cow productivity or health is also a possibility. Diet and management-related treatments appear to affect some of the proteins present in milk, providing a mechanism to naturally shift the milk proteome. This lecture will review the overall makeup of the proteome, some examples of known bioactive proteins, and what is currently known about management and nutritional factors that can influence the proteome.

WHAT IS THE MILK PROTEOME?

Simply put, the milk proteome encompasses all proteins present in milk. However, these proteins are diverse in their origin, function, and proportions, and this simplistic definition juxtaposes the complexity of the protein profile highlighted through characterization of the proteome. In terms of the basic protein profile, approximately 80% of milk proteins are caseins. Caseins, along with the two major whey proteins, α-lactalbumin and β-lactoglobulin, are considered to be the high abundance milk proteins. These are synthesized in the mammary gland and are expelled from the secretory cells by simple exocytosis (Mercier and Gaye, 1980). The term low abundance milk proteins encompasses all other whey proteins. It is this low abundance protein fraction that has garnered the most attention over the past several years, with high throughput techniques such as mass spectrometry (MS) being included in the laboratory workflow as a method to identify thousands of milk proteins present in a milk sample. Separating these different milk proteins through phasic separation is perhaps the simplest and most crude method for categorizing milk proteins. Centrifugation of a milk sample can accomplish this basic separation such as described by Nissen et al. (2013), yielding three different categories of proteins: 1) milk fat globule membrane (MFGM)-associated proteins, 2) skim milk-associated proteins, and 3) cell pellet-associated proteins. As reported by Nissen et al. (2013), there is some overlap in the proteins identified across

the different fractions; however, these fractions also each contain their own unique protein profiles and can, in a sense, each be considered as their own unique proteome.

Low abundance proteins have been identified within the milk fat globule membrane (MFGM) and are secreted by the mammary secretory cells in association with MFGM secretion (Reinhardt and Lippolis, 2008; Pisanu et al., 2011). Endogenous losses likely also contribute a large portion of the milk low abundance protein profile through sloughing of secretory cells and through secretion of cytosolic crescents, which are portions of the secretory cell cytosol that become entrained within the MFGM prior to its secretion (Heid and Keenan 2005). The average number of cytosolic crescents entrained within the MFGM ranges across species (Huston and Patton, 1990). Proteins associated with the MFGM include a large proportion of membrane-associated proteins, with research reporting a wide variation in this proportion. For example, Yang et al. (2015) reported that approximately 35% of the proteins identified in the MFGM were membrane-associated proteins, while Reinhardt and Lippolis (2008) reported that approximately 70% of identified proteins being membrane-associated in their trial. In terms of functionality, a high proportion of proteins isolated with the MFGM are associated with binding function (Yang et al., 2015, identified 49% of the MFGM-associated proteins being involved with binding).

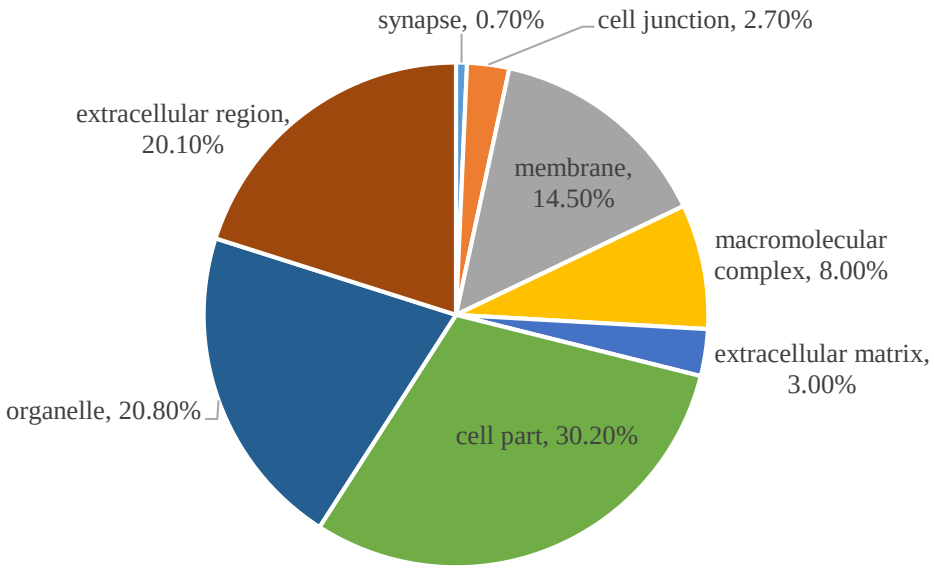

Figure 1. Cellular component analysis of low abundance proteins identified in the skim milk fraction by Tacoma et al. (2016).

While the skim milk fraction includes the high abundance proteins as well as many low abundance proteins, not all skim milk-associated proteins seem to be mammary cell-derived, some having a high concentration in blood (example, serum albumin). Evidence of protein secretion into the alveolar lumen primarily via transcellular routes (similar to casein secretion) but also paracellular routes (through ‘leaky’ tight junctions) exists (Peaker and Taylor, 1975). Gene ontology analysis of the skim milk

fraction helps to highlight the diversity of cell component localizations of these low abundance proteins within the cell. Using a data set listing skim milk-associated low abundance proteins published by Tacoma et al. (2016), we can more closely examine the cell component analysis of this proteome. PANTHER classification analysis (Mi et al., 2017) of this data set classified the majority of proteins as associated with the cell (Figure 1). This cellular component breakdown is similar to that identified through MFGM gene ontology classification (see Reinhardt et al., 2013, for example).

D'Alessandro et al. (2011) suggested that the majority of identified low abundance proteins appear to be involved in 1) host defense and immunity, 2) structure, 3) lipid transport, or 4) cellular growth and differentiation. These categories all do appear to be repeatedly represented across the different published data sets; however, some variation in their dominance is evident. Using again the Tacoma et al. (2016) data set, examination of the biological process classification of these proteins identified a lesser proportion of immune-associated proteins in skim milk from cows that were nearing mid-lactation (Figure 2). Conversely, the number of proteins with known immune system function are higher in colostrum. To visualize this, Figure 3 depicts the same biological process analysis using proteins identified in the skim milk fraction of colostrum by Tacoma et al. (2017b).

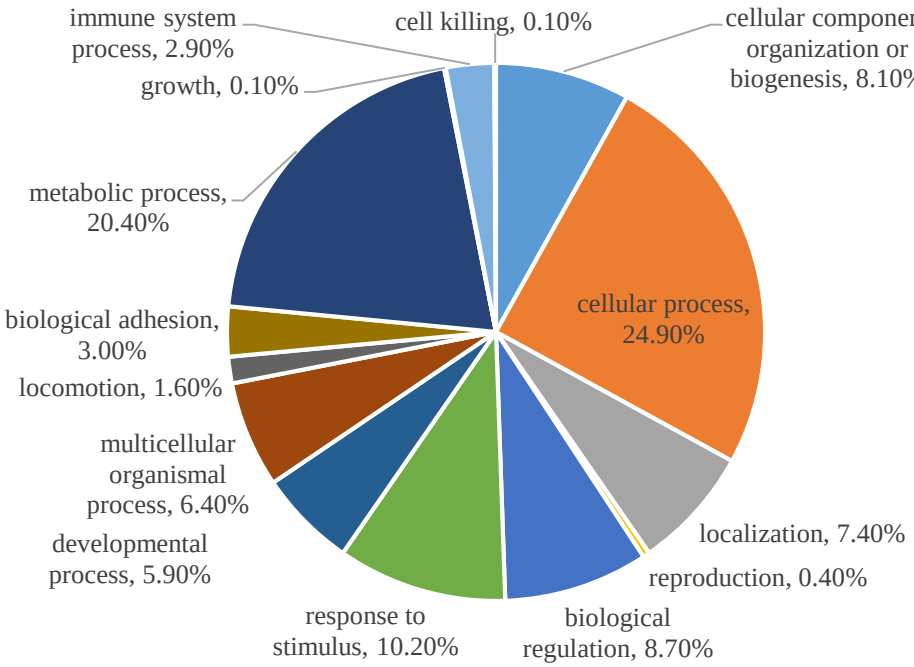

Figure 2. Biological analysis of low abundance proteins identified in the skim milk fraction by Tacoma et al. (2016). These milk samples were collected from Holstein cows nearing mid-lactation.

The cell pellet is formed from centrifugation of a milk sample, whereby the larger cellular debris is separated as the heavier phase of the three. Of the protein fractions directly compared by Nissen et al. (2013), it was the cell pellet fraction that yielded the

highest number of proteins, as well as the protein profile that had the least overlap with the non-fractionated milk, the cell and fat free fraction, or the whey fraction. In this cell pellet fraction, the exosomal proteome is also captured and methods to further isolate this specific group are published (Reinhardt et al., 2012). As expected, the exosome proteome contains a higher proportion of organelle-associated proteins compared the MFGM or skim milk proteomes, but also includes a higher number of proteins with molecular and binding functions compared to the MFGM and skim milk proteins (Reinhardt et al., 2012).

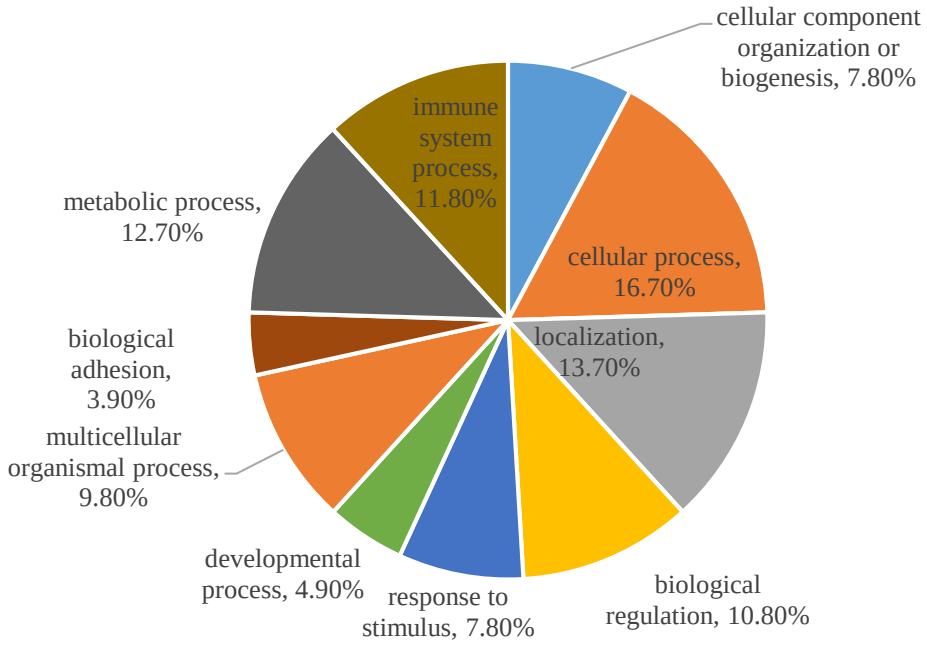

Figure 3. Biological analysis of low abundance proteins identified in the skim milk fraction by Tacoma et al. (2017b). These milk samples were collected from Holstein cows during the colostrum period.

KNOWN BIOACTIVES

While the gene ontology classifications of the milk proteome provide a sense of the likely biological targets, research focusing on the bioactivity of these proteins is underway. From this research, it is evident that the bioactivity of proteins and peptides is diverse in terms of target and potency. A comprehensive list of the bioactive proteins and peptides within milk has not been published for some time, but some examples of the diversity are well documented by Clare and Swaisgood (2000). Lactoferrin is a specific low abundance protein in milk that highlights the potential biological strength of these proteins. It stimulates intestinal epithelial cell proliferation and differentiation (Buccigrossi et al., 2007; Lönnerdal et al., 2011), intestinal epithelial cell sucrase and lactase activity (Buccigrossi et al., 2007) and antimicrobial activity (Lönnerdal, 2014). Additional low abundance proteins such as osteopontin, lysozyme, haptocorrin and lactoperoxidase all act on the milk-fed animal to stimulate biological activity (Lönnerdal, 2003; Lönnerdal, 2014).

Importantly, the bioactivity of bovine milk proteins affects not only the milk-fed calf, but also the human consumer due to cross-reactivity. Casein-derived peptides created from the digestion of cow-milk based infant formulas in the infant gut have functionality (Raikos and Dassios, 2014), while low abundance proteins present in these cow milk-based infant formulas also show human cross-bioactivity. For example, Lönnerdal et al. (2011) were able to enhance intestinal epithelial cell (Caco-2 cell) differentiation by 27% through the addition of 50 µg/mL of bovine lactoferrin to the media, while Buccigrossi et al. (2007) observed 20% higher Caco-2 cell lactase activity when treated with bovine lactoferrin compared to the human lactoferrin. In addition, Jiang and Lönnerdal (2013) have identified the regulation of 284 genes in human intestinal cells (HIEC cells) by bovine osteopontin, with the majority of affected genes being related to cell proliferation and immune function. Crucially, the presence and bioactivity of milk low abundance proteins even persists after pasteurization of milk regardless of species of origin (Maga et al., 2013; Sousa et al., 2014), providing the feasibility of large-scale commercialization to take advantage of these milk proteins in the human food market.

MANAGEMENT AND NUTRITIONAL MANIPULATION

The ability to change the milk protein profile has been validated in both our laboratory and by others. Some of the earliest research in this area demonstrated the potential for diet manipulation of the milk protein profile by reporting milk casein content. Ostersen et al. (1997) reported an increase in γ-casein and total whey protein in milk in cows with a higher calving body condition score (BCS), and early research by Christian et al. (1999a and b) reported shifts in the casein content of milk from cows given different pasture allowances or offered different base rations. While these two published works demonstrate the potential to alter the milk protein profile, they preceded MS technology, and hence could not give an indication of the dietary impacts on the greater milk proteome. Later research published by Danowski et al. (2013) uncovered some of the first evidence of diet-induced manipulation of low abundance proteins in cow's milk. In this study, milk lactoferrin concentrations were 52.6% lower (77.5 µg/mL less) from cows fed an energy restricted diet (49% of requirement) compared to milk samples from control cows, translating to 4.5 versus 1.9 g of lactoferrin secretion per day from control versus restricted cows, respectively, based on milk yield of the respective treatments. Focusing on the impact of energy balance, Lu et al. (2013) found that shifts in energy balance during the transition period affected the abundance of 10 milk proteins and numerous milk serum metabolites associated with energy utilization, including Acyl-CoA synthetase, NADH-cytochrome b5 reductase, β-hydroxybutyrate, carnitine, N-acetyl sugars, and acetoacetate. Research by Sun et al. (2015) reported the impact of low- and high- forage quality inclusion in the diet on the milk proteome. Along with a negative impact on milk production and milk efficiency, 8 milk proteins were affected by the diet, including creatine.

Manipulation of the diet beyond restriction is scarcer. Li et al. (2015) reported a shift in the abundance of zinc-α-2-glycoprotein (ZAG), a bioactive protein in milk, by manipulating the rumen degradation rate of the diet. These researchers used steam-flaked or finely ground corn, and either solvent-extracted soybean meal or heat-treated

soybean meal, to create diets that were considered to either undergo fast or slow rumen fermentation rates. Providing a diet that can be rapidly degraded was reported to increase the abundance of ZAG in milk. Manipulating the dietary RDP: RUP ratios does not appear to be the causative factor of such as shift, as Tacoma et al. (2017a) observed no difference in the 595 milk proteins identified by MS when cows were fed isoenergetic and isonitrogenous diets that included a 10% swing in the RDP: RUP ratio across the dietary treatments. While these results combined could indicate that the milk proteome is most reactive to energy balance or total intake, it is plausible that it could also be that other non-nitrogenous dietary components, such as the carbohydrate shifts used in Li et al. (2015), which may be the more dominant drivers of the milk proteome.

SUMMARY

The dairy industry could benefit from our knowledge of milk proteomics on several levels, including calf health, biomarker development, and commodity markets. The bioactivity of milk proteins, both at the calf and human level, appear to include a diverse array of functions; however, the variability of the protein profile and our understanding of how to manipulate it is less elucidated. Ongoing research to solidify our understanding of this unique aggregate is helping to tease out the possible drivers that impact the proteome, and will help in our exploitation of the healthfulness of milk or use of this fluid as a diagnostic tool.

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OPTIMIZING PRODUCTIVITY FROM PASTURE BASED SYSTEMS – A CASE STUDY FOR HIGH FORAGE FEEDING LEVELS

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INTRODUCTION

The global population is expected to increase from 7.2 to 9.6 billion by 2050 (UN, 2013). This 33% rise, coupled with increased demand for dairy products worldwide due to increasing global standards of living, means that the demand for agricultural products will increase by about 70% in the same period (FAO, 2009). However, this increased demand comes concurrent with the realization that consumers want agricultural products that are produced in a sustainable and environmentally benign manner (Godfray et al., 2010). To help meet this 70% increase in demand it is expected that milk production will increase from 644 million tons (in 2006) to 1,077 million tons (by 2050; Alexandratos and Bruinsma, 2012). Livestock products provide 17% of global kilocalorie consumption and 33% of global protein consumption and are therefore important agricultural commodities for global food security (Rosegrant et al., 2009). The return on human edible protein inputs, for dairy cattle, is larger than 1, with a typical range of 1.4 to infinite; infinite being those diets containing no human edible protein i.e. grazing (Dijkstra et al., 2013; Karlsson et al., 2018). This indicates that dairy cattle add to the total human food supply in a manner that does not compete with food resources for humans.

Irrespective of this powerful contribution to food security, popular press continuously associates milk production with inefficient use of natural resources. Livestock production uses 75% of grassland land (Foley et al., 2011) of which one third of the land area is arable and two thirds are grasslands and rangelands (Steinfeld et al., 2006), consumes 35% of grain products (Alexandratos and Bruinsma 2012) and emits 14.5% of global greenhouse emissions (Gerber et al., 2013). As the population of the planet and demand for human-edible plant resources is increasing rapidly, livestock production in the future might not have access to this arable land and inventory of grain products. Therefore, the ability of ruminants to turn human inedible fibrous feed resources and by-products from the human food chain, into edible human food of high biological value, may become more significant. In a recent analysis, forage, particularly pasture, was the largest component of the Irish cow diet, typically accounting for 82% of the diet on a dry matter basis (O'Brien et al., 2017). Grazed pasture was the dominant source of forage from March to October and usually contributed 95% to 97% of the diet as fed in the summer period. Of course, there were periods of the year when average contribution of concentrate was substantial such as the early spring months of January and February (30% to 35% of dry matter). This high utilization of non-edible human resources can be achieved in synchrony with maintaining a profitable farm business structure. Due to climatic factors, such dependence on grazing is not possible in many parts of the world however, there is significant opportunity to increase forage inclusion levels in many

ruminant diets. This trend is already being seen in the industry as in a feed industry professionals survey (Chase, 2017), 91.5% of responders stated that in the last 10-15 years, the level of forages fed in the dairy herds they work with has increased. By describing certain management practices, and new insights recently gained in efficient pasture-based systems, strategies to achieve higher forage (human inedible) diets will be proposed.

