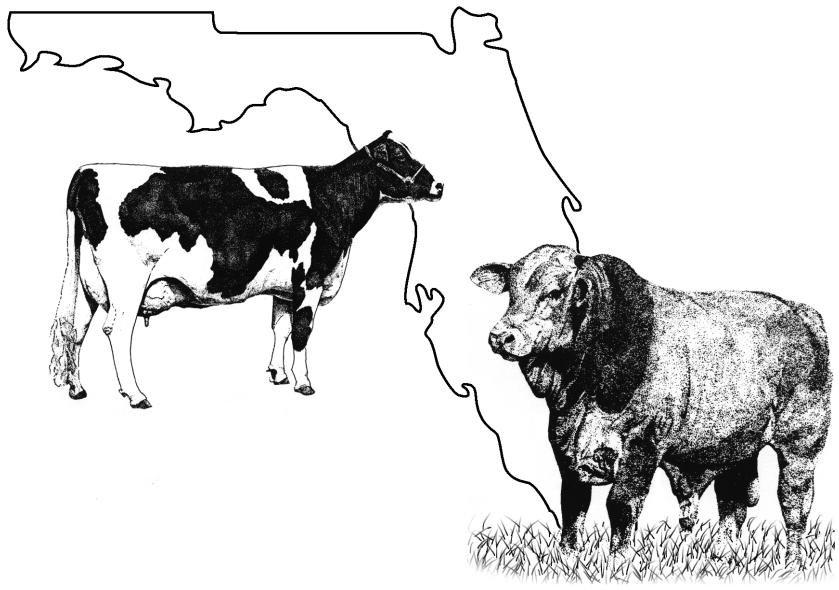
2018 Florida Ruminant Nutrition Symposium

29th Annual Meeting



February 5-7, 2018
Best Western Gateway Grand
Gainesville, Florida

PROCEEDINGS

UF UNIVERSITY of FLORIDA
IFAS
Department of Animal Sciences

2018

29th ANNUAL FLORIDA RUMINANT NUTRITION SYMPOSIUM

February 5 - 7, 2018
Best Western Gateway Grand Hotel
Gainesville, Florida

Department of Animal Sciences
University of Florida
Institute of Food and Agricultural Sciences
Gainesville, Florida 32611

Florida Ruminant Nutrition Symposium – February 5 to 7, 2018

February 5, Monday – 1:30 to 5:30 pm

"Challenges With Assessing Nutrient Bioavailability in Ruminants?"
Pre-Symposium sponsored by Balchem Corporation

1:30 pm - Clay Zimmerman, Balchem Corp. *Welcome and Introductions*

1:35 pm - **Bill Weiss**, The Ohio State University. *"Assessing Bioavailability of Minerals"*

2:25 pm - **Richard Erdman**, University of Maryland. *"What Do We Know About Balancing Dairy Rations for Methyl Donors"*

3:15 pm – Refreshment Break

3:30 pm – **Charlie Staples**, University of Florida. *"Challenges in Assessing Choline Bioavailability"*

4:20 pm - **Mark Hanigan**, Virginia Tech. *"Methods of Assessing Amino Acid Bioavailability – What Works and What Doesn't"*

5:10 pm – Clay Zimmerman. *Summary and Wrap-up.*

5:30 pm – Poolside barbeque

February 6, Tuesday - 8:30 to 11:45 am

"*Capitalize on Insights Into Amino Acid Balancing*"
Pre-Conference Sponsored by Ajinomoto Heartland

8:10 am - *Welcome and Objective*

8:15 am - **Luiz Ferraretto**, University of Florida. *"Impact of Essential Amino Acid Balancing Postpartum on Lactation Performance by Dairy Cows"*

8:55 am - **Hugo Ramirez**, Iowa State University. *"Gut Integrity During Periods of Stress and Its Implications on Performance"*

9:35 am - **Johan Osorio**, South Dakota State University. *"Amino Acid Balancing and Its Role on Metabolism, Inflammation, and Oxidative Stress: Future Molecular Implications"*

10:15 am - **Monty Kerley**, University of Missouri, Emeritus. Consultant to Gladwin A. Read Co (GARCO). *"Influence of Amino Acid Nutrition on Milk Efficiency"*

10:55 am - **Jessica Tekippe**, Ajinomoto Heartland, Inc. *"Setting Yourself Up for Success With Amino Acid Balancing"*

February 6, Tuesday - 1:00 to 5:00 pm

1:00 pm – **Charlie Staples**, University of Florida. *Welcome.*

1:10 pm - **Jose Santos**, University of Florida. *"Prepartum Negative DCAD Diets – They're Not Just for Milk Fever Anymore - A Meta-analysis"*

1:50 pm – **Josh McCann**, University of Illinois. *"New Perspectives on Adapting Cattle to Finishing Diets Without Compromising Rumen Health"*

2:30 pm – **Kevin Folta**, University of Florida. *"Communicating With the Public About Animal Agriculture Technology"*

3:10 pm – Refreshment Break

3:40 pm – **Victor Cabrera**, University of Wisconsin. *"Are There Financial Advantages of Grouping and Feeding Dairy Cows by Nutritional Need?"*

4:20 pm – **Bill Weiss**, The Ohio State University. *"Trace Minerals and Vitamins for Dairy Cows"*

5:00 pm – Welcome Reception

February 7, Wednesday - 8:00 to 11:50 am

8:00 am – **Adam Lock**, Michigan State University. *"Fat Supplementation to the Periparturient Dairy Cow: Does Fatty Acid Profile Matter?"*

8:40 am – **Anne Laarman**, University of Idaho. *"Dietary Effects on Ruminal Papillae During Periparturient Transition in Holstein Cows – Is Cow Performance Affected?"*

9:20 am – **Ian Lean**, Scibus, New South Wales. *"Ruminal Acidosis – Much More Than pH."*

10:00 am – Refreshment Break

10:30 am – **Jon Schoonmaker**, Purdue University. *"Use of Novel Feed Additives in Beef Cattle Production"*

11:10 am – **Antonio Faciola**, University of Florida. *"Canola Meal as a Protein Source for Lactating Dairy Cows"*

11:50 am - Adjourn

2018 Symposium Speakers

Guests

Victor Cabrera, University of Wisconsin-Madison
Richard Erdman, University of Maryland
Mark Hanigan, Virginia Tech
Monty Kerley, Nutritional Counsultant
Ann Laarman, University of Idaho
Ian Lean, Scibus New South Wales
Adam Lock, Michigan State University
Josh McCann, University of Illinois
Johan Osorio, South Dakota State University
Hugo Ramirez-Ramirez, Iowa State University
John Schoonmaker, Purdue University
Jessica Tekippe, Ajinomoto Heartland, Inc.
Bill Weiss, The Ohio State University

University of Florida
Department of Animal Sciences

Antonio Faciola
Luis Ferraretto,
Kevin Folta
José Santos
Charles Staples

29th Annual Florida Ruminant Nutrition Symposium

Table of Contents

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Dr. Victor E. Cabrera is an associate professor and extension specialist in dairy management at the University of Wisconsin-Madison Dairy Science Department. Dr. Cabrera combines applied research, interdisciplinary approaches, and participatory methods to deliver practical, user-friendly, and scholarly decision support tools for dairy farm management. These scientific tools are aimed to improve dairy farm profitability, environmental stewardship, and long-term sustainability of the dairy farm industry. During his short career, Dr. Cabrera has developed more than 40 decision support tools, published 54 refereed articles, and 5 book chapters, presented in more than 100 scientific sessions, and given talks in more than 170 extension meetings in Wisconsin, other States, and several other countries. Dr. Cabrera's work in the past 8 years has been pivotal to attract more than $4.0 million to support his research and extension initiatives. Dr. Cabrera has been distinguished with the University of Wisconsin-Madison Vilas Faculty Mid-Career Investigator Award, Second Mile Extension award of the Wisconsin Association of County Agricultural Agents, the Pound Extension Award and the Alfred Toepfer Faculty Fellow Award from the University of Wisconsin College of Agriculture and Life Sciences, the Distinguished Achievement Award from the University of Florida School of Natural Resources and Environment, and the Foundation Scholar Award in Dairy Production from the American Dairy Science Association.

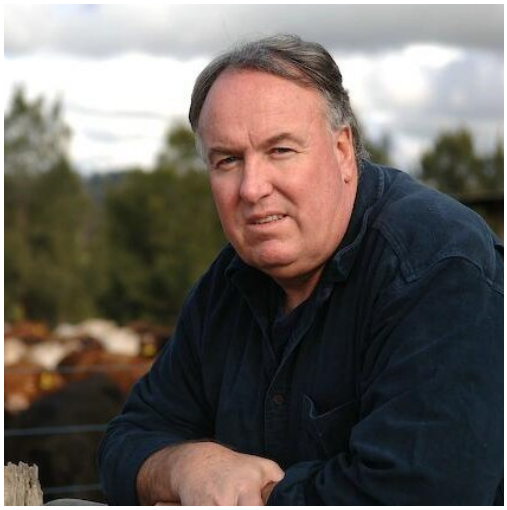
Dr. Antonio Faciola is an Assistant Professor of Livestock Nutrition in the Department of Animal Sciences at the University of Florida. Prior to joining UF in the summer of 2017, Dr. Faciola served on the faculty at the University of Nevada for 4 years. He grew up in a ranch in the Brazilian Amazon, where his family raised water buffaloes, beef and dairy cattle for over 100 years. He received B.S. and M. S. degrees in Animal Sciences from the Federal University of Viçosa, Brazil, a Ph.D. in Dairy Science from the University of Wisconsin-Madison, and was a postdoc at the ARS-USDA U.S. Dairy Forage Research Center. The overall research goal of his laboratory is to further our understanding of ruminant nutrition to improve the efficiency of nutrient utilization in order to enhance animal production and minimize environmental impact of livestock operations. Projects in his lab include evaluating canola meal as a protein supplement for dairy cows, feeding different oilseeds, and determining the nutritional value of different forages for dairy cows. Methodological approaches in his lab include the dual-flow continuous culture system and the omasal sampling technique. He has been an invited speaker in the Brazil, Canada, Italy, Kazakhstan, South Africa, Turkmenistan, U.S.A., and Uzbekistan, and is an Ad Hoc reviewer for over a dozen scientific journals including the Journal of Dairy Science and Journal of Animal Science, and is currently an Associate Editor for Frontiers in Microbiology and Scientia Agricola. He was awarded the 2016 Researcher of the Year and the 2017 Early Career Innovator in Nevada. For more details on his research projects, lab personnel, teaching, and publications, please visit his website at: www.faciola.com

Dr. Luiz Ferraretto is originally from Brazil where he earned his B.S. degree in Animal Science from São Paulo State University in 2008. Immediately after completion of his B.S. Degree, Luiz joined the University of Wisconsin-Madison for an internship (2009) followed by earning the M.S. (2011) and Ph.D. (2015) degrees in dairy science with focus on applied dairy nutrition. After completion of his Ph.D., Luiz joined The William H. Miner Agricultural Research Institute as a Post-doctoral Research Associate. Currently, Luiz is an Assistant Professor of Livestock Nutrition in the Department of Animal Sciences at the University of Florida. His research interests include applied dairy cattle nutrition and management with emphases on starch and fiber utilization by dairy cows, corn silage and high-moisture corn quality and digestibility, the use of alternative by-products as feed ingredients, and supplementation of amino acids and feed additives to lactating dairy cows.

Kevin M. Folta is a Professor and the Chairman of the Horticultural Sciences Department. His research program examines how light signals are sensed in plants and his group uses novel genomics approaches to identify genes related to flavor and disease resistance in small fruits. An innovative new project is testing a method to create new small-molecule drugs for use in everything from plant growth regulation to MRSA. In 2016 he was recognized with the prestigious CAST Borlaug Award in Agricultural Communications, in recognition of his workshops to train scientists, ag producers, and medical professionals about contentious issues communication. He also hosts the weekly podcast *Talking Biotech* (www.talkingbiotech.com).

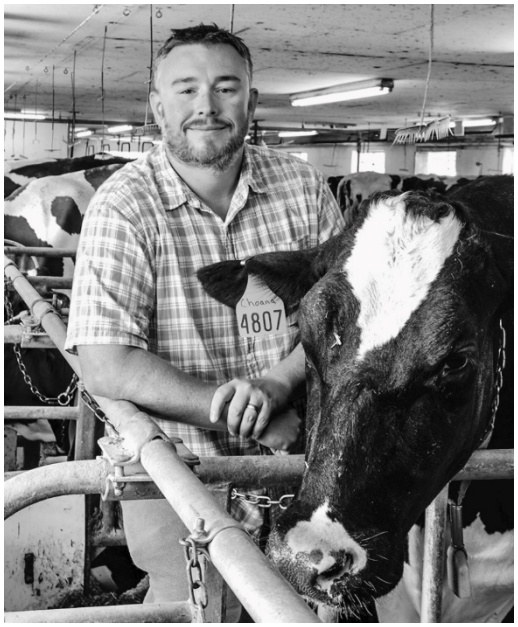
Dr. Monty Kerley, PhD, earned his Bachelor of Science degree from Southern Illinois University-Carbondale and his MS and PhD degrees from the University of Illinois. In 1987 he joined the Animal Science Department at the University of Missouri. His major research focus has been understanding the nutritional and metabolic influences on gain efficiency of cattle. The two major thrusts of this focus has been (1) studying mitochondrial relationship to residual feed intake (RFI) differences among individual animals and impact of selection for RFI on growth performance and (2) development of a diet formulation approach to meet amino acid requirements of cattle based upon energy consumption. Dr. Kerley retired in 2017 and is currently involved in cow-calf production and consults for beef and dairy nutrition entities.

Dr. Anne Laarman is an assistant professor in the Department of Animal and Veterinary Science at the University of Idaho. He received his BSc (physiology and developmental biology) and MSc (animal science) at the University of Alberta and his PhD (animal physiology) at the University of Guelph. He joined the University of Idaho in 2015. Dr. Laarman's expertise is on the interface of nutrition and physiology, focusing on development and dietary adaptation of the ruminant gastrointestinal tract. Dr. Laarman's research often targets the weaning transition and the calving transition, when dietary changes place great strain on the rumen. Current and past research experience includes short-chain fatty acid transport mechanisms and their effect on ruminal acidosis, epithelial integrity, and immune response. His current research program focuses on the development and adaptability of nutrient uptake and gut health, and the role feed additives and management strategies in optimizing cattle performance during dietary transitions.



IAN J. LEAN BVSc (Syd), DVSc (Syd), PhD (Calif), MACVSc
Ian's general interests are in improving the profitability of ruminant production. He is Managing Director of Scibus, a company that conducts research and consults to dairy and beef producers, within and outside of Australia. Scibus is recognized for leadership and excellence in acidosis, meta-analytic and transition cow research. Scibus work with public and private research organizations and consult to a substantial part of the Australian Dairy Industry and to large beef herds. Ian is:

- a past president of the Australian Association of Cattle Veterinarians and the Cattle Chapter of the Australian College of Veterinary Scientists and sits on boards of other industry bodies.
- He has been on faculty at Universities of California and the University of Sydney.
- Ian completed his PhD in 1990 at University of California in Davis with majors in Nutrition and Epidemiology.
- Awarded the Gilruth Prize, the Australian Veterinary professions highest honor and
- in 2010 awarded the Australian Dairy Science Award.
- Awarded DVSc from the University of Sydney in 2012 for excellence of published works.
- an Adjunct Professor at the University of Sydney (since 2000).

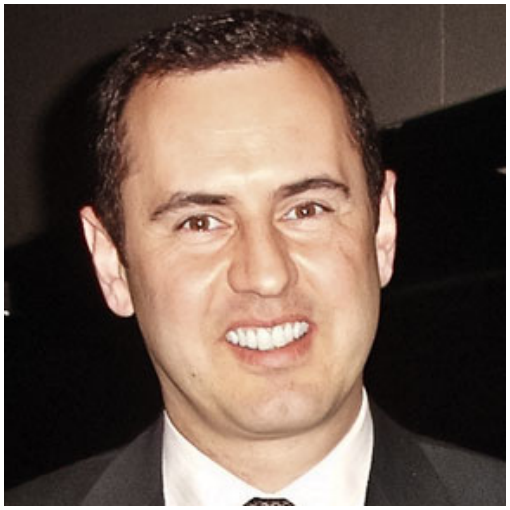


Dr. Adam Lock is an associate professor in the Department of Animal Science at Michigan State University. Originally from a dairy farm in the southwest of the United Kingdom, Dr. Lock received his PhD from the University of Nottingham and completed a post-doc at that institution as well as at Cornell University. He had a research and teaching appointment at the University of Vermont from 2006 to 2009 before moving to his current research and extension appointment at Michigan State University in the fall of 2009. Dr. Lock has developed his expertise in ruminant nutrition and physiology and is recognized for his ability to communicate to many sectors, from dairy farmers to dietitians. His research and extension programs focus on both dairy production and human nutrition and health, and the interface between these two disciplines. The central theme is fatty acid digestion and metabolism in the dairy cow and the impact of bioactive fatty acids on animal production and human health. Current efforts concern the effect of diet on the production of biohydrogenation intermediates in the rumen, dietary strategies for maximizing milk fat synthesis, applying this knowledge to improve our ability to troubleshoot on farm issues related to milk fat depression, fatty acid absorption in the small intestine, fat supplementation opportunities, and the potential for omega-3 fatty acids to promote dairy cattle metabolism and health. The impact of milk and dairy products on human health, in particular the role of milk fat is also of special interest.

Dr. Josh McCann joined the Department of Animal Sciences at the University of Illinois at Urbana-Champaign in 2016 as an Assistant Professor. Dr. McCann grew up in the southeast on a small family farm. He obtained a B.S. in animal and food science at Texas Tech University, followed by a M.S. in animal science at Texas A&M University, and a Ph.D. at the University of Illinois. Dr. McCann's research centers on the influence of nutrition on metabolism and subsequent efficiency and performance of feedlot cattle. We are striving to understand the interplay between nutrition, the rumen microbiome, gastrointestinal epithelium, and muscle development, to connect this fundamental information to applied advances in the feedlot. To study these effects, Dr. McCann utilizes classic ruminant nutrition techniques coupled with molecular high-throughput technologies. His research goal is to leverage new nutritional insights to improve the efficiency, sustainability, and profitability of feedlot cattle operations.

Johan Osorio, Ph.D. Dr. Osorio joined South Dakota State University as Assistant Professor in the Dairy and Food Science Department in the summer of 2016. He received his undergraduate degree in Agricultural Sciences and Production at Zamorano University in Honduras in 2004 and his M.S. and Ph.D. in Animal Sciences from the University of Illinois, in 2010 and 2014, respectively. For his doctoral dissertation, Dr. Osorio worked extensively on the link between applied and molecular nutrition of periparturient cows and neonatal calves. On the cow front, he studied the interrelationships between dietary rumen-protected Methionine and inflammation, oxidative stress, immune function, and large-scale transcriptome profiles in the liver of transition dairy cows. His work with calves aimed to study the potential carryover effects of maternal plane of dietary energy or organic trace minerals prepartum on immune function and metabolism of the newborn calf. At South Dakota State University, Johan Osorio continues his efforts to expand the field of nutrigenomics in dairy cows by developing new techniques and methods to evaluate nutrient-gene interactions effects with the aim to improve health and performance.

Dr. Hugo Ramirez is originally from the state of Guanajuato in Mexico. He grew up surrounded by agricultural education because his father and older sister are faculty members at the Autonomous University of Chapingo, the largest agricultural university in Mexico. He attended the University of Chapingo where he completed an agricultural high school program and earned a Bachelor's degree in Animal Science. Upon graduation, Dr. Ramirez managed a state-of-the-art dairy farm in central Mexico that was equipped with a methane digester that fueled electric generators to supply energy to the farm. Subsequently, he completed his graduate education at the University of Nebraska-Lincoln conducting applied research in dairy nutrition including evaluation of corn silage hybrids and ethanol co-products for dairy cows. Dr. Ramirez joined the Texas A&M system in January of 2014 as Assistant Professor and Director of the Southwest Regional Dairy Center at Tarleton State University and Research Scientist with Texas A&M AgriLife Research. He joined Iowa State University in the autumn of 2015 and is working on developing a research-based extension program that is producer-focused in the areas of dairy herd management, forage quality and preservation, and nutrient utilization. In addition, he teaches Applied Dairy Farm Evaluation and coaches the Dairy Challenge Team for Iowa State University.



Dr. Jose E. P. Santos is a Research Foundation Professor in the Department of Animal Sciences at the University of Florida where he conducts research and extension in dairy cattle nutrition and reproduction. Jose earned his DVM degree from Sao Paulo State University in Brazil in 1992, completed the MSc and PhD degrees in 1995 and 1997 at the University of Arizona, and a clinical residency in Dairy Production Medicine in 2000 in the School of Veterinary Medicine at the University of California Davis. He spent 8 years as a faculty member with clinical and research responsibilities in the Department of Population Health and Reproduction in the School of Veterinary Medicine at the University of California Davis before moving to the University of Florida in 2008. Jose is a member of the committee for the National Research Council on nutrient requirements for Dairy Cattle. He has authored and coauthored 163 peer-reviewed manuscripts in the scientific literature, and trained 7 clinical residents in dairy production medicine, and has been the major professor of 9 PhD and 13 MSc students, co-major professor of 7 visiting PhD students, received 7 sabbatical visitors and 92 visiting students. His primary research efforts focus on the interface between nutrition and reproduction and methods to improve postpartum health and fertility of dairy cows.

Jon Schoonmaker, Ph.D. is an Associate Professor in the Department of Animal Sciences, Purdue University. Dr. Schoonmaker is originally from Wisconsin and received his B.S. degree from the University of Wisconsin-Madison. Dr. Schoonmaker received his M.S. and Ph.D. degrees from The Ohio State University, in the area of beef cattle nutrition and management, and spent 3 years at Iowa State University working on a post-doctorate focusing on genetics of fatty acid composition of beef and milk. Dr. Schoonmaker has been at Purdue since 2009 and his research centers on nutritional influences on growth and development, specifically how energy source, protein source, vitamins, minerals, and feed additives impact muscle and fat development in developing, growing, and finishing beef cattle.

Jessica Tekippe is the R&D and technical service manager at Ajinomoto Heartland Inc. She earned her Master of Science degree at Penn State University, and worked in industry in a technical role before joining Ajinomoto in 2013. She specializes in finding new ways to create economic nutrient solutions for the dairy farmer. She believes that the key to ensuring the next generation is finding a way to improve feed efficiencies and create solutions that are biologically and economically successful. In her free time she farms with her husband and chases after her two small children.

Dr. Bill Weiss is a Professor of Dairy Cattle Nutrition in the Department of Animal Sciences at The Ohio State University, located at OARDC in Wooster. His main research areas currently are: factors affecting digestibility in dairy cows and relationships between minerals and vitamins and health of dairy cows. He has authored 121 journal articles and more than 350 popular press and proceedings articles and has given invited talks in 42 states and 25 countries. He was a member of the 2001 Dairy National Research Council (NRC) committee and is currently serving as vice chair of the 2016 Dairy NRC committee. He also served as Interim Chair of the Department of Animal Sciences from 2016-2017.

Impact of Essential Amino Acid Balancing Postpartum on Lactation Performance by Dairy Cows

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Introduction

Precision feeding became an essential strategy to optimize income over feed cost in dairy herds, mainly through the increase in nutrient utilization and the corresponding benefits in milk and milk components production. Furthermore, environmental impact of dairy herds is lessened when nutrient utilization is improved. All these benefits may be achieved through the formulation of diets targeting for optimum amino acid (**AA**) balancing. For example, several research studies reported increases in milk and/or milk protein production with supplementation of rumen-protected amino acids (**RPAA**). In addition, RPAA allows dairy nutritionist to formulate diets with lower CP content while maintaining or sometimes improving performance; and these diets were often reported to reduce N excretion. Combined these results suggest that RPAA supplementation is desirable, especially in eras when or locations where protein feedstuffs are expensive. Thus, the objective of the present article is to discuss the relationship between dietary essential amino acids (**EAA**) concentration and lactation performance of early lactation dairy cows. Our focus will be to present some findings from a recent meta-analysis study from our group combined with recent literature.

Benefits of Balancing Essential AA on Performance of Dairy Cows

Meta-analysis description

Our meta-analysis study used an unconventional approach; instead of summarizing published literature data, a dataset comprised of 20 unpublished feeding trials was assembled and used (**Table 1**). This approach was selected due to the uniqueness of the dataset. All feeding trials were performed in collaboration between The William H. Miner Agricultural Research Institute (Chazy, NY) and Ajinomoto Heartland Inc. (Chicago, IL) in the 1990's and were designed as continuous lactation trials to evaluate the effect of lysine or lysine/methionine supplementation on early-lactation performance by dairy cows. Diets from all 20 feeding trials were formulated by the same nutritionist using CPM/CNCPS (version 2) which provided a complete dietary AA profile (**Table 2**). Furthermore, feed and milk samples from all 20 feeding trials were analyzed at the same commercial laboratory.

Data analysis were performed to evaluate: 1) the relationship between individual dietary essential AA concentration (g of AA/Mcal of ME) and lactation performance of each of

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the initial 4 weeks of lactation (Ferraretto et al., 2016); and 2) to evaluate various strategies of RPAA supplementation on lactation performance (Ferraretto et al., unpublished). Although AA concentrations are often reported as percentage of metabolizable protein (**MP**) supply, our dietary AA concentrations were expressed in g of AA/Mcal of ME based on the recommendations of Higgs (2014). Higgs (2014) observed a relationship between AA supply to ME, but not MP, and underscored that perhaps its use could improve predictions of AA utilization.

Rumen Protected Methionine and Lysine

It is well documented that diets based on corn silage and soybean meal (typical North American diets) are limiting in lysine and methionine for milk production and milk protein synthesis (NRC, 2001). Therefore, balancing dairy cow diets with limiting and/or essential AA can be an effective strategy to increase milk and milk protein production. Several studies demonstrated that supplementation with rumen-protected lysine (**RPL**) and methionine (**RPM**) may improve feed intake, milk production, and content and production of milk protein. Those responses are more likely to be observed in high-producing dairy cows rather than in low-producing cows, when rumen-undegraded protein (**RUP**) supplies a greater portion of metabolizable protein (**MP)**, and for cows in early than mid- and late-lactation (NRC, 2001; Socha et al., 2005). In addition, increased dietary concentrations of lysine and methionine in MP have indicated that milk protein content is more sensitive than milk protein production; and that increases in milk protein content are independent of milk production (NRC, 2001).

Our meta-analysis (Ferraretto et al., unpublished) compared the effects of RPL supplementation in early to mid-lactation cows (**Table 3**). Treatments were control, RPL plus dietary sources of methionine, combination of RPL and RPM, and 2x RPL plus a dietary source of methionine. Overall, AA balancing enhanced intake and yield of milk and milk components. However, improvements were greater for RPL plus RPM than other AA treatments. Socha et al. (2005) evaluated the effect of supplementing corn-based diets to pre-partum and early post-partum cows with RPM alone or with a combination of RPM plus RPL on lactation performance. They reported that cows that were supplemented with RPM plus RPL increased production of energy-corrected milk (**ECM**), milk true protein, and milk fat. However, no effects on performance were detected when RPM was supplemented alone compared to basal diet and basal diet plus RPAA combination. Wang et al. (2010) evaluated the effect of supplementing corn-based diets of mid-lactation cows with RPM, RPL, and in combination (RPM + RPL) in diets slightly limiting in MP. The authors observed an increase in milk production of 1.5 kg/d by cows fed RPL or 2.0 kg/d by cows fed RPM alone, and an increase of 3.8 kg/d when RPM and RPL were supplemented together compared to the basal diet (without RPAA supplementation). Also, increased milk protein yield and fat content were observed when diets supplemented with RPM alone or in combination with RPM plus RPL were fed.

Relationships between dietary methionine or lysine concentrations and yields of milk and milk protein are in **Table 4** (Ferraretto et al., 2016). Our meta-analysis revealed

positive relationships between dietary methionine and milk and milk protein yields during weeks 1 to 4. This is in agreement with a recent meta-analysis review which used CNCPS (version 6.5) to predict nutrient supplies from a large number of published experiments (Lean et al., 2018). These authors observed a positive relationship between metabolizable methionine (g/d) with milk protein yield and content. The supply of methionine estimated by Lean et al. (2018) was similar (57 vs. 54 g/d and 2.24 vs. 2.10% of MP) to that of Ferraretto et al. (2016).

Ferraretto et al. (2016) observed an increase in actual and ECM yields along with lysine concentration during weeks 1, 2 and 4, whereas milk protein yield increased during the 4 initial weeks of lactation (**Table 3**). Interestingly, no relationship was detected between lysine and milk protein or milk yield (Lean et al., 2018). However, the supply of lysine estimated by Lean et al. (2018) was lower (162 vs. 179 g/d and 6.38 vs. 6.88% of MP) than Ferraretto et al. (2016). According to CNCPS 6.5, the optimal lysine supply (% of MP) recommended to maximum protein yield is 6.68%. Perhaps the lack of a relationship in the review by Lean et al. (2018) is related to the supply of lysine being under the recommended concentration by CNCPS. Furthermore, there is a difference in lactation stage between the 2 meta-analyses (early- vs. mid-lactation) which may also have contributed to this difference as speculated in previous studies (Wang et al., 2010; Osorio et al., 2016). Overall, these results underscore the importance of balancing lysine and methionine when formulating diets for high-producing dairy cows; and this importance is increased during early lactation.

Reducing dietary CP with rumen-protected AA

The efficiency of N utilization for milk (milk N/N intake, %) in high-producing dairy cows can range from 25 to 35% with the remaining N excreted in feces and urine (Arriola Apelo et al., 2014b). Nitrogen can be a pollutant from animal operations, having a negative environmental impact (e.g., surface water eutrophication, ground water nitrate, and ammonia emissions; US EPA, 2011). Therefore, the US dairy industry is under pressure to reduce N excretion in dairy cow operations and, as a consequence, there is an increased interest to improve N utilization efficiency without compromising milk production and animal health (Lapierre et al., 2005; Wang et al., 2010). Previous studies highlighted that decreasing dietary CP concentration is an efficient way to reduce N excretion (Olmos Colmenero and Broderick, 2006; Agle et al., 2010). However, reducing intake of CP may result in a deficient supply of MP, and thereby reduce yields of milk and protein and milk protein content (Cabrita et al., 2011). A strategy to overcome this issue may be achieved by improving the balance of metabolizable AA supply (Lapierre et al., 2005; Lee et al., 2012a).

Ferraretto et al. (unpublished) evaluated effect of diets of low CP, RDP, or MP concentrations supplemented with and without RPL and RPM on performance of early-lactation dairy cows. Dry matter intake and yield of milk, milk protein, and ECM increased for cows fed diets supplemented with RPAA compared to the cows without supplementation (**Table 5**). Socha et al. (2005) evaluated the effect of supplementing corn-based diets of pre-partum and early post-partum cows with a combination of RPM plus RPL with two different concentrations of CP (18.5 vs. 16.0%, DM basis) on

lactation performance. These authors observed that cows fed the diet containing 16% CP numerically increased DMI (+ 0.4 kg/d), ECM (+ 1.5 kg/d), and had greater gross efficiency of N utilization (35 vs. 29%) compared with cows fed a diet with 18.5% CP. Recently, Nursoy et al. (2017) evaluated the optimal dietary CP concentration for mid-lactation cows fed corn-based diets supplemented with soybean meal (**SBM**) plus RPM. The authors tested 4 CP concentrations (11, 13, 15, and 17%, DM basis), and all diets were formulated to maintain a methionine to lysine ratio of 3.1:1 (% of MP). The authors concluded that feeding a corn-based diet supplemented with SBM plus RPM with 15% CP was adequate for mid-lactation cows producing approximately 40 kg/d of milk. Compared to a 17% CP diet, cows fed the 15% CP diet improved N efficiency (29.5 vs. 32.7%, respectively). On the other hand, Cabrita et al. (2011) evaluated the effects of dietary CP concentration (16 vs. 14%, DM basis) and balance of MP by manipulating the main protein sources (SBM and corn byproducts), and by adding RPM plus RPL on lactation performance of dairy cows fed corn silage-based diets. Overall, authors did not observe benefits supplementing RPM and RPL in reducing dietary CP from 16 to 14%, since no significant differences were observed for all production traits evaluated.