PLANT MATURITY

The timing of harvesting, which in pasture-based systems is achieved directly by the ruminant, is of vital importance to both plant and animal performance. Perennial ryegrass (PRG) is the predominant species of grass grown in Ireland. As PRG is a '3-leaf' plant, only 3 green leaves exist at any one time with the initiation of a new leaf coinciding with senescence of the oldest fourth leaf (Donaghy, 1998; Figure 1). If the plant matures past the '3-leaf' stage, pasture wastage will therefore occur with overall pasture quality also diminishing. Therefore, the time required for the plant to reach this stage sets the maximum grazing interval (i.e. rotation length). This onset of senescence drives implementation of management practices that aim to maintain the pasture in an immature stage. Fulkerson and Donaghy (2001) also show that, the metabolizable energy (ME) level declines gradually from the 1-leaf stage to the 4-leaf stage, 2.62 Mcals/kg dry matter (DM) to 2.15 Mcals/kg DM, respectively. This would suggest to harvest as early as possible however, studies have shown that subsequent regrowth is suppressed if plants are defoliated before the 2-leaf stage of regrowth. In intensive pasture based systems, high pasture utilization is favored and a post grazing sward height of 4 cm is often targeted. This forces the plant to rely on and deplete stored water-soluble carbohydrate (WSC) reserves to grow new shoots. When 75% of the first new leaf has regrown the plant then has adequate photosynthetic capacity for growth and maintenance and WSC content begins to replenish. If the grazing rotation is too short, and animals enter the paddock prior to the 2-leaf stage, there will not have been sufficient time for the concentration of WSC to replenish. This will affect overall productivity of the swards, as regrowth will be suppressed, while also having the potential to reduce sward persistence in the longer term. Thus, replenishment of WSC reserves and the gradual decline in ME sets the optimum harvesting interval at the 2–3 leaf stage. From the criteria set out above, the grazing interval can be expressed in a similar morphological stage of growth or pasture mass (discussed later), irrespective of season or location and has been shown to maximize growth and persistence of ryegrass and optimize the levels of most nutrients in pasture required by dairy cattle including protein, WSC, calcium, potassium and magnesium (Fulkerson and Donaghy, 2001).

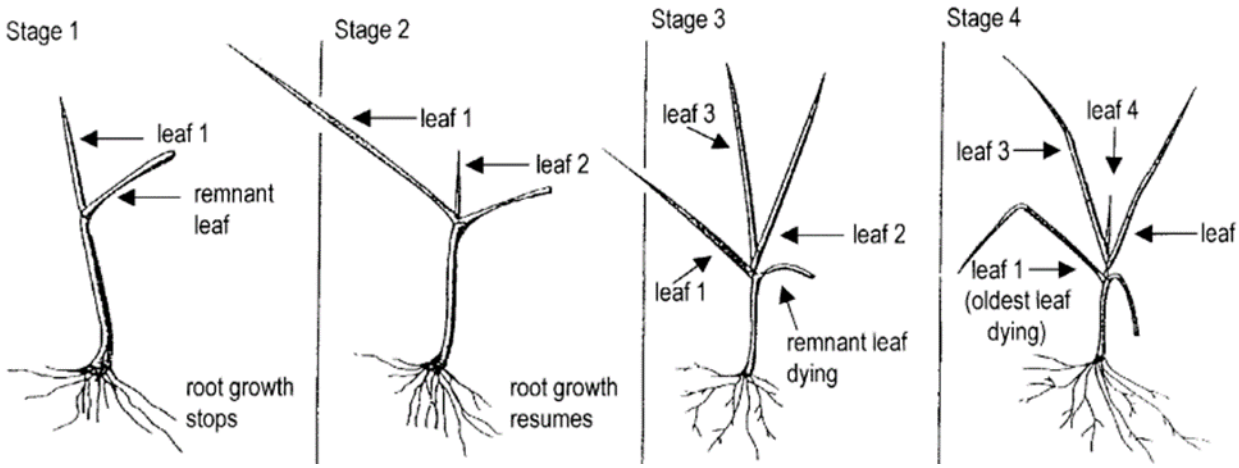

Figure 1. Regrowth of a ryegrass tiller following defoliation (Donaghy, 1998)

A similar approach for setting criteria for alfalfa/grass stands could be considered. The optimization of both yield and quality is of high importance when selecting a harvesting time. However, yield and quality is contradictory as the two variables are not in synchrony, maximal yield peaks around day 35 of regrowth whereas quality begins to decline from day 15 onwards (Undersander, 2017). Work in Italy has shown that indigestible NDF levels increase as alfalfa stands were harvested with a 21-d cutting schedule, at a pre-bloom stage; with a 28-d schedule, at about first-bloom stage; and at a 35-d cutting schedule, full bloom (15.5, 17.2, and 18.3 iNDF % DM, respectively; Palmonari et al., 2014). Further, it was shown that cows fed early-cut orchardgrass-based total mixed ration (TMR) had greater DMI, milk production, and milk protein than those fed late-cut orchardgrass-based TMR (Cherney et al., 2002). The fiber digestibility of early-cut orchardgrass was much greater than late-cut orchardgrass, contributing to the improved dry matter intake (DMI) of cows on the early-cut orchardgrass TMR. Therefore, if high forage rations are to be implemented optimization of harvesting time needs to account for forage maturity, yield, aNDFom content and digestibility.

HOW TO CONTROL PLANT MATURITY

To continuously achieve this optimum harvesting interval, both physical labor and mental commitment is required. In the context of pasture-based grazing systems, the PRG plant can have a large range in daily growth rates (0 - > 200 kg DM/hectare/day) depending on a number of factors such as climatic, soil temperature, nutrient administration etc. This makes the grazing management process very dynamic and complex. In recent years the development of reliable, easy to use web based decision support tools has facilitated improved feed budgeting and grazing management on grassland farms (e.g. PastureBase Ireland; PBI; Hanrahan et al., 2017). The farmer enters weekly pasture cover estimations, attained using a favored measuring technique, from which PBI produces a series of daily and periodic outputs (Figure 2). Some of these outputs include:

- Spring rotation planner - used from turnout until grass growth equals herd demand
- Pasture wedge - used to control grass supply during mid-season taking into account herd demand, rotation length and post-grazing residual. Allows early identification of pasture surpluses and deficits
- Autumn feed budgets - used to maximize the amount of grazed grass utilized while at the same time ensuring that the grazing season is extended into late-November/early-December

Outputs such as above can support the farmer in the day-to-day decisions required such as pasture allocation, concentrate supplementation and winter forage preservation. These types of tools allow farmers to enhance their grazing management skills through grazing pasture at the right stage ultimately increasing DMI and quality and the achievement of higher performances from pasture-based systems (O'Donovan and Dillon, 1999). An additional benefit, as each individual farms database develops, evaluation of DM yields at the paddock level can occur which can help determine high or low performing paddocks and associate different causes such as cultivar, soil fertility, etc.

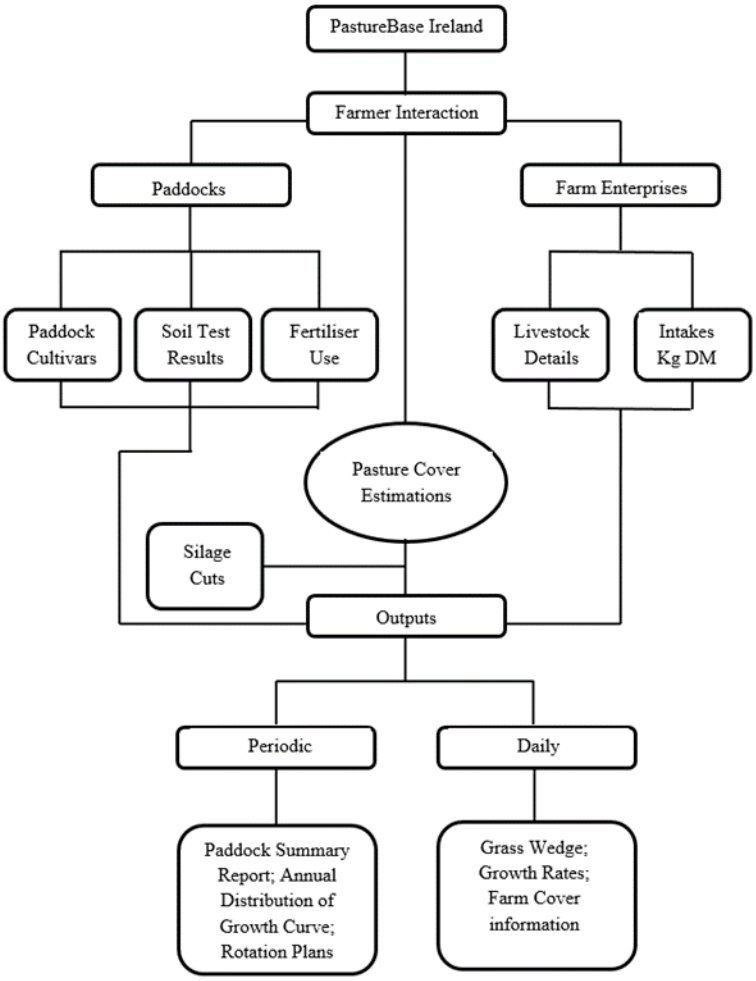

Figure 2. Flow diagram of grazing management web based decision support tool, PastureBase Ireland (Hanrahan et al., 2017)

Similar strategies have been proposed and can be implemented to maintain high quality forage mixtures in TMR systems. “Dynamic harvesting” is a field organizational method that aims to capture each field when forage quality is high with requirement for the process to be dynamic not static (Lawrence, 2018). This strategy helps mitigate some of the weather related issues with harvesting time. The ‘dynamic harvesting’ method aims to reduce the number of predetermined fields for non-lactating or heifer feed (poorer quality) and therefore help achieve the required high quality forage needed for high-producing animals. Another example of this involves ‘sequential’ versus ‘staggered’ cutting system for alfalfa stands. A study in California demonstrated that the number of high quality cuttings was increased using a ‘staggered’ cutting order. Other areas of opportunity exist such as forage storage strategies to help allocate high quality forage for high producing animals.

ANIMAL PERFORMANCE AND FORAGE DIGESTIBILITY

Once targets are set and implemented successfully, highly digestible forage can be consistently grown and harvested. Pasture mass is a quantitative measure, utilized to indicate plant maturity. It is a measure of the quantity of pasture DM, above 4 cm, in a given area and typical expressed as kg DM/ha. O’Donovan (2000) developed targets for average pasture mass based on the factors described above (see plant maturity) with the 3-leaf stage or optimal entry time typically reached at a pasture mass of 1,500 kg DM/ha. Many experiments utilizing lactating dairy cattle at Teagasc Moorepark, Ireland, have shown that this pasture mass (or lower) improves animal performance (McEvoy et al.,2010; Wims et al., 2010). Further a number of in-vivo digestibility studies using sheep have shown that there is a decline in DMI, DM digestibility (DMD), organic matter digestibility (OMD) and NDF digestibility (NDFD) as pasture mass increases past the 3-leaf stage (Garry et al., 2014; Beecher et al., 2018). In the Beecher et al., (2018) experiment, on the high quality PRG swards, the authors noted the importance of fiber digestibility, as the measured NDFD had a significant positive relationship with DMI of the sheep. Pasture mass is not the only factor influencing sward digestibility. Many agronomic conditions (e.g. light, heat, water stress, soil type), plant genetics and season can impact digestibility and must be accounted for. The effect of season on feed chemistry and aNDFom fractionation is shown in Table 1. Pasture mass and CP were not significantly different across season however, the aNDFom concentration in autumn swards was elevated. The rate at which the potentially digestible (pdNDF) pool degraded was faster for spring compared to autumn pasture (9.53 versus 7.76 ± 0.6% $hour^{-1}$, respectively). Furthermore, the extent to which aNDFom was digested was greater for spring compared to autumn (9.75 versus 15.50 ± 0.44% uNDF, respectively). Predictions of the ME per kg of DM of the swards showed that spring pasture had a higher energy density ($P < 0.01$) and also supplied a higher amount of grams of Metabolizable Protein (MP; $P < 0.01$) to the animal.

Table 1. Effect of season on pre-grazing yield and nutritive value of pasture swards

Variable[1]	Spring	Autumn	S.E.	*P*-value
Pasture mass (kg DM ha^{-1})	1,691	1,494	137	0.228
Crude protein (g kg^{-1} DM)	214	190	10	0.106
aNDFom[1] (g kg^{-1} DM)	325	355	8	0.006
Rate of degradation (% hour^{-1})	9.53	7.76	0.60	0.021
uNDF[2] (% aNDFom)	9.75	15.50	0.44	<.001
ME[3] (Mcal kg^{-1} DM)	2.68	2.52	0.04	0.003
MP[4] (g kg^{-1} DM)	121	112	2.2	0.004

[1]aNDFom = Neutral Detergent Fibre; [2]uNDF = undigested NDF; [3]ME = Metabolisable energy; [4]MP = Metabolisable protein

This analysis shows that aNDFom as a fraction can behave differently, even within plant species. The fraction can differ in degradation rates and the extent to which it degrades. Season can affect these characteristics, independent of pasture mass. Increased degradation of aNDFom can affect energy supply to the animal through increased volatile fatty acid production and increased flow of microbial protein, while also affecting DMI. Therefore, the measurement of pasture mass alone, is not dynamic enough to capture the variation and it is essential that determination of these aNDFom fractions are included in all basic pasture feed analyses. When swards such as those described above are fed, impressive performance can be achieved. Previous research indicates that with high yielding dairy cows in early lactation on pasture only, grass DMI of 17 kg can be achieved supporting milk production of 30 kg/d, under good grazing conditions (Kennedy et al., 2003, McEvoy, 2008). Additionally, multiyear lactation long grazing experiments have demonstrated exemplary performance per cow (410 kg milk solids; MS; kg fat + protein) and per hectare (1,165 kg MS), when lactating cows were maintained on a > 95% pasture diet throughout lactation (Dineen, 2017). When a mixed sward of PRG and white clover was available, performance per cow and per hectare increased further (460 kg MS/cow and 1265 kg/ha). These animals will typically weigh 500 to 530 kg and can consistently consume 18 kg DM/d from pasture (3.4% to 3.6% BW) with a number of the animals within these populations producing their body weight in milk solids on a > 95 % forage diet. To achieve such performance from high forage diets, forage digestibility is key. Therefore, the above data supports that we should maximize harvesting highly digestible feeds to help support high performance.

In the ‘high forage feeding’ survey (Chase, 2017) mentioned previously, 85% of responders cited ‘forage quality not being good enough’ as the main reason that they would be hesitant to feed higher levels of forages in lactating cow ration. Similar data exists for the effect of NDF digestibility on animal performance in TMR systems. Both Dado and Allen (1996) and Oba and Allen (2000) have identified increased NDF digestibility as being positively associated with DMI and milk production. Considerations are required for both timely harvest and selection of forage varieties when implementing high forage diet rations.

RUMEN TURNOVER

Over the past 2 years, we have conducted some studies in Ireland on cattle consuming high quality PRG pastures. The average PRG pasture used in the experiments varied in aNDFom and was generally grazed between 30% and 35% aNDFom with sugar levels between 10% and 15%. In grasses, the veins with their associated sclerenchyma strands, especially in an enlarged midrib when present, provide the leaf with tensile strength. These strands can link to the vascular tissue and form strong 'I' girder engineering structures. This acts to strengthen epidermis attachment, and slow the splitting of leaves when consumed. To allow for more microbial digestion and passage out of the rumen, rumination and particle size reduction are considered essential functions to increase surface area of these feed particles. However, in the immature temperate grasses, this 'I' structure is not completely formed. Instead, epidermis cells are attached by mesophyll cells, which are readily digested or break allowing the epidermis to be shed. Also, temperate grasses have straight-sided epidermal cells which allows easy splitting along the middle lamella. This allows the leaf to rapidly fragment into long vascular strands. In the immature pastures, rumination and chewing is still important, but the rate of digestion of the fast pool of aNDFom and the size of the pool alter the relationship between particle size reduction and digestion due to the speed of digestion. For example, when we fractionate the aNDFom of the spring pasture into pools, the kd for the fast pool aNDFom was 0.24/h and represents 66% of the aNDFom in this forage. Using the aNDFom passage rate equation in the CNCPS v6.55, an equation developed for the NorFor model (NorFor, 2011), the calculated passage rate for these cattle was 0.017/h. Thus, when calculating the simple integration of this relationship (kd/(kd+kp), (0.24/(0.24+0.017)) = 0.934, the result is nearly 94% of the fast pool is digested in the rumen. The rate with which disappearance is occurring, can have a dramatic effect on rumen emptying and consequently intake, due to the space created by microbial degradation of the forage independent of particle size reduction. Since the epidermis cells are attached by mesophyll cells, there is lower resistance against the microbes to digest the aNDFom and this is described mathematically by the size of the fast pool of digestible aNDFom. This will ultimately result in a lower proportion of the digestible aNDFom escaping fermentation. These calculations are in close agreement with the sheep in-vivo total tract NDF digestibility (>80%) reported in Beecher et al., (2018) at similar pasture quality and pasture mass.

COMPLEMENTING HIGHLY DIGESTIBLE FORAGES

Despite these advances in grazing management technologies and cultivar selection, pasture availability can still be limited in the spring, which leaves a requirement to supplement the diet of the lactating cow during this period. This supplementation management practice, can also be utilized tactically in regards to the early spring feed-budgeting scenario, as offering concentrate has the additive effects of maintaining the grazing rotation at the target length until grass growth exceeds herd demand. Nationally the average contribution of concentrate is substantial for the early spring months of January and February (30% to 35% of DMI; O'Brien et al., 2017). Additionally in mid-season and autumn periods of the year pasture availability is dependent on temperature

and precipitation which subjects pasture-based production systems to climatic variations (Roche et al., 2009). In these circumstances, supplements can be utilized to ensure that cows are not underfed when her requirements are not met by pasture availability (Bargo et al., 2003). In a modeling exercise, to establish the financially optimum strategy for Irish dairy farms, it was concluded that on average across a number of stocking rate levels, a concentrate supplementation of 600 kg of DM/cow/yr was the most profitable for most of the different concentrate, silage, and milk price scenarios (Ruelle et al., 2017). However, there was a tipping point in their analysis as continuing to increase concentrate supplementation to 900 kg of DM/cow/yr led to a decrease in the farm profit. In the future the analysis should consider the potential human edible proportion of this supplementation and its availability and not just the profitability.