Lee et al. (2012b) conducted 2 experiments evaluating the effects of supplementation of MP-deficient or MP adequate diets with RPM and RPL on milk production, milk components, and N utilization. In experiment 1 dietary treatments were: (1) MP-adequate diet without RPAA supplementation; (2) MP-deficient diet (approximately 12% of MP-adequate diet) plus supplementation with RPL; and (3) MP-deficient diet supplemented with RPL plus RPM. In experiment 2, dietary treatments were: (1) adequate MP supplemented with RPL; and (2) adequate MP supplemented with RPL plus RPM. Overall, authors did not observe significant differences in milk yield and components among the dietary treatments (albeit milk yield decreased by about 1 kg/d for both deficient-MP diets). When the MP-deficient diet was only supplemented with RPL, milk protein content decreased compared to adequate-MP diet.

Overall, these results indicate that supplementing RPL and RPM along with a reduction in dietary CP may be an important nutritional strategy to improve N utilization efficiency and minimize potential environmental pollution by dairy cow operations.

Other Essential AA for high producing dairy cows

Although lysine and methionine are the two most limiting AA when feeding corn-silage based diets, recent studies suggest that increasing the supply of other EAA may improve lactation performance of dairy cows. For example, recent studies suggested that histidine may be a limiting AA after lysine and methionine (Vanhatalo et al., 1999; Lee et al. 2012a,b; Giallongo et al. 2016). Furthermore, other studies indicate that other EAA may play an important role to improve yields of milk and protein (Haque et al., 2013; Arriola Apelo et al., 2014a).

Ferraretto et al. (2016) reported that dietary histidine had a positive relationship with milk yield, but a negative relationship to milk protein content on weeks 2 to 4 of lactation (**Table 6**). This is in agreement with previous research that suggested production benefits related to histidine (Lee et al., 2012a, b; Giallongo et al., 2016; Lean

et al., 2018). For example, Giallongo et al. (2016) evaluated the effects of supplementation of RPAA (methionine, lysine, and histidine) in MP-deficient diets on performance of dairy cows. The diet deficient in MP supplemented exclusively with rumen-protected histidine increased milk protein content, and when supplemented with rumen-protected methionine, lysine, and histidine together, it further increased yields of milk fat, protein, and ECM and ECM feed efficiency compared to the diet deficient in MP without RPAA supplementation. Lean et al. (2018) observed that histidine elicited a positive response in milk yield despite the small difference between treatments; control and treatment groups supplied 2.57% and 2.61% of MP, respectively. These authors suggested that this response indicates that histidine plays a role as a co-limiting AA in dairy cow diets.

Dietary valine was positively related to ECM, but negatively to milk protein concentration on weeks 2 and 3 (Ferraretto et al., 2016). On weeks 3 and 4, a positive relationship between milk yield and valine was observed. Haque et al. (2013) evaluated milk protein responses to changes in post-ruminal infusion of EAA and depletion of arginine, isoleucine, and valine in dairy cows. The authors observed that when cows did not receive post-ruminal infusions of valine, milk protein synthesis was decreased compared to cows that did receive post-ruminal infusions of all EAA. They concluded that the lower level of valine (4.5% of MP) may explain the negative effect on milk protein. In our study the average concentration of valine was 5.7% of MP which is within the range reported by Doepel et al. (2004).

Other EAA were also related to lactation performance in our meta-analysis study (Ferraretto et al., 2016). Dietary concentration of arginine and threonine were negatively related to milk fat content and yield on weeks 3 and 4 of lactation. Moreover, quadratic relationships between milk or milk protein yields and dietary concentrations of leucine and phenylalanine were observed during weeks 1 to 4. Isoleucine concentration of the diet was negatively related to ECM and milk protein yields during weeks 3 and 4 and to milk and milk fat yields on week 3. Dietary concentration of tryptophan was negatively related to ECM, milk fat content, and milk fat yield. Leucine was associated with greater milk protein yield in the study by Lean et al. (2018). In addition, tryptophan and threonine affected milk yield positively (Lean et al., 2018).

Summary

Overall, benefits on lactation performance were observed with supplementation of RPAA to early lactation dairy cows, particularly when RP sources included both, methionine and lysine. In addition, RPAA supplementation is a viable tool to reduce concentration of CP, RDP or MP in dairy cow diets. Increased dietary concentrations of methionine, lysine, valine, and histidine enhanced lactation performance in lactating cows during the initial 4 weeks of lactation. In contrast, isoleucine and tryptophan were negatively related to lactation performance whereas arginine and threonine depressed milk fat. These results underscore the importance of amino acid balancing beyond the lysine to methionine ratio when formulating diets for early lactation dairy cows.

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Table 1. Cow numbers, supplementation period, length of collection of milk yield, and amino acid supplementation strategy of the 20 studies used for the meta-analysis.

Study	n[1]	Supplementation Period[2]	Lactation[3]	Lysine[4]	Methionine[5]
1	41	-21 to 140	140	+	+
2	7	-21 to 56	140	+	+
3	27	-14 to 42	112	+	-
4	17	-14 to 42	112	+	-
5	35	-21 to 42	112	+	-
6	20	1 to 28	56	+	+
7	11	1 to 28	112	+	+
8	16	1 to 28	112	+	-
9	40	-21 to 56	168	+	+
10	40	-21 to 42	70	+	-
11	13	-14 to 42	168	+	+
12	20	1 to 28	112	+	+
13	11	-21 to 70	308	+	+
14	45	-21 to 42	112	+	-
15	14	-21 to 42	168	+	+
16	14	1 to 42	168	+	-
17	30	-14 to 28	112	+	+
18	24	-21 to 42	168	+	-
19	14	-21 to 42	140	+	+
20	15	-21 to 301	308	+	+

[1] Number of lactating dairy cows used in trial per treatment.
[2] Period of rumen-protected amino acid supplementation, presented as days in milk.
[3] Days in milk at the end of lactation performance assessment.
[4] With or without rumen-protected lysine (+, -).
[5] With or without rumen-protected methionine (+, -).

Table 2. Selected nutrient composition of diets fed in studies used in the meta-analysis.

Item	Average	SD	Minimum	Maximum
Forage, % of DM	44.1	4.5	37.1	56.9
NDF, % of DM	30.6	2.4	26.8	37.0
NFC, % of DM	39.2	2.2	35.7	44.2
Fat, % of DM	5.5	1.3	2.6	7.2
Metabolizable energy, Mcal/d	61.8	3.0	55.3	68.1
NEL, Mcal/kg of DM	1.76	0.88	1.56	1.94
CP, % of DM	18.4	1.0	15.9	20.6
RDP, % of CP	60.1	4.7	50.1	69.9
RUP, % of CP	39.9	4.7	30.1	49.9
Metabolizable protein, g/d	2601	168	2381	2930
Methionine, g/d	54	7	44	67
Lysine, g/d	179	19	137	223
Arginine, g/d	157	10	134	176
Threonine, g/d	117	7	105	131
Leucine, g/d	220	25	180	287
Isoleucine, g/d	124	8	111	143
Valine, g/d	148	13	131	174
Histidine, g/d	69	6	56	82
Phenylalanine, g/d	132	10	115	158
Tryptophan, g/d	36	3	29	41
Essential amino acids, g/d	1236	80	1077	1396

Table 3. Effect of supplementation of rumen-protected amino acids on lactation performance by early lactation dairy cows.[1]

Item	CON	RPL	RPLM	RP2LM	SEM[2]	*P*-value
DM intake, kg/d	19.9^{c}	21.6^{a}	20.6^{b}	21.6^{a}	0.7	0.001
Milk, kg/d	41.3^{d}	42.1^{c}	45.0^{a}	43.2^{b}	1.8	0.001
ECM, kg/d	41.9^{c}	42.8^{b}	45.1^{a}	44.2^{a}	1.8	0.001
Milk fat, %	3.70	3.69	3.60	3.76	0.09	0.06
Milk fat, kg/d	1.51^{c}	1.52^{bc}	1.61^{a}	1.58^{ab}	0.07	0.001
Milk protein, %	2.85^{b}	2.90^{a}	2.81^{c}	2.92^{a}	0.03	0.001
Milk protein, kg/d	1.18^{c}	1.23^{b}	1.36^{a}	1.28^{b}	0.06	0.001
Milk lactose, %	4.74	4.77	4.76	4.76	0.03	0.13
Milk lactose, kg/d	1.80^{b}	1.82^{b}	1.98^{a}	1.81^{b}	0.13	0.001
MUN, mg/dL	17.5	16.0	17.8	18.2	1.5	0.28

[1] Treatments were control diet (CON), rumen protected lysine plus a dietary source of methionine (RPL), rumen protected lysine and methionine (RPLM), and 2x rumen protected lysine plus a dietary source of methionine (RP2LM).
[2] Standard error of the mean.

Table 4. Relationship between dietary intake of methionine or lysine (g/Mcal of ME) and yield of milk and milk protein by dairy cows during the initial four weeks of lactation.[1]

Item	Intercept	SE	Slope	SE	P-value	RMSE[2]
			Methionine			
Week 1						
Milk yield, kg/d	25.28	3.59	6.494	3.714	0.10	1.75
Milk true protein, kg/d	0.97	0.14	0.245	0.126	0.07	0.11
Week 2						
Milk yield, kg/d	30.69	3.88	7.883	3.869	0.06	1.89
Milk true protein, kg/d	0.89	0.13	0.341	0.131	0.02	0.05
Week 3						
Milk yield, kg/d	33.87	4.05	8.116	4.428	0.08	2.19
Milk true protein, kg/d	0.97	0.10	0.225	0.087	0.02	0.07
Week 4						
Milk yield, kg/d	33.22	3.78	10.901	3.242	0.01	2.26
Milk true protein, kg/d	0.87	0.11	0.323	0.103	0.01	0.07
			Lysine			
Week 1						
Milk yield, kg/d	23.93	3.55	2.423	1.073	0.04	1.85
Milk true protein, kg/d	0.85	0.16	0.119	0.052	0.03	0.10
Week 2						
Milk yield, kg/d	28.79	3.94	3.050	1.135	0.02	1.86
Milk true protein, kg/d	0.84	0.14	0.120	0.042	0.02	0.04
Week 3						
Milk true protein, kg/d	0.92	0.15	0.088	0.047	0.08	0.05
Week 4						
Milk yield, kg/d	32.77	4.48	3.498	1.289	0.02	1.93
Milk true protein, kg/d	0.88	0.14	0.108	0.046	0.03	0.03

[1] Adjusted for the random effect of study.
[2] Root mean square error.

Table 5. Effect of supplementation of rumen-protected methionine and lysine in diets formulated with low amounts of CP, RDP, or MP on lactation performance by early lactation dairy cows.[1]

Item	CON	RP-	SEM[2]	*P*-value
DM intake, kg/d	21.6	22.7	1.0	0.001
Milk, kg/d	31.6	33.9	3.0	0.001
ECM, kg/d	33.0	33.9	3.3	0.001
Milk fat, %	3.82	3.53	0.10	0.001
Milk fat, kg/d	1.21	1.18	0.14	0.10
Milk protein, %	2.89	2.92	0.08	0.52
Milk protein, kg/d	0.91	0.98	0.06	0.001
Milk lactose, %	4.71	4.73	0.02	0.36
Milk lactose, kg/d	1.50	1.60	0.13	0.001

[1] Treatments were control diet (CON) and low CP, RDP or MP diets with rumen protected methionine and lysine (RP-).
[2] Standard error of the mean.

Table 6. Effects of dietary histidine (g/Mcal of ME) on milk yield and milk protein content by dairy cows during the initial four weeks of lactation.[1]

Item	Intercept	SE	Slope	SE	P-value	RMSE[2]
Week 2						
Milk yield, kg/d	24.87	5.98	11.39	5.780	0.07	2.13
Milk true protein, %	3.65	0.16	-0.441	0.141	0.01	0.05
Week 3						
Milk yield, kg/d	22.31	7.16	16.629	6.781	0.03	2.34
Milk true protein, %	3.24	0.12	-0.334	0.103	0.01	0.06
Week 4						
Milk yield, kg/d	26.76	7.19	14.554	6.805	0.05	2.24
Milk true protein, %	3.07	0.16	-0.274	0.139	0.06	0.06

[1] Adjusted for the random effect of study.
[2] Root mean square error.

SESSION NOTES

Gut Integrity During Periods of Stress and its Implications on Performance

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Introduction

Livestock production relies heavily on nutrition and feeding programs that are designed to promote maximal or optimal production. Quality of feedstuffs included in animal diets influence nutrient supply, digestibility, and absorption, and will ultimately have a great impact on animal performance. In addition to feed quality, intrinsic animal factors also determine how animals respond to dietary manipulations. Of these factors, the gastrointestinal tract (**GIT**) is of critical importance for efficient animal production because of its obvious role in digestion and absorption; however, the GIT also plays a substantial role in immune status of the animal. Therefore, the objective of this article is to highlight the interaction of the GIT and nutritional management of livestock and the impacts of this interaction on animal performance.

The Roles of the Gut

In ruminants, the sum of reticulorumen, omasum, abomasum, and intestines accounts for as much as 71% of body weight (Holstein cows, Beecher et al., 2014). The large mass of the GIT, relative to total body mass, involves physiological processes that demand a great deal of nutrients for maintenance and turnover of tissue. The GIT has a clear and obvious role in digestion of feed and absorption of nutrients. The gut presents a diversity of large anatomical features such as a multi-chamber stomach in ruminants as wells as microscopic differences in arrangement and number of cell layers in the different segments of the GIT. Morphological features such as villi and microvilli magnify the surface area of the intestines by several orders of magnitude which increase digestion and absorption potential.

In addition to the role in digestion and absorption processes, the gut serves a physical and chemical barrier to prevent intrusion of foreign substances or organisms into the body. In fact, the gut is the first line of defense against pathogens and toxins and represents the largest organ of the immune system. The gut has a mucus layer composed of threonine-rich glycoproteins called mucins (Perez-Vilar and Hill, 1999). This layer is a physical barrier that impedes direct contact between the enterocyte and the contents in the lumen of the intestine which include digestive secretions, toxins, and microorganisms (Forstner, 1995). In addition to these glycoproteins, the integrity of the gut is reinforced by tight junction proteins between enterocytes. This barrier protects against the infiltration of ions, toxins, and other molecules through the paracellular pathway (González-Mariscal et al. 2003).

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Overall, the gut has a paramount role in protecting the body through various mechanisms including chemical signaling pathways, physical mucosal barrier, and tight junction proteins. Alteration of any of these mechanisms can lead to reduced integrity of the gut barrier, reduced thickness of the mucosal layer, or altered synthesis and function of tight junction proteins.

Stress Factors and Gut and Immune System Responses

Livestock species experience stressful events at various points in their productive cycles. Some of the events are weather-related, heat being the most common factor associated with stress. Other stressful events include weaning and shipping or transportation. Each one of these factors may elicit a variety of ethological and physiological alterations, but they all share reduced feed intake as a commonality. Thus, it is important to highlight that other situations in which feed intake is compromised can also elicit a response similar to that of major stressful events. High stocking density in pasture or pens, drought, prolonged periods away from pen due to milking or extended times in a palpation rail, or animals running out of feed for a few hours per day are common farm scenarios that can lead to restricted feed intake.

Recent work by Kvidera et al. (2017a) has shown that feed restriction in dairy cows compromises gut integrity. The response to increasing feed restriction resulted in altered morphology of the intestinal epithelium so that animals that were feed-restricted had decreased villus height and width as well as reduced crypt depth. These alterations in gut histology not only imply altered digestion and absorption processes but can also lead to loss of effective barrier function. In the same study, the authors also reported increased concentration of circulating biomarkers of inflammation which may be a direct result of increased permeability due to loss of architectural integrity in the gut.

Heat is a common stress factor that can alter integrity of different tissues, Weng et al. (2017) reported that heat-stressed dairy cows had reduced expression of proteins involved in barrier function in the mammary gland. Similarly, Pearce et al. (2013) demonstrated deleterious effects on epithelial architecture due to heat stress and increased gut permeability in as little as 1 day of heat stress in pigs. A reduction in feed intake during periods of heat stress may be a response to reduce metabolic heat production (Baumgard and Rhoads, 2012) and commonly associated with subpar animal performance. However, there is evidence that feed restriction alone accounts for only 35 to 50% of reduction in milk yield during experimental hyperthermia (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011) which indicates that there are other mechanisms responsible for reduced productivity in dairy cows. During heat stress, blood flow is preferentially diverted toward the peripheral circulation as a strategy to dissipate heat (Lambert et al., 2002). This shift causes less irrigation to the splanchnic area (Hall et al., 1999) resulting in hypoxia of the gut, reduced nutrient supply, and depletion of ATP accompanied by increased oxidative and nitrosative stress (Hall et al., 2001).

Impacts of Gut Health on Animal Production

There is no clear diagnosis for gut barrier dysfunction, therefore, it is important to highlight that the animal industry would benefit from a generalized definition of gut health so that dysfunctional gut barrier can be subsequently defined. Animals that suffer from dysfunctional gut barrier can present a myriad of signs that can affect animal performance. More importantly, there is a cascade of physiological changes that are brought on upon disruption of gut barrier and translocation of bacteria or toxins from the lumen of the GIT into the general circulation. As a result, the body mounts an immune response which is known to divert energy and nutrients away from production towards the immune system. Namely, there is a hypoglycemic response which is thought to be a way to spare glucose from other tissues towards the immune system; however, it was unknown how much glucose was utilized during an immune challenge. It was only until recent discoveries that it was possible to obtain an estimate of the energy diverted towards the immune system upon activation. Kvidera et al. (2017b) conducted a study in which a euglycemic clamp was used to determine the energy requirements of an activated immune system. The authors estimated that the immune system can use slightly more than 1 kg of glucose in a 12 h period. In terms of milk production, that amount of glucose would be enough to synthesize close to 15 kg of milk since 72 g are required for every kg of milk produced (Kronfeld, 1982). Furthermore, the energy contained in that amount of glucose is approximately 4,100 kcal, considering a rate of 10 kcal for each gram of protein synthesis; the glucose diverted toward the immune system would have been enough to synthesize 410 g of protein. This amount of protein would be found in ~1,366 g of lean tissue.

In addition to energy being diverted away upon reduced gut integrity, it is reasonable to expect an increase in the requirements for certain amino acids. Tight junctions and mucins are proteins, therefore when alterations occur so that gut permeability is increased, the animal may respond by mobilizing amino acids or diverting dietary amino acids towards re-establishment of gut permeability. Maintaining mucin synthesis seems to be a primary function of the GIT. Rémond et al. (2009) induced inflammation of the ileum in minipigs and reported increased intestinal mucin synthesis with a concomitant increased requirement for threonine. Interestingly, the increased demand for threonine was ameliorated from mobilization of endogenous proteins rather than luminal supply of this amino acid. Furthermore, recent discoveries in minipigs confirm that intestinal tissues retain a disproportionate amount of threonine to maintain mucin synthesis even during periods of deficiency (Munasinghe et al., 2017). This indicates that protein synthesis in non-mucin producing organs or tissues would have a lower metabolic priority; from a livestock producing perspective, this would translate in lower production of animal protein. The discoveries in energetic and amino acid trafficking indicate that animals may redirect glucose to supply energy to the immune system while breaking down endogenous proteins to supply threonine for mucin synthesis. Both of these metabolic adaptations would translate into subpar animal performance and inefficient use of nutrients.

Even though most of the studied responses involving induced gut barrier dysfunction involve an acute stimulus, it is reasonable to think that less intense

situations are more commonly present in farm scenarios (animals running out of feed, long distance transport, heat stress) with less severe but similar partitioning of nutrients away from production for a few days or even a few hours throughout the day. These non-acute but frequent situations may represent cumulative inefficiencies in production similar to sub-clinical diseases or disorders.

Summary

The GIT serves a major role in digestion and absorption of nutrients and it also has a substantial barrier function to protect the animals from pathogen intrusion. Integrity of the gut may be negatively affected when animals undergo stressful events; because of its large mass and its close interaction with the immune system, gut health should be considered paramount for efficient animal production. Because an activated immune system utilizes substantial amounts of glucose and alters amino acid utilization, it is important to highlight that management and feeding practices should not only consider nutrient supply for the animal but also promote and support gut health and integrity. Doing so can lead to partitioning energy and other nutrients more efficiently towards animal production.

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SESSION NOTES

Amino Acid Balancing and Its Role on Metabolism, Inflammation, and Oxidative Stress: Future Molecular Implications

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Introduction

The modern dairy cow has been selected over generations for high milk production, with many successful dairy operations averaging over 13,500 kg of milk per lactation cycle per cow. This amount of nutrient output in the form of milk components puts a tremendous amount of pressure on the metabolism of the dairy cow, especially during early lactation. In fact, it is common to all mammals, including the dairy cow, to undergo marked physiologic and metabolic changes during the transition from pregnancy to lactation. For instance, it has been estimated that in dairy cows the energy and protein requirements can dramatically increase up to 5 times from late pregnancy to lactation (i.e., transition period). Therefore, during the past 3 decades, a substantial amount of research has been conducted to understand how these biological adaptations can predispose dairy cows to negative effects on health and consequently on the lactation performance of transition dairy cows.

Metabolizable protein (**MP**) is comprised primarily of ruminally synthesized microbial CP (**MCP**) and rumen undegradable protein (**RUP**) and is the true protein digested postruminally and absorbed by the intestine in the form of amino acids (**AA**) and peptides (Schwab and Broderick, 2017). Dairy cows in early lactation commonly experience a negative MP balance condition, where the dietary MP supplied does not meet the requirements for maintenance, growth, and milk synthesis (Bell et al., 2000). Among AA, the availability of methionine (**Met**) in MP across a wide range of diets for dairy cows is low (NRC, 2001), hence, limiting its use for mammary and liver metabolism and also for the synthesis of the methylated compound S-adenosylmethionine (**SAM**) (Martinov et al., 2010). Therefore, supplementation of rumen-protected Met to transition dairy cows has consistently improved milk yield and DMI (Osorio et al., 2013; Zhou et al., 2016b; Batistel et al., 2017), milk protein yield (Ordway et al., 2009; Osorio et al., 2013; Zhou et al., 2016b; Batistel et al., 2017), and milk fat yield (Osorio et al., 2013; Zhou et al., 2016b). The latter effects have been associated at the metabolic level with considerable improvements in liver function and antioxidant precursor synthesis (Osorio et al., 2014b; Zhou et al., 2016a; Batistel et al., 2018). At the molecular level the effects of Met as a precursor of SAM has been previously investigated (Osorio et al., 2016a). Due to the multiple biological processes that require SAM, including transsulfuration, polyamine biosynthesis, DNA methylation (Lu and Mato, 2012), and histone methylation (Shima et al., 2017), the requirements for

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methyl donors, such as choline and Met, increases at the onset of lactation (Preynat et al., 2009). Histone methylation is one of the mechanisms by which the genetic information contained in the DNA is made available or unavailable (i.e., chromatin status) for transcription and translation into proteins. Within the context of the dairy cow, Bionaz and collaborators (2012) observed marked alterations associated with the chromatin status (i.e., euchromatin or available DNA or heterochromatin or unavailable DNA) in bovine mammary tissue from late pregnancy to lactation indicating that the mammary gland undergoes substantial changes in the available genetic information during the transition period. Taken together, is it conceivable that Met availability can affect the chromatin status at the molecular level through histone methylation, since Met is the primary source for SAM, and, in turn, SAM is the main methyl donor for histone methylation. Thus, the objective of this article is to present and discuss the effects of Met on metabolism, inflammation, and gene regulation.

Methionine and the Transition Dairy Cow

It is well-known that the most challenging stage in the lactation cycle of a dairy cow is the transition from late pregnancy to lactation, where most metabolic and infectious diseases occur. Primarily, this is due to several conditions including immunosuppression, changes in endocrine status, and decrease in DMI that collide during this relatively short period of time (Grummer, 1995; Drackley, 1999). In late pregnancy and early lactation, the nutrient demand increases quite considerably. In late pregnancy nutrient demand increases as a result of fetal development, then, at the onset of lactation, the nutrient demand further increases dramatically for milk synthesis (Ingvartsen, 2006). This demand for nutrients triggers a coordinated response in different tissues such as liver, adipose, and mammary gland resulting in endocrine and metabolic alterations to ensure high milk yield concurrently with maintenance of physiological homeostasis (Ingvartsen, 2006; Loor, 2010). Such endocrine and metabolic alterations include decreased insulin while increasing glucocorticoids, growth hormone, and NEFA. At the same time tissue sensitivity to glucocorticoids increases while insulin sensitivity decreases (Bell, 1995; Ingvartsen and Andersen, 2000). Contrasting to the energy demands, transition dairy cows commonly experience a reduction in DMI around calving time, therefore limiting the supply of dietary nutrients to sustain milk production in early lactation. This scenario renders transition dairy cows in a negative balance of nutrients not only from an energy standpoint but also from a negative protein balance, specifically negative MP balance (Bell et al., 2000). Thus, it is within this time frame that improving not only MP balance, but also the AA profile comprising the MP supplied, where the greatest beneficial effects can be obtained by supplementing key AA such as Met and lysine (**Lys**).

Methionine is commonly characterized as one of the most-limiting AA in dairy cow rations, therefore it is not surprising the amount of attention this nutrient has received over the years (**Figure 1**; PubMed search using keywords “methionine dairy cows” on January 14, 2018). And more recently, the primary focus has been in transition cows where the evident and consistent beneficial effects of Met supplementation indicate that it is during this period when dairy cows have the most benefit from this nutrient (Schwab and Broderick, 2017). From a biological standpoint,

the importance of Met resides on the plethora of biological processes that it is involved in beyond the synthesis of milk proteins, and such importance has been exposed during fragile metabolic and physiologic conditions as the transition period of dairy cows. At the metabolic level, some of the main biological areas affected by Met supplementation are the lipid metabolism, inflammation, and oxidative stress.

The Lipotropic Effect of Methionine

In transition dairy cows, the common lipolytic state of the adipose tissue is partially driven by the decreased insulin levels along with decreased insulin sensitivity, which eventually leads to elevated blood NEFA concentration. This excess NEFA will then be transported through the bloodstream to peripheral tissues for use as an energy source. The liver is the most important site for removal of NEFA from circulation (Bell, 1979). Extreme rates of NEFA or lipid mobilization lead to increased uptake of NEFA, hence, increasing the susceptibility to hepatic lipidosis (Bobe et al., 2004). The hepatic assembly/export of very-low density lipoproteins (**VLDL**) is one of the potential mechanisms utilized by this organ to limit the lipid accumulation or hepatic lipidosis (Drackley, 1999). However, the rate of hepatic VLDL synthesis has been demonstrated to be lower in ruminants than monogastrics (Pullen et al., 1990). Interestingly, McCarthy et al. (1968) hypothesized that Met deficiency in ruminants might limit hepatic VLDL synthesis and be a causative factor of ketosis. Similarly, Grummer (1993) proposed that limiting AA such as Met can have a potential impact on VLDL assembly and secretion in ruminants. Several studies have assessed the role of Met as a potentially limiting AA in the regulation of hepatic VLDL synthesis in dairy calves (Auboiron et al., 1994; Auboiron et al., 1995) and dairy cows (Durand et al., 1992). More recently, the Met effect on hepatic VLDL assembly/export has also been reported in transition dairy cows (Osorio et al., 2013), where a mild increase was observed in blood ApoB-100, a key protein for the assembly/secretion of VLDL. Similar results have been observed by Sun et al. (2016), where transition dairy cows supplemented with rumen-protected Met had an overall increased blood concentration of ApoB-100 and VLDL.

Among the potential mechanisms for the effect of Met on VLDL synthesis, is the improvement in liver function. In fact, it is commonly understood that liver functionality is depressed during the transition period of dairy cows (Trevisi et al., 2013). Albumin is primarily synthesized in the liver and is one of the main blood biomarkers associated with liver function. Albumin is commonly observed to decrease in blood during the transition period (Bertoni et al., 2008). Then, the limiting effect of Met as an AA for protein synthesis is evident when a consistent increase in blood albumin has been observed when supplementing Met to transition dairy cows (Osorio et al., 2014b; Zhou et al., 2016a; Batistel et al., 2018). An increased liver function level during the transition period will likely ensure that the synthesis of key proteins such as albumin and ApoB-100 will not be impaired or at least maintained. Therefore, it is conceivable that Met as a limiting factor for protein synthesis in the liver will improve liver function and, in turn, this will improve the VLDL assembly and secretion through ApoB-100.

Methionine Alterations on Inflammation and Oxidative Stress

Contrary to liver function, inflammation and oxidative stress commonly increase during the peripartal period in dairy cows (Bionaz et al., 2007; Trevisi et al., 2012). The

peripartal inflammatory response is characterized by an increase in the production of positive acute-phase proteins (**posAPP**) such as haptoglobin and serum amyloid A (SAA), and a concomitant decrease in the production of negative APP (**negAPP**) such as albumin (Bertoni et al., 2008). The well-established triggers of the acute-phase response are the cytokines interleukin-6 (**IL**-6), IL-1, and tumor necrosis factor-alpha (**TNF-α**) (Kindt et al., 2007). They also mediate the inflammatory response by activating leukocytes and endothelial cells (Bannerman et al., 2009). Oxidative stress is driven by the imbalance between the production of reactive oxygen metabolites (**ROM**) and the neutralizing capacity of antioxidant mechanisms in tissues and blood. Such antioxidant mechanisms include glutathione, taurine, superoxide dismutase, and vitamins A and E (Bernabucci et al., 2005). During the transition period of dairy cows, there is a common degree of oxidative stress associated with the onset of the lactation (Grohn et al., 1989). But, if there is excessive lipid mobilization in the form of NEFA reaching the liver this will likely overwhelm the cellular antioxidant capacity (Bernabucci et al., 2005); then excessive ROM can induce an inflammatory response which is controlled at the molecular level by gene expression regulators or transcription factors (**TF**) (e.g., STAT3 and NFKB) (Huang et al., 2016).

At the crossroads between inflammation and oxidative stress, Met can have profound alterations on these biological processes through improved liver function and glutathione metabolism. The latter is a major antioxidant, and structurally it is a tripeptide mainly synthesized in the liver. Glutathione is the most abundant endogenous antioxidant due to its marked ability to scavenge ROM and free radicals and consequently is commonly used as a biomarker in oxidative stress-related diseases (Romeu et al., 2010; Vetrani et al., 2013). In transition dairy cows, Met supplementation has consistently increased the concentration of glutathione in the liver (Osorio et al., 2014b; Zhou et al., 2016a; Batistel et al., 2018), which has been associated with Met being incorporated upstream in the *de novo* synthesis pathway for glutathione (Halsted, 2013). Glutathione can not only serve as an important hepatic antioxidant but also it can be exported into the bloodstream, where it can aid in the control of systemic oxidative stress response. In the liver, glutathione is commonly depleted during the transition period, primarily after calving (Osorio et al., 2014b; Zhou et al., 2016a; Batistel et al., 2018). The liver glutathione has been described as a reservoir for supplying AA such as Cys to the γ-glutamyl cycle (Lu, 2009). Then, the postpartal depletion of liver glutathione indicates that the metabolism of the transition dairy cow relies on this reservoir of AA (i.e., liver glutathione) for vital functions such as oxidative stress. In summary, the decrease of important proteins synthesized in the liver such as glutathione and albumin suggests that liver protein synthesis is compromised during the transition period and the supply of limiting AA such as Met can potentially reverse these conditions and prepare dairy cows for a smooth transition from pregnancy to lactation.

Methionine and Gene Regulation in Transition Dairy Cows

The DNA contains the genetic information to synthesize all the proteins in the body, but this information must be transcribed into mRNA (i.e., transcriptome) prior to being utilized as the template for protein synthesis. The ability to obtain transcriptomic information has facilitated the characterization of the behavior of molecular networks at multiple points during the onset of diseases or stress periods such the transition period

of dairy cows. However, major gaps in knowledge of the molecular adaptations during this crucial life state of the dairy cow remains. As mentioned above, the liver plays an essential role in the physiological adaptations of the transition dairy cow, therefore transcriptomic information from this organ has been a major focus in transition dairy cow research (Loor, 2010).

Preynat and collaborators (2010) published one of the first experiments evaluating transcriptomic changes in the liver of transition dairy cows supplemented with rumen-protected Met. This study showed that cows supplemented with Met had an upregulated transcription of genes associated with Met and the methylation cycle including phosphatidylethanolamine transferase (***PEMT***), responsible for the synthesis phosphatidylcholine (**Figure 2**), the latter being an important structural component in the assembly of VLDL in the liver. More recently, studies conducted at the University of Illinois in transition dairy cows supplemented with Met revealed a common upregulation of genes related to the Met cycle (Figure 2) such as *PEMT*, S-adenosylhomocysteine hydrolase (***SAHH***), and Met adenosyltransferase 1A (***MAT1A***) (Osorio et al., 2014a; Zhou et al., 2017). The SAHH is a substrate-dependent enzyme and might play an important role in the availability of both SAM and homocysteine (**Hcy**). In fact, the inhibition of SAHH causes the accumulation of SAH and, subsequently, suppresses SAM-dependent transmethylation via feedback inhibition (Lee et al., 2011). The *MAT1A* gene encodes both MATI and MATIII isoenzymes in mammals, which are responsible for the first step in the hepatic synthesis of SAM from Met (Martinov et al., 2010). The importance of Met in the synthesis of SAM is confirmed by the upregulation of *MAT1A* and *SAHH* genes related to the Met cycle.

The essential role of SAM within the context of the transition cow relies on multiple biological processes that require this methyl donor, including transsulfuration, polyamine biosynthesis, DNA methylation (Lu and Mato, 2012), and histone methylation (Shima et al., 2017). Among these, the epigenetic modifications caused by DNA and histone methylation are particularly important in order to understand the potential transcriptomic alterations due to Met supplementation. DNA methylation occurs through specialized enzymes called DNA methyltransferases, which utilize the methyl group provided by SAM to methylate cytosines within a Cyt-phosphate-Gua (**CpG**) region (“island”) in the DNA and eventually creating methylated CpG patterns in the mammalian genome (Kass et al., 1997). The final epigenetic effect of DNA methylation is to override the predetermined genetic information in the DNA, and then the phenotype in mammals, the methylation of the DNA can induce significant modifications to the transcriptome. Previously, we observed a prepartal upregulation of *DNMT3A*, a gene that encodes for a DNA methyltransferase in charge of the *de novo* methylation of the DNA (Osorio et al., 2014a). And, more recently the significance of these findings was confirmed by observing significant alterations due to Met supplementation in the liver of transition dairy cows in terms of global DNA methylation and specific region methylation of an important TF, the peroxisome proliferator-activated receptor alpha (**PPARα**; Osorio et al., 2016a). The uniqueness of this gene regulator or **TF** within the context of the transition dairy cow was initially presented by Drackley (1999), and since then this nuclear receptor has become an interesting area of research in dairy cattle nutrigenomics (i.e., nutrient-gene interaction) (Bionaz et al., 2013). Therefore, the

connection between Met and PPARα upregulation through DNA methylation during the transition period is another suitable mechanism to explain the consistent improvements in performance (e.g., milk yield and DMI) observed in transition dairy cows supplemented with Met.

Since the initial application of high-throughput transcriptomic analysis such as a microarray platform in the dynamic adaptations of the liver during the transition period of dairy cows (Herath et al., 2004), a number of studies have followed utilizing even more advanced techniques such as RNA sequencing (**RNA-seq**) encompassing the whole transcriptome (Loor et al., 2013). The use of these techniques has revealed the biological signatures in the liver involving complex networks through numerous regulatory mechanisms with the aim to respond accurately to metabolic and physiologic cues during the transition period (Loor et al., 2006; Bionaz and Loor, 2012). From the initial experiment conducted at the University of Illinois on Met supplementation to peripartal dairy cows (Osorio et al., 2013), a microarray analysis of the liver transcriptome was performed (Osorio et al., 2012). This study revealed transcriptomic alterations in 2,663 genes [differentially expressed genes (**DEG**)] in the liver of cows supplemented with Met during the peripartal period (**Figure 3**). The functional analysis of these DEG showed not only an expected overall impact on the metabolic pathways of Cys, Met, and glutathione but also on less known cyanoamino acid and taurine metabolisms. Additionally, the high impact of gene networks associated with AA metabolism in control cows in this experiment underlined the key role of AA metabolism in the adaptations that occur during the transition period. From a nutrient-gene interaction standpoint, the results from this microarray analysis underpin the fact that dietary nutrients such as Met can have profound effects on the molecular makeup of dairy cows through gene expression alterations and subsequently promoting a better outcome in their performance during the transition period.

The importance of nutrigenomics in dairy cows has been previously reviewed (Bionaz et al., 2015). This relatively new area of research focuses on how dietary nutrients and compounds can affect gene expression directly or indirectly via interactions with TFs. Although, a specific TF that responds directly to AA or even specifically for Met still unknown, the potential interactions between AA and TFs have been discussed (Osorio et al., 2016b). The fact that Met can produce changes in the transcriptome add another layer of complexity to the metabolic, inflammatory, and antioxidant effects discussed above. Although the gene expression alterations by Met supplementation in dairy cows are consistent and evident, the actual molecular mechanisms by which this nutrient cause such alterations remain unclear.

A Model for Gene Regulation of Methionie in Dairy Cows

A proposed model for transcriptional alterations by Met supplementation is presented in **Figure 4**. This model rests on the well-established fact that Met is a precursor for SAM, that, in turn, can cause alterations in DNA and histone methylation. However, the effects of Met on gene expression through a specific TF via intermediate metabolites or cell membrane transporters are less understood.

- Intermediate metabolites of Met, for instance, cysteine downstream in the Met cycle could potentially interact with unknown TFs (e.g., zinc finger proteins) or be

essential for the final conformational structure (e.g., protein folding) of a TF through disulfide bonds, and increasing the available functional form of such TF; then this effect could cause a transcriptomic alteration.

- The PPAR belong to a family of TF that can bind and be activated by nutrients and compounds in the diet (i.e., ligand-dependent TF) (Bionaz et al., 2015). In the case of PPAR, it is well-known that this TF responds to fatty acids by increasing the transcription of genes related to metabolism and inflammation (Bionaz et al., 2013). Similar to PPAR, it is plausible that other TF in this family could potentially respond to Met, and then generate a change in gene expression, but such TF remains unknown.
- In recent years, advances in cell physiology have broadened our understanding of cell membrane AA transporters, making evident that these transporters may have dual receptor-transporter functions and act as “transceptors” to sense AA availability. As part of this sensor activity, transceptors can potentially initiate a cascade of cell signaling to result in a transcriptomic alteration through a TF.
- The use of Met as a precursor of SAM is widely accepted, and this methyl donor has been observed to cause significant alterations in DNA methylation in transition dairy cows supplemented with Met (Osorio et al., 2016a). Until now the effects of Met on histone methylation and subsequently, on gene expression have not been evaluated in the context of dairy cows.

Histone Methylation

In the cellular nuclei, the DNA is normally packed in condensed structures called chromatins, consisting primarily of histone proteins, which serve as spools where the DNA winds around. Then, the genetic information contained in the DNA exists in two states: unavailable or wind around histone proteins, and available or unwound. Chromatin remodeling is the main mechanism by which DNA is wind or unwound from histones and these dynamic modifications occur by enzymatic modifications including acetylation, phosphorylation, ubiquitination, and methylation (Singh et al., 2010). The latter is a potential mechanism through which Met can alter gene expression in dairy cows (**Figure 3**). Currently, the limited amount of data on histone methylation in dairy cows has been conducted using immune cells (He et al., 2012) primarily related to subclinical mastitis (He et al., 2016). This work has provided nuances on the interactions between mastitis-related pathogens and histone methylation, however dietary effects on histone methylation have not been investigated.

The use of fluorescent proteins to track biological events at the cellular level has been vastly exploited and impacted the fields of biochemistry, biotechnology, and cell biology. Within the context of nutrigenomics in dairy cattle, the use of fluorescent proteins was initially proposed and reviewed by Bionaz et al. (2015). One of the first papers utilizing this technique from a nutrigenomic approach in bovine mammary epithelial cells (i.e., **MacT** cells) was published (Osorio and Bionaz, 2017). Among the advantages to using fluorescent proteins is the ability to collect “true” real-time data on a specific cellular process without harvesting or extracting cells for each time point.

Recently, we have utilized a dual-fluorescent proteins system developed at the Massachusetts Institute of Technology (Lin et al., 2004) to track histone methylation with high spatial and temporal resolution in bovine cells. This fluorescent protein reporter allows for the analysis of specific methylation sites such as K9 and K27 in histones (i.e., regions of high methylation activity). For this analysis, we used the well-established bovine mammary epithelial cells, MacT cells, and treated them with 4 levels of Met in the media (0, 125, 250, and 500 µM) for 24 hours. The final parameter utilized for this type of experiment was relative fluorescent intensities analyzed through the CellProfiler (Kamentsky et al., 2011), and these data are presented in **Figure 5**. The results on K9 show an evident increase in histone methylation since 12 h post-treatment, and by 24 h the cells treated with 125 and 500 µM of Met expressed a greater ($P < 0.01$) histone methylation than control (Figure 5A). In contrast to K9, histone methylation at K27 site seems less receptive to methylation; in fact, cells treated with 500 µM of Met had a lower ($P < 0.01$) methylation status than control (Figure 5B). The viability results indicate a consistent improvement in K9 (Figure 5C) and K27 (Figure 5D) when cells were treated with 250 µM of Met. These preliminary data confirm the potential of Met to create histone modifications through methylation and consequently altering the transcriptome of dairy cows. The fact that histone methylation is not affected in a dose-dependent manner suggests that other unknown factors governing this biological process might override the effect of Met. Further ongoing analyses on global DNA methylation, region-specific DNA methylation, and gene expression profiling on the Met cycle and cell membrane transporters will help to get a better picture of the implications of the manipulation of this biological process through Met.

The importance of the method outlined above should not be restricted to an in-lab highly-controlled environment, but rather expanded by utilizing this as a complementary analysis for on-farm research experiments, or hybrid experiments (**Figure 6**). For instance, blood serum can be isolated from dairy cows supplemented with Met, with the aim to use it as a medium for bovine cells (e.g., liver, mammary, etc.). Prior to the incubation, the genetic information of the histone methylation reporters can be introduced into the bovine cells so these specialized proteins can be present at the incubation time. Through fluorescent microscopy methods the histone methylation data can not only be tracked in real-time, but also, these qualitative data can be transformed into quantitative data via imaging analysis software (i.e., CellProfiler). The ability to combine on-farm and in-lab data can be a new approach to broaden our understanding of dietary effects at the molecular level while providing actual data from an on-farm setting where importance sources of variation such as animal and environment can be considered.

Summary

Data collected in recent years on the effects of Met supplementation during the transition period of dairy cows have delineated a clearer picture of the multiple effects exerted by this nutrient at the level of performance, metabolism, and transcriptional. At the performance and metabolic level, the supplementation of Met to peripartal dairy cows consistently improved DMI, milk yield and components, and energy balance by enhancing liver function and antioxidant capacity while ameliorating the inflammatory

response. In contrast, the effects of Met at the transcriptional level are less understood. However, the substantial number of genes altered in the liver of transition dairy cows supplemented with Met is indicative of a fundamental change at the molecular level that, in turn, can be associated with a favorable metabolic status and performance. Future research related to AA balancing in dairy cows should focus on the nutrigenomic aspect of these nutrients and how they can impact and manipulate the genetic makeup of dairy cows and consequently associate this with performance and health status.

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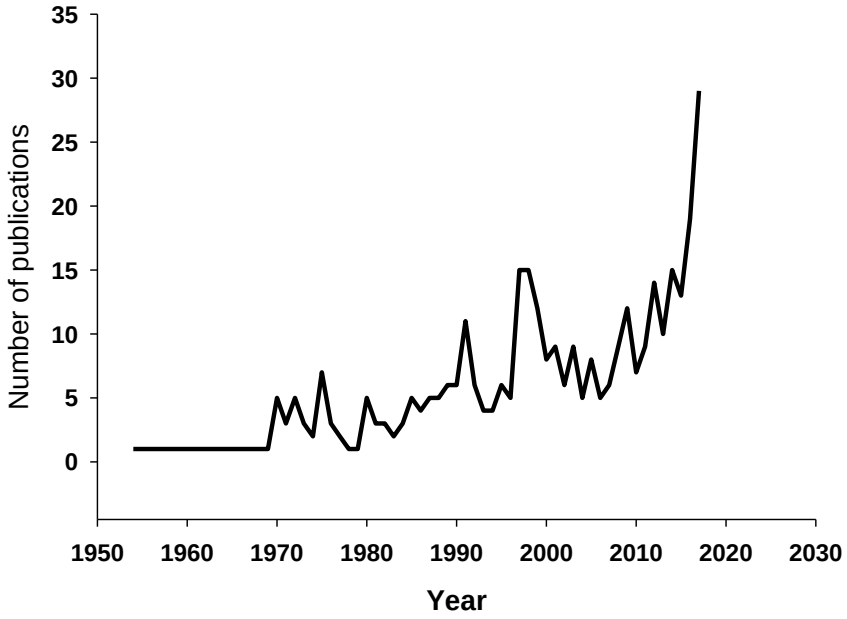

Figure 1. Number of scientific publications found in PubMed per year. The search was performed using the keywords “(methionine) AND (dairy) AND (cows)”. The search was performed on January 10, 2018.

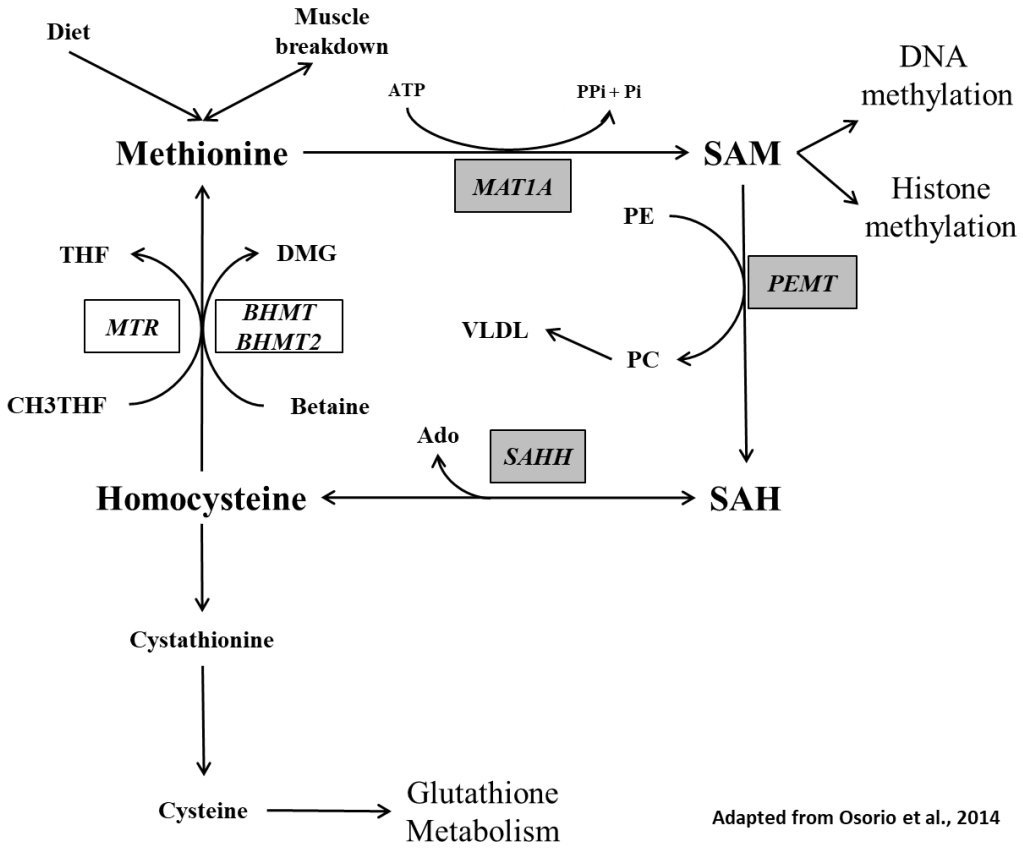

Figure 2. Key genes (squares) encoding for enzymes related to the Met cycle: Met adenosyltransferase 1A (*MAT1A*), phosphatidylethanolamine methyltransferase (*PEMT*), *S*-adenosylhomocysteine hydrolase (*SAHH*), betaine homocysteine methyltransferase (*BHMT* and *BHMT2*), and 5-methyltetrahydrofolatehomocysteine methyltransferase (*MTR*). Genes upregulated by Met supplementation in the liver of transition dairy cows are denoted by gray squares. PPi = pyrophosphate; Pi = inorganic P; SAM = *S*-adenosylmethionine; PE = phosphatidylethanolamine; PC = phosphatidylcholine; SAH = *S*-adenosylhomocysteine; Ado =adenosyl; THF = tetrahydrofolate; CH3THF = 5-methyltetrahydrofolate; DMG = dimethylglycine.

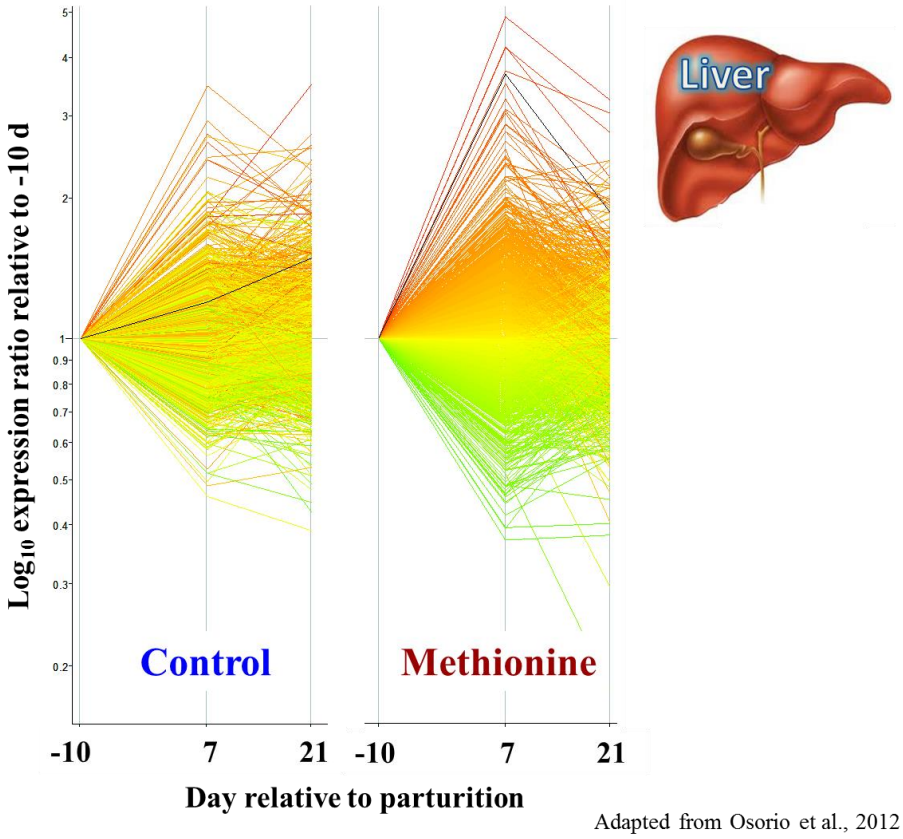

Figure 3. Overall transcriptomics adaptations in the liver of peripartal dairy cows fed a baseline diet (Control) or a baseline diet plus Met (Methionine). Shown in the image generated by Genespring GX7 (Agilent) of the 2,663 genes deemed to be differentially expressed with Time × Treatment with false discovery rate < 0.05. Green and red lines denote genes with an expression ratio lower or higher at 1 relative to -10 d, respectively.

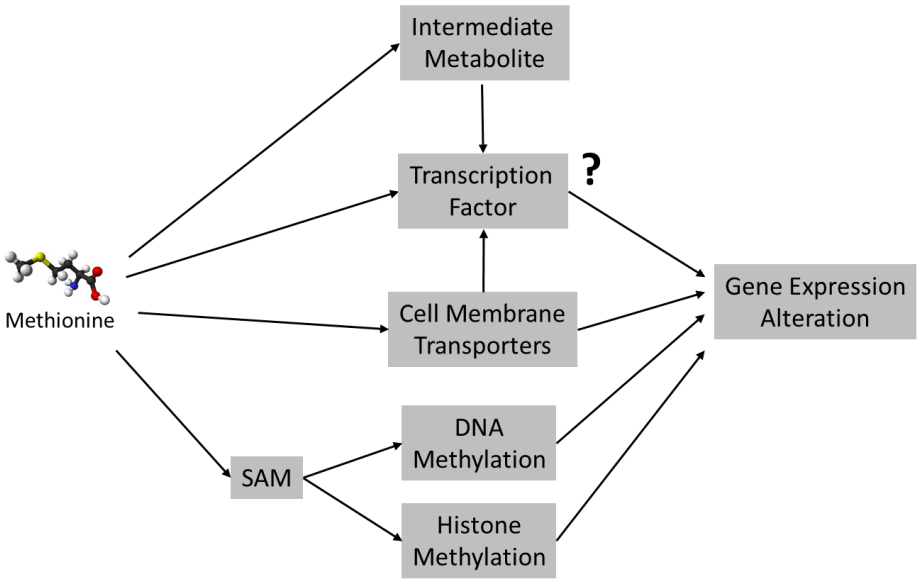

Figure 4. Proposed model for transcriptional alterations by Met supplementation in dairy cows.

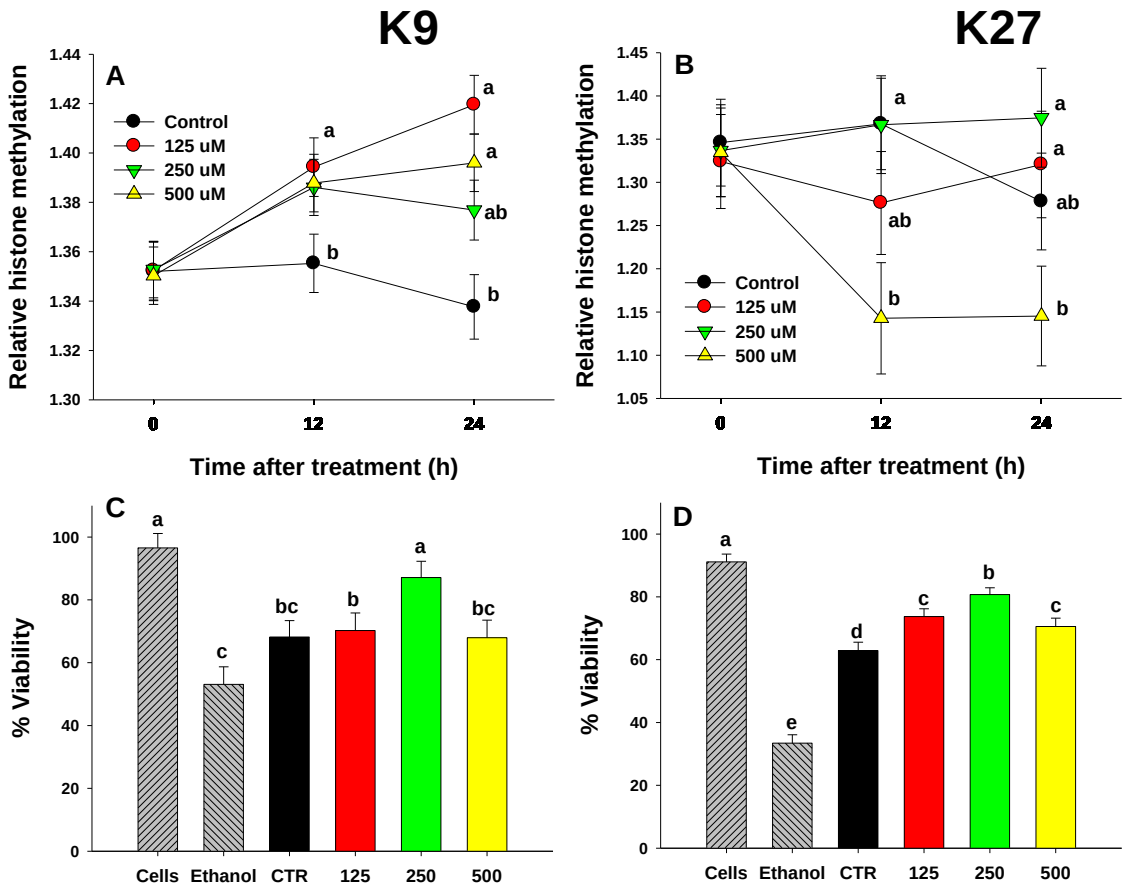

Figure 5. Relative histone methylation and cell viability in MacT cells treated with 0, 150, 250, and 500 uM of Met. MacT cells were seeded at 30,000 cells/well in a 96-well plate 24 h prior to transfection. Cells were transfected with either a K9 (**A**; Cat#22866, Addgene) or K27 (**B**; Cat# 22865, Addgene) plasmids to track histone methylation using a dual-fluorescent protein system (i.e., Fluorescence resonance energy transfer (FRET)]. Plasmids were transfected with 0.3 µL/well of Lipofectamine® 3000 (Cat# L30000001; Life Technologies) and 50 ng/well of DNA plasmid. The relative histone methylation was measured with an inverted fluorescent microscope for live imagining (Life Technologies) equipped with a motorized scanning stage. Viability of cells for K9 (**C**) and K27 (**D**) were measured at 24 h post-treatment using a staining for live cells (NucBlue®; Life Technologies) and a far-red nuclear staining (NucRed®; Life Technologies) for dead cells. The open-source software CellProfiler (Kamentsky et al., 2011) was used to analyzed each picture to quantify cell number and fluorescent intensities.

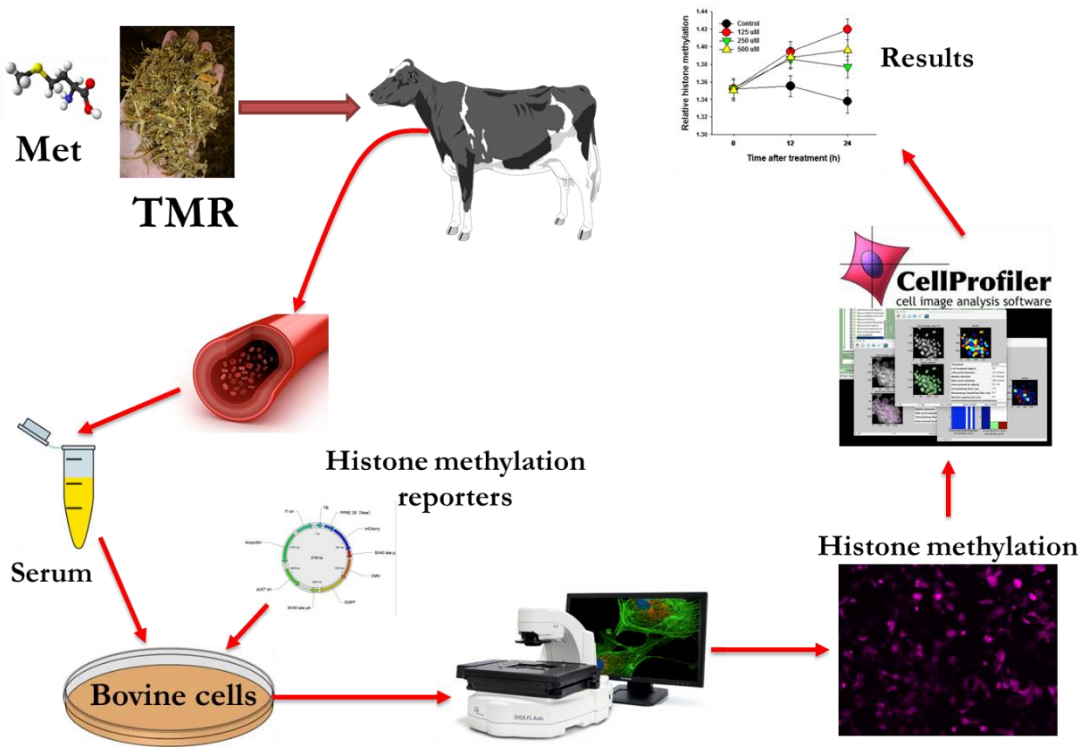

Figure 6. Schematics of a hybrid experiment for the study of histone methylation. This proposed method combines in-lab analysis of specific molecular events such as histone methylation and the tangible aspect of an on-farm experiment. Blood serum isolated from cows supplemented with Met can be utilized to incubate bovine cells. Prior to the incubation the genetic information encoding for a histone methylation reporter can be inserted (i.e., transfection) into the bovine cells. Then, the response to Met supplementation can be tracked through high-resolution imaging by multiple pictures taken by a fluorescent microscope in real-time. The qualitative data from each image taken during the incubation can be transform into quantitative data (i.e., relative intensity) through an open-source cell imaging software (CellProfiler). Finally, the relative intensity data can be statistically analyzed.

SESSION NOTES

Setting Yourself Up for Success with Amino Acid Balancing

Jessica Tekippe[1]
Ajinomoto Heartland Inc.

Introduction - Why Protein Nutrition is Important

Of the nitrogen fed to dairy cows, only 21 to 38 percent actually is exported as milk or meat. Consequently, 62 to 79 percent of the nitrogen fed is excreted in urine and feces. Can we increase the efficiency of nitrogen utilization? Yes, we can via amino acid balancing. What started as a catchy phrase has become a best practice when formulating dairy rations. Our counterparts in the swine and poultry industries, in monogastric livestock production, have long realized and capitalized on the production and efficiencies that occur when balancing for an animal's specific amino acid requirements.

In our defense, the ruminant animal is much more complex. With ruminants, amino acids need to be protected enough to get past the rumen, so they can be available for digestion and absorption in the small intestine. The "crude" protein we originally balanced for was actually a composite of different amino acids. As an industry, we want to move the dairy business forward. Amino acid balancing provides additional opportunities for the dairy to profit, i.e., higher milk and component production or reduced protein needs.

Ideally, each amino acid's supply and requirements would be identical matches. However, the amino acid composition of milk protein differs from the amino acid composition of feed ingredient protein. This mismatch often results in deficiencies of amino acids in dairy rations. These deficiencies, in turn, result in production or profit inefficiencies. When amino acids are supplied at levels below cow requirements, milk production is limited. Two amino acids are typically first limiting, methionine and lysine. It is absolutely necessary that these two amino acids make up a certain portion of the dietary protein content. Without them, dairy cows simply cannot produce at peak potential.

Transition Potential

Transition management directly impacts milk production and the bottom line. Poor transition periods can result in the loss of 10 to 20 pounds of peak milk per cow per day. That's significant – because for every pound of potential milk unrealized at peak production, the herd's total milk production for the lactation decreases approximately 200 pounds. This could represent 2,000 to 4,000 pounds of potential milk lost per cow per lactation. Cow management at transition is critical. It is closely linked to not only lactation performance but also to clinical and subclinical postpartum diseases and reproductive performance. Why? Transition is a critical turning point. Cows move

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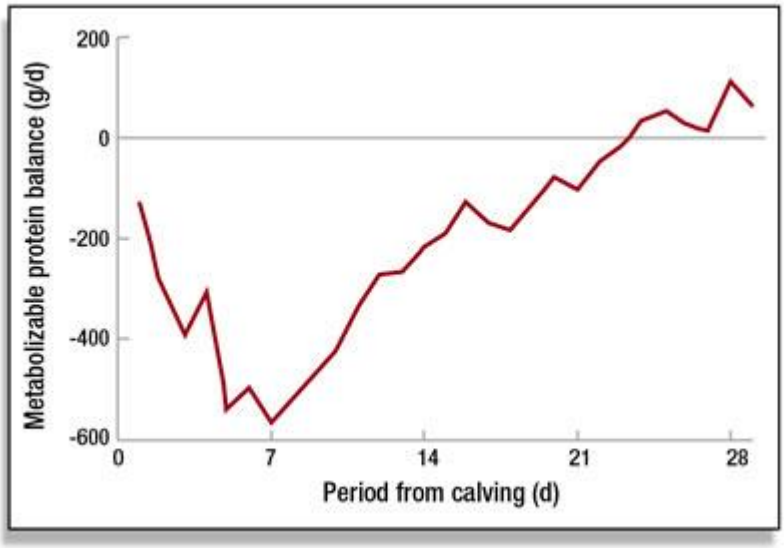
from non-lactating to lactating. There truly is a transition in the cow's physiology, metabolism, and nutritional requirements for three weeks prepartum until three weeks postpartum. During the critical transition period, several factors deserve management's focus. Prominent among these is the normal decrease in dry matter intake (**DMI**) as calving approaches. As DMI is compromised, a negative energy balance develops. DMI can decrease at a rate of approximately 2 percent of body weight during the first several weeks of the dry period. This ramps up to 1.4 percent of body weight during the seven days prior to calving. As cows approach transition, an overall 30 percent decrease in DMI may seem to have occurred rapidly.