In practice, the mean response to concentrate supplementation is extremely variable. Some of this variation is attributed to a reduction in pasture intake (i.e., substitution effect) when supplementary feeds are consumed (Leaver, 1985; Stockdale, 2000; Bargo et al., 2003; Sheahan et al., 2011). Studies have also shown animals reduce their time spent grazing when fed a supplement; 12 min/kg supplement (Bargo et al., 2003). Milk response (MR) to concentrate supplementation, which is the increase in milk yield per kilogram of concentrate offered, is reportedly lower in spring compared with summer (Stockdale, 1999) because of the higher energy content of spring grass. Low substitution rates will result in greater MR to the supplement offered, thus making it more economical to offer the supplement to the animals. A reduction in fiber digestion, with the inclusion of starch in the ration when pasture cows are offered supplement, has also been suggested as a cause of the variation in response and potentially a causative link to the substitution effect (Bargo et al., 2003; Doyle et al., 2005; Nousiainen et al., 2009). As suggested earlier, when cattle are fed these highly digestible forages the extent of digestion and the rate with which disappearance occurs allows for faster rumen emptying because of the space created by microbial degradation of the forage. This may have important effects on the cattle stimulation to ruminate and chew. While pasture-based animals achieve a satisfactory intake of aNDFom the amount of supplementation and substitution effect can dramatically alter this. For example, preliminary data from an omasal flow study conducted during mid-lactation (Dineen et al., unpublished), show that the animals on a grass only diet achieved an intake of 16.25 kg DM of which 5.9 kg was aNDFom (1.16% of BW). Through utilization of the rumen evacuation technique, we were able to determine that ruminal aNDFom levels were on average 4.8 kg (0.95% BW). When a starch treatment (3.32 kg DM/cow rolled barley) was offered to the animals, a large substitution effect occurred. Total DM intake was still higher 16.82 kg DM however, grass DMI reduced to 13.5 kg DM. This resulted in a lower overall aNDFom intake (5.5 kg; 1.10% BW). Ruminal aNDFom content was higher at 5.4 kg (1.09 % BW) indicating reduced fiber degradation capability as total aNDFom intake was lower for this treatment. As we know, aNDFom intake is an important variable in terms of allowing the animal to self-buffer, a highly fermentable starch load and a reduction in aNDFom intake might overwhelm this capacity and contribute to a reinforcing loop of reducing rumen pH and consequently a reduction in aNDFom digestion. In a project carried out this past summer, the supplementation of a feed ingredient rich in soluble fiber to grazing dairy cows was assessed. As Ireland experienced its worst drought in 40 years the diets formulated where

different to what was observed. However, when the rain returned and the grass turned back to its lush green self, there was a beneficial effect to supplementing this type of ingredient. A similar example in the TMR system approach is when highly digestible brown midrib corn silage is included in the ration. At equivalent diet inclusion rates as a conventional corn silage variety that has a slower rate of degradation, they will have very different effects on rumination and chewing (Oba and Allen, 2000). If this is exacerbated by poor management practices such as sorting, overcrowded head rails, long time away from pen, the impact on the animal's ability to buffer the system could potentially be detrimental. To overcome these situations, commonly chopped straw has been introduced into the diets. While this may be successful to mitigate the issue, is it the optimal way to complement these highly digestible forages? More research is required into this area. Therefore, considerations are required and rethinking of current recommendations when formulating diets including highly digestible forages.

CONCLUSION

Pasture-based systems and high forage diets have the potential to play a significant role in meeting the increasing global demand for food. The demand for human-edible plant resources is increasing rapidly as the population of the planet also increases. These management practices have the ability to utilize and convert non-human edible forages into high quality human edible protein. However, there is a requirement to enhance the efficiency and productivity of pasture-based and high forage inclusion systems through refinement of certain management and nutritional practices that have been discussed in this paper.

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CREATING NUTRIENT SUSTAINABILITY INDICATORS FOR DAIRIES NATIONWIDE

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SUSTAINABILITY IN THE AGRICULTURAL SECTOR

Sustainability of agricultural production is a discussion issue that occurs on agendas more and more often. On a global scale, this is born out of necessity, as the world population has been increasing gradually and is projected to expand further over the coming decades. By 2050, the planet will be home to more than 9 billion people, which all need to be fed. It is clear that this demands a major increase in agricultural production.

The resources required for the intensification of agriculture, such as land, water, and nutrients, are often scarce and/or non-renewable. Simultaneously, inadequate use of these resources may not only lead to waste, but also to degradation of the environment. Soil erosion, salinization, and eutrophication of water bodies like rivers, streams and lakes are ubiquitous problems that can decrease the suitability of agricultural land for future cropping and negatively affect terrestrial and aquatic ecosystems across various spatial and temporal scales. In the Northeastern US, the recurrence of water quality and air quality challenges has farmers, farm advisors, industry partners and policy makers wondering about long-term solutions. Proper resource use is key to achieve a sustainable increase in productivity necessary to feed a growing population.

It is hardly surprising that many ag-related organizations, companies, and governmental agencies have adopted a focus on sustainable production in recent years. The manner in which this occurs varies widely by organization. The Food and Agriculture Organization (FAO, 2014), for example, has identified five principles that should guide the process of transitioning towards a more sustainable agricultural sector:

"The principles which can collectively guide the process of transition to greater sustainability are summarized as:

- *Improving efficiency in the use of resources is crucial to sustainable agriculture*
- *Sustainability requires direct action to conserve, protect and enhance natural resources*
- *Agriculture that fails to protect and improve rural livelihoods and social well-being is unsustainable*
- *Enhanced resilience of people, communities and ecosystems is key to sustainable agriculture*
- *Sustainable food and agriculture requires responsible and effective governance mechanisms"*

The USDA Sustainable Agriculture Program wields a legal definition for the term 'sustainable agriculture' (USDA, 2007):

"The term "sustainable agriculture" (U.S. Code Title 7, Section 3103) means an integrated system of plant and animal production practices having a site-specific application that will over the long-term:

- *Satisfy human food and fiber needs.*
- *Enhance environmental quality and the natural resource base upon which the agriculture economy depends.*
- *Make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls.*
- *Sustain the economic viability of farm operations.*
- *Enhance the quality of life for farmers and society as a whole."*

Many organizations in the dairy industry, such as FrieslandCampina, Dairy Farmers of America, Ben & Jerry's, and others have sustainability programs, either individually or in collaborative efforts such as the Sustainable Agriculture Initiative (SAI) platform that includes suppliers, farmers, and external stakeholders. These programs have often adopted their own definition of sustainability.

Although many organizations have sustainability definitions and mission statements, few developed key performance indicators for sustainability. Those who have developed metrics or are in the process of doing so, tend to focus on environmental and socio-economic indicators. One example is the Fields to Market organization that created national indicators for environmental sustainability (Field to Market, 2016), which include environmental indicators (biodiversity, energy use, greenhouse gas emissions, irrigation water use, land use, soil carbon, soil conservation, and water quality), economic indicators (farm financial health, farm profitability, and generation of economic value), and social indicators (work safety and labor productivity).

Much of the focus of various sustainability platforms relates to external outcomes. While important, these overlook the fact that dairy farmers are by and large, nutrient managers. By looking within the dairy farm system, and attempting to utilize nutrients as efficiently as possible, significant source reduction can be obtained, providing important complementarity to minimizing external impacts, such as nutrient loss to water bodies.

There are 17 essential nutrients of which nitrogen (N), phosphorus (P) and potassium (K) are the three primary macronutrients for plant growth and animal performance, taken up in large amounts. Especially N and P are recognized as potential contributors to environmental degradation of air and/or water. Thus, if we are to move forward with assessment and documentation of sustainable food production in the dairy sector, it is crucial to have key indicators of performance and sustainability for N and P use, in addition to other indicators (e.g. for water use and greenhouse gas emissions).

Current programs, including Field to Market, do not address sustainability indicators for the dairy sector at this point. In addition, the nutrient indicators that are used, often do not address performance on a whole-farm scale. Sustainability indicators can provide a means to see instantly whether a system, such as a farm, has opportunities to improve its resource allocation to become more sustainable over time. The nutrient use of a farm is particularly suited for the use in sustainability indicators and such dairy sustainability indicators are needed to aid in implementation of improved management practices, to develop such practices, and to monitor the progress made in the industry.

There are several advantages and uses to the implementation of a broadly supported nutrient sustainability indicator. It can inform consumers about the origin of the product they buy and it can be used by dairy companies as an incentive to reward producers for sustainable practices. Simultaneously, it can give farmers insight in their nutrient use and how to improve it. It allows them to see whether they can produce the same amount of milk, using fewer nutrients and it may help reduce losses of valuable nutrients to the environment.

A DAIRY NUTRIENT SUSTAINABILITY INDICATOR

Compared to resources like land, soil, or energy, the use and transport of nutrients on dairy farms can be quantified with relative ease. For many parts of a dairy farm, there are indicators or tests that assess the efficiency of nutrient use. Examples include Milk Urea Nitrate (MUN) in milk, the corn stalk nitrate test (CSNT), soil test analyses, and the availability of manure nutrients. A whole farm indicator recognized that animal, the land, manure, feed storage, and barn management are all interconnected but such indicators are far less common.

The success of a dairy nutrient sustainability indicator relies on its characteristics. In short, a successful sustainability indicator:

- is easy to interpret;
- gives a measure of how sustainably the system is managed;
- sets targets for farmers to strive for;
- is responsive to significant management changes;
- takes a limited time to calculate/conduct.

One of the additional advantages of assessing nutrient management on a whole farm scale is that the boundaries (of the farm) are clearly marked. Furthermore, the errors at the farm level are typically smaller and less variable than for indicators that operate on a smaller scale.

THE WHOLE-FARM NUTRIENT MASS BALANCE

The whole-farm nutrient mass balance (NMB) is a nutrient assessment tool for nitrogen (N), phosphorus (P) and potassium (K) that meets the requirements for a successful sustainability indicator as described in section 2. A whole-farm NMB is

calculated by subtracting the annual sum of nutrients exported from a farm from the nutrients imported onto the farm (Figure 1). The difference between these imports and exports is called the balance, and this is a measure of how many nutrients remain on the farm or are lost to the environment. The balance is expressed per tillable farm acre or per cwt of milk sold. The balance per tillable acre gives an indication on how many nutrients remain per acre, and is thus a measure of the environmental impact of a farm. The nutrient balance per cwt of milk sold is a measure for the amount of nutrients "used" to produce a unit of milk. This tells us something about the efficiency at which the farm operates.

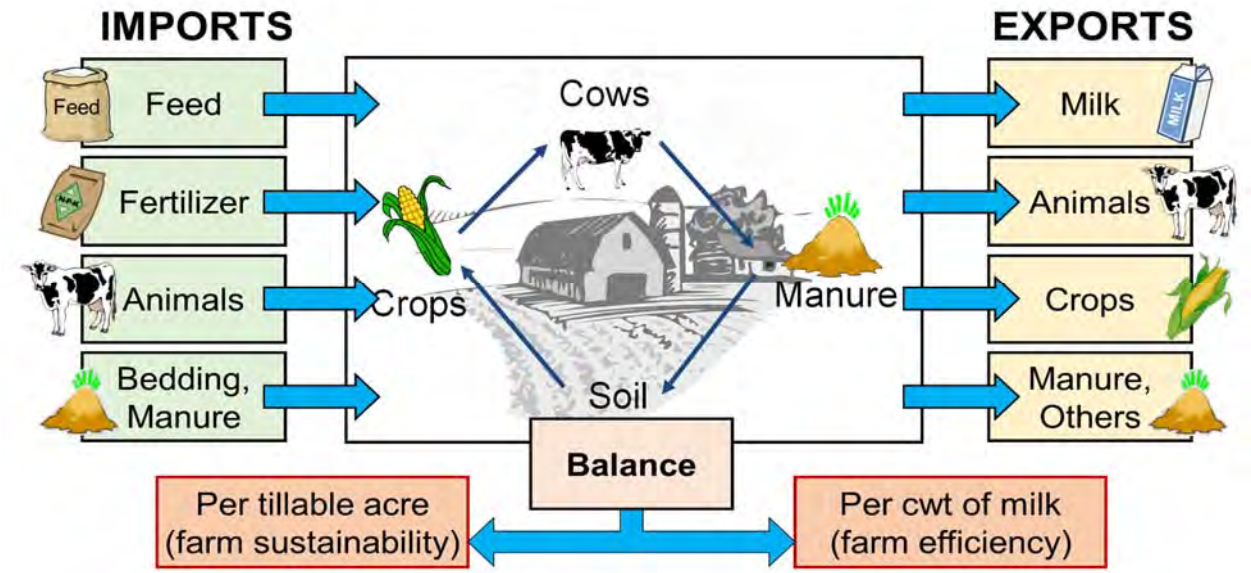

Figure 1. Overview of farm imports and exports included in the NMB. Only easily measurable components are considered. The nutrients in the resulting balance either remain on the farm system, or are lost to the environment.

The whole-farm NMB estimates a solid indicator from a relatively small amount of data. To conduct a NMB assessment, farmers are asked to fill out four sheets of paper with information on the size of the farm (number of acres and number of cows) and on any nutrient-containing imports and exports that entered or left the farm during a calendar year. These numbers are entered in the Cornell Nutrient Mass Balance software, which calculates the N, P, and K balances for the farm (Soberon et al., 2013).

For imports, the NMB distinguishes between feed imports, fertilizer imports, purchased animals, and bedding/manure. For exports, nutrients sold in milk, animals, crops, and manure or other products are considered. Information on the quantity and the nutrient content of all these commodities is needed to calculate the total amount of N, P, and K that enters and exits the farm. Furthermore, information is gathered on the forage and grain feed grown on the farm. Although this does not contribute to the balance itself (as homegrown feed is neither an import nor an export) it allows for the calculations of key performance indicators that can tell us more about the efficiency of the farm and opportunities to improve over time where feasible.

We can roughly distinguish three types of nutrient balances: negative balances, slightly positive balances, and largely positive balances. Negative balances have higher exports than imports, which means that there is a net flux of nutrients off the farm. On the short term this can be desirable, especially if nutrient levels in the soil are high. However, if negative balances are sustained over a long time, soil mining of nutrients (such as P and K) may occur and homegrown crops may not yield optimally. Slightly positive balances are desirable, as biological processes always need inputs that are a little larger than the outputs. It is therefore expected that nutrient imports are often larger than the exports, and as long as the difference remains small enough, this is a sustainable practice. However, when the nutrient imports are a lot larger than the exports, there is an increased risk of environmental losses. Although the difference can temporarily be stored in feed stocks on the farm, the remainder is often lost (this is mainly the case for N) or stored in the soil and slowly lost over time (for P and to a lesser extent for K). Sustained large balances can result in loss of N, P, and K to surface waters.

Table 1. Feasible balances for dairy farms in New York.

Nutrient	Lbs per acre	Lbs per cwt milk
N	0 ≤ 105	0 ≤ 0.88
P	0 ≤ 12	0 ≤ 0.11
K	0 ≤ 37	0 ≤ 0.30

The ideal level of an NMB is thus larger than zero, but not so large that the nutrients are used inefficiently, as this costs money and is potentially harmful to the environment. As a good indicator sets targets, it is essential here to have a range of balances per nutrient to aim for. To find this range, 102 balances from New York dairies were investigated. Feasible balances per acre were set at the third quartile of the farm distribution. In other words: if 3 out of 4 dairy farms in New York can achieve to be below a certain balance, this should be feasible for the other farm as well. For the balance per hundredweight (cwt), farms were divided in two groups, those below and those above the average balance per cwt for all farms (Cela et al., 2014).

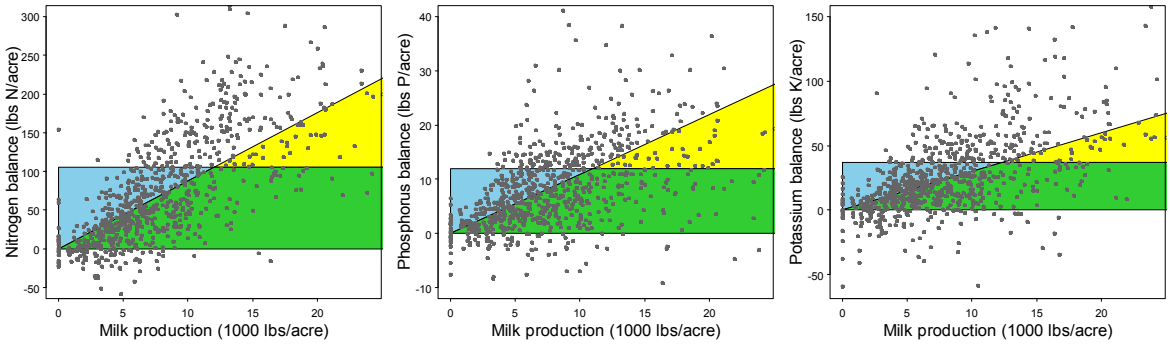

Figure 2. Optimal operation zones for N, P, and K. The grey dots represent farm records collected over the past decade. Farms in the green box have feasible balances per acre and per cwt.

The limits for balances per acre and per cwt (Table 1) can be combined per nutrient into one figure, to describe an optimal operation zone, or Green Box (Figure 2). This allows for a quick indication of a farm's sustainability. Farms that are in the green box have both feasible balances per acre and per cwt. Long-term records from a number of farms indicated that balances can be maintained in the "Green Box" for many years in a row (Soberon et al., 2015; Cela et al., 2015).

Because there is information on the crops and nutrient amounts grown on-farm as well, the NMB assessment can provide efficiency indicators beyond the balances. These indicators can give insight in why a farm operates within or outside the green box. For most of these indicators, a threshold value is derived. Should a farm cross this value, it is likely that it will operate beyond the feasible balances. This way we can indicate where the farm is likely to have the largest opportunities of improving the balances. In the example below (Table 2), the Example Farm has high N fertilizer imports (64 lbs/acre) which may have contributed to the high N balances (106 lbs/acre and 1.09 lbs/cwt). The N imported through feed (107 lbs/acre), however, is not crossing the indicator. This farm may thus have more opportunities for reducing the N balances by re-examining fertilizer use, than by decreasing feed imports.

Table 2. Efficiency indicators and the threshold values beyond which farms risk exceeding feasible balances. Orange cells are indicators that exceed the threshold values.

	Indicator to predict likelihood of exceeding feasible balances	Example Farm 2016			High risk of exceeding the feasible balances if		
		N	P	K	N	P	K
1	Balance per acre (lbs/acre)	106	13	29	> 105	> 12	> 37
2	Balance per cwt milk (lbs/hundredweight milk)	1.09	0.14	0.30	> 0.88	> 0.11	> 0.30
3	Milk per cow (lbs/cow/year)		25575		-	< 20000	-
4	Animal density (animal units/acre)		0.8		-	> 1.0	-
5	Whole-farm nutrient use efficiency. (%)	38	45	45	< 44	< 51	< 39
6	Purchased feed (lbs/acre)	107	19	35	> 121	> 20	> 38
7	Feed (tons dry matter/animal unit)		3.7		-	3.5 to 7.5	-
8	Feed use efficiency (milk, %)	28	31	19	< 20	< 25	< 11
9	Homegrown feed (% dry matter)		58		-	< 62-65	-
10	Homegrown forage (%)		40		-	-	-
11	Homegrown grain (%)		23		-	-	-
12	Homegrown nutrients (% dry matter)	41	34	59	< 50	< 50	-
13	Crude protein (CP) and P in all feed (%)	20	0.50	1.51	> 17	> 0.40	-
14	CP and P in purchased feed (%)	28	0.79	1.47	> 30	> 0.60	-
15	CP in homegrown feed (%)	14.5			< 11.8	-	-
16	Fertilizer (lbs/acre)	64	6	18	> 39	> 6	> 38
17	Crop exports (lbs/acre)	11	1	8	< 1	< 1	< 1
18	Manure exports (lbs/acre)				< 1	< 1	< 1
19	Overall crop yield (tons dry matter/acre)		3.3				
20	Acres receiving manure (%)		78				
21	Land in legumes with manure (%)		21				

Although this table of indicators give a general indication of where improvements in nutrient management can be made, it does not provide a detailed and guaranteed protocol to increase production with fewer nutrients. To make improvements in farm management, these results need to be discussed with the farm's nutritionist, crop specialist and planner. Collaboration between these experts can provide a level of detail that is lacking in the NMB. Alternatively, an integrated whole-farm model that links compartments with different farm components can provide a more detailed analysis of nutrient flows and efficiencies on a dairy farm. However, the simplicity of the NMB assessment makes it easy to interpret and relatively quickly to conduct, which is why it is suitable as a key sustainability indicator. This simplicity also makes it an easy exercise to repeat yearly to track a farm's progress, and see the results of management changes over calendar years. Multiple years of NMB assessments also help to filter out the effect of extreme years with bad yields, which may necessitate larger feed imports and would thus result in higher balances.