After calving, cows enter negative energy balance. For example, the energy requirement of a cow producing 55 pounds of milk per day just two days after calving is about twice as high as her energy requirement two days before calving. The three weeks after calving, DMI is increasing at the rate of 3.3 pounds (1.5 kg) to 5.5 pounds (2.5 kg) per week, and negative energy balance corrects. How do we assist cows in maintaining energy balance? One strategy is to use concentrated nutrients that take up less room in the already limiting dry matter space and to balance amino acid levels. Balancing for amino acids is an increasingly common ration management tool that benefits all cows. Proteins are built from amino acids and have numerous and varied roles in cows. Today's rumen-protected amino acid products are concentrated nutrients that take comparatively little space in the ration. Their positive effects on transition cows are continuing to be revealed by research. At transition (Figure below), if rations contain insufficient levels of amino acids and proteins, cows mobilize their limited protein reserves. These reserves are located in peripheral tissues and muscle.



At transition, the synthesis of proteins and the efficiency of protein synthesis both increase, yet the diet should theoretically require a higher concentration of amino acids due to the reduced DMI. Rumen-protected amino acids can help ease this nutritional burden. During the last seven to 10 days of pregnancy, cows mobilize 1,000 grams of tissue every day. At the end of gestation, the uterus itself is extracting 72 percent of the amino acids in circulation. Methionine and lysine are the first two limiting amino acids. In

lactating cows, either histidine or arginine appears to be the next limiting. In transition cows, however, arginine is considered the next limiting after lysine and methionine.

Insufficient supplies in the wrong quantities can limit herd production and performance. For example, methionine is important for immune function, milk protein synthesis, and the formation of cysteine, an amino acid necessary for milk protein synthesis and immune system antioxidants. When methionine is lacking, and cysteine becomes limiting, cows can start to suffer from stress, inflammation, and immunosuppression. A shortage of lysine would be expected to first affect production, then reproduction, and then the immune system. Eventually, it could affect all body functions and systems, as lysine is a building block of several proteins with widely varying purposes in the body. Excess amino acid supplies have consequences too, so precision is important. An oversupply of methionine, for instance, throws off the balance of the lysine-to-methionine ratio and can have profound effects by further reducing DMI. This is supported by the hepatic oxidation theory. This theory suggests cows will stop eating based on the signals carried from the liver to the brain. These signals can be triggered when an excess supply of methionine is present and is not being used due to the limited lysine supply. An approximately 3-to-1 ratio of lysine to methionine is strongly recommended for cows through the transition period. The exact ratio varies somewhat among the various commercial ration formulation software programs. The corresponding grams of lysine and methionine would be around 96 to 32 for prefresh and 180 to 60 after calving.

Several transition studies on amino acid nutrition have been presented at the annual meetings of the American Dairy Science Association in recent years. Let's consider highlights: In one study (Table below), cows were fed zero, 16, and 32 grams of supplemental lysine for the four weeks following calving (Bell et al., 2000).

	0 - 4 weeks in lactation			5 - 8 weeks in lactation		
	Control	Lysine, 16 g	Lysine, 32 g	Control	Weeks 0-4, 16 g lysine; Weeks 5-8, control diet	Weeks 0-4, 32 g lysine; Weeks 5-8, control diet
Milk, lbs	81.8[a]	84.9[b]	86.4[b]	94.4	94.8	92.7

[a,b] Means are different, $P < 0.05$.

The resulting milk production was 81, 84 and 86 pounds, respectively. The milk volume response to lysine plateaued when the percent of lysine to metabolizable protein reached 7.67 and 8.36 – meaning the current recommendations might be too low. Further research is needed. Following the treatments, all groups were fed the control diet. Interestingly, milk production for the cows initially supplemented with amino acids and the control cows converged. This indicates that proper use of amino acid nutrition is vital as lactation progresses.

In another transition study, from three weeks before calving until three weeks post-calving, cows were assigned treatments as follows:

Control treatment pre- and post-calving;

Supplemental lysine pre- and control treatment post-calving;

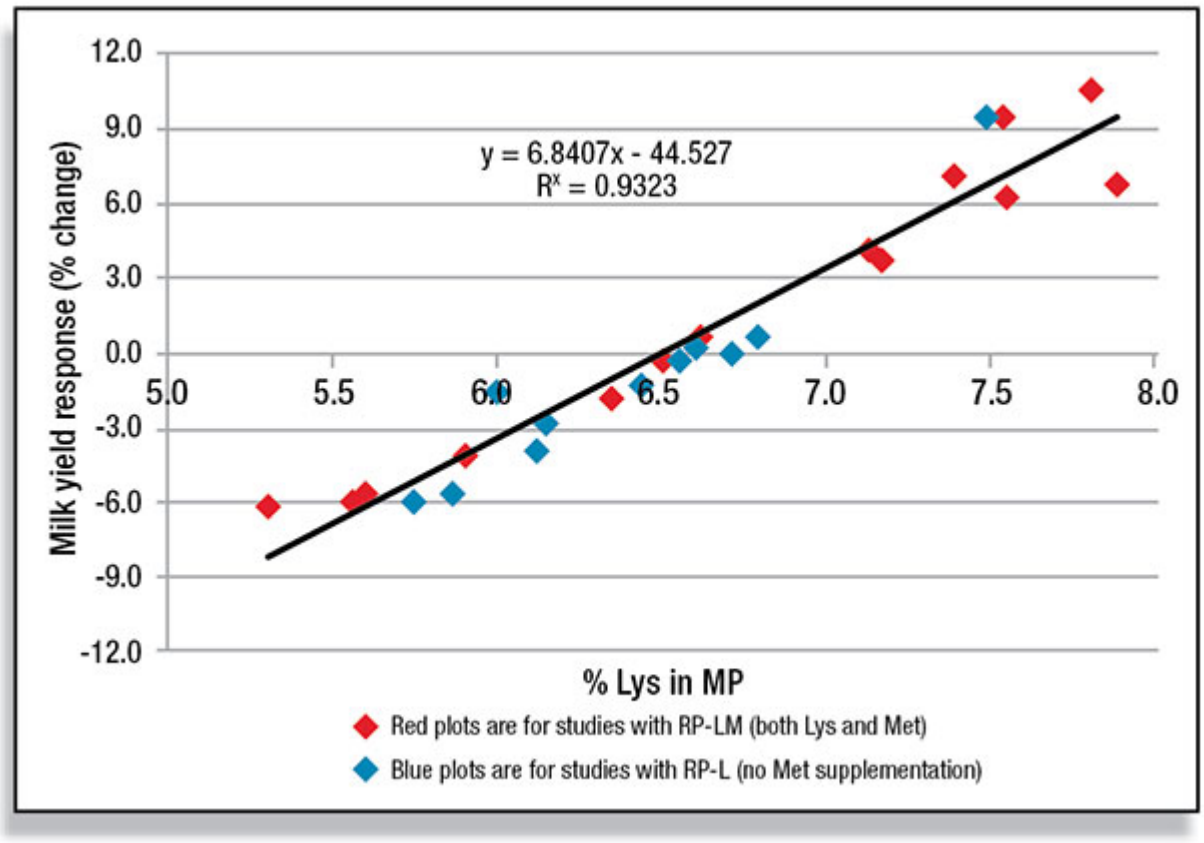
Control treatment pre- and supplemental lysine post-calving;

Supplemental lysine pre- and post-calving

The results? The group receiving supplemental lysine pre-calving had a greater DMI after calving with a significantly reduced incidence of disease. The group that received supplemental lysine pre- and post-calving produced the most milk, with the control group producing the least amount of milk. Future discussions of amino acid requirements through transition will likely expand beyond methionine and lysine as the industry focus shifts to which of the remaining 10 essential amino acids is next limiting – which is next in holding back production?

Lactating Animal

Supplementation with rumen-protected methionine (**RP-Met**) and lysine (**RP-Lys**) is now common. With methionine supplementation, we are seeing a trend to a 0.1 percent increase in milk protein for every 10 g of methionine supplementation when rations are methionine deficient. With lysine, a comparative newcomer, usage trends and recommended practices are emerging. Several published abstracts show an increase in milk flow from lysine supplementation. The figure below depicts the



abstracts resulting from research by Ajinomoto Heartland, Inc. company on our RP-Lys product. Several of the trials were conducted using only RP-Lys; others included RP-Met too.

As you can see, as the percentage of lysine in the metabolizable protein (percent Lys MP) increases, there is a constant increase in milk yield response. Many of these trials were done in early and high-lactation groups and resulted in a consistent response. From this dataset, we can derive that every 1 percentage point increase in Lys MP could result in a 6.8 percent increase in milk volume. How does this translate in the field? After observing and tracking several herds, the following lysine-usage practices are becoming apparent:

1. Adding amino acids to the ration; no other alterations.
2. Removing some blood meal or protein feed; adding RP-Lys.
3. Removing all blood meal; adding RP-Lys.

Adding Lysine; No Other Alterations

Adding lysine on top of the existing ration typically fails, based on our on-farm experience. Herds typically saw no response. Why? The diet likely was not limiting in lysine; consequently, the lysine added was metabolized into something else the cow needed, such as energy. While feed costs increased, no additional revenue was generated. Those herds that did sense a response had rations deficient in lysine (g less than 165).

Remove Some Blood Meal or Protein Feed

Removing some blood meal or protein feed typically provides cost savings thanks to the use of a synthetic form of lysine. Space also opens up in the diet for other, cheaper feeds. The ingredient changes that occur with this approach appear in the table below (expressed in pounds/cow per day). As you can tell, blood meal was reduced. In the new space, soybean products were increased for this 1,500-cow herd. The methionine source was adjusted to maintain the proper lysine-to-methionine ratio.

Ingredient	Pre-supplementation	Post-supplementation
High bypass soybean meal	1.32	1.42
Blood meal	0.90	0.54
Soybean hulls	0.07	0.28
Rumen-protected lysine	0.0	0.063
Rumen-protected methionine	0.035	0.04

Research recently completed comparing the models have indicated that lysine to methionine can be 2.7 to 1 for optimal results when utilizing a Cornell-based system and closer to 3 to 1 when utilizing a ration formulation system based on the National Research Council (NRC) recommendations. *Diet specifications* – The crude protein of this diet was reduced by 0.3 percent, the lysine was reduced by 2 g, methionine by 4 g, and the lysine-to-methionine ratio increased from 2.61 to 2.76. Some things stand out.

The total grams of lysine and methionine did decrease in this diet. This occurred because the lysine-to-methionine ratio was corrected, and the amount of lysine being provided by the blood meal is unknown. So the diet may have resulted in an increase in lysine that caused the production response. *Return on investment* – Positive results were obtained from both a cost savings and production standpoint. The diet cost decreased from $5.75 to $5.74. Milk yield increased from 80.5 pounds to 81.5 pounds. Butterfat percent increased from 3.58 percent to 3.7 percent, and protein increased from 3.03 percent to 3.13 percent. Milk urea nitrogen decreased from 10.4 to 9.2.

Remove Animal Byproduct Feeds

With the true methionine and lysine needs for the high-producing dairy cow being somewhat of a mystery, some nutritionists raised the question of what the next limiting amino acid is and whether we need to make sure we are meeting those requirements. Does this mean there is a need to keep some animal protein in the diet?

Research conducted by professor Alex Hristov at Pennsylvania State University has shown that at low crude proteins (13 to 14 percent), histidine likely becomes limiting. It would be unlikely to see levels that low in the field. However, environmental concerns could become an influencing factor here.

Ingredient	Pre-supplementation	Post-supplementation
Expeller meal	2.50	3.25
Blood meal	1.00	0.00
Rumen-protected methionine	0.01	0.02
Rumen-protected lysine	0.00	0.10
Supplemental methionine with cherry flavoring	0.05	0.06

This table (expressed pounds/cow per day) shows an example of a multi-thousand-head dairy whose nutritionist took the above approach. He felt consumer sentiment would prevent the use of animal proteins at some point. The extra diet space was filled with lysine supplementation and expeller meal. The methionine level was adjusted to maintain the proper ratios. *Diet specifications* – With this diet, crude protein decreased from 16.7 percent to 16 percent. The lysine grams went from 175 to 185, methionine from 59 to 61, and the lysine-to-methionine ratio changed slightly from 2.96 to 3.01. *Return on investment* – The return on investment was twofold. Price decreased from $7.29 to $7.02, and milk flow increased from 97.5 to 97.8 pounds. Butterfat increased from 3.5 to 3.6 percent, and protein increased from 3.03 to 3.05 percent. Milk urea nitrogen remained at 10.0.

What we know – What we don't know

Do we know everything about amino acid balancing? We have come a long way. Trends and key principles for amino acid balancing are developing.

- Protein level targets are: prefresh 1,300 grams of MP, and fresh cow and high cow MP target at 98 percent of required model predictions.
- Provide sufficient rumen-degradable protein to maximize microbial yield.
- Confirm that your RP-Met and RP-Lys match the manufacturers' specifications.
- Make sure starch levels provide enough energy to drive the production of amino acids.
- Focus on prefresh cows too. Ample research shows methionine improves immune function. However, maintaining the proper lysine-to-methionine ratio is essential to ensure excess methionine is not converted to something else needed by the cow.
- Know your ration formulation system to know if your lysine-to-methionine ratio should be 2.7 to 1 or 3 to 1.

Amino acid balancing will be critical in the future if markets are down, up, or in between. Amino acids are essential nutrients. They directly impact animal production and performance.

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SESSION NOTES

Prepartum Negative DCAD Diets – They're Not Just for Milk Fever Anymore

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Introduction

Hypocalcemia is presented in two forms, the less common clinical disease called milk fever and the more common form called subclinical hypocalcemia (**SCH**). Depending upon how it is defined and the frequency of blood sampling for diagnosis, SCH can affect 25 to 40% of primiparous and 45 to 80% of the multiparous cows. Cows with SCH have reduced dry matter (**DM**) intake, suppressed measures of innate and acquired immune function, compromised energy metabolism, and increased incidence of other periparturient diseases. This is why hypocalcemia is considered a "gateway" disease for dairy cattle.

Cows develop hypocalcemia because of the sudden irreversible loss of calcium (**Ca**) during synthesis and secretion of colostrum and milk. Most cows are able to cope with the loss of Ca with the onset of lactation, but many, if not all, will experience a decline in blood concentrations of total (**tCa**) and ionized (**iCa**) Ca in the first 2 to 3 days of lactation. Multiple strategies are available to minimize the risk or prevent hypocalcemia in dairy cows and they often include manipulations of the prepartum diet. One of such strategies is the manipulation of the mineral content of prepartum diets to induce a compensated metabolic acidosis. Extensive research has characterized the benefits of feeding acidogenic diets prepartum on reducing the incidence of milk fever, but less has been known about the potential benefits of these diets on productive performance and incidence of other periparturient diseases. One would think that preventing milk fever would consistently result in improved yields of milk and milk components and reduced risk of other common diseases in early lactation. This manuscript reviews recent research on methods to control and reduce the impact of hypocalcemia in dairy cows and the extended benefits of acidogenic diets prepartum.

Hypocalcemia

During the last month of gestation, the absorbable Ca requirements in the typical dairy cow are estimated at 16 g/day to meet the needs for accretion into the pregnant uterus and endogenous fecal losses. As lactation initiates and cows synthesize colostrum, the needs for Ca increase substantially. The concentration of Ca in

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colostrum is range from 2.2 to 2.4 g/L and it remains elevated during the period in which the cow produces transition milk.

Dairy cows develop hypocalcemia because of the inability to promptly restore blood concentrations of Ca with the onset of colostrogenesis and lactation. In general, blood concentrations of Ca start to decline 1 or 2 days before calving in old Jersey cows fed an alkalogenic diet that predisposes to hypocalcemia (Goff et al., 2002) or at least 9 h before calving in multiparous Holstein cows fed an acidogenic diet to prevent hypocalcemia (Megahed et al., 2018). This decline in blood concentrations of Ca before calving is thought to be mediated by the sequestration of Ca in the gland for synthesis of colostrum. When Jersey cows underwent mastectomy, they did not experience any noticeable decline in blood tCa despite the typical decline in DM intake on the days preceding calving (Goff et al., 2002). These findings by Goff and colleagues (2002) reinforced the concept that changes in blood Ca concentrations around calving are the result of irreversible loss of Ca for synthesis of colostrum and milk, and not related to the endocrine changes associated with parturition. As cows age, they become more susceptible to hypocalcemia (DeGaris and Lean, 2008), in part because older cows produce more colostrum (Martinez et al., 2018a) and, therefore, have greater loss of Ca immediately after calving. Multiparous cows secrete larger quantities of Ca in the first milking (52% more or 9 g) than primiparous cows, which poses increased strain on Ca homeostasis. Risk of hypocalcemia depends, in part, on the amount of colostrum synthesized and secreted by cow. Depending on the amount produced, the irreversible loss of Ca in the first milking might represent 5 to 10 times the total plasma Ca pool. If milked twice in the first day postpartum, the total Ca loss might be as much as 22 to 30 g. This means that a dairy cow has to replenish the equivalent of total amount of circulating Ca at least 5 to 10 times daily to be able to supply all the Ca for colostrum and transition milk synthesis on the day of calving.

It is known that milk fever is a problem that affects primarily older cows and prepartum nulliparous cows are almost never affected by the clinical form of hypocalcemia. It is thought that the greater colostrum synthesis associated with a less dynamic bone Ca resorption mechanisms and, perhaps, less active Ca transport in the gastrointestinal tract favors a greater incidence of hypocalcemia in multiparous than primiparous cows. The risk of milk fever increases 9% for every lactation in the life of a cow (DeGaris and Lean, 2008). Although milk fever can be life-threatening, the most common presentation of hypocalcemia is the subclinical form, in which blood Ca concentrations are below a particular threshold that consequently predisposes cows to other diseases. Most commonly, SCH characterized by blood tCa ≤ 2.0 m*M* (Reinhardt et al., 2011), although the rationale for such a cut-off has not been clearly defined. Others have proposed different thresholds such as tCa of < 2.15 m*M* (Martinez et al., 2012) based on increased risk of metritis and other diseases, or iCa < 1 m*M* (Oetzel et al., 1988). Prevalence of SCH within the first 2 days postpartum, based on serum tCa ≤ 2.0 m*M*, was 25% in primiparous and 45% in multiparous cows (Reinhardt et al., 2011), whereas incidence can vary not only with threshold selected, but also frequency of sampling of blood postpartum.

Impacts of Hypocalcemia

One of the imminent effects of milk fever is the risk of death. Cows that suffer from milk fever are more likely to die either because of the consequence of low blood Ca and recumbence with subsequent muscle-skeletal lesions, but also because of the risk of therapy with Ca, particularly intravenously, might result in cardiac arrest.

The importance of hypocalcemia goes beyond its clinical symptoms. It is well established that hypocalcemia increases the risk of other diseases. Subclinical hypocalcemia reduces DM intake and rumination, and impairs insulin secretion (Martinez et al., 2014), leading to increased lipolysis. Those effects likely explain the increased risk of displaced abomasum, ketosis, dystocia and uterine prolapse. Furthermore, hypocalcemic cows have increased plasma concentrations of cortisol (Horst and Jorgensen, 1982), reduced proportion of neutrophils with phagocytic activity (Martinez et al., 2012; 2014) and reduced concentrations of cytosolic iCa in neutrophils (Martinez et al., 2014) and mononuclear cells (Kimura et al., 2006). Therefore, reducing the risk of hypocalcemia is thought to minimize its consequences, i.e., by reducing the incidence of SCH and milk fever, one expects that the incidence of the periparturient problems mentioned above would also be reduced.

Dietary Methods to Reduce Hypocalcemia

There are three main dietary strategies evaluated to reduce the risk of SCH and milk fever in dairy cows. They include limited gastrointestinal Ca absorption either by feeding limited amounts of Ca or by sequestering Ca in the lumen of the digestive tract with the use of zeolites; supplementing Ca orally or through intravenous or subcutaneous administration immediately after calving; and manipulation of the mineral content of prepartum diets. For this manuscript, we will focus on the effects of altering the dietary-cation anion difference (**DCAD**) of prepartum diets.

Altering the dietary cation-anion difference

Although clear statistics are not available, it is likely that in the US today, manipulations of the DCAD are the most widely implemented dietary method to reduce the risk of hypocalcemia in dairy cows. Feeding acidogenic diets induce a state of compensated metabolic acidosis that increases the concentrations of iCa in blood through different pathways. The reduction in blood pH increases the ionized fraction of Ca by displacing it from albumin. Metabolic acidosis increases parathyroid hormone secretion (Lopez et al., 2002), increases sensitivity of tissues to PTH (Goff et al., 2014), and increases the expression of PTH receptor in the kidney of cattle (Rodriguez et al., 2016). In general, the maximal PTH response to Ca chelation occurs during metabolic acidosis compared with normal blood pH (Lopez et al., 2002).

When salts containing strong anions are fed, the premise is that the rate and extent of absorption of the anion is greater than that of the cation in the salt or that the cation is metabolized such that it is not absorbed as a cation. In the latter case, the

cation in the salt can be utilized by the rumen microbes such as in the case of ammonium chloride. The strong anions most commonly supplemented in diets are chloride (**Cl^-**) and sulfur (**S^{2-}**) in the form of sulfates (**SO_4^{2-}**). It is thought that Cl^- is more bioavailable than S^{2-} so it has greater acidifying power. When salts of Cl and SO_4 are fed, for instance $CaCl_2$, more equivalents of the anion (Cl^-) from the molecule is absorbed than the cation (Ca^{2+}). This causes an imbalance in charges in the epithelial cell of the gut (increase in negative charges), which forces secretion of bicarbonate (**HCO_3^-**) into the intestinal lumen or retention of H^+ ions. The end result is a loss of HCO_3^- and an increase in H^+ concentration which ultimately results in a state of metabolic acidosis. If compensated, then minor changes in blood pH occur, with changes in blood HCO_3^- and the partial pressure of carbon dioxide (**CO_2**) caused by changes in respiration rate. A common finding is aciduria because of the increased proton excretion in urine as part of the compensatory mechanism.

Overwhelming evidence from controlled experiments demonstrate that feeding acidogenic diets prepartum reduce the risk of milk fever and SCH in dairy cows (Ender et al., 1971; Block et al., 1984; Martinez et al., 2018b); however, less certainty exists about the effects of acidogenic diets on lactation performance and incidence of diseases other than hypocalcemia.

We recently completed a meta-analysis of the published literature with randomized controlled experiments with transition cows in which prepartum diets fed as totally mixed rations had the mineral composition altered to manipulate the DCAD or cows were fed diets with acidogenic products in which the contents of Ca, phosphorus (**P**), or magnesium (**Mg**) were manipulated (Santos et al., 2018).

A total of 42 experiments were included in the data base, with 5 experiments reporting data for nulliparous totaling 151 cows and 41 experiments that reported data for 1,652 parous cows. The DCAD ([mEq of K+ + mEq of Na+] – [mEq of Cl− + mEq of S^{2-}]) of prepartum diets ranged from -246 to 1,094 mEq/kg and concentrations of dietary Ca (0.16 to 1.98%), P (0.18 to 1.58%), and Mg (0.09 to 0.68%) had wide ranges that allowed us to evaluate their associations with production and health. Diets were fed for an averaged (± SD) of 21.9 ± 1.8 and 25.6 ± 1.0 d for nulliparous and parous cows, respectively.

Effects of altering the prepartum DCAD on DM intake pre- and postpartum

Data were analyzed with 115 treatment means from 36 of the 42 experiments that reported DM intake prepartum. Manipulating the DCAD resulted in a quadratic response with a decrease in prepartum DM intake. As the DCAD decreased, DM intake prepartum also decreased in both nulliparous and parous cows with no interaction between DCAD and parity group. Reducing the prepartum DCAD from 200 to -100 mEq/kg resulted in a 0.7 and 0.4 kg/d reduction in DM intake in nulliparous and parous cows. Concentrations of Ca, P, and Mg in prepartum diets did not influence DM intake prepartum. We then analyzed data on prepartum DMI incorporating type of acidogenic product fed as none for diets without any acidogenic product, salts for diets in which

acidogenic salts were used, or commercial products for diets in which the acidogenic product was a designed commercial product to determine if the depression was induced by use of a source of strong ions. Inclusion of salts or commercial product reduced (P < 0.01) DMI prepartum irrespective of the source of strong ions fed (none = 9.0 ± 0.4 vs. salts = 8.5 ± 0.4 vs. commercial products = 8.4 ± 0.4 kg/d).

Intake postpartum was reported in 26 experiments with 86 treatment means. As opposed to prepartum intake, a reduction in prepartum DCAD increased postpartum DM intake in both parity groups. An increment of approximately 1 kg/d in DM intake was estimated by reducing the prepartum DCAD from 200 to -100 mEq/kg (Santos et al., 2018).

It is often debated the reason acidogenic diets influence prepartum DM intake in dairy cows. In some cases, authors suggested that palatability of acidogenic salts is the culprit for such depression, although the decrease is observed even when cows are fed commercial products. We recently completed an experiment to address this question (Zimpel et al., 2018). We used 10 nulliparous pregnant non-lactating Holstein cows that were subjected to a replicated 5 x 5 Latin square design. The experiment was composed by 5 periods of 14 days each and all 10 cows received all 5 treatments. Diets were fed as total mixed rations and composed of corn silage, Bermuda hay, and concentrates. Diets were manipulated by replacing a portion of the grain in the concentrates with an acidogenic product or salts containing potassium (**K**), sodium (**Na**), and Cl. Dietary treatments were:

T1. (K = 1.42%, Na = 0.04%, Cl = 0.26% of DM) a base diet containing 55% corn silage, 10% grass hay, and 35% concentrate that resulted in a DCAD of +200 mEq/kg;
T2. (K = 1.83%, Na = 0.42%, Cl = 1.23% of DM), the control diet with 2% added mixture of 1:1 NaCl and KCl to result in a DCAD of +200 mEq/kg;
T3. (K = 1.71%, Na = 0.54%, Cl = 0.89% of DM), the control diet with added acidogenic product and a mixture of K_2CO_3 and $NaHCO_3$ to result in a DCAD of +200 mEq/kg;
T4. (K = 1.29%, Na = 0.13%, Cl = 0.91% of DM), the control diet with added acidogenic product to reduce the DCAD to -120 mEq/kg; and
T5. (K = 1.78%, Na = 0.53%, Cl = 2.03% of DM), the control diet with added acidogenic product, KCl, and NaCl to result in a DCAD of -120 mEq/kg.

Therefore, T1, T2 and T3 had different contents of Cl and addition or not of acidogenic product, but the same positive DCAD, whereas T4 and T5 had distinct amounts of Cl, but the same negative DCAD. Intake of DM and water was monitored daily and feeding behavior was evaluated for 48 h in each period. Blood and urine samples were collected multiple times from each cow in each period for measurements of acid-base status and urinary excretion of minerals.

A summary of some important findings in the experiment is presented in **Table 1**. Adding chloride salts, including the acidogenic product without altering the acid-base status of cows did not affect dry matter intake (see T1, T2 and T3); however, when the

acidogenic product reduced the DCAD from +200 to -120 mEq/kg in treatments T4 and T5, then cows experienced a compensated metabolic acidosis with reduced blood and urinary pH, increased respiratory rate, and reduced blood bicarbonate (HCO_3^-) and partial pressure of CO_2 (**pCO_2**), which reduced dry matter intake. It is important to note that addition of acidogenic product per se, as in T3, did not reduce dry matter intake. In fact, if one compares intake in treatments T1, T2 and T3, it is clear that not only they did not differ statistically, but they were numerically very similar, ranging from 10.2 to 10.3 kg/day (or 1.76 to 1.74% of body weight). On the other hand, when adding the acidogenic product induced a metabolic acidosis, like in the case of T4 and T5, regardless of level of Cl in the diet, then DM intakes decreased to 9.7 and 9.5 kg/d (1.68 and 1.64% of body weight), respectively (Zimpel et al., 2018). These results demonstrate that depression in intake is not necessarily related to the inclusion of acidogenic products but caused by the metabolic acidosis induced by the acidogenic diet.

Effects of altering the prepartum DCAD on production performance

Numerous experiments have evaluated the impact of manipulating the prepartum DCAD on postpartum performance in dairy cows and, in many cases, numerical differences were observed without statistical effect.

Interactions between level of DCAD and parity group were observed for yields of milk, fat-corrected milk, fat, and protein. Reducing the DCAD increased yields of milk, fat-corrected milk, fat, and protein in parous cows; however, a similar manipulation in DCAD either did not influence yields of milk but tended to reduce those of fat-corrected milk and protein and reduced that of milk fat in nulliparous cows (**Table 2**; Santos et al., 2018).

It is clear that parous cows respond positively to acidogenic diets with increased yields of milk and milk components. Nevertheless, heterogeneity in response to manipulations in DCAD prepartum also have been reported by others (Lean et al., 2014), and productive responses of nulliparous to acidogenic diets do not seem to be the same as that observed for parous cows. Parous cows are more prone to disturbances of Ca metabolism with the onset of lactation (Lean et al., 2006), and hypocalcemia is known to depress appetite (Martinez et al., 2014), which can compromise lactation performance. One of the limitations of the meta-analysis by Santos et al. (2018) is that only 5 experiments with 15 treatment means reported production performance for nulliparous cows. This limited data base might have precluded detection of a clearer productive response of nulliparous to manipulation of prepartum DCAD.

Effects of altering the prepartum DCAD on incidence of diseases in early lactation

Milk fever and retained placenta were reported in most experiments, whereas metritis, mastitis and displaced abomasum were reported in only half of the experiments reviewed in the meta-analysis. Because milk fever affected only parous cows, nulliparous were not included in the statistical models. As expected, DCAD had a

profound effect on incidence of milk fever. The predicted incidence in parous cows reduced from 11.7 to 2.8% by reducing the DCAD from 200 to -100 mEq/kg (**Table 3**). Moreover, the benefits of diets with negative DCAD were also observed for retained placenta and metritis. Incidence of retained placenta and metritis decreased with a reduction in DCAD and the benefits were observed in nulliparous and parous cows (**Table 3**). The incidence of mastitis and displaced abomasum were not influenced by DCAD of prepartum diets and no interaction between DCAD and parity group were observed for those two diseases. Number of disease events per cow declined with a reduction in DCAD of prepartum diets in both nulliparous and parous cows.

We had previously shown that cows that develop SCH have suppressed innate immune function and increased risk of uterine diseases (Martinez et al., 2012). When SCH was induced in dry cows, neutrophil function was suppressed for at least 72 h after concentrations of tCa and iCa had been reestablished in blood of cows. It is well described that innate immunity is critical for shedding of the placental tissues and protection of the reproductive tract against invading pathogens. Because cows fed acidogenic diets maintain increased tCa and iCa concentrations on the day of calving and the first days postpartum, they are less likely to develop SCH, which likely improves innate defenses of the uterus, thereby minimizing the risk of retained placenta and metritis.

Although cows fed acidogenic diets had reduced incidence of milk fever and metritis and consumed more DM postpartum, the risk of displaced abomasum did not change. It is important to mention that only 14 experiments reported displaced abomasum incidence. Also, some experiments removed cows from the data base if they developed issues at calving. Therefore, it is possible that lack of differences in risk of displaced abomasum might have been influenced by either the limited data base or by potential exclusion of cows from experiments.

Duration of feeding acidogenic diets and level of DCAD

To our knowledge, only three experiments have evaluated the impact of duration of feeding of acidogenic diets prepartum (Weich et al., 2013; Wu et al., 2014; Lopera et al., 2018). Sixty cows were fed one of 3 treatments starting at 42 d relative to the expected calving date. Treatments were a control diet (+120 mEq/kg), a positive DCAD in the first 21 d followed by a negative DCAD diet in the final 21 d of gestation (+120 mEq/kg followed by -160 mEq/kg), or 42-d of feeding a negative DCAD diet (-160 mEq/kg). The authors found feeding acidogenic salts for the last 21 d of gestation improved Ca homeostasis and milk yield (5.6 kg/d). They also found that extending the feeding of acidogenic salts from 21 to 42 d had no statistically significant effect on the subsequent lactation, although cows fed the diet for the extended period produced 2.3 kg less milk (44.8 vs. 42.5 kg/d). Wu et al. (2014) showed no differences in postpartum performance when prepartum cows were fed a diet with a DCAD of -210 mEq/kg for the last 3, 4 or 6 weeks of gestation.

We have recently explored this question and evaluated the effects of feeding acidogenic diets for the last 21 or 42 d of gestation at two levels of DCAD, -70 or -180 mEq/kg (Lopera et al., 2018). We enrolled 114 parous Holstein cows at 230 d of gestation, 48 that completed lactation 1 and 66 that completed lactation > 1. Cows were randomly assigned to 1 of 4 treatments arranged as a 2 x 2 factorial; treatments varied by level of DCAD, -70 or -180 mEq/kg, and by length of feeding, the last 21 d (Short) or the last 42 d (Long) prepartum. Therefore, the 4 treatments were Short -70 (n = 29), Short -180 (n = 29), Long -70 (n = 28) and Long -180 (n = 28). Cows in the Short treatments were fed a diet with positive DCAD of +110 mEq/kg of DM from -42 to -22 d relative to calving. After calving, cows were fed the same diet and lactation performance and incidence of diseases were evaluated for the first 42 DIM, whereas reproduction and survival was evaluated for 305 d postpartum. Reducing the DCAD linearly decreased prepartum DM intake between -42 and -22 d relative to calving (+110 mEq/kg = 11.5 vs. -70 mEq/kg = 10.7 vs. -180 mEq/kg =10.2 ± 0.4) and the diet with -180 mEq/kg fed in the last 21 d of the dry period reduced intake by 1.1 kg/d compared with the diet containing -70 mEq/kg (-70 mEq/kg = 10.8 vs. -180 mEq/kg = 9.7 ± 0.5 kg/d). Cows fed the -180 mEq/kg diet had increased concentrations of iCa in blood on the day of calving (-70 mEq/kg = 1.063 vs. -180 mEq/kg = 1.128 ± 0.020 m*M*), but no differences were observed in the days following calving. Extending the duration of feeding the diets with negative DCAD from 21 to 42 d reduced gestation length by 2 d (Short = 277.2 vs. Long = 275.3 d), milk yield by 2.5 kg/d (Short = 40.4 vs. Long = 37.9 ± 1.0 kg/d) and tended to increase days open because of reduced pregnancy per AI after all inseminations (Short = 35.0 vs. Long = 22.6%).

Although other experiments would suggest some flexibility in how long acidogenic diets should be fed prepartum, the results of Lopera et al. (2018) suggest that feeding these diets for longer than 21 d might not be advised. In any case, there seems to be no advantage of extending the feeding of acidogenic diets for the entire dry period and a potential loss in production and, perhaps, reproduction. Although the meta-analysis did not reveal a value of negative DCAD that optimized postpartum performance and health (Santos et al., 2018), the results of Lopera et al. (2018) suggest that there is no need to reduce the DCAD to -180 mEq/kg.

Conclusions

Hypocalcemia is a prevalent metabolic disorder in dairy cows in early lactation. Cows develop SCH and milk fever because of inability to either mobilize Ca from bones or readjust gastrointestinal absorption at the onset of colostrogenesis and lactation to replenish the blood Ca pool. Hypocalcemia increases the risks of numerous other health problems resulting in economic losses to dairy producers. Dietary manipulation by feeding acidogenic diets remains the method of choice for prevention of hypocalcemia. The foundation of acidogenic diets should be based on limiting the intakes of Na, K, at the same time that strong anions, particularly Cl, are supplemented. The metabolic acidosis induced by acidogenic diets is expected to depress DM intake prepartum, but prevention of clinical and SCH have long-last benefits to dairy cows, particularly those that become multiparous cows. Acidogenic diets reduce the risk of milk fever, SCH,

retained placenta, and metritis; they increase DM intake postpartum, and productive performance in parous cows. The ideal DCAD remains to be established, but it is likely that for parous cows it does not need to be less than -150 mEq/kg, whereas for nulliparous the data are scarce, and the potential benefits remain unclear.

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Table 1. Effect of manipulating the dietary cation-anion difference on acid-base status and intake in dry cows[1]

Item	T1	T2	T3	T4	T5	SE[2]
Intake DM, kg/d*§	10.3	10.2	10.2	9.7	9.5	0.2
Intake DM, % of BW*§	1.76	1.75	1.74	1.68	1.64	0.03
Intake of water, L/d§‡ †	25.4	31.0	30.5	26.5	32.3	0.8
Urine pH*§‡	8.1	7.9	7.9	5.7	5.6	0.06
Blood						
pH*§‡	7.450	7.436	7.435	7.420	7.416	0.005
Base excess, m*M* *§	1.85	1.20	1.45	-0.20	-0.95	0.32
HCO_3^-, m*M* *§	25.9	25.5	25.8	24.3	23.7	0.3
pCO_2, mm Hg§	37.4	38.2	38.4	37.0	36.6	0.7
Respiratory rate, n/min§	27.6	27.3	26.8	28.4	29.0	0.4

[1] Data from Zimpel et al. (2018)
[2] SE = standard error.
* Effect of adding acidogenic product: T1 vs. T4 ($P < 0.05$).
§ Effect of metabolic acidosis: T2 + T3 vs. T4 + T5 ($P < 0.05$).
‡ Effect of adding Cl salts to alkalogenic diet: T1 vs. T2 ($P < 0.05$).
† Effect of adding Cl salts to acidogenic diet: T4 vs. T5 ($P < 0.05$).

Table 2. Estimated effect (means and SE) of reducing the DCAD from +200 to -100 mEq/kg on intake and yields of milk and milk components in Holstein cows[1]

		Nulliparous		Parous		*P*-value[2]	
Item	Means (Exp.)[3]	+200	-100	+200	-100	DCAD	DCAD x parity
DM intake, kg/d							
Prepartum	115 (36)	10.3 ± 0.5	9.6 ± 0.5	12.4 ± 0.4	12.0 ± 0.4	0.02	0.49
Postpartum	86 (26)	12.9 ± 0.9	13.7 ± 0.9	17.6 ± 0.7	18.6 ± 0.7	0.03	0.81
Yield, kg/d							
Milk	90 (28)	25.9 ± 1.3^{c}	24.5 ± 1.3^{c}	36.2 ± 1.1^{b}	37.9 ± 1.1^{a}	0.74	0.03
Fat-corrected milk	84 (25)	26.6 ± 1.9^{c}	24.5 ± 1.9^{d}	38.8 ± 1.8^{b}	39.9 ± 1.8^{a}	0.90	0.002
Fat	84 (25)	0.995 ± 0.073^{c}	0.888 ± 0.073^{d}	1.438 ± 0.066^{b}	1.512 ± 0.066^{a}	0.60	0.006
Protein	84 (25)	0.755 ± 0.053^{c}	0.695 ± 0.053^{c}	1.115 ± 0.047^{b}	1.139 ± 0.047^{a}	0.44	0.07

^{a,b,c,d} Within a row, superscripts differ ($P < 0.05$).

[1] Data from Santos et al. (2018).

[2] DCAD = effect of DCAD; DCAD x parity = interaction between DCAD and parity.

[3] Number of treatment means and number of experiments (Exp.) for each response analyzed.

Table 3. Estimated effect (means and SE) of reducing the DCAD from +200 to -100 mEq/kg on incidence of diseases in Holstein cows[1]

		Nulliparous		Parous		*P*-value[2]	
Item (% ± SEM)	Means (Exp.)[2]	+200	-100	+200	-100	DCAD	DCAD x parity
Milk fever, parous cow	99 (35)	0	0	11.7 ± 2.8	2.8 ± 0.9	< 0.001	NE[3]
Retained placenta	73 (22)	12.7 ± 2.7	3.5 ± 2.7	17.0 ± 1.6	9.0 ± 1.6	0.05	0.61
Metritis	42 (12)	34.4 ± 5.6	12.0 ± 5.6	16.3 ± 2.7	9.9 ± 2.7	0.02	0.34
Cases per cow, n ± SEM	108 (35)	0.34 ± 0.07	0.20 ± 0.07	0.39 ± 0.04	0.17 ± 0.04	< 0.001	0.56

[a,b,c,d] Within a row, superscript differ ($P < 0.05$).

[1] Data from Santos et al. (2018).

[2] DCAD = effect of altering the DCAD; DCAD x parity = interaction between DCAD and parity (nulliparous or parous).

[3] Number of treatment means and number of experiments (Exp.) for each response analyzed.

[4] Non-estimable because of no incidence in nulliparous cows

SESSION NOTES

New Perspectives on Adapting Cattle to Finishing Diets Without Compromising Rumen Health

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Introduction

Adapting cattle to the finishing diet is a critical time during the feeding period that has long-term ramifications. The importance of this period has been reinforced by an increasing number of calf-feds and average days on feed in the cattle feeding sector. Successful adaptation to the finishing diet can result in cattle quickly reaching peak performance and consistent profitability. However, mistakes during the transition can persist and lead to digestive upsets, health complications, and unrealized growth during the remainder of the finishing phase. While weaned calves are functioning ruminants, the microbes in their forestomach have not reached their maximum digestive capabilities. As their microbial communities are maturing, avoiding management missteps can be key to preventing rumen-based maladies later.

The importance of the rumen and its microbes to cattle nutrition and production efficiency has long been established. However, a newfound understanding of the rumen microbiome and gut physiology has generated new emphasis in this area of livestock production. Recent research has investigated practical solutions to improving performance during the transition phase as well as understanding the development of the rumen and its microbial communities. To make profit-driven management decisions in this changing landscape, cattle feeders must understand the basics of rumen function that underlie best feeding practices to evaluate the consequences of market-based choices affecting cattle management.

Importance of Rumen Function and Health

Fermentation in the rumen is responsible for harvesting the majority of the energy for the ruminant animal. When it is functioning well, the rumen is the ideal place for anaerobic bacteria to efficiently digest feed; the rumen is warm, properly mixed, appropriately buffered, regularly provided with substrate (feed), and free of oxygen. Indicators of rumen function can include rate of VFA absorption, motility patterns, rumen papillae histology, and microbial digestion of feed and fiber. Beyond the digestive contributions of the rumen, it also serves an immune function as a protective barrier from microbial inhabitants. In the context of feedlot cattle, the rumen will experience more challenges to the natural equilibrium of rumen function. This is because maximizing weight gain potential by greater energy intake and minimizing of digestive

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upsets are antagonistic goals. To achieve both goals, a balanced diet must be complemented with proper feeding management.

A growing appreciation of rumen and gut health have led to novel techniques to monitor the rumen in commercial and research environments. Ruminal pH can be monitored continuously in cannulated and non-cannulated animals using an indwelling bolus equipped with a pH sensor. There are several types of commercially available pH boluses that are currently being marketed primarily to the dairy industry and researchers. Monitoring ruminal pH is one of most informative measures of fermentation and can indicate how animals are adapting to a new diet. The epithelial tissue is the rumen has garnered more interest recently from a development and functional standpoint. Tissue biopsies of the ruminal papillae are being used in research to understand the function of the rumen wall. While the epithelial tissue has multiple cell types, the overall gene expression pattern and individual protein abundance can be monitored using new sequencing platforms. Culture-independent methods have redefined our understanding of rumen microbial communities. As microbiome evaluations become routine with the maturation of the science, opportunities to use the technology in a production setting will increase.

Common Challenges to Rumen Health

Economic and genetic factors have altered common cattle feeding practices in recent years. From 2010 to 2016, average hot carcass weight increased from 835 lbs to 880 lbs (NASS, USDA) with greater days on feed and moderating feed prices. Over the same time span, the occurrence of liver abscesses increased 25% up to 19% of slaughter cattle evaluated using industry monitoring services. With additional regulations on feeding tylosin to feedlot cattle in the Veterinary Feed Directive and a host of new, “natural” feed additives being released, priorities within the cattle feeding sector have led to a renewed interest on rumen and lower gut health.

The feedlot sector has historically focused on gut health by preventing rumen-related maladies. Common challenges to rumen health include acute and subacute acidosis, bloat, laminitis, rumen ulcers, and liver abscesses. These conditions are often not observed in isolation but are often interrelated. Acidotic conditions in the rumen are driven by the rapid production of organic acids that exceed the rate of absorption by the rumen wall to result in a depressed ruminal pH. Generally, acute acidosis is defined by a pH below 5.0, while subacute acidosis is defined by a pH between 5.0 and 5.6. When ruminal pH is above 5.6, rumen health will be improved by greater motility, increased fiber degradation, and improved barrier function by the rumen wall. The difficulty of measuring pH in a production setting can make diagnosis more challenging. Acute acidosis results in more noticeable symptoms; these may include large decreases in feed intake, recumbent animals with their head in their flank, an absence of ruminal contractions, and severe dehydration. Lactic acid accumulates in the rumen during acute acidosis and further reduces pH while increasing osmolality. The osmolality gradient concentration causes water to diffuse from tissues into the rumen resulting in dehydration and diarrhea. The rapid influx of water can also damage the rumen wall and lead to a rumen ulcer or rumenitis. In contrast, subacute acidosis would typically

only cause a moderate reduction in feed intake, loose stools, and some signs of colic. The long-term occurrence of subacute acidosis will likely decrease performance and fiber digestion, but this has been difficult to document the magnitude of effect in a research setting.

Although commonly described as two distinct conditions, acidosis exists as a continuum of symptoms with greater severity often causing subsequent ailments. When acidosis disrupts the barrier function of the rumen wall, liver abscesses can occur. A breach of the rumen epithelium allows bacteria to enter the bloodstream to be transported to the liver. While not a predominant bacterium in the rumen, *Fusobacterium necrophorum* is an opportunistic pathogen found in liver abscess infections. Tylosin is a feed grade antibiotic fed to the majority of feedlot cattle (80%; Samuelson et al., 2017) to prevent liver abscesses. Tylosin is effective at reducing liver abscesses, but it does not change the precursor events that lead to the development of liver abscesses including a decreased rumen pH and damage to the rumen wall. Beyond the health implications of an active infection, severe liver abscesses decrease growth performance and cost slaughter facilities $20-80 in carcass value per animal (Brown and Lawrence, 2010). The recent implementation of the Veterinary Feed Directive and continued public pressure on the use of feed-grade antibiotics in livestock production will continue to impact nutritional management of cattle in the future.

Bloat is the easiest form of digestive upset to diagnose in feedlot cattle. An accumulation of gases trapped within the rumen causes distension on the left side of the animal that can range from mild to severe. Although several variations of bloat exist, frothy bloat is the most commonly observed in the feedlot and frequently occurs from 100-120 days on feed (Vogel et al., 2015). The formation of stable foam prevents eructation from expelling the gases from the rumen. Treatment of bloat includes passage of a stomach tube, administration of mineral oil, or use of a trocar for a rumenotomy. Because acidosis can affect ruminal contractions, saliva production, and the bacterial community, the stagnation of rumen can lead to gas accumulation and bloat (Meyer and Bryant, 2017).

Recent Research Findings

One of the primary risk times during the feeding period for digestive upsets is when animals are being transitioned to a finishing diet. Calves are typically adapted to a finishing diet during the 14 to 28 days after arrival. The goal of this period is to slowly adapt the rumen microbes to a higher concentrate inclusion in the diet. This can be successfully achieved by making moderate increases in feed calls while also making planned dietary adjustments. It is important not to increase the feed provided on the same day cattle are stepped up to a new diet. While a conservative approach is often used from a diet and management standpoint, there may be unrealized gain potential during this period since cattle are consuming diets with moderate energy. Also, these transition diets have the greatest inclusion of high-quality forage and can be the most difficult to mill. Feeding forage requires dedicated areas for proper storage, specialized machinery, and substantial time for grinding. Drought can have a major effect on regional forage prices. High levels of forage in the diet may also exceed the

requirements for rumen degradable protein in young, growing calves based on the new guidelines in the 2016 Beef Cattle Nutrient Requirements Model (BCNRM). The 2015 feedlot nutritionist survey revealed that the most common method for adapting cattle to a finishing requirement used 4 step-up diets with each provided for an average of 6 days (Samuelson et al., 2016). In smaller feedlots, using fewer step-up diets for a longer period may simplify feeding multiple groups of cattle and provide more acclimation time to each diet before the next change.

Recent research has also investigated the long-term consequences of different transition strategies on overall finishing performance. If cattle are truly more adapted for the finishing diet, then they should exhibit an advantage that extends beyond the transition period. Work conducted at the University of Illinois has shown that coproducts can replace most of the forage in transition diets to increase the energy content without adding starch and greater risk of digestive upset (McCann, unpublished). Multiple experiments from the University of Nebraska support the fact that management and nutritional decisions over this adaptation period can have long-lasting effects during the remainder of the finishing phase. Huls et al. (2016) observed that cattle adapted to a silage-based finishing diet using corn gluten feed (Sweet Bran, Cargill Corn Milling) had increased growth performance and feed conversion compared with cattle adapted using primarily alfalfa. Another experiment evaluated the ability of a complete starter feed (RAMP, Cargill Corn Milling) to adapt cattle to the finishing diet (Schneider et al., 2017). Cattle performance increased when fed RAMP compared with a more traditional, alfalfa-based adaptation diets. Collectively, this body of work indicates nutritional strategies during the transition period can improve the adaptation of the rumen microbiome and translate to a performance advantage.

Many non-nutritive feed additives such as direct-fed microbials have also been evaluated early in the feeding period. The diversity in the strain and species of the organisms present in these additives coincides with the diverse potential modes of actions and highly variable animal responses observed. There are many yeast-based products on the market, and they have been most extensively studied in the dairy industry. While there is some evidence yeast-based products can ameliorate aspects of subacute ruminal acidosis (Chiquette et al., 2015), many of the proposed modes of action (Jouany, 2006) have not been evaluated in a feedlot cattle context. Additionally, many of the additives may not target changes in rumen fermentation, but rather affect intake, stress, morbidity, or lower gut populations. Although most direct-fed microbial strains are not of rumen origin, recent work has evaluated the effect of dosing a robust, rumen-derived strain of *Megasphaera elsdenii*, a well-characterized lactate utilizer in the rumen (Henning et al., 2010). In a receiving cattle study, dosing with the *M. elsdenii* strain allowed cattle to be rapidly adapted to a finishing diet in only 10 days and reduced ruminal lactate concentrations (Ellerman et al., 2017). As the market for microbial feed additive continues to expand, evaluating strain-specific responses in the appropriate animal context will be important to demonstrate consistent effects and value to producers.

Reducing the incidence of digestive upsets in the feedlot will increase cattle performance and health to drive profitability, but many challenges exist. The latest

National Animal Health Monitoring System survey indicated 71% of feedlots were affected by digestive problems. However, it also described the greatest challenge with these issues: diagnosis prior to death. The ratio of mortality to morbidity for digestive problems was 159% compared with pneumonia which was 3.79%. Prevention of digestive upsets is critical considering our poor ability to detect their early onset.

It is well established among nutritionists that most of the problems with digestive upsets are rooted in management rather than the diet formulation. Although their opinion may have some level of bias, many implementation steps do alter the diet composition from the formulation to what is actually consumed by the cattle. In essence, variation or change is the enemy when feeding cattle a high concentrate diet. A range of management factors can reduce the risk of digestive upsets if done well and include bunk calls, ration mixing, ration delivery, feedstuff management, grain processing, and monitoring of cattle sickness. These are the primary opportunities to reduce man-made variation and prevent it from compounding the animal-to-animal variation that already exists. The level of individual animal variation in cattle on feed can be evaluated using the GrowSafe feed bunks that measure each animal's feed intake. While feed intake may remain consistent for a large group of cattle on feed, within the group, feed intake changes significantly on a day-to-day basis. Research at the University of Illinois has indicated some cattle may be particularly inconsistent, fluctuating more than 30% in dry matter intake on nearly 50% of the evaluated days. Recognizing the inherent animal variation further emphasizes the need for consistent management practices.

The transition to the finishing diet was historically considered the time with the greatest risk for acidosis. However, recent findings have indicated that the occurrence of acidosis increases with additional days on feeds (Castillo-Lopez et al., 2014). While the study was not large scale, it was able to collect consistent ruminal pH measurements throughout the finishing phase. Cattle are clearly adapted to the finishing diet near the end of the feeding period, so there must be a different factor initiating the acidotic events. During the finishing phase, minor acidotic insults accumulate and appear to condition the microbial community and the ruminal epithelium. Additional days on feed also increase the opportunity for an off-feed event to occur. A repeated subacute acidosis challenge was conducted at the University of Illinois to further understand the etiology of the acidotic events (McCann et al., 2016). During the initial two challenges, only one of the 12 cattle actually acquired acidosis despite different levels of challenges implemented. However, during the third challenge, all but one animal experienced subacute acidosis. The results indicate that minor events can prime the system over time for an acidotic event to occur later.

Conclusions

Ever-changing market and consumer signals will continue to drive our cattle feeding decisions, but nutritionists must be prepared to make the necessary adjustments to maintain and improve cattle performance levels. Challenging the status quo in preparing cattle for a finishing diet may be one opportunity to meet these

demands. Seeking and obtaining improvements in rumen health can also demonstrate our commitment to animal health and well-being to beef consumers.

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SESSION NOTES

Communicating with the Public about Animal Agriculture Technology

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Introduction

Consumers are asking questions about their food, and the processes leading from farm to table. We have seen unprecedented discussion about hormones, antibiotics, genetic engineering (familiarly "GMO") and other issues in animal husbandry. In addition, the internet provides substantial misinformation, propagated by parties unfriendly towards animal captivity and/or use for human sustenance. All of these variables create a perfect storm for animal agriculture, as misunderstood practices are frequently distorted in the familiar media.

Unfortunately, this confusion comes at a great time in animal genetics. Animal agriculture is at an important precipice. New genetic technologies are poised to impact the genetic improvement of livestock, creating targeted improvements in critical traits, such as size and disease resistance. The technologies known as 'gene (or genome) editing' stand to allow agile adjustment of important traits, customizing genetics to improve animal care and productivity. However, the public has expressed special disdain for laboratory-mediated intervention in animal genetics. While cloning and artificial insemination are the norm, modern excursions into genetic improvements are viewed with great skepticism or even fear. The internet is always glad to further the distortions that spawn the controversy and cloud the issues.

Realities do not reflect the claims. Hormones have a minor role and antibiotics are key in the treatment of bacterial infections. The concerns come at a time of great innovation in genetics, with new technologies available for use in medical application, crop biology, and animal improvement. Medical applications are lauded for their precision and speed, can capacity to solve pressing health challenges. Crop biology is blistering forward, with gene edited crops to hit the field in 2018. But animal gene editing remains locked in a special scientific purgatory. While the technology exists to solve grand problems, the overreaching and archaic regulatory climate arrests innovation that can reach the field.

The process of traditional animal breeding is a slow process, and remedies for today's problems must come at a much faster pace. Fortunately, scientists around the world have answered that call. With the use of genetic engineering (GE; synonymous with the colloquial, scientifically imprecise term 'GMO'), scientists can make precise changes to a plant's genetics to transfer much needed new traits with unprecedented speed. Plants solving deficiencies in vitamin A and iron exist. Plants resistant to disease, drought and pests exist. Most of all, these plants hold the promise of helping farmers produce abundant and predictable yields, and do it with more sensitivity to the

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environment. These are outstanding breakthroughs that satisfy the core tenets of sustainability.

Many public and private institutions have made great progress in animal agriculture innovation. Gene editing (also known as genome editing) has been used to introduce specific changes in DNA that target precise genes, leading to predictable outcomes. These animals exist today. Other animals, like the AquaAdvantage Salmon, use older technologies to generate the improved animal product. Sadly, while new innovations are being generated quickly, this massive public and private investment in improved animal genetics has not advanced to the field.

Why is innovation arrested? Policy formed around animal genetic engineering cannot move at the speed of the innovation. Modern gene editing practices are extremely rapid and have been implemented in a variety of species. However, while farmers and ranchers, research scientists and extension agents can identify problems, the lack of social license and even pressure from anti-biotech interests slows the development of helpful technology that ultimately could benefit animal agriculture, the environment and the animals themselves.

The controversial issues in animal agriculture are exacerbated by the landscape of alternative facts. With an internet full of clueless authoritative expertise, the role of legitimate food and farming experts becomes even more difficult. This is why scientists, farmers and ranchers, and agricultural-related industries, we must gain the trust of the public. We have to earn more credibility than television physicians, food activists, animal rights groups, and the internet's many celebrities that tarnish the motivations and methods of animal agriculture.

The ball is in our court. We know the evidence and we are the best authorities to communicate the science of our farms and industries. We just have to do it. We don't.

New Innovations

Genetic engineering in animals is surprisingly rapid, and the issues are no longer technical barriers. Aquaculture has wrestled with the introduction of the AquaBounty salmon, a fish that grows to market size in about half the normal time. Fewer inputs create the same output, which is the basis of sustainability. However, the technology has looped in endless regulatory discussion and even today, 28 years after the first fish was created, this sustainable technology has not reached the consumer. Avian influenza resistant chickens and low-phosphorous-emitting pigs are also old news. The next-generation 'genome editing' technologies are poised to impact animal agriculture. Genetically polled cattle, virus resistant hogs, and animals with greater meat production have all been created with a minor genetic tweak. Today we do not suffer a deficit in agricultural innovation. Agriculture worldwide faces a deficit in leadership, social license, and trust. The way to solve the problem is rethinking our strategy in education and communication, with communication being the main way we'll create durable change.

Put simply, ***we don't have a scientific or innovation problem. We have a communication problem***, and that can be corrected by scientists, farmers, ranchers and agricultural interests taking part in the public discussion.

Revising the Agriculture Communication Strategy

Farmers, ranchers and scientists must be part of the conversation. While they clearly are the most knowledgeable about the topic, those closest to farming and ranching are the least likely to step into public interaction. Similarly, scientists tend to remain on the sidelines. Additionally, when we do talk to the public we tend to make mistakes, as we speak from a heavy-hand of expertise and authority rather than providing an empathetic response to concerns. How can we change the way we engage the public to be more effective? It must be emphasized that this is not some plug-and-play formula for insincere conversions. You must always be honest, always share your true ambitions. Again, we're simply getting better at explaining what is true in a way that resonates with the listener.

1. **Audience.** The first rule of effective communication is to know who your audience is. Focus on those that have honest questions and concerns and avoid engaging people with deeply- entrenched ideas that cannot be changed. It is impossible to change people that do not accept data by applying more data. Identify audiences that are seeking answers and don't know who to trust.

2. **Establish rapport; Listen to understand their concerns**. Trust built from credibility and intimacy, meaning authentic feelings. Rapport is the connection between two parties where trust is established, and communication can flow. Our job as agricultural producers and scientists must start with listening to concerns—listening to understand, not listening to debate. While it seems overly simple, it actually is difficult to actively listen to someone and attempt to understand their point of view. ***The goal is to understand their position, not necessarily agree with them***, and show them that you understand their perspective.

3. **Trust from credibility.** What is your background, your expertise, your training? What is your perspective? Be transparent. Why do you favor one technology, product or approach? Does your business, or do you stand to profit? Never use your authority as a reason that they should accept your position—use it to demonstrate that you understand their questions and position.

4. **What are your concerns and interests?** Trust builds fast when others understand our goals and values. Discuss your interests in food and farming. Remember that sustainability includes profitable farming for producers. Describe your interests in seeing technology help farmers raise more nutritious, high-quality food. Discuss your feelings on the environmental impacts of farming. Talk about the new techniques, and how genetic innovation is just a part of achieving sustainability.

What you will find is that you actually are significantly in alignment with those harboring other opinions, and that the differences are relatively minor, and come from your deep understanding of food and farming.

5. **Share your story.** Describe the situation as it relates to you, your family, your city, or nation. What are some examples of how your solutions, once implemented, can create the change that satisfies everyone's shared values? Farmers and scientists make a common mistake when we talk about HOW. We discuss the details, we speak in the absolutes, and command agreement from our authority. Farmers and scientists don't communicate by bragging or exaggerating data. Scientists and farmers communicate with the facts. We communicate by describing ***how*** it works, ***how*** we do it, ***how*** we make it better.

   Unfortunately the consumer doesn't want to know ***how***, the consumer wants to know ***why***? Why do we do what we do? Why is it important? The consumer wants to know how on-farm decisions sync with our common interests and values. ***It is not about how we do it, it is why we choose to do what we do.***

   If we share the stories of the human element of new technology and then avoiding the sloppy language devised by anti-agriculture activists, we wage a more effective campaign of truth telling with impact.

6. **Say exactly what you mean.** While it is true that the American farmer feeds many worldwide, the "Feed the World" rhetoric comes off as disingenuous and inflammatory. Focus on specific examples of where technology helped others meet a production goal, or perhaps rescue a challenging situation. Talk about the story of the Hawaiian papaya, and how biofortification of crops like bananas, cassava and rice could benefit those facing malnutrition. These are stories of how biotechnology and next generation genetics have served people through improved crops. The same technology can eventually benefit animal agriculture.

7. **Be a friend, before an authority.** Experts like to remind non-experts that they are in fact the experts. Expertise is sometimes worn like a badge of authority, and that creates distance with the public we are trying to connect with. In medicine consumers are excited to trust authority. They want to know that those in command of the newest technology are trained and skilled.
   This is not true about food and agriculture. Consumers don't want to talk to an authority about food and farming. They want to talk to someone that eats, someone that farms, someone whose family lives on the farm. A friendly internet contact is more influential than a well-published scientific author. This phenomenon is rooted deep in the brain. Food technology is perceived as a threat whereas medicine appeals to our rational thinking.

   In wealthy industrialized nations medical technology is hope, food technology is a threat.

   It all distills down to ***how humans process information.*** This is why the final step in revising the agricultural communication process must make food and

farming technology a place of hope, a place of common dreams, a means to reinforce our mutual values and address our common concerns.

Summary

Go out and engage. The public has concerns they feel are very real, and they are looking to the media and to the internet for answers. They are not sure who to trust. When they don't know who to trust they make decisions that are over precautionary. These decisions ultimately negatively impact farmers, people in the world's emerging economies, the poor in the industrialized world, and the environment. Technology that exists is slow to meet the needs.

The solution is a simple one. Communicate. It is critical that experts step into the conversation, and describe the promise of new technology. Consumers love innovation—if it is not a threat to their families and appeals to their values.

That's where we classically have made mistakes. Rather than speaking to people to earn their trust, we provided a landslide of authoritative evidence. If agricultural producers want access to the best new technology communication will have to happen first to earn trust and gain social license to use them. In a way, it is a much more simple solution than we make it out to be.

SESSION NOTES

Are There Financial Advantages of Grouping and Feeding Dairy Cows by Nutritional Need?

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Introduction

Nutritional grouping is a herd management strategy that provides different diets to different groups of lactating cows to better fulfill their nutrient requirements. Hence, it can be beneficial by saving feed costs, improving productivity, improving herd health, and decreasing nutrient emissions to the environment (Cabrera and Kalantari, 2016). Total mixed rations have become an industry standard for feeding management. For example, 58% of Wisconsin and Michigan dairy farms used the same TMR for all lactating cows (Contreras-Govea et al., 2015). When feeding only 1 TMR diet, it is usually formulated for high-producing cows to ensure these cows reach their full milk production potential (Weiss, 2014), which results in overfeeding lower-producing cows (Cabrera and Kalantari, 2016). A strategy to relieve this problem is adopting nutritional grouping with more precise diets, which will result in financial advantages due to the better-tailored diet to the cow requirements in a group even when it could require more capital management and labor costs (VandeHaar, 2011). Within this context, Kalantari et al. (2016) studied by simulation modeling the economic efficiency of nutritional grouping in 5 Wisconsin commercial herds. This paper is an adapted excerpt of that study highlighting its practical results.

Materials and Methods

A daily dynamic stochastic Monte Carlo simulation was developed to model individual cows after first parturition in a dairy herd. The next-event scheduling approach (De Vries, 2001) scheduled stochastic events that could happen to cows during each reproductive cycle. First, a data set of all the cows in a herd and their current status were loaded (i.e., lactation number, day postpartum, reproductive status). Then, a list of possible stochastic events was scheduled for each cow at the beginning of the simulation and the list was renewed after starting their next lactation. For each cow, milk, fat and protein production, BW and BCS changes, and NE_L and MP requirements were simulated and monitored according to diets. The BCS was restricted to 2.0 and 4.5 in a scale of 1 to 5. If BCS was calculated to go below or above these limits, milk production or DMI was decreased, respectively, to maintain BCS within these limits. For all specific details of the underlying simulation model algorithms, please refer to Kalantari et al. (2016).