THE FUTURE OF THE NUTRIENT MASS BALANCE: NEW YORK AND BEYOND

Several other countries have adopted some form of NMB as a tool or a legislative instrument. Dairy farms in The Netherlands are required to assess the nutrient flows on their farm using de Kringloopwijzer, a software model that calculates the movement of nutrients through farm components and estimates specific environmental losses. This approach originates from the mineral accounting system (MINAS), which was introduced at the end of the 1990s (Van den Brandt and Smit, 1998). In New Zealand, the Overseer software is available, which allows for mass balance and nutrient flow calculations for a broader range of farms. Additionally, other countries with a large dairy industry, such as Ireland and Australia use a NMB as indicators as well, although there are differences in approaches and level of detail in the assessments. The exact method may thus vary per location, but the general idea is the same and has proven to be an efficient way to express and monitor sustainable use of nutrients.

In New York, the NMB assessment project has already documented improved whole-farm nutrient balances. Based on NMB assessments from New York farms collected between 2004 and 2013, it was estimated that N and P imports decreased by 20-30% (comparing 2004 and 2013) without a decrease in milk production (Cela et al., 2017). In 2013, imports onto farms were an estimated 66 million lbs of N and 6.6 million lbs of P lower than in 2004. Measures such as precision feeding, improved fertilizer management, and crop management for higher percentage of home-grown forage helped improve the nutrient efficiency and sustainability of farms statewide.

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PRECISION FEED MANAGEMENT – WHAT HAVE WE LEARNED?

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INTRODUCTION

Precision feed management (PFM) is a process that provides dairy producers the opportunity to improve the efficiency of nutrient use, decrease ration nitrogen (N) and phosphorus (P) content, lower feed N and P imports, decrease excretion of N and P into the environment, lower whole farm nutrient balance and improve farm profitability. The use of PFM on dairy farms is increasingly important as animal agriculture is under more pressure from environmental concerns and regulations. Water quality, nutrient runoff and emissions of ammonia and methane into air are the primary concerns. The dairy industry has already made significant progress in altering feeding programs and herd management to address these issues. However, there are still opportunities to make additional progress by increased use of PFM.

What is the potential impact of adjusting ration crude protein (CP) and P levels? A 1 unit decrease in ration CP lowers N excretion in the manure by 27.5 pounds/cow/year for a cow producing 70 pounds of milk with a dry matter intake of 47 pounds per day. This level of milk production represents the average dairy cow in New York. A reduction of ration P of 0.05% lowers manure P excretion by 8.5 pounds/cow/year. On a statewide basis, this would be a reduction of 17.1 million pounds of N and 5.3 million pounds of P excreted in manure. White and Capper (2014) used a modeling approach to examine the impact of precision diet formulation. They reported that balancing diets weekly rather than seasonally improved income over variable costs by $83/cow/year in a 300-cow herd. A margin of error in the feeding system of <1% was required to achieve this result.

WHAT HAVE WE LEARNED?

A PFM Working Group was established in New York to develop a unified approach to evaluating the impact of PFM in field research using commercial dairy herds. The initial impetus for this was the need to evaluate nutrient intake and excretion for herds located in the New York City Watershed. This group consisted of university faculty, cooperative extension agents and NRCS personnel. In addition, input was obtained from feed industry representatives. The working definition of PFM used by this group is “providing adequate, not excess, nutrients to the animal while maintaining environmental and economic sustainability through the integration of feeding and forage management.” One result was the development of PFM benchmarks that could be used as an initial evaluation of farms (Table 1). A spreadsheet was also developed to calculate these benchmarks. This spreadsheet also included milk and feed prices and calculated income over both total and purchased feed cost. Excretion of N and P in the manure was also calculated.

This approach has been used in several field studies with commercial dairy herds. Cerosaletti (2012) reported results for 34 herds enrolled between 2008 and 2011 in Delaware County. These herds decreased purchased grain by about 2 lbs./cow/day and increased forage in the ration from 59 to 65.4%. Manure P decreased by 18.6% (11 g/cow/day) while manure N was 9.8% (42 g/cow/day) lower. Milk income over purchased feed cost increased by 50 cents/cow/day.

Table 1. New York PFM Benchmarks

Benchmark	**Goal**
Forage NDF intake, % of BW	≤0.9%
Forage, % of total ration DM	≥60
Homegrown feed, % of total ration DM	≥60
Ration P, % of NRC requirement	<110
Ration CP, %	<16.5
Milk urea nitrogen, mg/dl	8 – 12
Cows dead or culled <60 days in milk, %	<8

A 2017 report contained the results for 8 herds that lowered ration P intake as a results of participating in a PFM program (Cerosaletting and Dewing, 2017). Manure P decreased by 23% (15 g/cow/day) and manure N was 7% lower (28 g/cow/day). Milk income over purchased feed cost increased by 46 cents/cow/day. Ganoe (2011) reported information from 40 herds using the PFM program. Manure P excretion decreased by 8.7% while manure N excretion went down by 6.3%. in the herds that exceeded the PFM benchmarks at the initiation of the study.

An 8-month study was conducted in 2 western New York herds using the CNCPS model (Higgs et.al., 2012). Ration CP was lowered by 1.7 units and milk urea nitrogen decreased by 2 mg/dl in these herds. Manure N excretion was decreased by 6% (28 g/cow/day) and 17.8% (89 g/cow/day). Milk production was maintained in these herds. Total daily feed cost decreased by 21 and 72 cents/cow/day. Income over purchased feed cost increased by 0.27 and 1.27 dollars/cow/day.

DELAWARE COUNTY PROJECT

Cornell Cooperative Extension of Delaware County received an agricultural nonpoint source pollution control grant to evaluate the impact of PFM on dairy herds in the Upper Susquehanna watershed. This grant was from the New York State Department of Agriculture and Markets. Ten dairy herds in Delaware, Broome and Tioga counties were enrolled in the project. This was a cost share grant and each herd provided a portion of the total grant cost. One herd was a custom heifer grower and one of the dairy herds fed 100% forage and no grain. The results contained in the following tables are from the 8 herds that fed both forage and concentrates. This project was conducted over a 3- year period. The PFM benchmark spreadsheet was used to track milk production, ration information and feed costs. Rations were formulated by the feed industry professional working with the farm. The CNCPS program was used to evaluate the initial (highest CP) and final (lowest CP) on each farm. There were 5 feed companies and 2 nutrition

consultants working with these herds. Table 2 contains information on herd size, housing and feeding system. Table 3 contains information on milk production and milk price. The milk price information is based on January 2017 data calculated from the Northeast Federal Milk Marketing Order #1

Table 4 contains information on ration CP and manure N. Ration CP decreased by 1.68% ration CP which was associated with a 61 grams per day of manure N excretion. Manure N excretion decreased by 14% (range = -5.2 to -29). Manure N excretion was 49.2 lbs./cow/year lower (range = (17.7 to 119.1). Figures 1 and 2 provide information on Income over feed cost. Feed prices used in these calculations are based on January 2017 information. The average increase in income over total feed cost was $147/cow/year (range = 62 to 299). Income over purchased feed cost had an average increase of $158/cow/year (range = 33 to 361.). The increased income over purchased feed cost in herd C is primarily related to the decrease in ration CP. In both herds F and G, the change in income over purchased feed cost is a combination of lowering ration CP and implementing a specific low group ration,

Table 2. Herd Information

Herd	Number of Cows	Housing	Milking Frequency, times/day	DHI	Feeding System
A	30	Tie-stall	2	No	Component
B	54	Tie-stall	2	No	Component
C	88	Tie-stall	2	Yes	TMR
D	76	Tie-stall	2	Yes	TMR
E	188	Free-stall	2	Yes	TMR
F	435	Free-stall	3	Yes	TMR
G	565	Free-stall	3	Yes	TMR
H	265	Free-stall	2	No	TMR

Table 3. Milk Production and Milk Price

Herd	Milk, lbs./cow/day	Milk Fat, %	Milk True Protein, %	Milk Price, $/cwt
A	50	3.92	3.02	19.34
B	65	3.9	3.1	19.46
C	65	4.2	3.3	20.46
D	75	4.6	3.6	22.31
E	74	3.8	3.2	19.77
F	86	4	3.2	19.73
G	87	3.9	3.0	19.25
H	75	3.56	3.0	18.41

Milk price is calculated for each herd using January 2017 Northeast Federal Milk Marketing order #1 data.

Table 4. Ration Crude Protein and Manure N Excretion

Herd	Initial CP, %	Final CP, %	Initial Manure N	Final Manure N	Manure N Excretion	Manure N Excretion

			Excretion, g/cow/day	Excretion, g/cow/day	Change, %	Change, lbs./cow/year
A	16.0	14.9	358	323	-9.7	28.2
B	16.3	14.9	319	282	-11.5	29.8
C	20.5	16.0	510	362	-29	119.1
D	17.1	16.0	385	344	-10.6	33.0
E	19.0	16.2	465	379	-20.4	75.6
F	17.4	16.5	456	423	-7.2	26.6
G	16.7	15.7	424	345	-18.6	63.6
H	16.9	16.2	422	400	-5.2	17.7

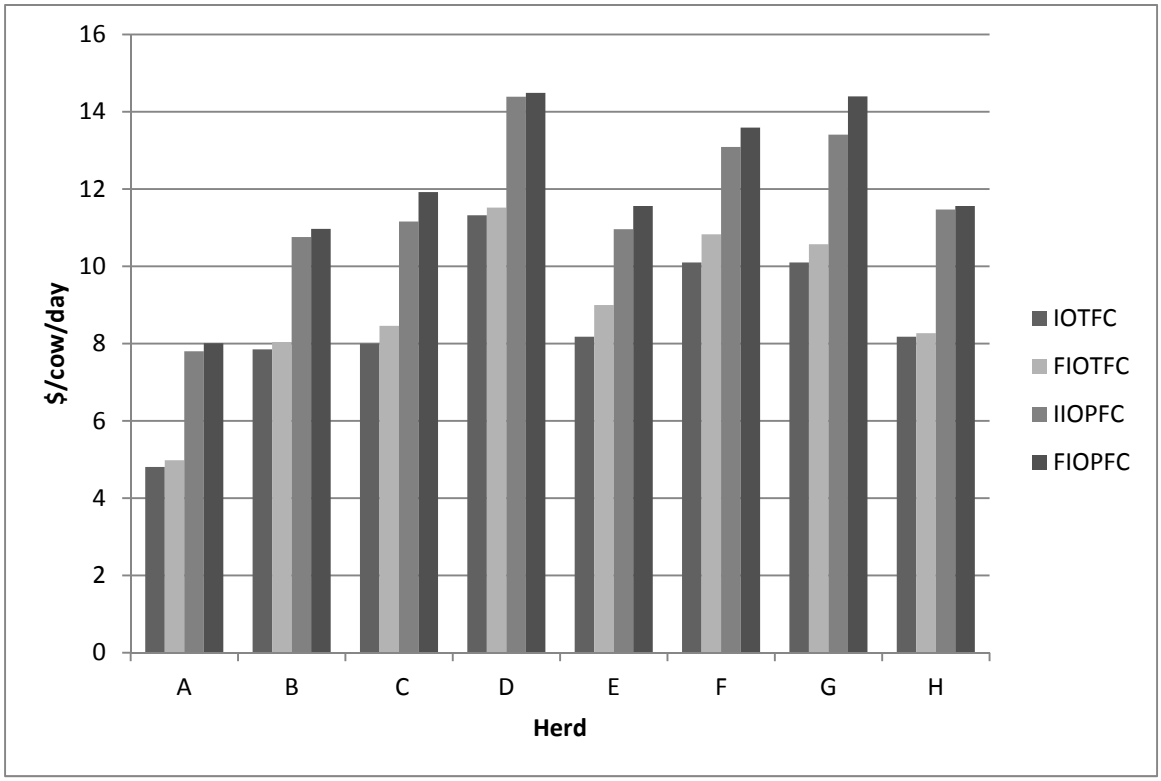

Figure 1. Income over total and purchased feed cost, $/cow/day (IOTFC = initial income over total feed cost, FIOTFC = final income over total feed cost, IIOPFC = Initial income over purchased feed cost, FIOPFC = final income over purchased feed cost)

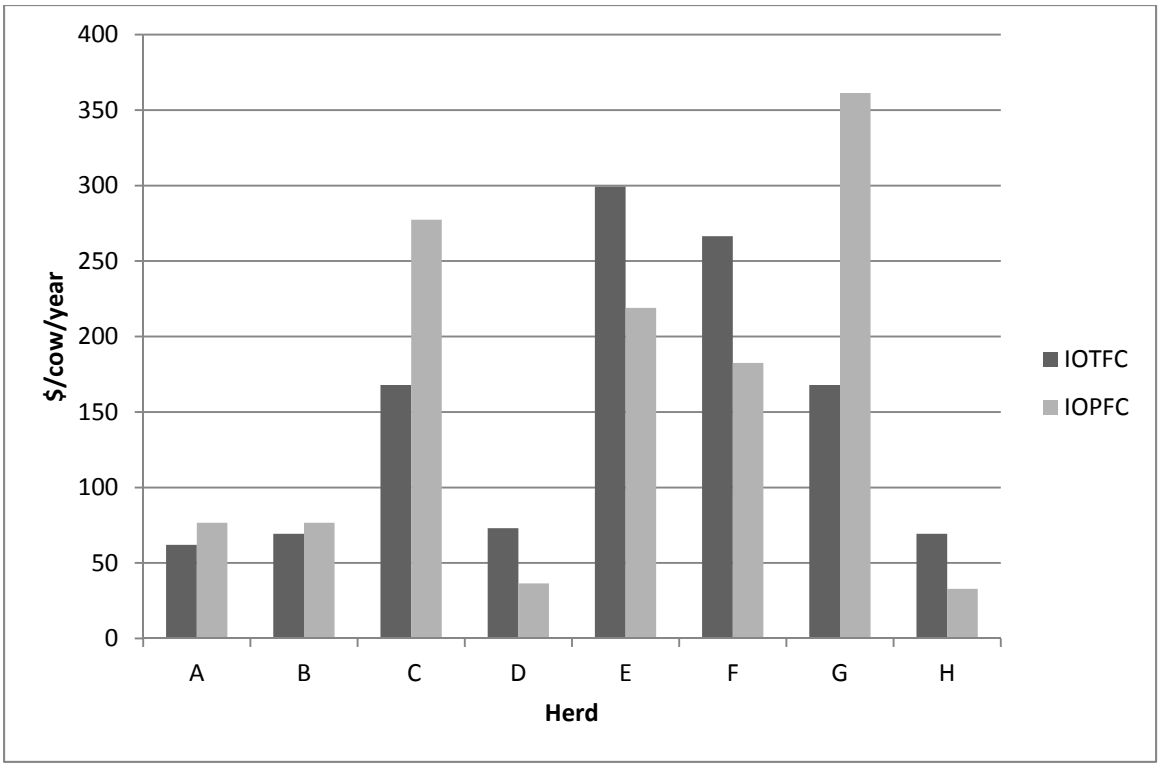

Figure 2. Change in Income over Total and Purchased Feed Cost, $/cow/year (IOTFC = income over total feed cost; IOPFC = income over purchased feed cost)

WHOLE FARM IMPLICATIONS

A whole farm modeling approach was used to evaluate PFM strategies for P farm planning strategies (Ghebremichael et. al, 2007). Two case study farms in Delaware county were used. A combination of more accurate feeding, increasing forage in the ration and improved yields of the of the grass forages lowered the whole farm P balance from 4.7 to 0.45 lbs./acre for one farm and 8.6 to 0 for the other farm. Decreasing ration P from 0.48 to 0.38% was reported to lower the acres needed for manure P application by 44% (Powell et. al., 2001).

The results of a pilot project using 2 dairy herds was reported by Cerosaletti et. al. (2003). In these herd, there was a decrease of 28% in feed P imports, a decrease of 33% in manure content and a decease in farm P mass balance from 60 to 42%. The results of a 5-year trial on a commercial dairy herd were reported (Tylutki et. al., 2004). This study incorporated changes in ration, feed management procedures, forage production and forage storage. Total animal numbers increased about 23% while total milk shipped per day increased by 45%. The percent of home-produced feeds increased from 43 to 59% and purchased N and P decreased by 37 and 40%. The shifts made in the cropping program resulted in more forage being available to incorporate into the feeding program. Daily purchased feed cost went down by 34% and manure N and P excretion decreased by 17 and 28%.

Results from a study evaluating the changes in whole farm mass nutrient balance (WFNB) on 4 New York dairy farms was recently reported (Cela et. al., 2015). These herds were used since they had 8-10 years of WFNB data. Herd milk production was maintained or improved during this time while improving nutrient use efficiency on a whole farm basis. All 4 farms lowered the CP an P content in purchased feeds, fed more closely to animal requirements and decreased manure P and N excretion. The authors indicated that precision feeding was the largest single component of the N and P changes made.

SUMMARY

The results of these studies consistently indicate that implementation of PFM practices on dairy farms can decrease purchased N and P imports, improve the efficiency of nutrient use, lower N and P excretion into the environment and improve dairy farm profitability. This requires integration of areas including ration formulation, feed purchasing decisions, feed and forage analysis, feeding management practices, forage production and forage storage. A key component for success is nthe development of a team including the dairy producer, key dairy employees, feed and crop advisors. The goal is to provide a consistent ration with minimal day to day variation.

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INNOVATIONS TO CREATE NEW NUTRITIONAL VALUE FROM FIBER

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INTRODUCTION

Sustainable conversion of forages and crop residues into ruminant protein presents multiple benefits. Grasslands and permanent pastures store vast amounts of carbon, reduce wind / water erosion and replenishment ground water reserves. Crop residues and protein-rich legume forages can reduce the use of human edible grains and proteins in ruminant diets. The fiber in plant cell walls is rich in polysaccharides including glucose, xylose, arabinose, galactose and mannose, all of which can be fermented by rumen microorganisms and provide energy for microbial protein synthesis and to the host. However, only a fraction of the sugars in most forages are in a free form, with the majority being contained within cellulose, hemicellulose and pectin. Furthermore, these carbohydrate complexes, particularly hemicellulose, are decorated with a range of side groups including acetyl, ferulic/coumaric acid and glucuranoic acids with covalent linkages to lignin. These linkages with lignin must be oxidized to be hydrolysed and therefore lignin is not degraded within the rumen. Even with this caveat, ruminants are unique in their ability to convert lignocellulosic biomass into high quality meat and milk protein. However, in spite of the rumen's reputation as the most efficient biological system at converting plant cell wall biomass into usable fermentation products, often less than 50% of the energy in low quality forages is available for cattle. This apparent inefficiency in what is deemed to be a highly efficient system arises primarily as a result of lignin / phenolics restricting the access of rumen microbes and enzymes to the carbohydrate stores within plant cell walls. Development of technologies to enhance the conversion of low quality forage fiber into energy within the rumen is essential for cost effective and sustainable dairy and beef production.