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Nutritional Grouping

Nutritional grouping strategies were studied on post-fresh lactating cows (DIM > 21) to test their effect in the overall milk income over feed cost (**IOFC** = milk value minus RDP, RUP, and NE_L costs). The sizes of nutritional groups were chosen to be approximately equal among them (total available cows divided by the number of defined nutritional groups). The monthly regrouping process of groups started by ranking the cows based on their NE_L and MP requirements (clustering method; McGilliard et al., 1983). Kalantari et al. (2016) used individual cow's daily NE_L and MP requirements to formulate more precise diet nutrient concentrations in simulated groups of cows. Average NE_L and MP + 1 SD concentrations were used to formulate the group diets.

Economic Parameters

Economic parameters for the base scenario were set as 10-yr Wisconsin average prices from 2005 to 2014. Thus, milk price was set to $0.39 per kg of milk. FeedVal 6.0 decision support tool (http://DairyMGT.info: Tools) was used to calculate the nutrient prices of NEL, RDP, and RUP. The calculated nutrient prices were: $0.1/Mcal of NEL, $0.18/kg of RDP, and $1.04/kg of RUP.

Scenario Analyses

Two extreme scenarios were analyzed. The worst-case scenario was designed by coupling the lowest milk price with the highest nutrient costs and vice versa for the best-case scenario. Ten-year annual average of milk price was used to set the highest ($0.52/kg) and lowest milk ($0.29/kg) prices. The highest (lowest) nutrient costs were set at $0.14/ Mcal of NE_L ($0.05), $0.26/kg RDP ($0.09), and $1.52/ kg RUP ($0.52). Considering the large differences among studies regarding milk losses when grouping cows (Smith et al., 1978; Hasegawa et al., 1997; Zwald and Shaver, 2012), possible milk loss due to regrouping lactating cows was explored with a base scenario without any milk loss and another scenario with extreme milk losses of 1.82 kg/d during 5 d after grouping (Cabrera and Kalantari, 2014). In addition, the effect of having first-lactation cows as a separate nutritional group was studied.

Case Study Herds and Projection Timeline

Five Holstein herds from Wisconsin using a TMR feeding management system were studied (**Table 1**). The model captured current cow and herd profiles (d = 0 of the simulation) and then projected individual cow and herd performance daily for a year (d = 365) with 1,000 replications.

Results and Discussion

Grouping

Post-fresh lactating cows (592) from the 787-cow herd at 300 d in the simulation are shown in **Figure 1A** ranked according to their NE_L concentration requirements. It is clear that lactating cow requirements vary substantially on a given day because of differences in lactation stage, pregnancy status, BW, and milk production. In this example, the highest NE_L concentration requirement was from a cow in third lactation, 23 d postpartum, with milk yield 20% above herd average. The lowest NE_L

concentration requirement was from a cow in third lactation, 385 d postpartum, with 10% below average milk yield. The effect of grouping these 592 post-fresh lactating cows is illustrated in **Figure 1B** where the difference between offered and the required NE_L concentrations are depicted for 3 cases of nutritional groupings. **Figure 1B** shows that increasing the number of groups decreases the variability among the cows within the group, which is especially beneficial in offering the cows a diet closer to individual cow requirements in terms of health, environment, and economics. This benefit is more pronounced in the case of large herds and when the distribution of the requirements is not normal (McGilliard et al., 1983).

Financial advantages of nutritional grouping

The economic value of nutritional grouping measured in terms of IOFC is displayed as the difference from 2 to 4 TMR and 1 TMR in **Figure 2**. It is clear that an economic gain results from nutritional grouping. These gains depended on the number of groups and varied from ($/cow per yr) $39 for 2 groups, to $46 for 3 groups, to $47 for 4 groups (**Figure 2**).

The gain in IOFC with more nutritional groups was due to higher milk production and lower feed costs. Higher milk production for more than 1 group was due to fewer cows having milk loss for low BCS (BCS < 2.0). The lower feed costs with 2 and 3 groups were mainly due to less RUP cost (**Figure 2**). Compared with RUP cost, other components of IOFC (RDP and NE_L costs and milk revenue) were more stable across different grouping numbers and MP concentrations in the diet. The largest relative IOFC gain was obtained when moving from 1 group to 2 groups. Economic gains found in other studies are different because of differences in model and input values used in those studies. For example, Williams and Oltenacu (1992) reported that the mean annual IOFC ($/cow per yr) of 3 nutritional groups were $21, $33, and $40 higher than that of 2 groups at production levels of 8,000, 9,000, and 10,000 kg per cow per 305-d lactation, respectively. St-Pierre and Thraen (1999), using economic optimized lead factors for CP and NE_L for different group numbers, calculated average economic gains ($/ cow per yr) of $44 and $77 when comparing 2 and 3 groups with 1 group, respectively. These values are comparable to those found in this study. The other important factor in financial evaluation of grouping lactating cows is the extra labor needed to formulate, prepare, and deliver feeds, and the extra costs of running mixers for preparing the TMR for each group separately. In addition, there is a labor cost related to moving cows among groups. These costs are usually farm specific and vary among herds (Østergaard et al., 1996), and for simplification were not included in Kalantari et al. (2016). Overall, profitability and feasibility of nutritional grouping are highly farm and market dependent. Farm size has an effect on the feasibility of nutritional grouping. For example, the extra labor for regrouping and moving cows might be less important in larger herds than in smaller herds (Østergaard et al., 1996). Also, when market conditions determine high feed costs and low milk prices, nutritional grouping could be more economically appealing (Allen, 2008; Hutjens, 2013). Simulation studies (Pecsok et al., 1992; Williams and Oltenacu, 1992) have suggested dividing lactating cows into 3 nutritional groups for optimal efficiency. Results from this

study corroborate those previous reports indicating that economic gain and efficiency increase up to 3 nutritional groups.

Formulated Diet

The average NEL, RDP, and RUP concentrations in DM under 3 levels of offered MP concentrations are summarized in **Table 2**. The formulated diet for 1 group had a concentration of 1.5 Mcal/kg of DM. Having more groups divides the cows into more homogeneous NE_L concentration groups and hence higher and lower concentrations of NE_L in the diet. A similar pattern was observed in RDP and RUP percentages in the diet. The reported NE_L concentrations by McGilliard et al. (1983) using a clustering method with 2 groups were 1.62 (high) and 1.42 (low) Mcal/kg, which are comparable to those obtained here (1.59 and 1.41 Mcal/kg, respectively). The optimum allocation of NE_L found in the St-Pierre and Thraen (1999) study was 1.78 (Mcal/kg) in the 1-group case and remained above 1.7 even in the case of 3 groups. Previous studies have used CP to estimate required protein in the group, whereas this study used the MP requirement of the cows. The CP% (RDP + RUP/0.8) in this study was higher than the reported optimum allocation of CP% by St-Pierre and Thraen (1999), which used milk production as the proxy for diet formulations. In 1 group, the estimated range of CP was 18, 18.5, and 19% for average, 0.5 SD, and 1 SD above average, respectively. In the current study, the difference of CP% in different group numbers were approximately 2, 3, and 3.8 percentage points for 2, 3, and 4 groups, respectively. The differences for the optimum allocation of CP reported by St-Pierre and Thraen (1999) were 1 and 2 percentage points for 2 and 3 groups, respectively.

Nutrients Captured in Milk and BCS

The results of the Kalantari et al. (2016) could be explained by studying the detailed charts of the NE_L concentration in the diet (**Figure 3**) and the distribution of the retained body energy in terms of BCS (**Figure 4**). A greater proportion of the cows in the herd were underfed in the case of 1 group than with more groups and therefore the total NE_L consumption and milk yield (milk yield depended on the energy in the body as captured in BCS) for just 1 group was less than that with 2 and 3 groups. Utilizing 2 or 3 groups increased the diet NE_L concentration in early lactation (the time that is most needed) until around 150 d postpartum (**Figure 3**). After this point, 2 and 3 groups had a lower NE_L concentration in the diet than did 1 group. The overall lower NE_L concentration required for late-lactation cows was generally lower than the higher NE_L concentration required for early-lactation cows, and therefore the total NE_L consumed was higher for multi-groups than for 1 group. Two and 3 groups assure that late-lactation cows have enough energy in the diet but not much more than required. Overall, it is clear that use of 2 or 3 groups distributes NE_L more efficiently based on DIM and productivity, which might increase overall NE_L consumption in the herd.

Excess energy in late-lactation cows is associated with greater BCS and over-conditioned cows that can have complications in the next lactation (Cameron et al., 1998). The effect of several nutritional groups on BW and BCS can be seen in **Figure 4**, which compares the effect of 1 and 3 nutritional groups on BW and BCS distributions of the 787-cow herd. The left panel of **Figure 4** shows that the BW density plot of 2

grouping strategies (1 vs. 3 groups) does not differ considerably; they both have similar distributions. The stable BW among different grouping numbers has also been reported in field trials (Smith et al., 1978; Clark et al., 1980; Kroll et al., 1987). The right panel of **Figure 4** illustrates the effect of nutritional grouping on the distribution of the cows' body energy content (BCS). The 1 group represented by a dark-shaded density plot has a different distribution than 3 groups (light shading). With 1 group, the distribution is thick-tailed, which means the model projects that many cows that are either under-conditioned (BCS = 2.0) or over-conditioned (BCS = 4.5), and it has a mode around BCS = 2.75. On the other hand, use of 3 groups shows a rather normal distribution curve with the mode around BCS = 3.25. Similar distribution was observed in the case of 2 groups and in the other studied herds (data not shown). Having 2 or 3 groups appears to ensure that the consumed energy is better-distributed promoting healthier cows.

The overall MP trend is similar. In the 1 group case, the MP consumption decreased to 11 g/100 g of DM post freshening, and stayed at the same level until about 300 d postpartum, when it decreased consistently through the rest of the lactation (**Figure 3**). However, in 2 and 3 groups, the provided MP in the diet was closer to the actual requirements. Therefore, with 2 or 3 groups, cows were fed more MP until about 100 d postpartum and thereafter fed lesser MP than the 1-group case. This higher N consumption in late lactation for 1 group compared with more groups is consistent with the literature (VandeHaar, 2014). Having 3 groups and formulating the diet at 1 SD above the MP average improved N efficiency by 2.7% on average. The main economic gain of having more groups could be attributed to an increased percentage of N captured in milk, which in turn decreases feed cost related to RUP. Having more groups clearly improves the percentage of N captured in milk, which, at the same time, improves environmental stewardship by decreasing the amount of N excreted (VandeHaar, 2014).

Scenario Analyses

Results from scenario analyses on the input price, inclusion of milk loss, and separation of the first-lactation cows from older cows are depicted in **Table 3**. The results show that even in the worst economic conditions (lowest milk price with highest nutrient costs), grouping cows had a similar average IOFC gain compared with the base scenario. Comparing the base and best case scenarios over all herds, the average IOFC gain ($/cow per yr) was $6 higher in 2 groups and $4 in 3 groups. Comparing the IOFC gain ($/cow per yr) of 2 and 3 groups, the relative gain was highest in the worst case scenario ($10) and the lowest relative IOFC gain of having 3 groups instead of 2 groups was under the best case scenario ($6). This emphasizes the importance of grouping lactating cows in tough economic conditions, when the milk price is low compared with feed price. Even though the relative IOFC gain was greater in the worst conditions, the highest IOFC gain in absolute terms was when the milk price was high compared with feed costs (i.e., best case; **Table 3**). Assumed milk loss (1.82 kg/d for 5 d) due to regrouping decreased the average 5 herds' IOFC of 2 groups by $18 across all the herds and by $20 for 3 groups compared with 1 group (**Table 3**). The data showed that even under the assumption of milk loss because of regrouping, there is still

an overall economic gain. However, considering milk loss for all cows, as was assumed in this study, resulted in the lowest economic gain among all the scenarios, including the worst-case scenario. The amount of IOFC gain ($/ cow per yr) ranged from $14 to $32 when comparing 1 and 2 groups and the IOFC gain ranged from $19 to $38 when comparing 1 and 3 groups. The amount of loss depended on the number of times cows were reassigned to a different group, and it was affected by cow characteristics (i.e., milk production and DIM that determine cow requirements) and the nutrient requirement variations among the cows in the groups. The trend when having milk loss because of regrouping was consistent with the base scenario in that the largest gain was observed between 1 and 2 groups. Smith et al. (1978), in a field study, compared lactating cows grouped into 1 and 2 groups. In that study, the average decline in milk production was found to be 2 kg/cow per day for 7 d, and this amount was affected by parity (less milk loss for first-lactation compared with older cows). Even with this amount of milk loss, the IOFC of 2 groups was $30/cow per year greater than that of 1 group, as a result of less concentrate fed (Smith et al., 1978). This amount of gain in IOFC is in the range of values found in this study. In another field study by Zwald and Shaver (2012) the milk loss due to change in groups was reported to be insignificant. Overall, the effects of grouping on the milk production of the cows is inconclusive (Clark et al., 1980) and, based on those field studies mentioned above, it seems that the assumed amount of milk loss in this study (total of 9.1 kg in 5 d) could be either underestimated or overestimated. Thus, the true amount of milk loss is unknown, and studies have shown that it could be affected by parity (Smith et al., 1978) and could vary among cows based on their DIM (Kroll et al., 1987) and other characteristics. It seems safe to assume that not every cow might experience the same amount of loss and the duration could vary among cows based on their characteristics. However, the amount of saving in the feed cost due to grouping could exceed the loss in milk production (Smith et al., 1978; Clark et al., 1980).

Adding first-lactation cows as a separate group also affected the economics of nutritional groupings and is summarized in **Table 3**. The average IOFC gain among all the herds was lower than that of the base scenario by $7/cow per year. This smaller gain when separating first-lactation cows was mostly due to the fact that having a separate group of first-lactation animals ensures a diet tailored more closely for those cows and older cows, similar to having a separate nutritional group. **Table 2** summarizes the formulated diet when separating first-lactation cows into their own group. Regardless of the number of groups, the formulated diet of first-lactation cows was the same across different group numbers and herds. However, separating the first-lactation cows into a group increased the nutrient concentration of the diet of older cow groups, thus the higher feed costs (higher RUP costs) and smaller IOFC gain in this scenario. It should be mentioned that the model did not consider the possible benefit of separating first-lactation animals due to social hierarchy among the younger cows and older cows, which could result in decreases in feed intake and milk production of first-lactation cows (Botheras, 2007). Considering this issue could increase the reported economic gain of separately grouping first-lactation cows.

Conclusions

Financial gains of nutritional grouping measured as milk income minus NE_L and MP costs were 15.2 ± 5.5, 30.5 ± 6.0, and 46.6 ± 6.6 for 2, 3, and 4 nutritional groups compared to 1 group. Financial gains were explained mainly due to higher milk production and lower RUP costs when grouping, and gain was emphasized during tough economic conditions. The effect of a possible constant milk loss when regrouping cows would have a deleterious economic effect, but not high enough to overcome the gains. The percentage of total NE_L consumed and captured in milk for >1 nutritional group was slightly lower than that for 1 nutritional group due to better distribution of energy throughout the lactation and higher energy retained in body tissue, which resulted in better herd BCS distribution.

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Table 1. Studied dairy herds.

	Herd Size (Lactating + Dry)				
Characteristics	331	570	727	787	1,460
Average Herd ME305[1] (kg/cow/yr)	13,348	16,140	13,897	12,884	14,188
1st Lactation (%)	38	43	39	39	45
Average days in milk[2] (d)	193	169	181	165	174
Average days in pregnancy (d)	134	140	141	133	157
Average lactation number (#)	2.03	1.99	2.29	2.21	2.02
21-d pregnancy rate[3] (%)	17	18	19	19	18
Conception rate[3] (%)	35	32	36	37	40
Estrus detection[3] (%)	49	57	51	51	45
Culling[3] (% per yr)	35	32	36	37	40
Abortion[3] (% per gestation)	16	7	11	11	7

[1] 305 day mature equivalent milk production.
[2] Average days in lactation.
[3] As defined and calculated in DairyComp305 (Valley Agricultural Software, Tulare, CA).

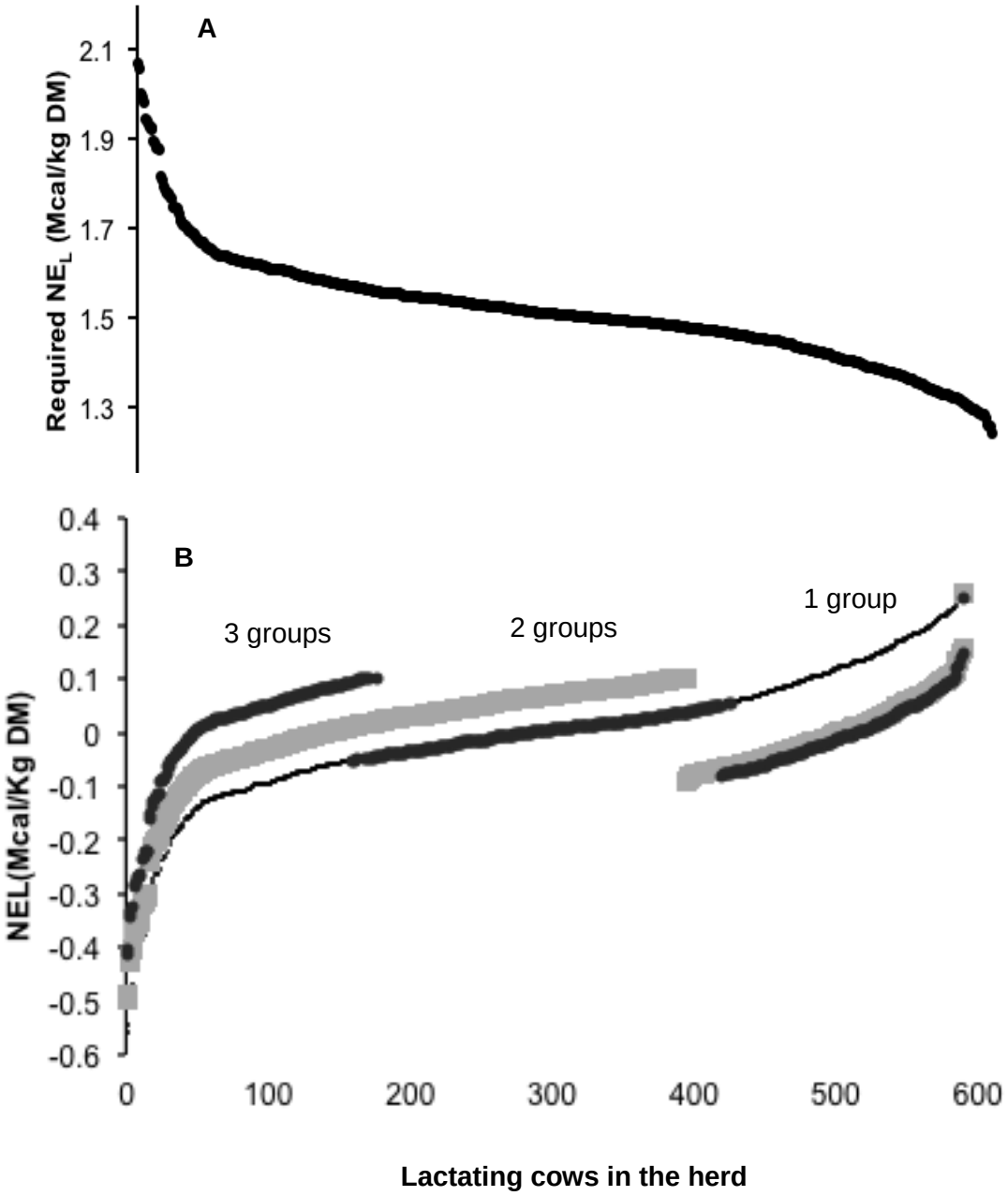

Figure 1. Nutrient NE_L required and provided to 592 post-fresh lactating cows from the 787-cow herd at d=300 in simulation. **A)** NE_L concentration of the requirements. **B)** Difference between provided and required NE_L concentration (offered NE_L – required NE_L, Mcal/kg) under 1, 2, and 3 nutritional groups based on the diet offered at the average NE_L concentration of the group.

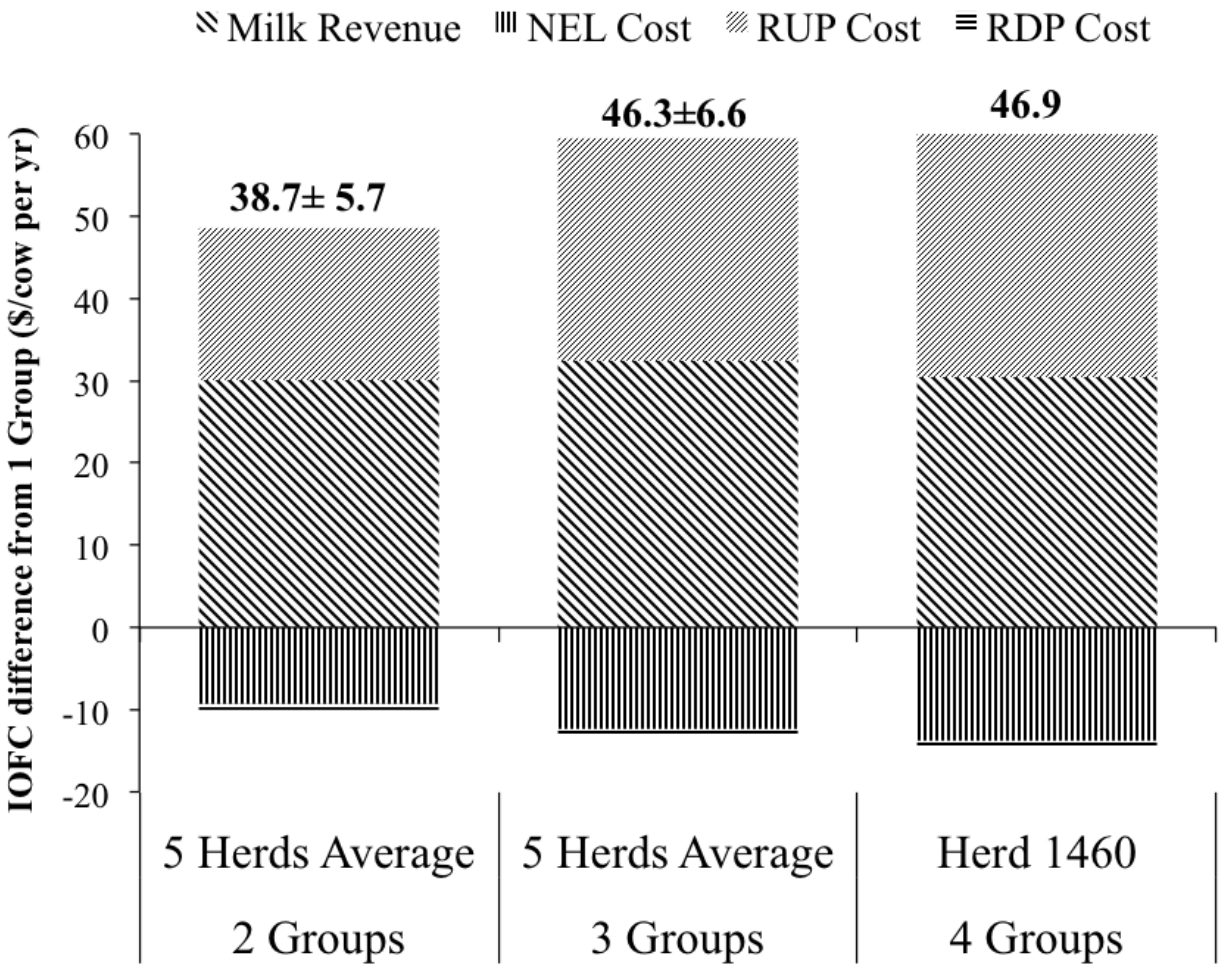

Figure 2. Difference in income over feed cost (IOFC) of 2, 3, and 4 nutritional groups and 1 nutritional group disaggregated in its components: cost of rumen degradable protein (RDP), cost of rumen undegradable protein (RUP), cost of NE_L, and milk revenue. The zero line is the average IOFC obtained by 1 group was equal to $2,822 for diet formulated at average MP + 1x SD. The labels on top of the bars are the additional IOFC (± SD among the herds) above 1 group. Four nutritional groups were applied only to the largest herd (1,460-cow herd).

Table 2. Formulated diet components for different nutritional group numbers and scenarios obtained by averaging 5 herds (± SD within herds) throughout the simulation of 12 monthly grouping periods.

Group number	Groups	NE_L (Mcal/kg DM)	RDP (% of DM)	RUP (% of DM)		
				0xSD	0.5xSD	1xSD
Grouping post-fresh lactating cows						
1	G1	1.5±0.004	9.34±0.0002	5.06±0.0004	5.46±0.0004	5.85±0.0005
2	G1	1.59±0.005	9.89±0.0003	5.35±0.0004	5.63±0.0005	5.90±0.0005
	G2	1.41±0.005	8.83±0.0003	4.78±0.0005	5.01±0.0005	5.22±0.0006
3	G1	1.66±0.006	10.27±0.0003	5.42±0.0005	5.68±0.0005	5.95±0.0006
	G2	1.48±0.005	9.25±0.0003	5.15±0.0003	5.27±0.0005	5.36±0.0004
	G3	1.38±0.006	8.67±0.0003	4.67±0.0004	4.85±0.0006	5.02±0.0006
4 [1]	G1	1.72	10.60	5.42	5.68	5.95
	G2	1.52	9.49	5.24	5.38	5.50
	G3	1.45	9.07	4.99	5.08	5.18
	G4	1.37	8.59	4.61	4.75	4.93
Separating first lactation cows from older lactating cows						
First lactation[2]		1.5±0.008	9.34±0.0005	4.93±0.0007	5.24±0.0006	5.55±0.0005
1	G1	1.5±0.003	9.35±0.0002	5.15±0.0003	5.57±0.0004	6.00±0.0005
2	G1	1.61±0.005	9.97±0.0002	5.46±0.0004	5.75±0.0005	6.03±0.0005
	G2	1.40±0.002	8.77±0.0002	4.85±0.0002	5.08±0.0002	5.31±0.0002
3	G1	1.67±0.006	10.33±0.0004	5.53±0.0005	5.80±0.0006	6.07±0.0006
	G2	1.48±0.003	9.24±0.0002	5.24±0.0003	5.35±0.0003	5.46±0.0004
	G3	1.37±0.004	8.60±0.0002	4.72±0.0003	4.90±0.0002	5.09±0.0002
4 [1]	G1	1.72	10.6	5.54	5.81	6.08
	G2	1.52	9.49	5.28	5.46	5.60
	G3	1.44	9.03	4.95	5.13	5.28
	G4	1.35	8.55	4.62	4.78	4.98

[1] 4 groups were studied only on the largest herd (1,460-cow herd)

[2] The average formulated diet for first lactation cows separated from older cows was similar across all the grouping numbers and herds.

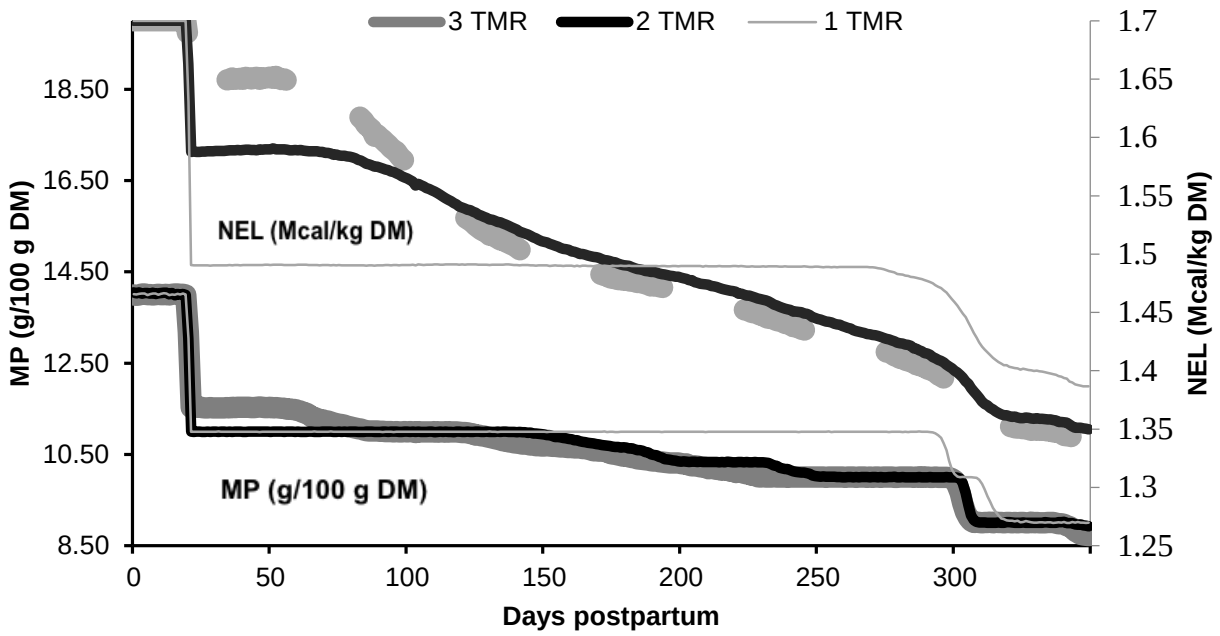

Figure 3. Offered diet average NE_L (light shade) and metabolizable protein (MP; dark shade) after calving for the 727-cow herd under different number of nutritional groups.

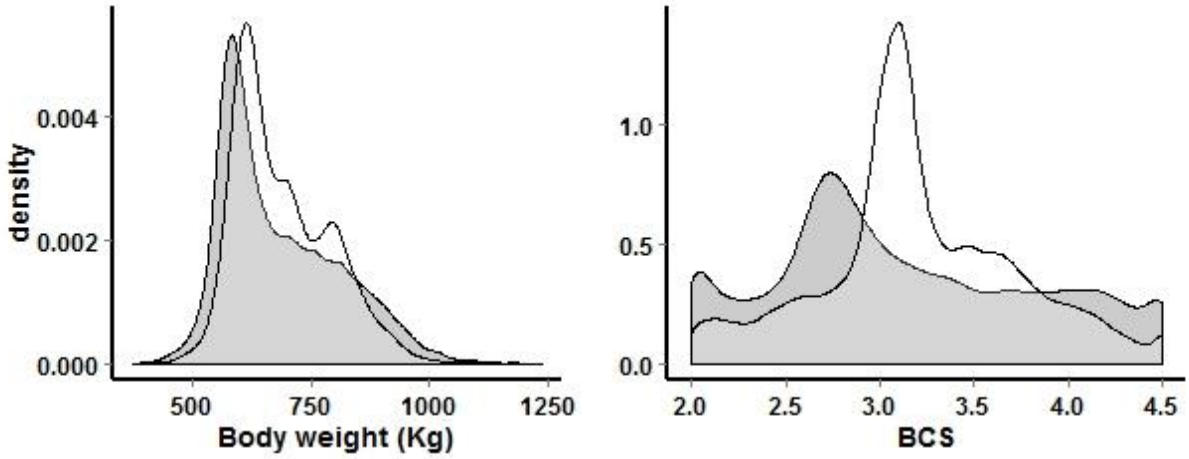

Figure 4. Body weight (left) and BCS (right) density plot from the 787-cow herd for 1 (dark shade) and 3 (light shade) nutritional groups. The average ± SD for 1 and 3 nutritional groups are 3.0 ± 0.7 and 3.25 ± 0.5, respectively. Total area under the curves adds to 1.

Table 3. Average economic gain in IOFC of grouping strategies of 5 studied herds.