FIBER STRUCTURE AND CHEMISTRY

Plant cell walls perform several functions, ranging from the simple to the complex. Their architecture provides a system for the movement and retention of water and nutrients within the plant. They provide structural integrity to the plant, enabling it to stand upright, maximizing the capture of CO_2 from the atmosphere by photosynthesis. They store carbohydrates and regulate plant growth and act as an important barrier to plant pathogens. There are two types of plant cell walls; primary and secondary. During growth, the primary wall is constructed first and surrounds the growing plant cells. The secondary cell wall surrounds plant cells that differentiate and undertake specialized functions (i.e., wood, xylem and phloem cells). The secondary plant cell walls are more lignified than primary cell walls and thus have a disproportional impact on the digestibility of forages. The plant cell wall is almost entirely composed of cellulose, hemicellulose, pectin and lignin (Figure 1).

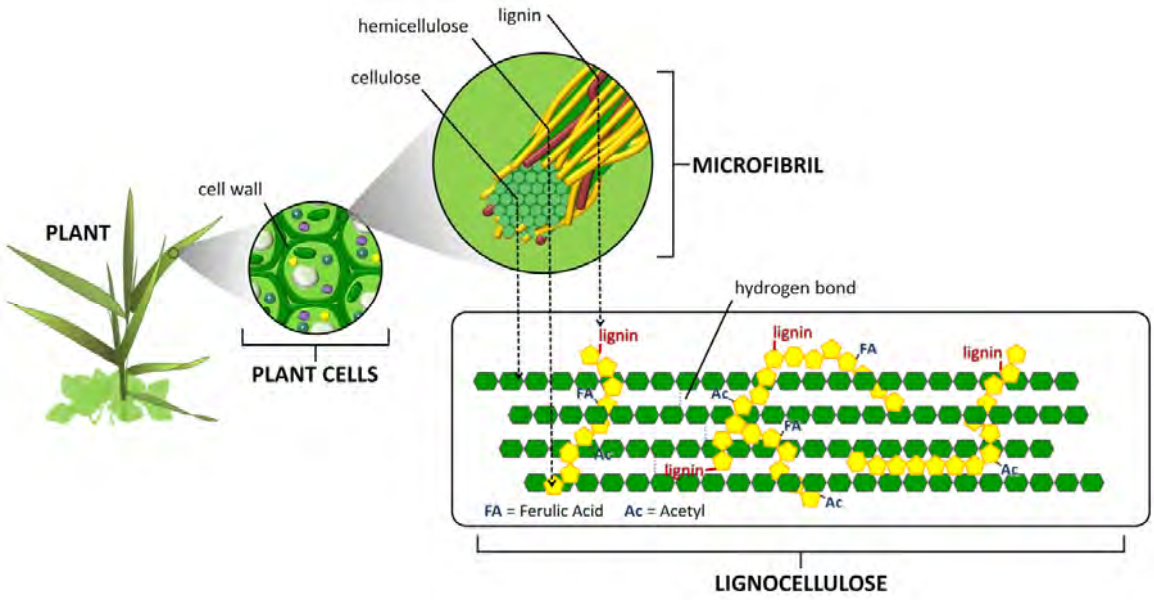

Figure 1. A simplified structure of plant fiber, showing cellulose in green and hemicellulose in yellow along with its linkages to lignin, and associated branches of ferulic acid and acetyl groups (Ribeiro *et al*., 2017).

Cellulose is a linear polymer of glucose connected via ß-1, 4- linkages to form of a linear chain and typically accounts for 35-50% of the plant cell wall. Hydrolysis of the ß-1, 4- linkages in cellulose is more difficult than the hydrolysis of the α-1, 4- linkages in starch, partially accounting for the differences in energy value between forages and grains. Adjacent cellulose chains interact via hydrogen bonding and hydrophobic interactions. The hydrophobic interactions between the flat surfaces of the pyranose rings hold the chains together to yield crystalline microfibrils. Generally, the higher the crystallinity of the cellulose, the lower the digestion, as amorphous regions within cellulose are more susceptible to enzymatic attack. Unlike cellulose, the backbone of hemicellulose is composed of a variety of sugars (i.e., glucose, xylose, galactose and mannose) with a number of different branch types. It accounts for 20-30% of the plant cell wall and its branching and variable linkages preclude it from forming a crystalline structure. Pectin is a group of highly complex polysaccharides with α-1,4-linked galacturonic acids or alternating α-1,2-rhamnopyranosyl residues as its backbone, with methyl-esterified or substituted acetyl groups. It plays an important role in plant cell wall permeability and defence against plant pathogens. Finally, lignin is a complex polymer of aromatic alcohols that fills the spaces in the cell wall between cellulose, hemicellulose and pectin. Typically, lignin accounts for 15 to 20% of the cell wall and is a barrier that impedes the access of microbes and enzymes to cellulose and hemicellulose. As a result, the linkages between lignin and hemicellulose are viewed as the primary factors that limit plant cell wall digestion in the rumen. As plants mature, the lignin content increases, accounting for the reduction in the digestibility of forages harvested at advancing maturity.

Secondary plant cell walls are composed primarily of ß -1,4-linked xylose residues. The xylan backbone may contain additional acetyl, coumaric or ferulic side groups depending on the plant species and tissue type. The frequency and type of branching, as well as the three dimensional orientation of these branches, affects the ability of the xylan to form hydrogen bonds with cellulose. In dicots, the glucuronoxylan backbone of hemicellulose consists of xylose residues that are often branched with α-glucuronic acid or 4-methyl glucuronic acid and acetyl groups. In contrast, monocots have a glucuronoarabinoxylan backbone that contains a large number of arabinose and methyl glucuronic acid side chains. Some of the arabinose branches are further ester linked to ferulic acid, or other hydroxycinammic acids, which connect hemicellulose to lignin.

RUMEN MICROBIOME AND FIBER DIGESTION

Feed that enters the rumen undergoes rapid colonization by rumen microbes and the digestion of the plant cell wall carbohydrates begins within minutes (Edwards *et al.*, 2008). Whereas rumen bacteria preferentially colonize damaged sites on the plant surface, rumen fungi physically penetrate the cell wall of the ingested material via rhizoidal growth (Gruninger *et al*., 2014). The attachment of rumen microbes is essential for the establishment of the complex microbial populations that are required for feed digestion. Feed colonization occurs in a multi-step process that involves: (1) displacement of the epiphytic microbiome by rumen microbes (Time <1 h), (2) establishment of a primary colonizing community of generalist microbes that primarily metabolize accessible carbohydrates (Time 1 – 4 h), (3) loss of some primary colonizers and selection of a secondary colonizing community specialized in digesting hemicellulose and cellulose (Time > 4 h; Figure 1) (Huws *et al.*, 2016). Within the microbial community attached to the surface of feed are taxa whose abundance does not change significantly during primary and secondary colonization including *Butyrivibrio*, *Fibrobacter*, *Olsenella*, and *Prevotella* (Liu *et al.*, 2016). Populations of *Prevotella* peak on the surface of fiber within 1 h of introduction to the rumen (Huws *et al.*, 2016). *Prevotella* sp, are ubiquitous in the rumen and have diverse metabolic capabilities (Rubino *et al.*, 2017). This metabolic plasticity is likely important for *Prevotella* to function in primary and secondary colonization. The ability to effectively utilize soluble carbohydrates, pectins, proteins, and hemicellulose provides *Prevotella* sp with a competitive advantage over more specialized microbes. Populations of Clostridia (*Pseudobutyrivibrio*, *Roseburia* and *Ruminococcus* sp.) peak after *Prevotella,* perhaps due to their role in targeting cellulose during the later stages of feed digestion (Rubino *et al.*, 2017). There is a minimal amount of biomass degraded during primary colonization, with the majority of plant cell wall digestion occurring in the later stages of colonization.

CHEMICAL APPROACHES TOO ENAHANCING FIBER DIGESTION

Alkali treatment of forages such as straw and crop residues has been used since it was first applied to paper processing in the 12th century. Bases (i.e., sodium hydroxide, calcium hydroxide, potassium hydroxide, ammonia) disrupt the chemical

structure of lignocellulose by cleaving the α-ether and ester bonds between lignin and hemicelluloses, and the linkages between hydroxycinnamic acids (i.e,. p-coumaric and ferulic acids) and hemicelluloses (Xiao *et al*., 2001). They also promote the swelling of cellulose, decreasing its crystallinity and promoting the microbial attachment which is essential for ruminal fiber digestion.

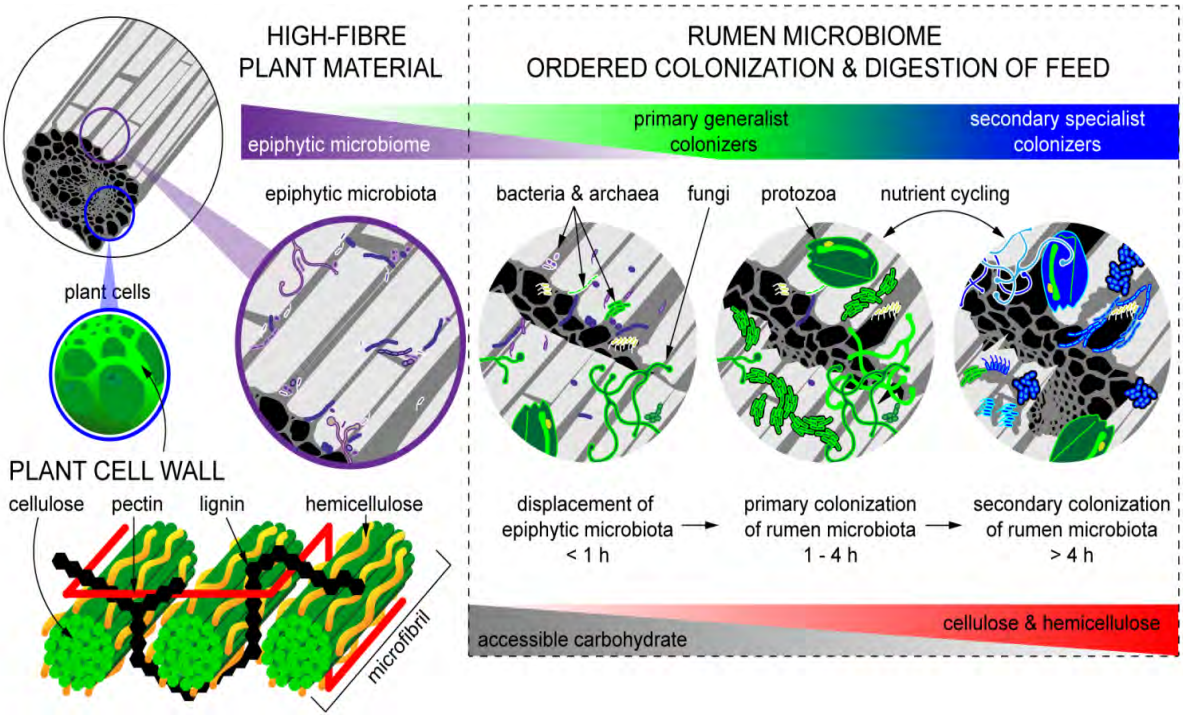

Figure 2. Fiber in plants is colonized by natural epiphytic microbes during growth in the field. Upon consumption, this epiphytic population is displaced by primary colonizing rumen bacteria that utilize sugars and soluble proteins. Primary colonizers are replaced by secondary colonizers which actively ferment the sugars arising from insoluble fiber (Gruninger *et al.*, submitted).

Researchers have examined the ability of NaOH, NH_3, $Ca(OH)_2$, KOH, and CaO to improve ruminal digestion of corn cobs and stalks, wheat, barley, rice and oat straws (Klopfenstein and Owen, 1981). However, safety, environmental, technical, and economical concerns have limited the widespread adoption of alkali treatment of forages for ruminants. However, there has been a renewed interest in some of these treatments as there has been considerable investment in their use for the production of biofuels from lignocellulose. Some of the accomplishments in this sector may have relevance to improving the utilization of forages by ruminants.

Calcium oxide (CaO)

The ability of calcium hydroxide to improve fiber digestion has not been investigated to the same extent as NaOH as it is a weaker base and did not prove as effective in in vitro digestibility studies. However, in the past 10 years, interest in the use of CaO as a source of $Ca(OH)_2$ for the alkali treatment of crop residues has increased. When added to water calcium oxide transforms into $Ca(OH)_2$, which can hydrolyze the

ester and either bonds described above. CaO is more practical and safer than NaOH, and has been shown to effectively improve the digestibility of crop residues in cattle (Shreck *et al.*, 2015). In addition, CaO can be used as direct source of Ca in the diet, reducing the need for Ca supplementation using other sources such as limestone.

Shreck et al. (2011) conducted in vitro rumen digestibility studies to evaluate the effect of applying 5% CaO on a DM basis to corn stover, corn cobs, and wheat straw at 35% and 50% moisture. Once the solution of CaO was added, the forage was stored under anaerobic conditions for 7, 14, or 28 days. In vitro DM digestibility was improved at 50% moisture, but no further improvement was achieved with a longer ensiling time. Shi *et al*., (2016) treated corn stover with three levels of CaO (3%, 5% and 7% of DM) at 40%, 50% and 60% moisture and stored it anaerobically for 15 days. Similar to Shreck *et al*., (2011), improvement in the fiber digestion of corn stover was the highest with 5% CaO at 50 or 60% moisture. Recently, Ciriaco *et al.*, (2018) ensiled bahia grass and bermuda grass hay for 7 or 14 d, after mixing with solutions of 5% or 10% CaO (DM basis) to achieve a moisture level of 50%. CaO at 5% increased forage digestibility, but no additional improvement was achieved with ensiling beyond 7 d or by increasing the level of CaO to 10% of DM. These results show that application of 5% CaO to forage at a moisture level of 50%, followed by ensiling for 7 d achieves the greatest improvement in in vitro and in situ fiber digestion.

In a study at the University of Nebraska, Shreck *et al*., (2015) examined the effect of treating corn cobs, wheat straw, and corn stover with 5% CaO (DM basis) at a moisture level of 50% on fiber digestibility and performance of feedlot steers. In this study crop residues were chopped through a 7.62 cm screen for corn stover and wheat straw, and a 1.91 cm screen for corn cobs. The chopped forage was treated with CaO, and ensiled for 30 d. The treated or untreated crop residues comprised 20% of the diet DM. These diets (36% Dry rolled corn, 40% wet distillers grains plus solubles, 20% crop residue, 4% supplement DM basis) were also compared to a control diet that had only 10% roughage composed of equal parts of untreated cobs, wheat straw, and corn stover. The control diet also contained 10% (DM basis) more dry rolled corn (46.0% vs. 36.0%) as compared to the experimental diets. Steers fed diets with 20% CaO treated wheat straw or corn stover exhibited improved ADG, DMI, and feed efficiency as compared to those fed untreated wheat straw or corn stover and had similar performance to those fed the control diet with 10% more dry rolled corn. The improved performance with CaO treatment of wheat straw or corn stover was attributed to the greater total diet DM and fiber digestibility of these diets. It is unlikely that CaO treated crop residues had a digestible energy value equivalent to dry rolled corn grain. However, a more favorable rumen environment (i.e., higher ruminal pH and longer retention time) for the activity of fibrolytic microorganisms may have led to an improvement in the digestibility of fiber in other diet ingredients. In fact, Nuñez *et al*., (2014) replaced limestone in a 60% dried distillers grain-based diet of feedlot steers with increasing CaO (0, 0.8, 1.6, or 2.4% DM basis) and observed reduced variation in ruminal pH, increased fiber digestibility, decreased acidosis, and increased growth performance.

We recently conducted in vitro, in situ, and in vivo studies looking at the effect of calcium oxide treatment of barley straw when used in a wheat grain-based finishing feedlot diet (Stehr *et al*., 2018). Barley straw was mixed with a solution of CaO and water to achieve 5% CaO (DM basis) at 50% moisture. Unlike previous studies, we did not ensile the forage, but stored it at room temperature and fed it 48 h after treatment. This approach avoided the expenses associated with ensiling the treated forage. However, to avoid spoilage, the treated straw could not be stored longer than 48 h. The CaO reduced NDF content of the straw, and improved the in vitro and in situ digestibility, and the effective rumen DM degradability of barley straw. Preliminary data showed that there was no difference in the growth performance of steers fed wheat-based finishing diets with barley silage (12% diet DM) vs those fed a diet where barley silage was replaced by CaO treated barley straw (unpublished data).

Ammonia Fiber Expansion (AFEX)

Although ammoniation has been widely researched as a method to enhance ruminal fiber degradation (Abdel-Aziz *et al*., 2015), it has not been widely used on farm due to potential health and safety hazards associated with volatilization (Mor *et al*., 2018). Efficiency and safety of ammoniation has been improved with the advent of ammonia freeze explosion (Dale and Moreira, 1982), later termed Ammonia Fiber Expansion (AFEX). Originally developed for the pre-treatment of biomass for cellulosic ethanol production, AFEX has recently received increased interest for the treatment of crop residues as animal feed (Mor *et al*., 2018). The AFEX treatment uses a reaction chamber where the recalcitrant fiber is subjected to steam and anhydrous ammonia at high pressure (200-400 psi) and temperature (80-150°C) for < 1 h, followed by a rapid pressure release and ammonia recovery (Figure 3; Mor *et al*., 2018). Bals *et al*., (2010) observed that AFEX was much more effective at increasing digestion of late harvest switchgrass as compared to traditional ammoniation methods (206% vs. 56% increase, respectively). We conducted a semi-continuous culture study using the rumen simulation technique (RUSITEC) and found that DM disappearance of AFEX barley straw improved 35% (62.4% vs. 46.2%) and total daily VFA production improved by 17.5% (58.5 vs. 49.8 mmol/d) (Griffith *et al*., 2016).

Similar to other alkali treatments, AFEX disrupts hydrogen, ether and ester bonds and solubilizes some hemicelluloses. However, AFEX treatment is more intensive than most other alkali treatments (Bals *et al*., 2010), enhancing the fragility of the fiber and increasing the surface area available for microbial attachment and ruminal degradation.

Few feeding trials with AFEX-treated straw have been conducted. In one of the first studies, Weimer *et al*. (2003) replaced 7% of the alfalfa hay in the diet of lactating dairy cows with AFEX treated rice straw and observed a 1.3 kg increase in milk production (38.3 vs. 39.6 kg/d). In a recent Indian study with lactating Murrah buffalo and Karan-Fries cattle, Mor *et al*. (2018) observed that replacing wheat straw with increasing quantities of AFEX treated wheat straw pellets in a high forage diet (70% wheat straw DM basis) increased milk production in cows, with no changes in body weight. Buffalo fed a wheat straw diet lost body condition, while buffalo fed AFEX

treated wheat straw did not. Including 50% of the diet DM as AFEX treated wheat straw pellets in the diet of the cows also increased total tract DM digestibility from 52.4 to 61.4% (17.2% increase) and milk production from 7.5 to 9.4 kg/d (25.3% increase). Mor *et al*., (2018) concluded that AFEX wheat straw increased the energy available for lactating dairy cattle with no problems with palatability.

We have recently conducted a study looking at the effect of replacing alfalfa pellets with AFEX wheat straw pellets on the digestibility and performance of lambs (Ribeiro *et al*., unpublished data). Replacing 30% of the diet DM as alfalfa pellets with AFEX wheat straw pellets promoted an increase in DMI of 7.4%, and improved ADG in the first 42 d, but not over the entire feeding period (298 and 305 g/d, respectively). Consequently, feed efficiency for the full feeding period was reduced (5.7%) for AFEX diet compared to control diet. Apparent total tract DM digestibility was not different between diets. There was no difference between diets in final BW as lambs were fed to a constant weight of 50 kg. However, days on feed were longer for the control diet (higher in alfalfa pellets) than the AFEX diet (97 vs. 91 d). As alfalfa is considered the 'gold standard' of forages for inclusion in lamb diets, the fact that lambs fed AFEX wheat straw pellets exhibited similar performance, attests to its ability to improve the nutritional value of low quality forages. .

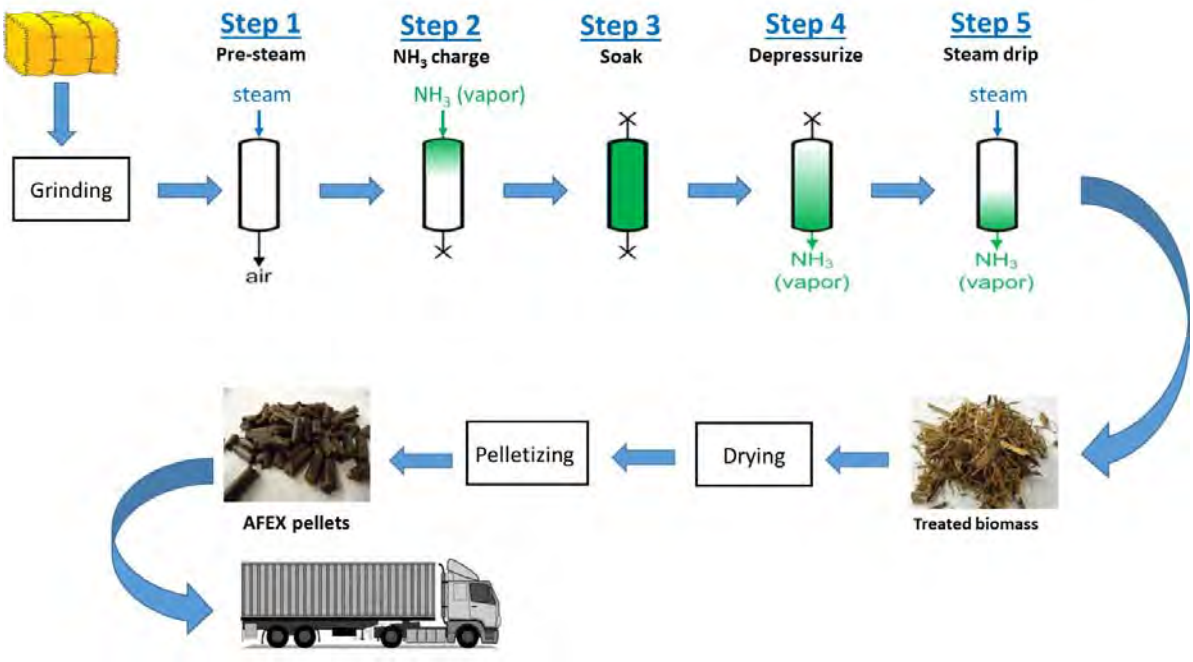

Figure 3. Ammonia fiber expansion involves the treatment of fiber residues with ammonia and steam under pressure. Depressurization and the alkali conditions promote the hydrolysis of ester and ether linkages and removes many of limits to plant cell wall digestion described above.

ENZYMATIC APPROACHES TO ENHANCING FIBER DIGESTION

Carbohydrate Active enZymes (CAZymes)

Ultimately, enhancing the nutritional value of low quality forages requires improving the accessibility of enzymes and microbes to both crystalline cellulose fibrils and hemicellulose. Lignin is not digested in the rumen and serves as a barrier that limits access to these digestible polymers. Through breaking the ester linkages between lignin and hemicellulose, fibrolytic enzymes can enhance degradation by gaining better access to core structure of the plant cell wall. Aerobic fungi have evolved to produce oxidative enzymes that hydrolyze lignin bonds during the digestion of lignocellulose. Due to the anaerobic environment within the rumen these enzymes do not function and rumen microbes must evolve other strategies to efficiently degrade the plant cell wall. The rumen is one of the richest sources of “Carbohydrate Active enZymes” or CAZymes. The development and adoption of molecular biology approaches (i.e. metagenomics and metatranscripitomics) that are independent of cultivation-based methods has yielded considerable information about the microbes and carbohydrate active enzymes involved in ruminal lignocellulose degradation. Although efforts at characterizing these activities has begun, researchers have yet to develop an effective ruminant fibrolytic feed enzyme cocktail that functions not only under rumen conditions, but also acts synergistically with the natural enzymes present in the rumen. A better understanding of the rate limiting enzymes to fiber deconstruction in the rumen will definitely play an important role in this process. An increasing number of rumen meta-omic studies are shedding light on potentially missing and/or limiting enzyme activities within the rumen. It is now known that cellulases of the classes GH7 and GH12 are not present in the rumen, but are found in aerobic environments (Dai *et al*., 2015). Furthermore, our work has shown that supplementing rumen enzyme mixtures with aerobic enzymes results in a synergistic release of glucose and xylose from barley straw and alfalfa hay (Badhan *et al*., 2014). The synergism between aerobic and anaerobic enzymes suggests that they may target unique sites within lignocellulose and thus do not compete for the same binding sites.

The complexity of the rumen makes “-omics” based approaches such as metagenomics and metatranscriptomics, ideal approaches to study the phylogeny and function of this community. Many of these studies have focused on characterizing the mechanism that the rumen microbiome has evolved to efficiently digest lignocellulose (Dai *et al*., 2015). The underlying goal of these studies is to uncover new ruminal fiber-degrading enzymes so as to expand the large database of CAZymes involved in the saccharification of lignocellulose and other carbohydrates. Combining the results of multiple omics approaches such as metatranscriptomics and metagenomics has provided novel insights into the roles of specific microbes that are active in lignocellulose digestion (Li and Guan, 2017). Metatranscriptomes was used to identify the most highly expressed CAZymes in the rumen were GH5, GH9, GH45, and GH48, that originated primarily from *Rumninococcus* and *Fibrobacter* in dairy cows fed a forage-based diet (Dai *et al.*, 2015). A shotgun metagenomic study by Hess and colleagues previously identified that cellulase families GH5 and GH9 were abundant in the rumen of forage fed dairy cows; however, GH45 and GH48 were not (Hess *et al.*, 2011). Differences in gene abundances observed between DNA or RNA based studies

have been reinforced by several other rumen metatranscriptome studies, thus highlighting the importance of using a combined -omics approach (Comtet-Marre *et al.*, 2017). These studies have pointed to the rumen fungi, *Prevotella* sp., *Ruminococcus* sp., and *Fibrobacter* sp. as being responsible for the production of a large proportion of the rumen CAZymes involved in lignocellulose digestion (Comtet-Marre *et al.*, 2017). Recent advancements in bioinformatics has led to researchers being able to reconstruct whole genomes of uncultured rumen microbes from shotgun metagenomic sequences. This has provided a novel approach to expand our understanding of the biological role of uncultured rumen microbes in ruminal fiber digestion (Svartström *et al.*, 2017).

Novel approaches to selecting fibrolytic enzymes for ruminants

Numerous studies have investigated the use of exogenous enzymes as a means of improving fiber digestion in ruminants (Meale *et al*., 2014). In some cases, increases in milk (Klingerman et al., 2009), ADG (McAllister *et al.*, 1999), DM and fiber digestion in situ, in vitro (Hristov *et al.*, 2008), and in vivo (Beauchemin *et al*., 2000) have been observed. However, in many cases, benefits to growth or milk production in ruminants have been inconsistent or nonexistent (Arriola *et al*., 2011). Past enzyme formulations were not designed specifically for use in ruminants, as this was viewed as an ancillary market for their primary use in pulp and paper, detergents or biobleaching. Consequently, these formulations likely just duplicated many of the fibrolytic enzyme activities that are endemic to the rumen ecosystem. Occasionally, the hydrolysis of fiber within the rumen may have been compromised, creating conditions where positive responses to exogenous enzymes occur. However, such preparations were not selected to act synergistically with the natural fibrolytic enzymes produced by the rumen microbiota.

Rumen Transfer experiments

It is well stablished that the rumen microbiome is the principal determinant of the efficiency of feed digestion, and consequently the ability of ruminants to extract energy from plant fiber. However, there is considerable variation in the ability of individual animals to digest different fiber sources (Griffith *et al.*, 2017). Feed additives including antibiotics, prebiotics, probiotics, enzymes, plant secondary compounds have been tested with the aim of altering the rumen microbiome and consequently improving feed digestion (Beauchemin *et al*., 2008; Benchaar *et al*., 2008). However, the results have been variable and consistent improvements throughout the feeding period have been largely unsuccessful.

The idea of introducing an improved bacteria strain with a highly efficient fiber digesting capacity into the rumen is not new, but has proven elusive (Miyagi *et al*., 1995; Varel *et al*., 1995). Reasons for this failure are many and may include the resilience and host-specificity of the rumen microbiome, the lack of fitness of these inoculated bacterial strains as compared to the indigenous rumen microbiota and adaptation of the microbial population that results in the reduction or elimination of the activity of the additive. Rumen transfaunation or the physical transfer of rumen contents from an individual that

is more efficient at digesting fiber to one that is less efficient, could potentially overcome the inherent fitness limits of cultured microbes. Rumen transfaunation has been successfully used to treat clinical indigestion and to degrade plant toxins to harmless or even beneficial compounds in cattle and sheep (DePeters and George, 2014). To test this hypothesis we conducted an experiment where rumen contents were transferred from bison to cattle, as bison have been shown to be more efficient at digesting low-quality forages (<7% crude protein) than cattle (Hawley *et al*., 1981). As we wanted to investigate if members of the bison rumen microbiome could colonize specific niches and potentially complement the microbial community in the rumen of cattle responsible for fiber degradation, we transferred bison rumen contents into the rumen of heifers fully adapted to a barley straw diet (Ribeiro *et al*., 2017). This process was repeated 2 weeks after in the same animals to increase the likelihood that microbial community associated with the degradation of fiber had the opportunity to establish in the rumen of heifers. Although inoculation with bison rumen contents successfully altered the cattle rumen microbiome and metabolism, and increased protein digestibility and nitrogen retention, fiber digestibility was not improved. One reasons for the lack of improvement in fiber digestion could have been that we were unable to adapt the bison to a low quality fiber diet prior to the rumen transfer. Others have proposed that the rumen microbiome may become irreversibly programmed once the host surpasses 14 months of age (Saro *et al*., 2018). However, our work would not fully support this hypothesis as it emphasizes the overwhelming impact that diet has on the activity of the rumen microbiome. Studies towards a better understanding of the development and establishment of the rumen microbial community in young ruminants, and conducting trasfaunation of these animals early in life with a highly efficient fibrolytic inoculum may be viable future strategies to improve fiber digestion in ruminants. Comparisons of the rumen microbiome of efficient vs inefficient fiber digesting hosts may also aide in the identification of microbes and their enzymes that ca lead to improvements in fiber digestion.

Selection for synergistic fibrolytic enzymes

It is obvious that progress in improving ruminal fiber digestion will be minimal if exogenous enzymes simply duplicate the natural fibrolytic activity of rumen microorganisms. To overcome this limitation, our laboratory developed a microassay to screen microgram quantities of recombinant carbohydratases for their synergy with the natural enzymes produced by rumen microorganisms (Badhan *et al.*, 2018a). The procedure involves the isolation of a profile of fibrolytic enzymes from cattle fed a low quality forage diet. The ability of this natural mixture of fibrolytic enzymes to release sugars from plant cell walls is then compared to the same mixture of fibrolytic enzymes in which a recombinant enzyme candidate has been added. An improvement in the release of sugars as a result of the addition of the recombinant enzyme is a reflection of the degree to which the candidate synergistically improves fiber digestion. This rapid throughput procedure lends itself to the screening of hundreds of recombinant candidate enzymes, an outcome that is impossible with traditional in vitro rumen batch culture assays (Figure 4).

The above procedure can also be used to identify those key components within enzyme cocktails that are responsible for improvements in ruminal fiber digestion (Figure 4). In this case the enzyme cocktail is fractionated use blue native PAGE and the bands corresponding to the various enzymes are excised. The microgram quantities of the enzyme are than used in the microassay with rumen mixed enzymes to measure the ability of the excised enzyme to enhance the release of sugars from the plant cell walls. Those excised bands that result in the greatest release of sugars reflect those enzymes within the cocktail that are responsible for the improvement in fiber digestion. LC-MS/MS can be used to further characterize those enzymes that are responsible for the improvement in fiber digestion. Phylogenetic analysis of these characterized enzymes can also be used to select closely related candidate enzymes from the CAZy database that may have further applications in improving the efficacy of enzyme cocktails.

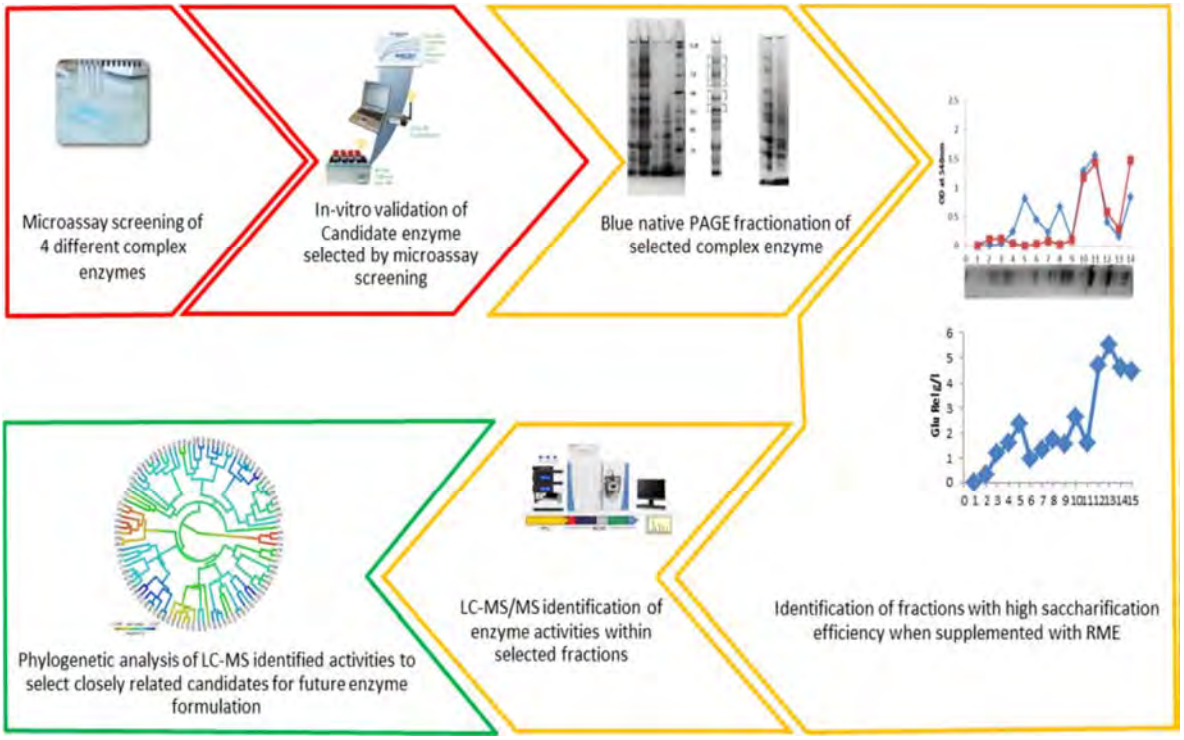

Figure 4. A novel strategy for the selection of recombinant fibrolytic enzymes or the identification of key enzymes in mixed enzyme cocktails that act synergistically with rumen mixed enzymes (RME) (Badhan *et al.,* 2018b).

FUTURE CHALLENGES AND CONCLUSIONS

Although we now have novel tools to identify fibrolytic enzymes that have the greatest potential to improve ruminal fiber digestion, challenges still remain. Supplementation of enzyme cocktails with those enzymes that act synergistically with rumen microorganisms to overcome the limits to fiber digestion can prove expensive, potentially yielding these methods as uneconomic. To overcome this, developing high yield recombinant expression systems will be central to the economic viability of this enzyme production strategy. Additionally, the formulation with a recombinant rate

limiting firbrolytic enzyme is likely to only prove effective until the next rate limiting enzyme hinders fiber digestion. This may require the addition of mixtures of recombinant enzymes to form complex formulations, which could further increase the cost of these additives. Once formulated, methods will still be required to apply the enzymes to the feed as contact with the feed prior to consumption still appears pivotal in ensuring enzyme efficacy. Structural disruption of the forage either through processing or during mastication will also still be necessary to ensure efficient forage digestion. There is also a possibility that forage specific formulations will be required to account for differences in plant cell wall composition between monocot and dicot plants. This could complicate enzyme treatment procedures in mixed forage diets.

Clearly, characterization of rumen microbiome using “omics” technologies is providing insight into the functionality of rumen microbiota and the construction of gene catalogues through metagenomics and genomic sequencing is expanding our understanding of the interactions between community members and the carbohydrate degrading enzymes they produce. These “omics” knowledge advancements are providing new insight into the formulation of exogenous enzymes that act synergistically with these rumen enzymes in a manner that enhances the efficiency of plant cell wall digestion.

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THE IMPORTANCE OF FIBER AS A SOURCE OF ENERGY DURING THE TRANSITION PERIOD

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INTRODUCTION

Carbohydrates comprise the majority of diets fed to transition cows and are the most important source of energy during this timeframe as in other parts of the lactation cycle. Much of the research conducted in carbohydrate nutrition of transition cows has focused on manipulating energy levels during the prepartum period by varying neutral detergent fiber (NDF) and starch concentrations in prepartum diets (e.g., Janovick and Drackley, 2010; Mann et al., 2015) or varying starch and digestible NDF levels during the postpartum period by varying concentrations of starch and nonforage fiber sources (NFFS) in the ration (e.g., Dann and Nelson, 2011; McCarthy et al., 2015). Comparatively little work has focused directly on the impact of fiber digestibility in diets fed during the prepartum and postpartum periods. In this paper we will summarize our current knowledge in this area and suggest future directions for research to further support additional recommendations for feeding transition cows.

RESEARCH REPLACING FORAGE NDF WITH NONFORAGE NDF IN DIETS FED DURING THE TRANSITION PERIOD

Several studies have been conducted that have focused on replacing forage with nonforage fiber sources in transition diets. Holcomb et al. (2001) fed multiparous cows high (70% forage) vs. low (28% forage) forage diets at two levels of intake for the last 28 d prepartum and fed a common lactation diet postpartum. To formulate the low forage diets, bermudagrass hay and corn silage were replaced by mostly soybean hulls and hominy feed. Concentrations of NDF (39.2 vs. 44.2%) and starch (25.1 vs. 22.0%) were somewhat different between the low forage and high forage diets. Cows fed the low forage diet for ad libitum intake had higher prepartum dry matter intake (DMI; 14.1 vs. 10.7 kg/d), lower overall plasma concentrations of nonesterified fatty acids (NEFA), and higher overall plasma concentrations of insulin; however, postpartum DMI (20.5 vs. 21.2 kg/d) was not different for cows fed low vs. high forage diets, respectively, during the prepartum period and milk yield was numerically higher (35.8 vs. 29.9 kg/d) for cows fed the high forage diet prepartum.

Dann et al. (2007) partially replaced oat hay in corn-silage based prepartum rations with beet pulp for the last 21 d before expected calving fed to multiparous and primiparous cows. Neither prepartum nor postpartum DMI, prepartum and postpartum serum NEFA, or milk yield were affected by treatment.