Scenario	Difference between grouping strategies and 1 group ($/cow per yr)		
	2 Groups	3 Groups	4 Groups[1]
Base[2]	38.66	46.24	46.9
Worst[3]	35.48	44.94	47.4
Best[4]	44.34	50.18	48.8
Milk loss[5]	20.46	25.9	23.5
1st lactation[6]	32.64	38.76	38.5

[1] 4 groups were studied only on the largest herd (1,460-cow herd).
[2] Base scenario running on the average NE_L concentration and average MP + 1 x SD with 10 years average annual milk price ($0.39/kg) and nutrient costs (NE_L = $0.1/Mcal, RDP = $0.18/kg, and RUP = $1.04/kg).
[3] Worst case scenario couples the lowest milk price with the highest feed price from historical 10 years annual average (Milk price = $0.29/kg, NE_L = $0.14/Mcal, RDP = $0.26/kg, and RUP = $1.52/kg).
[4] Best case scenario couples the highest milk price with the lowest feed price from historical 10 years annual average (Milk price = $0.52/kg, NE_L = $0.05/Mcal, RDP = $0.09/kg, and RUP = $0.52/kg).
[5] Adding 5 d of 1.82 kg/d milk loss for cows changing to another group under base scenario.
[6] Including 1st lactation cows as a separate obligatory group under base scenario. In this scenario the 1 group itself has 2 groups: 1st lactating cows and ≥ 2nd lactating cows. Thus, in addition to the number of groups for older cows one group is just for first lactation cows.

SESSION NOTES

Trace Minerals and Vitamins for Dairy Cows

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Introduction

Providing adequate trace minerals and vitamins to dairy cows is essential for high production and good health. However, feeding excess trace nutrients inflates feed costs and could be detrimental to production and cow health. Unfortunately quantifying the supply of available trace nutrients and their requirements is extremely difficult which leads to a high degree of uncertainty relative to diet supplementation. This paper provides suggested strategies for formulating diets to provide adequate but not excessive amounts of vitamins and trace minerals under a variety of conditions. When this paper was written (January, 2018), the NRC was in the process of updating the Nutrient requirements of Dairy Cows publication. The upcoming NRC may or may not reflect the opinions in this paper.

Mineral Supply

A major change that occurred in NRC (2001) was that requirements were calculated for absorbed mineral rather than total mineral. This was a major advance because we know mineral from some sources are more absorbable than minerals from other sources. However the use of absorbable mineral has limitations:

- measuring absorption of many minerals is extremely difficult;
- actual absorption data are limited; therefore most absorption coefficients (AC) are estimates;
- absorption is affected by physiological state of the animal and by numerous dietary factors (many of which have not been quantified); and
- for many of the trace minerals, the AC is extremely small and because it is in the denominator (i.e., Dietary mineral required = absorbed requirement/AC) a small numerical change in the AC can have a huge effect on dietary requirement.

Concentrations of Minerals in Basal Ingredients

For most minerals of nutritional interest good analytical methods that can be conducted on a commercial scale at reasonable costs are available. Assuming the feed sample is representative, a standard feed analysis (using wet chemistry methods for minerals) should provide accurate concentration data for Ca, P, Mg, K, Na, Cu, Fe, Mn, and Zn. Labs can also routinely measure sulfur and chloride but often these are separate tests. Most labs do not routinely measure Cr, Co, and Se because the concentrations commonly found in feeds are lower than what commercial labs can

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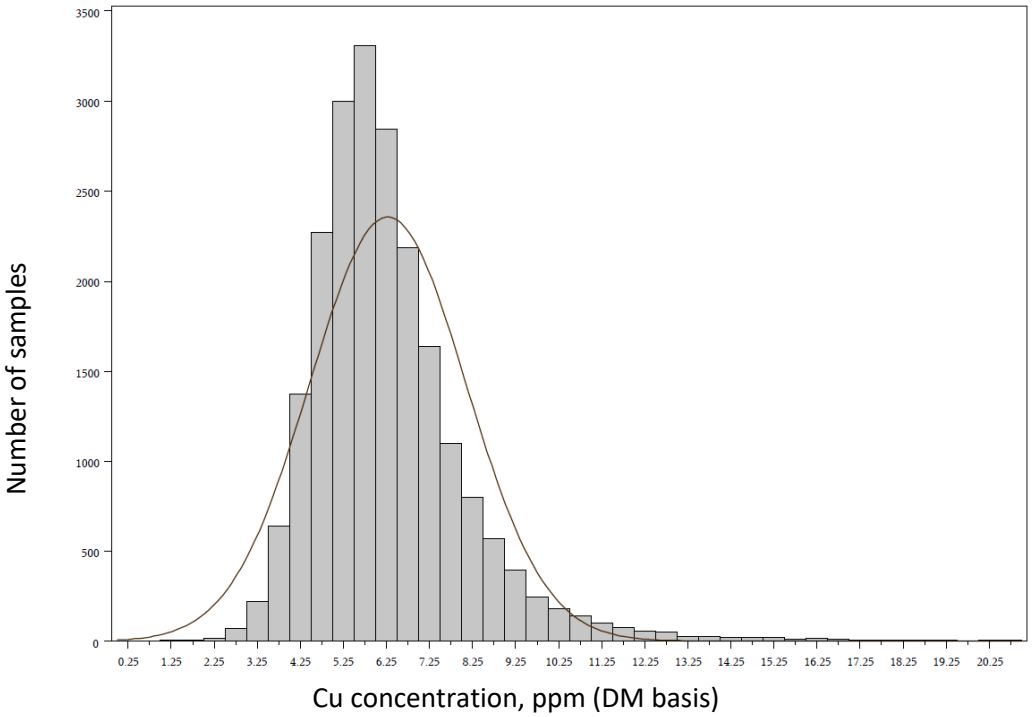

Figure 1. Distribution of Cu concentrations in corn silage grown throughout the U.S. The smooth line indicates a normal distribution would while the bars indicate the actual distribution. (Knapp et al., 2015).

reliably measure or because of contamination caused by routine sample processing such as using a steel feed grinder (a major concern for Cr). Although we can get accurate total mineral concentration data for basal ingredients, you must be careful when evaluating and using the data. Concentrations of minerals in feeds, even most macrominerals, are low. For example, 1 ton of average corn silage (35% dry matter) only contains about 2.5 grams of Cu (to put this in perspective a penny weighs about 2.5 g).

Sampling error is a problem for most nutrients and when concentrations are low, sampling error is usually larger. From a survey we conducted, sampling variation for trace minerals was greater than true variation. This means that mineral concentration data from a single sample should be viewed very suspiciously. Mineral concentration of soils is a major factor affecting the concentrations of most minerals in forages. Therefore, averages of samples taken from a farm over time (up to a few years) or from a group of farms within a small geographic area (e.g., a few counties) should be a truer estimate of the actual mineral concentration of a forage than a single sample.

In a normal distribution (the classic bell-shaped curve) about half the samples have less than the mean or average concentration, about half the samples have more than the average, and about 95% of the samples are within $\pm$ 2 standard deviation (SD)

units of average. This means that if you know the average concentration and the SD you have a good description of the population. This information helps with risk assessment. If a feed has an average concentration of Mg of 0.4% and an SD of 0.01% and the distribution is normal, about 95% of the samples of that feed should have between 0.38 and 0.42% Mg. With that information you should probably conclude it is not worth analyzing that feed for Mg, because even if your sample is 2 or 3 SD units from the mean it will have no effect on the diet or the animal. However, when distributions are skewed, the average and the SD may not be good descriptors of the population. For many minerals, concentrations within feeds are not normally distributed (**Figures 1 and 2**). Often the distributions have long tails because concentrations cannot be less than 0 but can be extremely high for various reasons. Some samples have high concentrations of certain minerals because of soil contamination. The more skewed the data, the less valuable the average and SD become in describing the feed. The median is the concentration where half of the samples have a lower mineral concentration and half of the samples have more mineral, and in a normal distribution the mean and the median are essentially equal. For concentrations of trace minerals and some macro minerals, the median is usually less than the average because their distributions are skewed. What this means is that for most situations, using average trace mineral concentration (e.g., feed table data), overestimates the trace mineral concentration in the majority of samples. For skewed populations, the median is a better descriptor of the population than the mean; however simply replacing average concentration with median concentration does not fix all the problems associated with a skewed distribution.

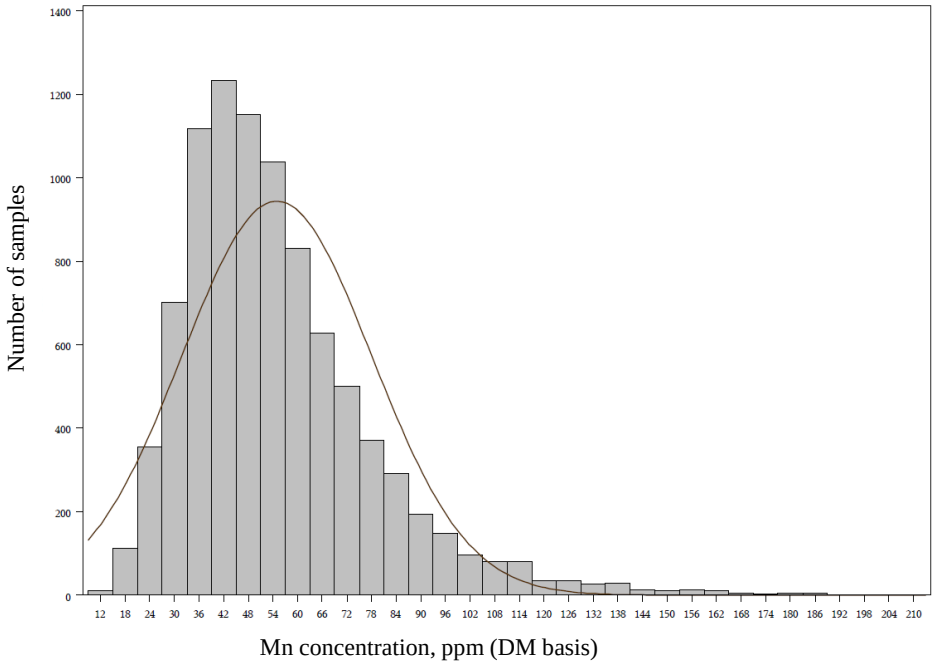

Figure 2. Distribution of Mn concentrations in mixed, mostly legume silage grown throughout the U.S. The smooth line indicates a normal distribution while the bars indicate the actual distribution (Knapp et al., 2015).

As a distribution becomes more skewed, the risk that a specific feed will contain excess mineral increases. The Mn data shown in **Figure 2** is a good example. That data has an average of 55 ppm and an SD of 23. Assuming a normal distribution, one would expect about 2.5% of the samples to have more than about 100 ppm (55 + 2 SD unit) and about 2.5% of the samples to have less than about 9 ppm. However, no samples had less than 9 ppm and 5.2% had more than 100 ppm. If your particular sample of mixed mostly legume silage was in the 5 out of every 100 samples with a very high Mn concentration, your diet would contain contain substantially more Mn than expected. Excess dietary Mn is rarely a problem for cows but excess dietary Cu can be (discussed below). Corn silage in **Figure 1** had a mean Cu concentration of 6 ppm with a SD of 1.8. With a normal distribution about 2.5% of the samples should have more than about 10 ppm Cu. However, about 5% of samples have more than 10 ppm Cu (i.e., twice the risk). If you formulate a diet assuming corn silage is 6 ppm Cu but it really has 12 ppm, and corn silage comprises a significant portion of the diet, over the long term (months) excess dietary Cu could become a problem. The bottom line is that averages for trace mineral concentrations in forages (and perhaps other feeds) found in tables should be used with caution. Because of substantial sampling variation, data from a single sample should not be used. The best advice is to generate median values for trace minerals for forages grown within a limited geographical area.

Do Trace Minerals in Feeds have Nutritional Value?

Essentially every feedstuff used in dairy diets contains some minerals. The question is, are those minerals biologically available to cows? Although survey data of nutritionists are lacking, based on personal experience it is not uncommon for nutritionists to set trace mineral concentrations in basal ingredients or at least forages, at 0. This approach would be valid if the trace minerals in feedstuffs were not biologically available to cows. Although substantial uncertainty exists regarding the absorption coefficients for most minerals in feeds, a portion of the trace minerals found in most (all?) feedstuffs is clearly available to cows. Tissues from wild ruminants such as deer (Wolfe et al., 2010) contain trace minerals indicating that absorption of basal minerals occur.

The NRC (2001) estimates that Cu, Mn, and Zn from basal ingredients are 4, 0.75, and 15% absorbable. The AC assigned to basal ingredients are usually lower than AC for the sulfate form of minerals even though most of the trace minerals contained within plant cells would be in an organic form. The lower AC for trace minerals in basal ingredients may reflect an adjustment for soil contamination. Some trace minerals in basal feeds, especially forages, are in soil that is attached to the feed and those minerals are often in the oxide form (low availability). Feeds with substantially greater ash and trace mineral concentration than typical likely have AC that are lower than the NRC values for trace minerals. Concentrations of trace minerals substantially greater than median value should be discounted but an exact discount cannot be calculated at this time, but those feeds would still contain some available mineral.

On average (and remember the issues with using averages), unsupplemented diets for lactating cows in the US based mostly on corn silage, alfalfa, corn grain, and soybean meal contain 7 to 9 ppm of Cu, 25 to 35 ppm of Mn, and 30 to 40 ppm of Zn (specific farms may differ greatly from these ranges). For an average Holstein cow (75 lbs of milk/day and 53 lbs of dry matter intake), basal ingredients supply about 80%, 235% (do not believe this), and 75% of requirements for Cu, Mn, and Zn (NRC, 2001). Ignoring minerals supplied by basal ingredients can result in substantial over formulation for trace minerals.

Recommendations

Chromium (Cr)

Chromium is a required nutrient, however, the NRC (2001) did not provide a quantitative recommendation. Furthermore, feeding diets with more than 0.5 ppm of supplemental Cr or from sources other than Cr propionate is not currently legal in the U.S. Cr is needed to transport glucose into cells that are sensitive to insulin. Because of analytical difficulties (e.g., normal grinding of feeds prior to chemical analysis can contaminate them with Cr) we do not have good data on Cr concentrations in feedstuffs. Some studies with cattle have shown that supplemental Cr (fed at 0.4 to 0.5 ppm of diet DM) reduced the insulin response to a glucose tolerance test (Sumner et al., 2007; Spears et al., 2012). Elevated insulin reduces glucose production by the liver and enhances glucose uptake by skeletal muscle and adipose tissue. These actions reduce the amount of glucose available to the mammary gland for lactose synthesis and this may be one mode of action for the increased milk yield often observed when Cr is supplemented. Most of the production studies evaluating Cr supplementation (studies used Cr propionate, Cr-methionine, Cr-picolinate and Cr yeast) started supplementation a few weeks before calving and most ended by about 6 wk. Supplementation rates varied but most were 6 to 10 mg/day (approximately 0.3 to 0.5 mg of Cr/kg of diet DM). The median milk response from 30 treatments from 14 experiments was +4.1 lbs/day (the SD among responses was 3.5 lbs/day). About 75% of the treatment comparisons yielded an increase in milk of more than 2 lbs/day. Although a comprehensive meta-analysis is needed, based on this preliminary analysis of studies, increased milk yield of at least 2 lbs/day is highly probably when approximately 0.5 ppm Cr is supplemented to early lactation cows. Whether this response would be observed throughout lactation is not known. The potential return on investment from milk can be calculated by using the value of milk and cost of feed plus the cost of the supplement and assuming a median response of about 4 lbs of milk and an expected increase in DMI of about 2.8 lbs. At this time, a milk response should only be assumed to occur up to about 42 DIM.

Cobalt (Co)

The current NRC requirement for Co is expressed on a concentration basis (i.e., 0.11 ppm in diet DM) rather than on a mg of absorbable Co/day basis. This was done because Co is mostly (perhaps only) required by ruminal bacteria and the amount they need is a function of how much energy (i.e., feed) is available to them. Although Co concentration data for feeds is very limited, the NRC requirement is for total Co and, in many cases, basal ingredients would provide adequate Co. In studies conducted in WA,

basal diets contained 0.2 to 0.4 ppm Co (Kincaid et al., 2003; Kincaid and Socha, 2007) but basal diets from WI contained 1 and 2 ppm Co (Akins et al., 2013). Data using growing beef animals (Stangl et al., 2000) found that liver B-12 was maximal when diets contain 0.22 ppm Co (approximately twice as high as the current recommendation). With dairy cows, liver B-12 concentrations continued to increase as supplemental Co (from Co glucoheptonate) increased up to 3.6 ppm (Akins et al., 2013). In that study elevated liver B-12 did not translate into any health or production benefits, indicating that maximal liver B-12 may not be necessary. Milk production responses to increased Co supplementation have been variable. One study reported a linear increase in milk yield in multiparious cows, but no effect in first lactation animals when supplemental Co increased from 0 to about 1 ppm. Older cows tend to have lower concentrations of B-12 in their livers which could explain the parity effect. Based on current data, the NRC (2001) requirement does not result in maximal liver B-12 concentrations in dairy cows. Across studies, when total dietary Co (basal plus supplemental) was about 1 to 1.3 ppm, maximum milk responses were observed. In some locations, basal ingredients may provide that much Co.

Copper (Cu)

The NRC (2001) requirement for Cu is expressed on a mg of absorbable Cu/day basis and over a wide range of milk yields (40 to 150 lbs/day). Requirements range from about 7 to 15 mg of absorbed Cu /day under normal conditions. As milk yield increases, the NRC requirement for Cu increases slightly because Cu is secreted in low concentrations in milk. However, DMI (and Cu intake) usually increase as milk yield increases to a greater extent than secretion of Cu in milk. Therefore the dietary concentration of Cu needed to meet the requirement may actually decrease as milk yields increase. Dry cows require less milligrams of Cu per day than a lactating cow, but because of dry matter intake differences, the concentration of Cu in dry cow diets may need to be greater than those for lactating cows.

Copper is stored in the liver and liver Cu concentrations are currently considered the gold standard for evaluating Cu status. Adult cattle liver Cu concentrations are deemed “adequate” between 120 and 400 mg/kg on a DM basis or approximately 30 to 110 mg/kg on a wet weight basis (McDowell, 1992). Over supplementation of Cu can result in Cu toxicity. Therefore, the range of adequate Cu status reflects both the minimum (110 or 30 mg/kg) and maximum (400 or 120 mg/kg) recommended concentrations of liver Cu on a DM or wet weight basis, respectively. The recommended range for liver Cu is the same for both Jerseys and Holsteins; however, livers from Jersey cows will usually have a greater concentration of Cu than those from Holsteins when fed similar diets. Liver Cu concentrations decrease when cattle are fed diets deficient in Cu and increase in a systematic manner as dietary Cu supply increases (Yost et al., 2002) which fits important criteria of a good marker of mineral status.

All trace minerals have antagonists that reduce absorption but often these do not occur in real situations. All trace minerals are toxic but for most of the minerals the intakes needed to produce toxicity are usually quite high. Copper, however, is unique among nutritionally important minerals in that it is toxic at relatively low intakes which

should dictate caution regarding over supplementation. On the other hand, Cu has numerous real world antagonists which mandate the need to over supplement in several situations. The NRC requirement assumes no antagonism (e.g., dietary S at 0.2% of DM); however, several situations commonly exists which result in reduced Cu absorption including:

- excess intake of sulfur (provided by the diet and water);
- excess intake of molybdenum (effect is much worse if excess S is also present);
- excess intake of reduced iron (may reduce absorption and increase Cu requirement);
- pasture consumption (probably related with intake of clay in soil); and
- feeding clay-based 'binders.'

Most of these antagonisms have not been quantitatively modeled, and specific recommendations cannot be provided. When dietary sulfur equivalent (this includes S provided by the diet and the drinking water) is greater than 0.25 to 0.30%, additional absorbable Cu should be fed. At higher concentrations of dietary equivalent S (0.4 to 0.5%), cows may need to be fed 2 to 3 times the NRC requirement when Cu sulfate is used. As a general guide for an average lactating Holstein cow, for every 100 mg/L (ppm) of S in water add 0.04 percentage units to the S concentration in the diet to estimate dietary equivalent S. For example, if your diet has 0.26% S and your water has 500 mg/L of S, dietary equivalent S = 0.26 + 5 × 0.04 = 0.46%. Note that some labs report concentrations of sulfate, not S. If your lab reports sulfate, multiply that value by 0.333 to obtain concentration of S. In most situations dietary S will be less than 0.25% of the DM. Diets with high inclusion rates of distillers grains and diets that contain forages that have been fertilized heavily with ammonium sulfate can have high concentrations of S. Water S concentration is dependent on source. Water should be sampled and assayed on a regular basis (at least annually) to determine whether water is adding to the S load in the diet.

Although the presence of antagonists justifies feeding additional absorbable Cu or using Cu sources that are more resistant to antagonism, no data are available indicating that the current NRC requirement is not adequate under normal conditions. Because of uncertainties associated with AC and the actual requirement, a **modest** safety factor should be used when formulating diets. Under normal situations, feeding 1.2 to 1.5 times the NRC requirement can be justified for risk management and it also should prevent excessive accumulation of Cu in tissues over the life of the cow. For an average lactating cow, the NRC requirement for absorbed Cu is about 10 mg/day. Applying the 1.2 to 1.5 times safety factor, the diet should be formulated to provide between 12 and 15 mg of absorbed Cu/day. For an average Holstein cow fed a diet without any antagonists and using Cu sulfate as the source of supplemental Cu, the diet should be formulated to contain 12 to 15 ppm of **total** Cu (i.e., basal + supplemental). If using a Cu source that has higher availability than Cu sulfate, the safety factor would be the same but because of a greater AC, the concentration of total Cu in the diet would be less because less supplemental Cu would be needed.

If antagonists are present, the NRC (2001) overestimates absorbed Cu supply and Cu supply will need to exceed NRC requirements. For an average Holstein cow fed a diet with substantial antagonists, total dietary Cu may need to be 20 ppm, or perhaps more, to provide 12 to 15 mg/d of absorbed Cu. Some specialty Cu supplements are less affected by antagonism (Spears, 2003) and under antagonistic conditions, those sources of Cu should be used.

Adequate absorbable Cu must be fed to maintain good health in dairy cows, however excess Cu is detrimental to cows. Acute Cu toxicity can occur but of a greater concern are the effects of long term overfeeding of Cu. When cows are overfed Cu, liver Cu concentrations increase. If Cu is overfed for a short period of time (i.e., a few weeks), the change in liver Cu may be insignificant but when Cu is overfed for many months, liver Cu concentrations can become dangerously elevated. Jerseys are at higher risk of Cu toxicity because they accumulate greater amounts of Cu in the liver than Holsteins (Du et al., 1996), however, toxicity can occur in Holsteins.

In non-lactating cows that were in good (or excess) Cu status and fed diets with approximately 20 ppm of total Cu, liver Cu accumulated at an average rate of 0.8 mg/kg DM per day (Balemi et al., 2010). Although milk contains Cu, because of differences in DMI (and subsequent Cu intake), this accumulation of liver Cu is likely similar to a lactating cow fed a diet with 20 ppm Cu. Over a 305-day lactation, a cow fed a diet with ~20 ppm Cu (without antagonists) could accumulate ~250 mg/kg DM in the liver. Over 2 or 3 lactations, liver Cu concentrations would become extremely high. Classic toxicity is thought to occur when liver Cu concentrations are greater than 2,000 mg/kg DM. Beef cattle are tolerant to extremely high liver Cu concentrations, and many of the studies used to establish the upper limit for liver Cu used beef cattle. However, beef cattle usually have short lifespans and may not be good models for dairy cows. Chronic copper poisoning is subclinical and can cause liver degeneration, which is evident based on elevated liver enzyme (AST and GGT) activities in plasma (Bidewell et al., 2012). Accumulating evidence suggests problems may start occurring at much lower concentrations of liver Cu (500 or 600 mg/kg DM). Activity of AST and GGT were significantly greater in heifers and bulls that had average liver Cu concentrations of 640 mg/kg DM compared with animals with average liver Cu of 175 mg/kg DM (Gummow, 1996). What was considered acceptable overfeeding of Cu (e.g., ~20 ppm of supplemental Cu) may result in problems because of the duration of the overfeeding.

Manganese (Mn)

The 2001 NRC greatly reduced the requirement for Mn compared with the earlier NRC. Based on NRC (2001) most lactating cows need between 2 and 3 mg/d of absorbable Mn and based on typical DMI translates to 14 to 16 ppm of total Mn in the diet. However, the 2001 NRC probably greatly overestimated the AC for Mn. Seventy percent of the calves borne from beef heifers fed a diet with about 16 ppm Mn for the last 6 month of gestation displayed signs of classic Mn deficiency (Hansen et al., 2006). Using Mn balance studies in lactating cows (Weiss and Socha, 2005; Faulkner, 2016), we estimated that lactating cows (average milk yield in the experiment was 84 lbs/day) needed to consume about 580 mg of Mn to be in Mn balance. Based on the DMI in

those experiments, that translated into a dietary concentration of ~30 ppm for total dietary Mn. As discussed above, uncertainty exists and reasonable safety factors (i.e., 1.2 to 1.5 multiplication factor) should be applied. For Mn, the starting point is 30 ppm and after the safety factor is applied, diets for lactating cows should have 36 to 45 ppm **total** Mn.

VITAMINS

Because of very limited data, the term 'requirement' should not be used for vitamins. Rather we should use the term 'Adequate Intake' or AI. This is the quantity of vitamin that has been shown to prevent health problems or result in statistically reduced prevalence or severity of disease. Some vitamins increase milk yields, but because effects on milk yields must be put into economic context (i.e., price of milk, price of feed and cost of the vitamin) milk yield response should not be a major factor when setting AI. However this does not mean that supplementation rates that increase milk yield but do not affect health should not be used in situations where they are profitable. Data on concentrations of vitamins in basal ingredients is extremely limited or lacking entirely which adds to uncertainty. Concentrations of certain vitamins in feeds can be extremely variable (e.g., concentrations of tocopherol in hay crop forages can range from almost 0 to more than 150 ppm). Because supply of vitamins from basal ingredients will almost never be known, AI are usually based on supplemental vitamins. Adequate data are available to determine AI for biotin, niacin, and vitamins A, D, and E.

Vitamin A

NRC (2001) recommendations for vitamin A appear adequate for average cows (i.e., 110 IU of supplemental vitamin A/kg of BW). For a typical Holstein cow that equals about 70,000 IU per day. Milk contains about 0.3 mg of retinol/kg; therefore, high producing cows can secrete substantial amounts of A into milk. The average cow in the NRC (2001) database produced about 35 kg of milk/day (77 lbs/day). For cows producing more than 35 kg of milk, feeding an additional 1000 IU of vitamin A/day per kg of milk greater than 35 kg will replace what is secreted in milk (about 450 IU/lb of milk above 77 lbs). For example for a Holstein cow producing 70 lbs of milk/d, the adequate intake of vitamin A is 70,000 IU but for a cow producing 100 lbs of milk, the adequate intake would be 70,000 + [(100-77)*1000] = 93,000 IU/day. No data are available that indicate that the NRC (2001) vitamin A recommendations for dry and prefresh cows are not adequate.

Vitamin D

Calcium homeostasis was long considered the primary function of vitamin D, but its effects on cells and animals go far beyond Ca including effects on immune function and health. The 2001 NRC recommendation (30 IU of supplemental vitamin D/kg BW or about 20,000 IU/day for a Holstein cow) is adequate with respect to Ca; however it may not be adequate for optimal immune function. Using a plasma concentration of 30 ng of 25-hydroxyvitamin D/ml to indicate sufficiency, 45 or 50 IU/kg of BW (about 30,000 IU/day) may be needed for lactating cows (Nelson et al., 2016). Cows that spend a few hours outside during summer months probably synthesize

adequate vitamin D but sun exposure in winter (in the US) probably lacks intensity for adequate synthesis rates.

Vitamin E

The 2001 NRC recommendations of 500 and 1000 IU/d of supplemental vitamin E for lactating and dry cows are adequate; however, sufficient data exists to justify increasing supplementation to 2000 IU/d during the last 14 to 21 d of gestation. This rate of supplementation has reduced early lactation mastitis and metritis.

Other Vitamins

Adequate consistent data exist to set the AI for supplemental biotin at about 20 mg/day. This inclusion rate often improves hoof health and milk production (Lean and Rabiee, 2011). Niacin has been extensively researched but data are equivocal; about half the studies report a benefit and half report no effect. Supplementation at 12 g/d is more likely to elicit a production response (increased milk yield and milk component yields) in early lactation cows than the commonly used rate of 6 g/d. The majority of data do not support the use of niacin to reduce ketosis. Therefore, in most situations, the AI of supplemental niacin is likely 0. Supplemental rumen-protected choline usually increases milk yield in early lactation (Sales et al., 2010) and may help reduce fatty liver. The common supplementation rate is 12-15 g of actual choline/d but the choline must be rumen protected. Because the data on health is equivocal at this time, choline does not have an AI, but it may often be profitable because of its effect on milk yield.

Conclusions

Adequate supply of trace minerals and vitamins improves the health and productivity of dairy cows; excess or inadequate trace nutrients can have the opposite effect. The 2001 NRC requirements for Cu, Zn, Se, and vitamin A are adequate in most situations and only a modest safety factor should be applied for risk management. Because of regulations, no safety factor can be applied to Se. For Cu, numerous antagonists exist and in those cases, diets need to provide substantially more Cu than recommended by NRC or a high quality organic Cu should be fed. Although many situations dictate higher concentrations of dietary Cu, be aware of excessive Cu supplementation. Modest overfeeding of Cu for months or years can result in high liver Cu concentrations that may be negatively affecting cow health. Manganese requirement is likely much higher than 2001 NRC and Co requirement also likely needs to be increased. Cows benefit from greater amounts of supplemental vitamin E during the prefresh period and lactating cows without great sun exposure may benefit from additional vitamin D supplementation.

Summary

- The NRC (2001) requirements for most trace minerals and vitamins appear adequate but modest safety factors (~1.2 to 1.5 times the NRC) should be used to reduce risk.

- The trace minerals contained in basal ingredients, including forages, have some degree of availability and concentrations should not be set to 0.
- NRC (2001) requirements for Co and Mn are too low and concentrations need to be increased substantially.
- Be wary of long term overfeeding of Cu. Health issues may develop at dietary concentrations as low as 20 ppm when fed over long periods.
- Supplying vitamin E in excess of NRC (2001) requirement to peripartum cows provides health benefits.
- Supplying vitamin D in excess of NRC (2001) to cows with limited sun exposure may be needed to maintain adequate D status relative to general health

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SESSION NOTES

Fat Supplementation to the Periparturient Dairy Cow: Does Fatty Acid Profile Matter?

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Introduction

The addition of supplemental fatty acid (**FA**) sources to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production. Recently, the effects of individual FA on digestibility, metabolism, and production responses of dairy cows has received renewed attention. In fresh cows, the high metabolic demand of lactation and reduced DMI during the immediate postpartum period result in a state of negative energy balance. Approaches to increasing energy intake of postpartum cows include increasing starch content of the diet and supplementing FA to increase the energy density of the diet. However, feeding high starch diets that promote greater ruminal propionate production during early lactation could be hypophagic and therefore further reduce DMI and increase the risk of ruminal acidosis and displaced abomasum (Allen and Piantoni, 2013). Regarding supplemental FA, some authors suggest that caution should be exercised when using dietary FA to increase the caloric density of diets in early lactation dairy cows, since a high lipid load may affect the endocrine system, feed intake, and increase the risk for metabolic disorders (Kuhla et al., 2016). However, just as we recognize that not all protein sources are the same it is important to remember that not all FA or FA supplements are the same. We will briefly review the biological processes and quantitative changes during the metabolism of FA, the digestibility of these FA, and their overall impact on performance. Our emphasis in the current paper is on recent research supplementing palmitic (C16:0), stearic (C18:0), oleic (*cis*-9 C18:1), omega-3, and omega-6 acids on feed intake, nutrient digestibility, and milk production.

Effect of Fatty Acids on NDF Digestibility

Changes in intake and digestibility of other nutrients, such as NDF, due to FA supplementation may affect positively or negatively the digestible energy value of any FA supplement. Weld and Armentano (2017) performed a meta-analysis to evaluate the effects of FA supplementation on DMI and NDF digestibility of dairy cows. Supplementation of supplements high in medium chain FA (12 and 14-carbons) decreased both DMI and NDF digestibility. Addition of vegetable oil decreased NDF digestibility by 2.1 percentage units but did not affect DMI. Also, feeding saturated prilled supplements (combinations of C16:0 and C18:0) did not affect DMI, but increased NDF digestibility by 0.22 percentage units. Overall, the authors concluded that the addition of a fat supplement, in which the FA are 16-carbon or greater in length,

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has minimal effects on NDF digestibility, but the effect of C16:0-enriched supplements were not evaluated.