Chung et al. (2008) fed multiparous cows either a conventional, high forage (70% of DM) ration or a lower forage (42% of DM) ration in which corn silage and grass hay were replaced with cottonseed hulls, soybean hulls, and alfalfa hay for the entire dry period. Feeding the ration higher in NFFS resulted in substantially higher prepartum DMI (15.7 vs 11.9 kg/d) along with higher plasma insulin and lower plasma NEFA concentrations; however, postpartum DMI (21.8 vs. 21.3 kg/d) and milk yield (45.2 vs. 43.4 kg/d) were not different for cows fed the low vs. high forage diets, respectively. Plasma NEFA were not different postpartum for cows fed the different prepartum treatments; however, interestingly, plasma B-hydroxybutyrate (BHBA) concentrations tended to be higher postpartum for cows fed the low forage diet prepartum.

Overall, results suggest that replacing forage NDF with NDF from NFFS sources during the prepartum period is likely to increase DMI during the prepartum period; however, these effects on prepartum DMI do not seem to translate into effects on postpartum DMI and milk yield. The increased prepartum DMI likely does contribute to better metabolic status for cows fed NFFS based upon higher circulating insulin and lower NEFA concentrations.

EFFECTS OF VARYING FORAGE DIGESTIBILITY DURING THE TRANSITION PERIOD

In contrast to results reported above in which replacement of forage NDF with NFFS during the prepartum period resulted in modest effects on postpartum outcomes, improved forage NDF digestibility in diets fed to transition cows generally does result in better postpartum performance and metabolic status.

Stone et al. (2012) replaced conventional corn silage with BMR corn silage in diets fed during the last 3 wk before calving through wk 3 to 4 postpartum, at which time all cows were fed the postpartum diet containing conventional corn silage through 15 wk postpartum. Diets were formulated to be the same in chemical composition and vary only in NDF digestibility. Cows fed BMR corn silage had higher DMI during both the prepartum (14.3 vs. 13.2 kg/d) and 3-wk postpartum (20.1 vs. 18.1 kg/d) periods than cows fed conventional corn silage. Milk yield was increased during the first 3 wk postpartum (37.5 vs. 34.3 kg/d) for cows fed BMR corn silage and there was a carryover response from wk 4 to 15 postpartum in that cows previously fed BMR corn silage tended to make more milk than cows previously fed conventional corn silage (37.3 vs. 35.3 kg/d). Metabolic status as reflected by plasma concentrations of NEFA and BHBA were similar across the two treatments.

Recently, LaCount et al. (2017) conducted a study similar to the one immediately above in that they replaced conventional corn silage with BMR corn silage in rations fed from d 21 before expected calving through 42 d postpartum; furthermore, cows were also randomized to control vs. monensin (0 mg/d vs. target 330 mg/d prepartum and 500 mg/d postpartum) treatments in a 2 x 2 factorial arrangement. In this study, cows fed BMR corn silage had higher prepartum DMI (14.7 vs. 14.0 kg/d) but similar postpartum DMI (23.3 vs. 23.0 kg/d) than cows fed conventional corn silage (Table 1). However, cows fed

BMR corn silage had higher milk yields during the first 42 days in milk (48.3 vs. 45.8 kg/d; Table 1) and had lower plasma concentrations of NEFA during both the prepartum and postpartum periods and lower BHBA during the postpartum period (Table 2).

Table 1. Main effect means for prepartum and postpartum intake and milk yield for cows fed conventional (CON) vs. brown midrib (BMR) during the transition period and 0 (NO) vs. 330 mg/d of monensin prepartum and 500 mg/d of monensin postpartum (MON). From LaCount et al., 2017.

	Corn Silage			Monensin			*P*-Value		
Item	CON	BMR	SEM	NO	MON	SEM	Corn	MON	C×M×T[1]
Prepartum									
Intake, kg/d	14.0	14.7	0.21	14.5	13.9	0.21	0.03	<0.01	0.85
Postpartum									
Intake, kg/d	23.0	23.3	0.39	23.3	23.0	0.39	0.57	0.55	0.44
Yield, kg/d	45.8	48.3	0.86	46.7	47.4	0.86	0.05	0.61	0.02

[1]Interaction of Corn silage × Monensin × Time, no other interactions for these variables were significant.

Table 2. Prepartum and postpartum NEFA and BHBA presented as geometric means with back transformed 95% confidence limits for cows fed conventional (CON) vs. brown midrib (BMR) during the transition period and 0 (NO) vs. 330 mg/d of monensin prepartum and 500 mg/d of monensin postpartum (MON). From LaCount et al., 2017.

	Corn		Monensin		*P*-Value		
Item	CON	BMR	NO	MON	Corn	MON	C×T[1]
Prepartum							
NEFA,	110.6	94.8	99.3	105.6	0.02	0.35	0.05
μEq/L	101.0-121.3	86.2-104.2	90.4-109.1	96.3-115.8			
BHBA,	0.72	0.70	0.75	0.68	0.62	0.04	0.53
mmol/L	0.68-0.77	0.66-0.75	0.70-0.79	0.64-0.72			
Postpartum							
NEFA,	458.9	369.1	440.0	385.0	<0.01	0.06	0.48
μEq/L	415.7-506.7	334.2-407.7	398.4-486.0	348.7-425.0			
BHBA,	1.22	1.00	1.21	1.00	<0.01	0.01	<0.01
mmol/L	1.10-1.35	0.90-1.11	1.10-1.34	0.91-1.11			

[1]Interaction of Corn × time, no other interactions for these variables were significant.

Evidence from these two trials demonstrates that increased forage fiber digestibility in the periparturient period can positively affect both performance and metabolism throughout the transition period.

TRANSITION COW PERFORMANCE AND METABOLISM AS AFFECTED BY NDF POOLS

During the past several years, the analytical capacity to determine different fractions of NDF (both digestible NDF and undigested NDF at varying timepoints (e.g., $uNDF_{240}$) has become common in commercial forage laboratories. However, little is known specifically about optimum fractions of digestible NDF and uNDF240 for the dairy cow during the immediate postcalving period.

Several years ago, we developed a case study within the context of an experiment designed to assess responses in higher vs. lower starch diets fed during the postcalving period (detailed in McCarthy et al., 2015). Cows originally enrolled in the experiment developed significant metabolic issues postcalving (primarily displaced abomasum and clinical ketosis). Upon investigation, we discovered that the NDF content of both the BMR corn silage and legume silage fed had decreased significantly since the samples used to formulate diets were collected. We modified the rations to decrease the inclusion rate of BMR corn silage and increase wheat straw (Table 3).

Table 3. Ingredient and chemical composition of diets before and after postpartum ration changes (adapted from McCarthy et al., 2015).

	Postpartum diet[1]			
Item	HSLF	LSLF	HSHF	LSHF
Ingredient, % of DM				
BMR corn silage	46.1	46.1	38.5	38.5
Wheat straw	3.8	3.8	11.5	11.5
Legume silage	9.6	9.6	9.6	9.6
Higher starch grain mix	40.5	. . .	40.4	. . .
Lower starch grain mix	. . .	40.5	. . .	40.4
Chemical[2]				
NDF, %	26.4	31.5	34.3	36.9
Starch, %	28.3	22.0	26.2	21.5
30-h dNDF, % of NDF	56.0	59.8	51.4	51.7
30-h dNDF, % of DM	15.9	20.4	17.5	18.5
$uNDF_{240}$, % of DM	7.7	8.9	10.5	10.9

[1] HSLF = high starch, low fiber (pre-change); LSLF = low starch, low fiber (pre-change); HSHF = high starch, high fiber (post-change); LSHF = low starch, high fiber (post-change).

[2] Determined using wet chemistry techniques for all variables (Cumberland Valley Analytical Services, Hagerstown, MD).

Performance and metabolic responses for cows fed higher and lower starch diets before and after the changes to both rations are presented in Table 4. The statistics and results should be interpreted with caution as the comparisons for the fiber main effect were not contemporaneous. However, an interaction of starch and fiber suggested that cows fed the combination of higher starch and high fiber had the highest

DMI, the combination of higher starch and low fiber had the lowest DMI, and DMI for cows fed the other two treatments was intermediate. Increased plasma concentrations of NEFA and BHBA for cows fed the combination of higher starch and low fiber were consistent with the lower DMI and poorer energy status of this group. Furthermore, concentrations of haptoglobin were increased for cows fed low fiber, suggesting greater systemic inflammation. Intakes of $uNDF_{240}$ were distinctly different for cows fed the low- and high fiber and both the $uNDF_{240}$ and 30-h dNDF values were markedly different between the low- and high fiber diets (Table 3).

Table 4. Postpartum DMI, $uNDF_{240}$ intakes, milk yield, and Ingredient and chemical composition of diets before and after postpartum ration changes (adapted from McCarthy et al., 2015).

	Postpartum diet[1]				
Item	HSLF	LSLF	HSHF	LSHF	Effect[2]
DMI, kg/d	17.8	19.9	21.4	20.1	F, S*F
$uNDF_{240}$ intake, kg/d	1.52	1.67	2.25	2.15	F, S*F
$uNDF_{240}$ intake, % of BW	0.26	0.28	0.37	0.36	F, S*F
Milk,kg/d	34.5	40.8	39.2	37.9	S*F
NEFA, uEq/L	646	528	406	493	F, S*F
BHBA, mg/dL	12.3	8.9	9.3	10.7	S*F
Haptoglobin, g/L	1.43	1.71	1.03	0.87	F
Displaced abomasum	4/7	2/10	0/38	0/39	
Clinical ketosis	4/7	1/10	4/38	6/39	

[1] HSLF = high starch, low fiber (pre-change); LSLF = low starch, low fiber (pre-change); HSHF = high starch, high fiber (post-change); LSHF = low starch, high fiber (post-change).
[2] F = effect of fiber (pre-change vs. post-change); S*F = interaction of starch and fiber.

These results led us to want to explore the relationships of NDF pools in postpartum diets in a more controlled manner, so LaCount et al. (2017) formulated diets to be either low fiber or high fiber through replacement of conventional corn silage with straw (Table 5). Respective treatment diets were fed through 28 DIM, after which all cows were fed the low fiber diet. In this case, cows fed the high fiber diet had lower postpartum DMI along with lower milk yield during the immediate postpartum period (Table 6). Furthermore, cows fed high fiber had higher plasma NEFA, higher BHBA, lower glucose, and a greater degree of negative energy balance while consuming the high fiber diet. After 28 DIM once cows assigned the high fiber diet were switched to the lower fiber diet, the cows were able to quickly meet the performance and metabolic status of cows fed low fiber throughout the study. Clearly, in this experiment we exceeded the $uNDF_{240}$ needs of the cows fed the high fiber diet such that we negatively influenced DMI and metabolism. Two important differences between this experiment and the previous case study were that we utilized conventional corn silage as the base forage in the controlled study and that retrospective analysis of the alfalfa hay that we utilized revealed that this particular hay had a $uNDF_{240}$ value of about 50% of the NDF. This increased the $uNDF_{240}$ value of both postpartum diets in excess of our targets.

Table 5. Ingredients and nutrient profile of rations (mean ± SD), obtained through wet chemistry analysis and in vitro fermentation (LaCount et al., 2017).

	Diet		
Item	Prepartum	Low Fiber (LF)	High Fiber (HF)
Ingredients, % of ration DM			
Conventional corn silage	45.21	42.31	38.46
Alfalfa hay	-	10.58	10.58
Straw	20.84	1.15	8.65
Corn meal	2.43	17.64	20.15
Soybean meal	-	6.03	4.73
Wheat middlings	-	4.82	1.58
Amino Plus	5.9	4.34	5.31
Canola meal	3.47	1.61	3.88
Corn gluten feed	1.74	1.61	0.47
Blood meal	2.43	0.95	1.09
Soybean hulls	6.95	2.41	-
Citrus pulp	4.52	-	0.79
Energy Booster	-	1.29	1.58
Rumensin, mg/d[1]	439	365	334
Other	6.4	2.3	2.3
Analyses, % of ration DM			
aNDFom	43.1 ± 0.3	32.8 ± 1.4	35.3 ± 2.3
ADF	29.0 ± 0.5	21.3 ± 1.1	22.9 ± 2.1
Starch	15.6 ± 0.3	24.8 ± 1.7	24.6 ± 2.3
Sugar	3.5 ± 0.4	5.0 ± 0.7	3.9 ± 0.1
Fat	2.3 ± 0.2	3.3 ± 0.2	3.2 ± 0.2
$uNDF_{240}$	12.8 ± 0.5	9.5 ± 0.4	12.2 ± 1.6
peNDF	33.3	21.6	23.2
MP, g/kg DM[1]	89.0	112.1	108.0

[1] Formulated value given by Cornell Net Carbohydrate and Protein System v. 6.55 using actual mean intakes

Table 6. The effect of low fiber and high fiber diets in the early postpartum period on intake, milk yield, and rumination for 1 to 6 wk postpartum (LaCount et al., 2017).

				P-Value	
Item	LF	HF	SEM	Trt	Trt×Time
Prepartum DMI, kg/d	15.5			-	-
Postpartum DMI, kg/d	23.6	22.2	0.3	0.002	<0.001
uNDF intake, %BW[1]	0.29	0.34	0.01	<0.001	0.047
Milk yield, kg/d	46.2	44.7	1.0	0.26	0.001
Fat, %	3.72	3.87	0.85	0.20	0.14
Protein, %	3.09	3.02	0.05	0.30	0.56
Total solids, %	12.57	12.64	0.13	0.70	0.33
ECM, kg/d	48.2	47.3	1.1	0.55	0.12
Rumination, min/d	544	543	8	0.90	0.14

[1]Calculated only for weeks where treatment diets differ (1 to 4 wk)

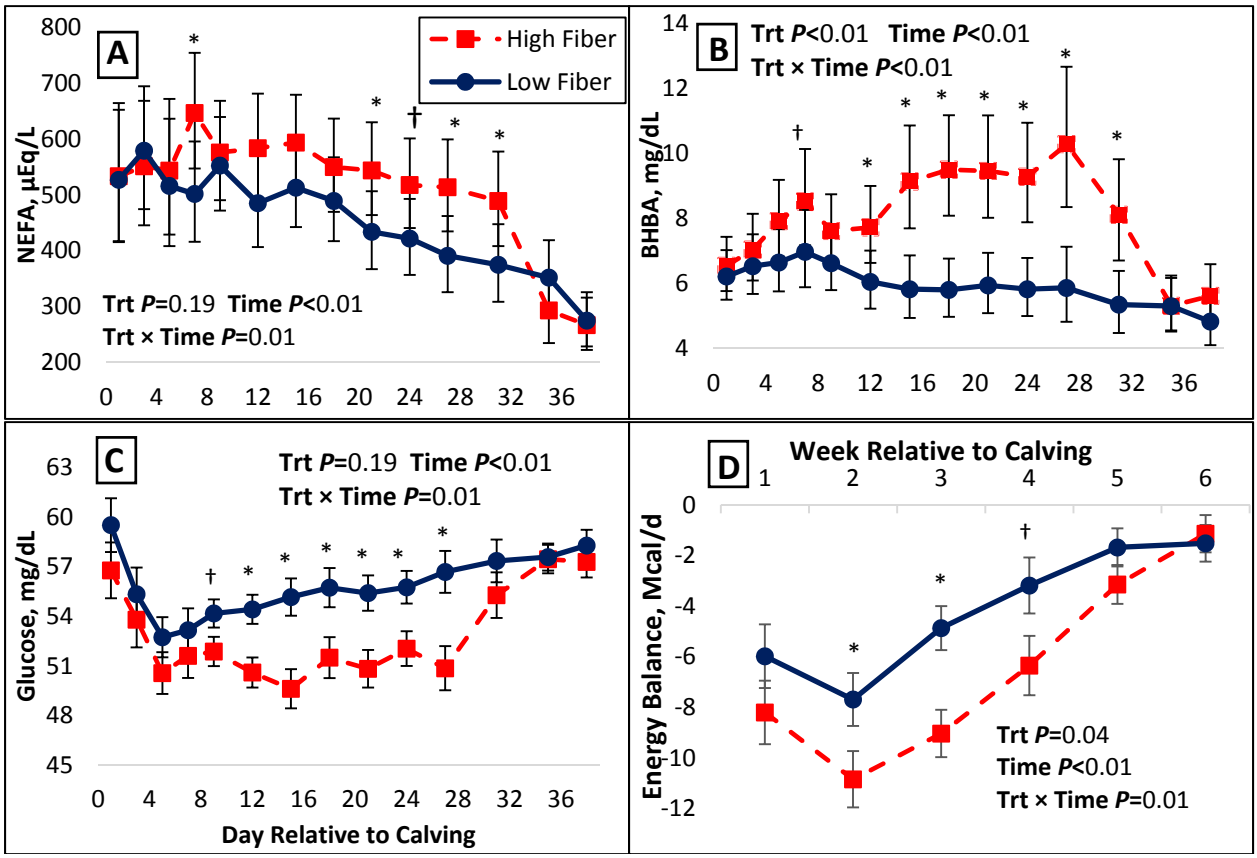

Figure 1. Plasma NEFA (A), BHBA (B), glucose (C), and energy balance (D) for cows fed high fiber and low fiber diets postpartum. Significant differences indicted with an asterisk (*), trends with a cross (†). From LaCount et al., 2017.

The results of these two studies, while not establishing definitive limits or ranges, are critical to begin understanding the role of $uNDF_{240}$ in fresh cow diets. More research in this area is needed, including characterization of all pools of NDF to understand how

we can utilize different pools to affect the performance and metabolism of the transition cow.

SUMMARY AND CONCLUSIONS

In summary, replacement of forage NDF with nonforage fiber sources during the prepartum period generally has increased prepartum DMI, but has translated into minimal differences in postpartum performance or metabolism. In contrast, research generally suggests that increasing forage NDF digestibility during the transition period results in higher prepartum DMI along with higher postpartum DMI, milk yield, and better energetic status. Increased fiber digestibility needs to be accompanied by sufficient $uNDF_{240}$ intake in order to maintain ruminal adaptation to postpartum diets, although more controlled research is needed to refine recommendations for NDF pools for use in diet formulation during the immediate postpartum period.

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IMPACT OF IMPROVED FIBER UTILIZATION ON PERFORMANCE

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Better genetics and improved forage management have helped to increase NDF digestibility on many U.S. dairy farms. The addition of supplemental fibrolytic enzymes at ensiling or feeding can further advance NDF digestibility. With better NDF digestibility of farm forages, ration grain levels can be reduced and rumen health can be improved, helping to control ration costs and enhance production efficiency.

Performance response to highly digestible NDF will depend on a number of factors including: rate and extent of NDF digestion, level of dietary undigested NDF (uNDF240om), physically effective NDF, and level of dietary starch as well as rate and extent of starch digestion. These factors will affect intake, nutrient availability, fiber and grain passage rate, rumen pH, and milk production. Physically effective fiber and digestible carbohydrate must be balanced to optimize rumen function, intake, milk and milk components. If diets with higher NDF digestibility do not maintain or improve this balance, production response will be compromised.

RATE AND EXTENT OF NDF DIGESTION

A number of factors influence NDF digestibility. Legumes have less total NDF but due to greater lignification, have lower NDF digestibility. Grasses, including corn silage, have less lignin and large ranges in maturity contributing to a large range in NDF digestibility. With plant maturation, fiber content increases and NDF digestibility decreases. Cooler weather promotes NDF digestibility. High corn plant density reduces NDF digestibility. Plant genetics also influences NDF digestibility, most notably in the highly digestible brown midrib (BMR) corn silage. Adding fibrolytic enzymes at ensiling or to the TMR is a relatively new tool on the market to improve NDF digestibility.

Table 1. Ranges in *In Vitro* NDF Digestibility (% NDF)

	48-hour NDF Digestibility	30-hour NDF Digestibility
Legume Hay	40-59	39-56
Grass Hay	31-78	22-62
Legume/Grass Silage	37-59	-----
Corn Silage	54-64	44-52
BMR Corn Silage	64-73	-----
Straw	------	23-31

Adapted from data bases from the University of Wisconsin and University of California-Davis

High fiber diets may physically limit DM intake due to increased chewing time requirements and reticulo-rumen distension in post-transition cows experiencing

negative or slightly positive energy balance (Allen, 1996; Allen et al., 2009). With higher NDF digestibility, plant cells should take up less space in the rumen and pass from the rumen more quickly, allowing greater NDF intake. In the past, many nutritionists predicted intake as a function of the NDF content of the ration. Dairy cows with high energy demands that were limited by rumen fill constraints were found to produce the most 4% fat-corrected milk when they ate 1.2% of their body weight as NDF (Mertens, 1985). It is now recognized that high-producing cows will eat more than 1.2% of their body weight as total NDF when the NDF is highly digestible. Gencoglu et al. (2010) found that cows ate up to 1.5% of their body weight as NDF when a large portion of the NDF came from highly digestible soy hulls (Table 2). Similar improvements in NDF intake have been seen with highly digestible forages, with NDF in a high BMR silage diet consumed at 1.53% BW and pasture at 1.80% BW (Grant, 2013).

Table 2. Effect of Added Dietary Soy Hulls on Intake and Performance (Gencoglu et al., 2010)

	Normal Starch Diet	Reduced Starch Diet	*P*-value
Soy Hulls (% Diet DM)	3.6	12.7	--
NDF (%DM)	30.6	36.6	--
Forage NDF (%DM)	20.7	20.7	--
Starch (%DM)	27.1	21.8	--
NDF Intake (kg/day)	8.2	10.7	0.01
NDF Intake (% of Body Wt.)	1.19	1.52	0.01
3.5% FC Milk Yield (kg/day)	46.2	49.1	0.02
Dry Matter Intake (kg/day)	26.7	29.1	0.02

UNDIGESTED NDF

uNDF240om is the undigested NDF remaining after fermenting a forage sample in a flask with rumen bacteria for 240 hours. There are three purposes for uNDF240om in ration balancing. First, uNDF240om can be used to better predict how much NDF is digested to yield energy for the cow. Second, uNDF240om can be used to predict DM intake. Third, uNDF240om can help assess acidosis potential and rumen passage of feeds. Higher levels of uNDF240om can limit intake while lower levels of uNDF240om can increase rumen acidosis and reduce feed efficiency. Researchers are currently suggesting that high-producing cows will consume an amount of uNDF240om equal to 0.35 to 0.40% of their body weight (Cotanch, 2015). Ideal amounts will vary depending on herd factors such as feeding management. Tracking intake of uNDF240om by farm pens can be very helpful for predicting intake and milk production when forages change.

PHYSICALLY EFFECTIVE NDF

Fiber with adequate (effective) length helps the cow in many ways. It stimulates chewing and saliva production. The saliva neutralizes rumen acids and increases rumen pH so that the rumen bacteria can function well. Physically effective NDF forms a rumen mat that slows passage of grains and increases their digestibility. Physically

effective NDF also facilitates movement of rumen contents to promote volatile fatty acid absorption out of the rumen.

Physically effective NDF does not explain all of the variation in the chewing value of forage. There are differences in the rate at which NDF is reduced in particle size during chewing. Mertens (1997) summarized research showing that cows had to chew (eat + ruminate) every kg of grass and alfalfa hay NDF between 111-165 minutes. However, for oat straw it took 200 minutes to chew a kg of NDF.

Fewer minutes of eating and ruminating per kg NDF have been observed with BMR corn silage (57% NDFd24h) versus conventional corn silage diet (42% NDFd24h) when fed at 43% of diet DM (Table 3) (Cotanch, 2010). This reduction in chewing time may have been the cause of higher DM intake with the BMR corn silage diet (McLeod et al., 1990). Furthermore, improvements in forage fiber digestibility may have increased rate of passage negating a portion of the benefits provided by increased digestibility and reducing feed efficiency. This indicates that with the higher NDF digestibility, a greater amount of physically effective NDF may be helpful (Allen,1995).

Table 3. Effect of Corn Silage Hybrid on Intake, Performance, and Rumination (Cotanch et al., 2010)

	Conv. Corn Silage	BMR Corn Silage	*P*-value
DM Intake, kg/d	25.2	27.8	0.001
NDF Intake, %BW	1.01	1.17	<0.001
Milk, kg/d	40.6	42.2	NS
Fat, %	3.62	3.71	NS
Milk/DMI kg/kg	1.60	1.50	0.03
Eating Min/kg NDF	31	26	<0.001
Ruminating Min/kg NDF	70	58	<0.001

DIETARY STARCH

The optimum rumen available starch level depends on many factors including: level of production, DIM, physically effective NDF, uNDF240om, fat, protein, soluble fiber, sugar, farm management, and feeding behavior. Too little digested starch yields insufficient propionate, lactose, energy, and microbial protein. Possible consequences of too much rumen digested starch include rumen acidosis, laminitis, milkfat depression, reduced intake, and reduced fiber digestion. With higher NDF digestibility, more energy will be derived from fiber, reducing the amount of energy needed to be supplied from starch. If uNDF240om is reduced with higher NDF digestibility, slightly lower dietary starch levels may improve rumen health.

IMPACT OF IMPROVED FIBER UTILIZATION ON PERFORMANCE

In the classic analysis of Oba and Allen (1999), increasing *in vitro* or *in situ* NDF digestibility of ration forage by one percentage point increased DM intake by 0.17 kg/day and 4% fat-corrected milk yield by 0.25 kg/day. Level of milk production will affect production responses with higher producing cows and early lactation cows

expected to improve the most with increased NDF digestibility (Grant, 2013). Furthermore, production response to improved NDF digestion will be highest when initial NDF digestibility is low and energy is the first-limiting nutrient in the diet (Beauchemin et al., 2003).

Unfortunately, many of the research studies with highly digestible NDF have not fully characterized the diets in terms of physically effective NDF, uNDF240om, rate and extent of NDF digestibility, and level of dietary starch as well as rate and extent of starch digestion. When improving NDF digestibility, characterizing and balancing diets for these fractions will help to optimize and understand rumen function, intake, milk and milk component responses.

When Oba and Allen (2000) replaced conventional corn silage (46.5% NDFd30h) with BMR corn silage (56% NDFd30h) at two dietary NDF concentrations (29 and 38%), milk production was increased (32 vs. 35.3 kg) but milk fat percentage was reduced (3.79 vs. 3.57%). Rumen pH was reduced with the BMR corn silage (5.84 vs. 5.68). Improvements with BMR corn silage were greater with the higher NDF diet.

The effect of BMR corn silage on performance during the first 180 days of lactation was tested using a diet containing 35% corn silage and 25% alfalfa hay (Holt et al., 2013). During the first 60 DIM, DM intake was not affected by treatment. However, post-peak DM intake tended to be higher for cows fed BMR corn silage (25.8 vs. 24.7 kg/d). Cows fed BMR corn silage maintained their body weight better during the first 60 DIM. Although milk yield was similar during the first 60 DIM, milk yield was higher for cows fed BMR corn silage from 61 to 180 DIM (41 vs. 38.8 kg/d). Post-peak milk fat concentration was lower for the BMR corn silage diet (3.47 vs. 3.80%).

Pre-treating with fibrolytic enzymes (0.75 mL of FETR/kg TMR) improved NDF digestibility (34.7 vs. 39.9% NDFD48h) in a diet containing 34% barley silage with 29.5% dietary NDF (Refat et al., 2018). This digestibility improvement resulted in greater milk fat yield (1.21 vs. 1.35 kg/d) and fat-corrected milk yield (36.35 vs. 38.87 kg/d) while DM intake was unchanged (27.26 vs. 27.64 kg/d).

A normal starch diet was compared to diets with a greater amount of either highly digestible NDF from BMR corn silage or non-forage fiber (Dann et al., 2015). The results are shown in the Table 4. Higher NDF digestibility in combination with a high forage diet resulted in fat-corrected milk yield similar to that of a lower forage diet. There was no effect of diet on total chewing, eating, and rumination time. However, rumination time per kg NDF was greatest for the control diet and least for the non-forage fiber diet. Mean rumen pH was not affected by treatment but the control diet resulted in more time below a pH of 5.5.

Table 4. Effect of Alternate Carbohydrate Sources on Intake and Performance (Dann et al., 2015)

	50% Forage Control	63% Forage BMR	50% Forage Non-forage Fiber	*P*-value
NDF, %	34.7	38.3	38.0	--
ADL, %	2.7	2.7	2.9	--
peNDF, %	18.6	24.5	22.3	--
Starch, %	26.0	21.4	21.3	--
NDFd24h, %NDF	54.1	53.0	51.6	--
DMI, kg/d	28.1^{x}	27.1^{y}	27.6^{xy}	0.08
NDF Intake, kg/d	9.1^{b}	10.0^{a}	9.9^{a}	0.001
NDF Intake, %BW	1.23^{b}	1.35^{a}	1.34^{a}	0.001
Milk yield, kg/d	51.6^{a}	48.4^{b}	50.5^{ab}	0.01
Fat, %	3.66^{y}	3.98^{x}	3.76^{xy}	0.07
3.5% FCM, kg/d	52.6	51.7	52.4	0.78
3.5% FCM/DMI	1.86	1.90	1.90	0.72

A meta-analysis was conducted using 17 experiments to determine the effect of fibrolytic enzymes on dairy cow performance (Arriola et al., 2016). Addition of fibrolytic enzymes tended to improve DM and NDF digestibility. Milk yield was slightly improved. However, as others have previously indicated (Beauchemin et al., 2003), there was considerable variation in milk and milk component responses. A number of proven fibrolytic enzymes are now commercially available. Further success of fibrolytic enzymes on commercial dairies will depend in large part on balancing diets for best rumen function and intake.

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BRINGING THE LAB TO THE FARM: DETERMINING FORAGE NUTRIENT COMPOSITION USING NEAR INFRARED TECHNOLOGY

C. Piotrowski
AB Vista

The ultimate objective of forage or feed analysis is to predict animal performance from the forage and feed used in a ration formulated to achieve an appropriate balance where the total rations nutrient composition meets the daily nutritional needs of the animal.

To achieve this on a daily basis, one needs to have some information as to the nutrient content of feed ingredients. Tremendous variation exists in nutrient composition between different feeds. Even within a feed ingredient, there is potential for significant variation in composition. This is especially true for forages. Forages harvested from the same field within the same year can have very different compositions, due to the influence of environmental conditions and cutting time. This variation can be clearly seen in work published by Trouw Nutrition in 2013 (Figure 1).

Figure 1: Schematic of 5 different sampling points across the silage clamp. (Trouw Nutrition 2013).

Table 1: The % Dry Matter of forage samples taken at 5 different sampling points across the silage clamp.

Sampling Point	% Dry Matter	Sampling Point	% Dry Matter
1	28.7	6	36.1
2	33.5	7	27.7
3	25.2	8	35.5
4	34.8	9	30.9
5	26.8		

Analyzing at different sampling points across the face of the silage shows how much variation can be seen in dry matter alone. Trouw went on to conclude: *'The level to which the grass silage quality changed through the winter was also significant. This had the potential to reduce milk yield by over 500 liters/cow/winter if diets were not checked and amended….'*

There is a wide variety of biological, chemical, enzymatic, and other sophisticated analytical methods that can be used to evaluate nutrient content and availability. Chemical methods can directly measure quantities of compounds associated with an essential nutrient such as nitrogen for protein determination; however, they tells us nothing about availability. Biological, enzymatic, and other sophisticated methods provide a more nutritional perspective helping us to better understand just how animal performance will be impacted. The downside to many of these methods is that it takes considerable time, money and effort to carry out the determinations. As an example, whilst protein analysis can be undertaken in a day, a full structural carbohydrate analysis including ADF and NDF can take over one week to complete.

Although wet chemistry analysis is considered the "gold standard" for feed testing, simpler and less expensive methods with shorter turnover time are needed. Near infrared reflectance (NIR) spectroscopy is a rapid, non-destructive method of analysis requiring minimal sample preparation that was first used to predict forage quality in 1976. NIR has been approved by the Association of Official Analytical Chemists (AOAC)[2] for use in determining moisture (AOAC 991.01), Kjeldahl nitrogen and acid detergent fiber (AOAC 989.03) for feed and forage analysis

In general, NIR analysis has high accuracy in measuring dry matter, crude protein and fiber fractions compared to wet chemistry, but is less accurate in measuring feed mineral content. Many certified feed analysis laboratories are capable of completing wet chemistry, NIR analyses, or both. The debate over wet chemistry versus NIR always revolves around accuracy, however in the case of forage analysis the real debate should be about sampling variability.

Dan Undersander, University of Wisconsin, in his paper entitled 'Uses and Abuses of NIR for Feed Analysis'[3] (Undersander, 2006) Reported that in a well-constructed ring test involving 135 laboratories showed Errors of 0.8, 1.4 and 2.3 % for CP, ADF and NDF, respectively. In the majority of cases, samples are not taken correctly and comparisons between testing labs using wet chemistry or NIR can be considerably higher than this. One way of increasing the accuracy of measurement is to take more samples, the accuracy of determining a nutrient is directly proportional to the number of samples measured.

In the UK alone over 60,000 samples of forage are analyzed in a season at an average cost of £15-£20 per samples this costs the industry over £1million. These costs are based on NIR analysis. If undertaken by wet chemistry the costs would be well over £100 per sample. Given the variability in the samples and analysis, the industry needs to be looking to more time and cost effective solutions. The need for analysis is clear; we know that if the forage is not rationed correctly based on its nutrient analysis then animal performance will be lost, either in terms of milk production, condition or fertility. The solution is obvious, a low cost accurate analysis that can be undertaken daily to maximize the benefits of the changing nutrients available.

In its report of 2006 and update of 2010, Strategic Directions[4] estimated the global market for laboratory NIR spectroscopy had grown by a compound annual growth rate (CAGR) of 4.5%. Over the same period, the market for portable and handheld spectrometers had grown from $12m to $37million, a CAGR of 45%. Portable spectrometers now represent nearly 20% of the total NIR instrument market.

The aforementioned 2010 report indicated that the major application area for portable instruments was Agriculture and Food (34%), mirroring the extensive use of other forms of NIR spectroscopy within the industry. Portable NIR, especially in a form that can be used for example, on farm, supports the overall precision farming agenda, delivering both financial and environmental benefits to the farmer through the more accurate use of nutrients, fertilizers etc., and their ability to lower the environmental impact through better management of farmyard waste. Whilst portable units are not new, they have to date been mostly used for qualitative purposes where their often more limited wavelength coverage has not restricted their usage. Exploitation of the true power of NIR in sectors such as food, feed and agriculture for quantitative purposes has been less rapid. However, change occurs rapidly and there are now a number of portable instruments such as the AgriNIR™ and the NIR4 system designed specifically for use in the agricultural market, representing a turning point in the use of NIR in this sector. Already used extensively in many other applications, these truly portable instruments are lightweight, yet extremely robust. Pre-loaded with calibrations, this new application of NIR presents a solution to the problem of accurate daily measurement and nutrient management.

NIR4 is an extremely light weight instrument linked to an android tablet and a web based application. Samples can be analyzed in real-time on farm or in a feed mill and the analysis shared with a nutritionist so that daily checks to the ration can be optimized for maximum benefits.

Figure 2: Portable NIR being used for Forage analysis on farm

This portable NIR can be used for a range of different forages (including fresh grass, ensiled grass, maize silage and whole wheat silage) providing accurate analysis for, Dry matter, protein, ADF,NDF, starch, digestibility, pH and fermentation characteristics including lactic and ammonia.

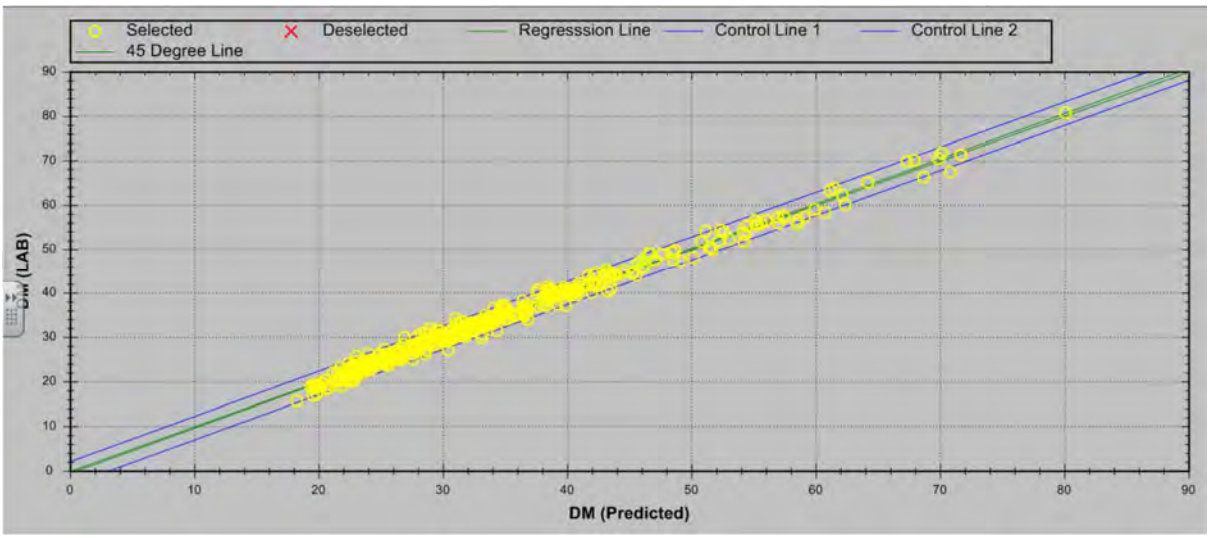

Figure 3: Graph plotting NIR predicted values (x axis) versus Lab wet chemistry values (y axis) illustrating the accuracy of this technique for dry matter in grass silage.

Dry Matter calibration plot for grass silage n=993, RSQ =0.986, SEC = 1.76, RPD = 8.4

In addition to forage analysis, extra value can be gained from the fact that the same hardware can be used to measure moisture, protein, oil, fiber starch and ash in a range of raw ingredients such as cereal, by-products, oil seeds and other protein sources along with compound animal feed.
This unique speed and versatility of NIR means that we can achieve the ultimate goal in assessing the total rations nutrient composition on a daily basis measuring the exact components being fed to the animal.

More importantly this new breed of miniature NIR instruments will eventually be marketed to the consumer as a means of checking the nutritional value of food and goods purchased from retailers. The announcement by the Fraunhofer Institute for Photonic Microsystems in Germany in May 2012 of a MEMS-based spectrometer is paving the way for NIR spectrophotometers embedded into the mobile phone units, testing your daily intake of calories will be as common as taking a snap shot.

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