We recently utilized a random regression model to analyze available individual cow data from 6 studies that fed C16:0-enriched supplements to dairy cows (de Souza et al., 2016). We observed that NDF digestibility was positively impacted by total C16:0 intake (**Figure 1A**) and DMI was not affected. This suggests that that the increase in NDF digestibility when C16:0-enriched supplements are fed to dairy cows is not explained through a decrease in DMI. Additionally, when comparing combinations of C16:0, C18:0, and *cis*-9 C18:1 in supplemental fat, we observed that feeding supplements containing C16:0 or C16:0 and *cis*-9 C18:1 increased NDF digestibility compared with a supplement containing C16:0 and C18:0 (de Souza et al., 2018).

With early lactation cows, Piantoni et al. (2015b) fed a saturated fat supplement (~ 40% C16:0 and 40% C18:0) and observed that fat supplementation increased NDF digestibility by 3.9% units in the low forage diet (20% fNDF) but had no effect in the high forage diet (26% fNDF). In our recent study that evaluated the effects of timing of C16:0 supplementation (PA) on performance of early lactation dairy cows (de Souza and Lock, 2017b), we observed that C16:0 supplementation consistently increased NDF digestibility ~ 5% units over the 10 weeks of treatment compared with control (**Figure 1B**).

Effects of C16:0, C18:0, and *cis*-9 C18:1 on Fatty Acid Digestibility

Our recent FA digestibility research has utilized and focused on C16:0, C18:0, and *cis*-9 C18:1. Of particular importance, Boerman et al. (2017) fed increasing levels of a C18:0-enriched supplement (93% C18:0) to mid-lactation dairy cows and observed no positive effect on production responses, which was likely associated with the pronounced decrease in total FA digestibility as FA intake increased (**Figure 2A**). Similarly, Rico et al. (2017) fed increasing levels of a C16:0-enriched supplement (87% C16:0) to mid-lactation dairy cows and even though a positive effect was observed on production response up to 1.5% diet DM, a decrease in total FA digestibility with increasing FA intake was observed (**Figure 2B**). However, considering that the range in FA intake was similar across both studies, the decrease in total FA digestibility was more pronounced when there was increased intake/rumen outflow of C18:0 rather than C16:0. This is supported by our meta-analysis, in which a negative relationship between the total flow and digestibility of FA was observed, with the decrease in total FA digestibility driven by the digestibility of C18:0 because of the negative relationship between duodenal flow and digestibility of C18:0 (Boerman et al., 2015). The exact mechanisms for these differences in digestibility are not understood; however, potential causes include the lower solubility of C18:0 compared to C16:0, which would be more dependent on emulsification for absorption (Drackey, 2000). Additionally, results have shown that *cis*-9 C18:1 has greater digestibility than C16:0 and C18:0 (Boerman et al., 2015). Freeman (1969) examined the amphiphilic properties of polar lipid solutes and found that *cis*-9 C18:1 had a positive effect on the micellar solubility of C18:0. To further understand what factors influence FA digestibility, we utilized a random regression

model to analyze available individual cow data from 5 studies that fed a C16:0-enriched supplement to dairy cows. We observed that total FA digestibility was negatively impacted by total FA intake, but positively influenced by the intake of *cis*-9 C18:1 (unpublished results). Finally, we recently evaluated the effects of varying the ratio of dietary C16:0, C18:0, and *cis*-9 C18:1 in basal diets containing soyhulls or whole cottonseed on FA digestibility. We observed that feeding a supplement containing C16:0 and *cis*-9 C18:1 increased FA digestibility compared with a supplement containing C16:0, a mixture C16:0 and C18:0, and a non-fat control diet. The supplement containing a mixture C16:0 and C18:0 reduced 16-, 18-carbon, and total FA digestibility compared with the other treatments (de Souza et al., 2018). This is displayed in **Figure 3** by using a Lucas test to estimate the apparent digestibility of the supplemental FA blends. The slopes (i.e., digestibility of the supplemental FA blends) in soyhull-based diets were 0.64, 0.55 and 0.75 and in cottonseed diets were 0.70, 0.56 and 0.81 for supplements containing C16:0, a mixture C16:0 and C18:0, and a mixture of C16:0 and *cis*-9 C18:1, respectively. This supports the concept that a combination of 16-carbon and unsaturated 18-carbon FA may improve FA digestibility, but reasons for this need to be determined.

In fresh cows, there is scarce information about the effects of supplemental FA on FA digestibility. We recently conducted a study to evaluate the effects of timing of C16:0 supplementation on performance of early lactation dairy cows (de Souza and Lock, 2017b). We observed a treatment by time interaction for C16:0 supplementation during the fresh period (1 to 24 DIM); although C16:0 reduced total FA digestibility compared with control, the magnitude of difference reduced over time (**Figure 4**). Interestingly, we also observed an interaction between time of supplementation and C16:0 supplementation during the peak period (25 to 67 DIM), due to C16:0 only reducing FA digestibility in cows that received the control diet in the fresh period. This may suggest an adaptive mechanism in the intestine when C16:0 is fed long-term. Understanding the mechanisms responsible for this effect deserves future attention, as does the impact of other supplemental FA during early post-partum on FA digestibility and nutrient digestibility.

Effects of C16:0, C18:0, and *cis*-9 C18:1 on Production Responses

We have recently carried out a series of studies examining the effect of individual saturated FA on production and metabolic responses of lactating cows. Piantoni et al. (2015a) reported that C18:0 increased DMI and yields of milk and milk components, with increases more evident in cows with higher milk yields, but the response occurred only in one of the two periods of the crossover design. Reasons why only higher yielding cows responded more positively to C18:0 supplementation and only in one period remains to be determined. Additionally, in a recent dose response study with mid lactation cows, feeding a C18:0-enriched supplement (93% C18:0) increased DMI but had no effect on the yields of milk or milk components when compared to a non-FA supplemented control diet, which was probably associated with the decrease in FA digestibility (**Figure 2A**, Boerman et al., 2017). Our results, and those of others, indicated that C16:0 supplementation has the potential to increase yields of ECM and

milk fat as well as the conversion of feed to milk, independent of production level when it was included in the diet for soyhulls or C18:0 (Piantoni et al., 2013; Rico et al., 2014). We recently utilized a random regression model to analyze available individual cow data from 10 studies that fed C16:0-enriched supplements to post peak dairy cows (de Souza et al., 2016). We observed that energy partitioning toward milk was increased linearly with C16:0 intake, as a result of a linear increase in yield of milk fat and ECM with increasing intake of C16:0.

When we compared combinations of C16:0, C18:0, and *cis*-9 C18:1 in FA supplements, a supplement containing more C16:0 increased energy partitioning toward milk due to the greater milk fat yield response compared with the other treatments (de Souza et al., 2018). In contrast, a FA supplement containing C16:0 and *cis*-9 C18:1 increased energy allocated to body reserves compared with other treatments. The FA supplement containing a combination of C16:0 and C18:0 reduced nutrient digestibility, which most likely explains the lower production responses observed compared with the other treatments. Interestingly, in a follow up study we compared different ratios of C16:0 and *cis*-9 C18:1 in FA supplements fed to post-peak cows and observed that supplements with more C16:0 favored energy partitioning to milk in cows producing less than 45 kg/d, while supplements with more *cis*-9 C18:1 favored energy partitioning to milk in cows producing great than 60 kg/d (de Souza and Lock, 2017a). Also, regardless of production level, supplements with more *cis*-9 C18:1 increased BW change. This may suggest that C16:0 and *cis*-9 C18:1 are able to alter energy partitioning between the mammary gland and adipose tissue, which may allow for different FA supplements to be fed in specific situations according to the metabolic priority and needs of dairy cows. Further research is needed to confirm these results in cows at different stages of lactation or other physiological conditions.

In early lactation cows, Beam and Butler (1998) fed a saturated FA supplement (~ 40% C16:0 and 40% C18:0) and observed that FA supplementation decreased DMI and did not affect yields of milk and ECM in the first 4 weeks after calving. Piantoni et al. (2015b) fed a similar saturated FA supplement (~ 40% C16:0 and 40% C18:0) and observed that FA supplementation during the immediate postpartum period (1to 29 DIM) favored energy partitioning to body reserves rather than to milk yield, especially in the lower forage diet. The high forage diet with supplemental FA increased DMI and tended to decrease BCS loss compared with the same diet without FA supplementation. Also, regardless of forage level, feeding supplemental FA increased DMI, decreased BCS loss, but tended to decrease milk yield. When cows were fed a common diet during the carryover period, the low forage diet with FA supplementation fed immediately postpartum continued to decrease milk yield and maintained higher BCS compared with the other treatments. On the other hand, Weiss and Pinos-Rodriguez (2009) fed a similar saturated FA supplement (~ 40% C16:0 and 40% C18:0) to early-lactation cows (21 to 126 DIM) and observed that when high-forage diets were supplemented with FA, the increased NE_L intake went toward body energy reserves as measured by higher BCS with no change in milk yield. However, when low-forage diets were supplemented with FA, milk yield increased (2.6 kg/d) with no change in BCS.

In our recent study, we evaluated the effects of timing of C16:0 supplementation on performance of early lactation dairy cows (de Souza and Lock, 2017b). During the fresh period (1 to 24 DIM), we did not observe treatment differences for DMI or milk yield (**Figure 5A**), but compared with control, C16:0 increased the yield of ECM by 4.70 kg/d consistently over time (**Figure 5B**). However, C16:0 reduced body weight by 21 kg (**Figure 6**), and BCS by 0.09 units and tended to increase body weight loss by 0.76 kg/d compared with control cows (CON). Feeding C16:0 during the peak period (25 to 67 DIM) increased the yield of milk by 3.45 kg/d, ECM yield by 4.60 kg/d (**Figure 5**), and tended to reduce body weight by 10 kg compared with control cows (**Figure 6**).

Interestingly, Greco et al. (2015) observed that decreasing the ratio of omega-6 to omega-3 FA in the diet of lactating dairy cows while maintaining similar dietary concentrations of total FA improved productive performance in early lactation. A dietary omega-6 to omega-3 ratio of approximately 4:1 increased DMI and production of milk and milk components compared with a 6:1 ratio. Approximately 1.3 kg of milk response could not be accounted for by differences in nutrient intake, which suggests that reducing the dietary FA ratio from 6:1 to 4:1 can influence nutrient partitioning to favor an increased proportion of the total net energy consumed allocated to milk synthesis. Further studies focusing on altering ratio of dietary FA are warranted, especially in early lactation cows.

Effects of Supplemental Fatty Acids on Reproduction

A recent meta-analysis of 17 studies reported a 27% increase in pregnancy rate in the first postpartum artificial insemination (**AI**) when dairy cows were fed fat supplements during the transition period (Rodney et al., 2015). In addition, the interval from calving to pregnancy was reduced. The inclusion of the very long chain omega-3 FA, eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) in the form of fish meal, fish oil, or algae in the diet, has been shown to improve either first-service or over-all pregnancy in 6 studies (Santos and Staples, 2017). A study conducted at the University of Florida (Silvestre et al., 2011) demonstrated that supplementation with Ca salts (1.5% of dietary DM) enriched in fish oil-derived FA starting at 30 DIM improved pregnancy rate/AI compared with Ca salts of palm FA (52.8 vs. 45.5%). Additionally, pregnancy loss between 32 and 60 d after AI was reduced by feeding Ca salts containing EPA and DHA (6.1 vs. 11.8%). Recently, Sinedino et al. (2017) observed that feeding 100 g/d of an algae product containing 10% of DM as DHA starting in the third week postpartum increased pregnancy rate by 39% and reduced days to pregnancy by 22 d (102 vs. 124 d). Therefore, polyunsaturated long-chain FA including omega-6 and omega-3 seem to be more effective at improving pregnancy in dairy cows than those containing mainly C16:0 and *cis*-9 C18:1. Furthermore, a meta-analysis indicated that the probability of pregnancy increased by 26% and the days from calving to pregnancy decreased by 34 d when *trans*-10, *cis*-12 conjugated linoleic acid was fed as a Ca-salt product across 5 studies involving 221 early lactation dairy cows (de Veth et al., 2009). Feeding long-chain FA might improve reproduction in dairy cattle through several potential mechanisms, including reducing negative energy balance, changes in follicle development and improvements in oocyte quality, improved early embryo

development, and reduced pregnancy loss. Since individual FA have a direct effect on several metabolic processes, research should focus on determining “ideal” combinations of FA for cows under specific physiological conditions and for specific purposes.

Conclusions

The addition of supplemental FA to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production. Although in general FA supplementation has been shown to increase milk yield, milk fat yield, and improve reproduction performance, great variation has been reported in production performance for different FA supplements, and indeed the same supplement across different diets and studies. Results are contradictory about the benefits of FA supplementation to early lactation dairy cows. We propose that this is a result of differences in FA profile of supplements used and the time at which FA supplementation starts. Further work is required to characterize the sources of variation in response to FA supplementation. Just as we recognize that not all protein sources are the same it is important to remember that not all FA sources and FA supplements are the same. The key is to know what FA are present in the supplement, particularly FA chain length and their degree of unsaturation. Once this information is known it is important to consider the possible effects of these FA on DMI, rumen metabolism, small intestine digestibility, milk component synthesis in the mammary gland, energy partitioning between the mammary gland and other tissues, body condition, and their effects on immune and reproductive function. The extent of these simultaneous changes along with the goal of the nutritional strategy employed will ultimately determine the overall effect of the FA supplementation, and the associated decision regarding their inclusion in diets for lactating dairy cows.

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Figure 1. Panel A: Relationship between C16:0 intake and NDF digestibility of dairy cows fed C16:0-enriched fatty acid (FA) supplements. Panel B: The effects of C16:0-enriched supplementation in early lactation cows on NDF digestibility.
Results in Panel A represent a combined data set evaluated using a random regression model from 6 studies feeding C16:0-enriched supplements on NDF digestibility of post-peak cows (de Souza et al., 2016). Results in Panel B utilized 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period) or from 25 to 67 DIM (peak period). From de Souza and Lock (2017b).

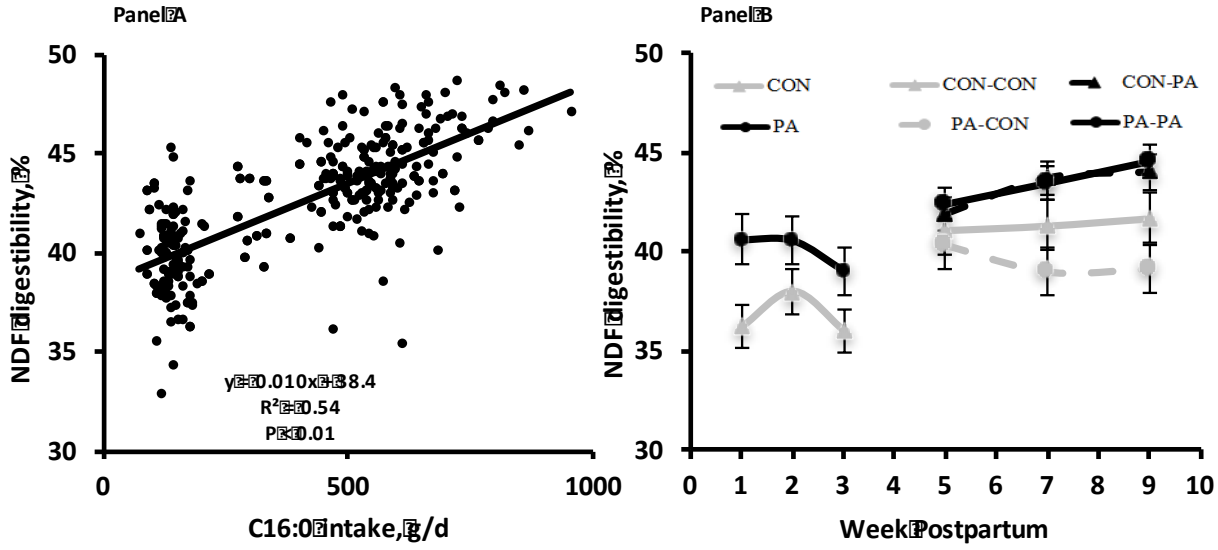


Figure 2. Relationship between total FA intake and apparent total-tract FA digestibility of dairy cows supplemented with either a C18:0-enriched supplement (Panel A) or a C16:0-enriched supplement (Panel B).
Results in Panel A utilized 32 mid-lactation cows receiving diets with increasing levels (0 to 2.3% dry matter) of a C18:0-enriched supplement (93% C18:0) in a 4 × 4 Latin square design with 21-d periods (Boerman et al., 2017). Results in Panel B utilized 16 mid-lactation cows receiving diets with increasing levels (0 to 2.25% dry matter) of a C16:0-enriched supplement (87% C16:0) in a 4 × 4 Latin square design with 14-d periods (Rico et al., 2017).

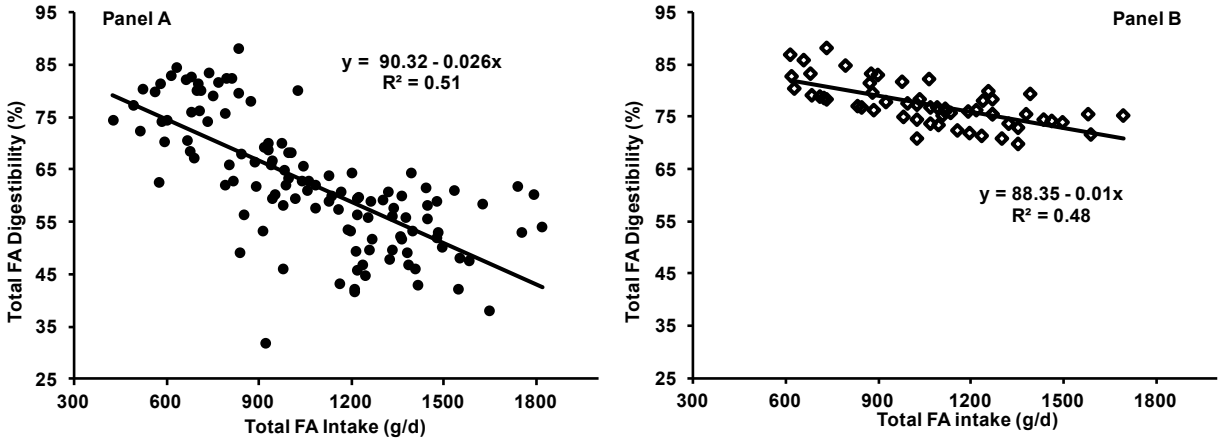


Figure 3. Lucas test to estimate total FA digestibility of supplemental FA treatments when cows received either a soyhulls-basal diet (Panel A) or a cottonseed-basal diet (Panel B). PA long-dashed line (1.5% of FA supplement blend to provide ~ 80% of C16:0); PA+SA solid line (1.5% of FA supplement blend to provide ~ 40% of C16:0 + 40% of C18:0); and PA+OA short-dashed line (1.5% of FA supplement blend to provide ~ 45% of C16:0 + 35% of C18:1 cis-9). Digestibility of supplemental FA was estimated by regressing intake of supplemental FA on intake of digestible supplemental FA. The mean intakes of FA and digestible FA when cows were fed the control diet were subtracted from the actual intakes of total FA and digestible FA for each observation. From de Souza et al. (2018).

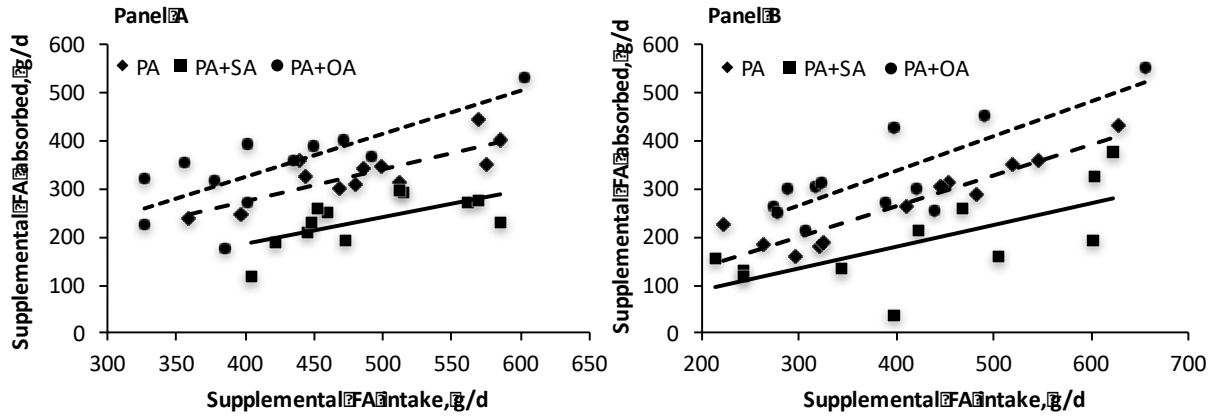


Figure 4. The effects of C16:0-enriched supplementation for early lactation cows on digestibility of 16-carbon (Panel A), 18-carbon (Panel B), and total FA (Panel C). Results utilized 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period) or from 25 to 67 DIM (peak period). From de Souza and Lock (2017b).

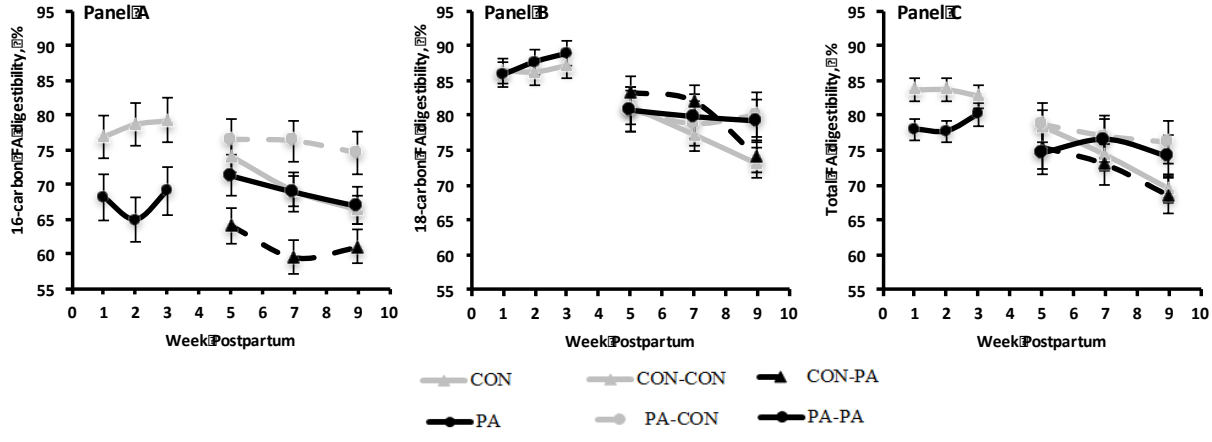


Figure 5. The effects of C16:0-enriched supplementation to early lactation cows on the yield of milk (Panel A) and ECM (Panel B).

Results from 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period) or from 25 to 67 DIM (peak period). From de Souza and Lock (2017b).

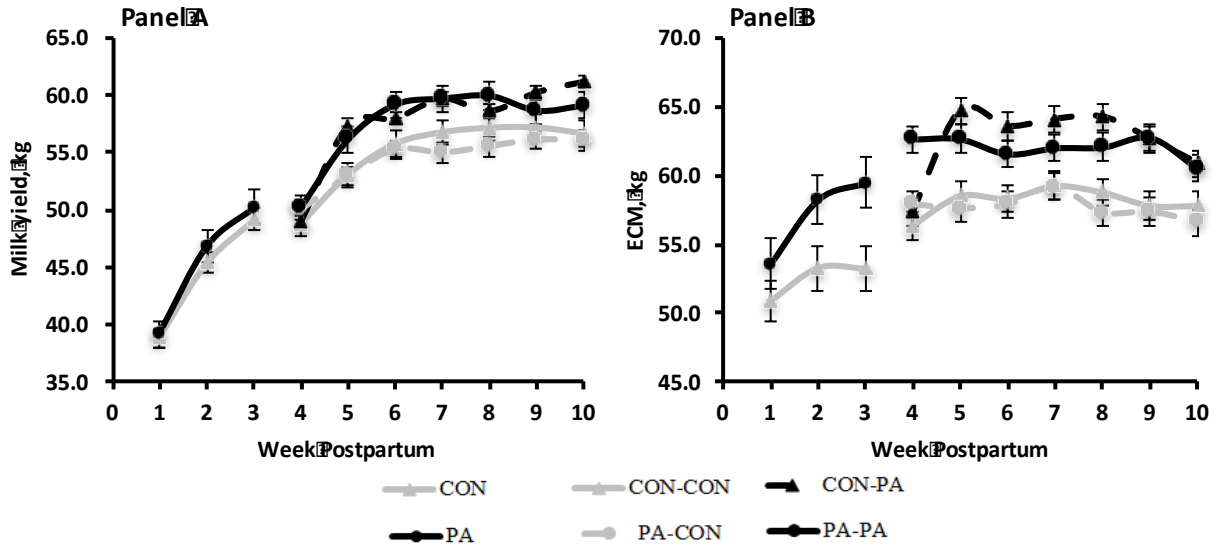
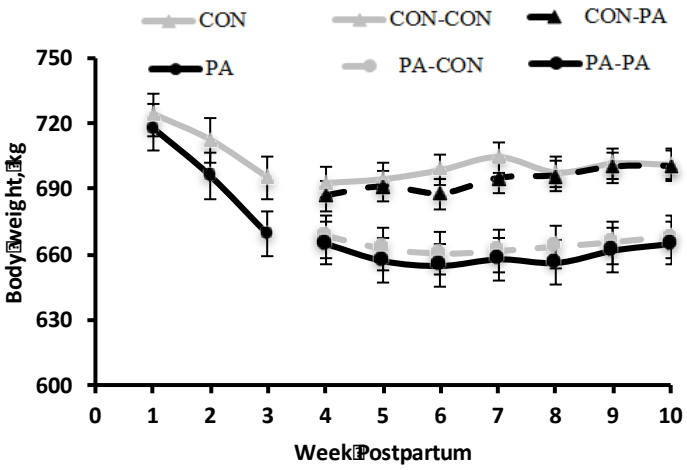


Figure 6. The effects of C16:0-enriched supplementation to early lactation cows on body weight.

Results from 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period) or from 25 to 67 DIM (peak period). From de Souza and Lock (2017b).



SESSION NOTES

Dietary Effects on Ruminal Papillae During Periparturient Transition in Holstein Cows – Is Cow Performance Affected?

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The Transitioning Cow

Starting lactation drastically increases energy needs for cows. For instance, a 1500 lb dairy cow has a maintenance requirement of 10.9 MCa/day (NE_M = 0.080 MCal/$BW^{0.75}$; NRC 2001). To produce 100 lb of milk (NE_L= 0.749 MCal/kg_{milk}) a day would require an additional 33.7 MCal (NRC 2001). Meeting the energy needs of cows during this transition remains a top priority for dairy producers. The principal challenge lies in the rumen's ability to absorb volatile fatty acids (**VFA**) from the diet to meet energy demands. When the absorption of VFA falls short of energy demands, cows go into negative energy balance and require mobilization of energy reserves.

Mobilizing energy reserves, principally triglycerides to non-esterified fatty acids (**NEFA**), must occur at an appropriate pace to avoid ketosis and fatty liver disease. If cows are underconditioned at calving, triglyceride reserves are insufficient, and ketosis results. If triglyceride mobilization is too fast, NEFA will accumulate in the liver and reform triglyceride, leading to fatty liver disease. Through balanced mobilization of triglycerides, the cow can meet the energy needs of lactation without suffering metabolic diseases.

In recent decades, research on optimal triglyceride mobilization focused on nutritional management strategies, primarily in the form of manipulating mobilization of energy reserves immediately prior to calving. For example, Rastani et al. (2005) varied the length of the dry period to investigate its impact on energy balance. They tested three dry period lengths: 0, 28, and 56 days. Their most noticeable finding was that cows without a dry period experienced almost no negative energy balance (**Figure 1**). In more recent research, Gross et al. (2011) induced a negative energy balance through feed restriction to cows at 100 DIM to determine how responsive blood metabolites were to negative energy balance. The researchers found that glucose, beta-hydroxybutyric acid (**BHBA**), and NEFA changes were much lower in induced negative energy balance than they were in early lactation. Together, these studies highlight the resilience of energy homeorhesis in cows that are already lactating. When lactation is uninterrupted, cows can mobilize energy reserves much more effectively than when lactation is turned off and on.

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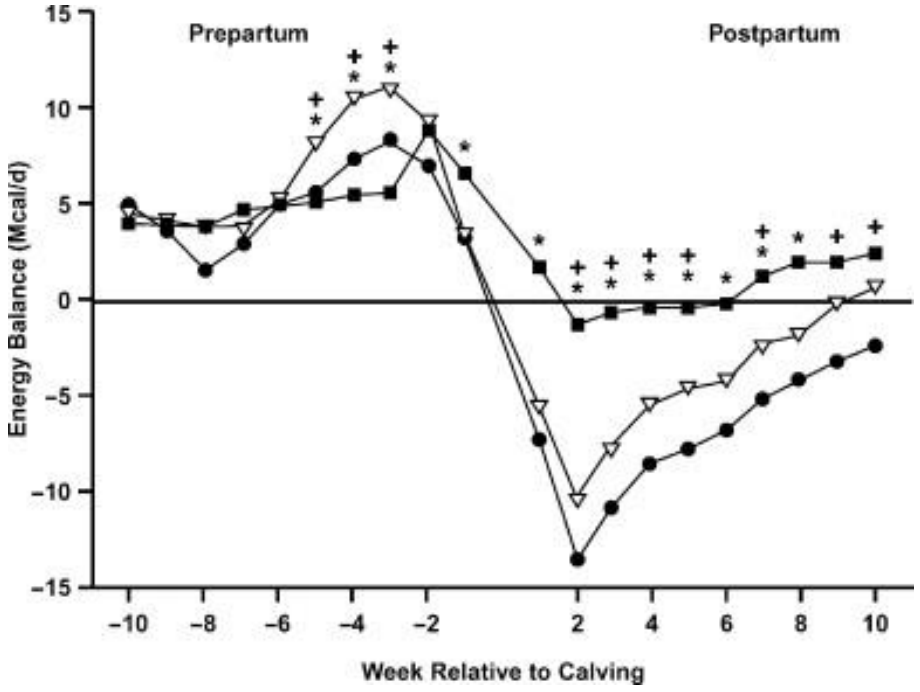

FIGURE 1. ROLE OF DRY PERIOD LENGTH ON NEGATIVE ENERGY BALANCE POST-PARTUM. A 56-DAY DRY PERIOD (CIRCLE) AND 28-DAY DRY PERIOD (TRIANGLE) CAUSED GREATER NEGATIVE ENERGY BALANCE THAN A 0-DAY DRY PERIOD (SQUARE). FROM RASTANI ET AL., 2005. ©JOURNAL OF DAIRY SCIENCE

When lactation needs to be turned on, cows need metabolic preparation to ensure a healthy transition to lactation. To facilitate preparation, cows can often be subjected to a “Goldilocks” diet, a low-energy diet fed pre-partum. Principally, low-energy diets during the dry period help mitigate postpartum negative energy balance, reduce circulating NEFA concentrations, increase plasma blood glucose, and reduce time to pregnancy (Janovick et al., 2011; Cardoso et al., 2013). While restricting energy intake pre-calving is important for performance during lactation, it sidesteps an important component of the calving transition: the adaptation of the rumen to improve VFA absorption. Increasing VFA absorption in the rumen, especially in the first weeks post-partum, holds much promise to improve negative energy balance via increasing energy intake rather than limiting energy expenditure.

The Transitioning Rumen

During the transition period, the cow may be switched from a dry period diet to a lactation diet in a matter of several days, but the rumen’s structural and cellular adaptations to the new diet takes weeks. The dietary non-fiber carbohydrate (**NFC**) content increases to as high as 45% (AlZahal et al., 2014), leading to elevated VFA production. While structural adaptations begin in the first week of the high NFC diet, the adaptation process persists to at least 6 weeks of lactation (Laarman et al., 2015, Steele et al., 2015). Until a cow fully adapts to the new diet, the need to increase energy

intake and DMI to mitigate negative energy balance elevates the risk of VFA production in the rumen exceeding VFA removal, placing considerable strain on rumen epithelial health.

The rumen epithelium carries out two important functions: absorption of nutrients from the rumen into the bloodstream and forming a barrier that prevents ruminal microbes from entering the bloodstream. Structurally, the rumen is a squamous epithelial layer consisting of four layers: the corneal layer facing the rumen, followed by the granular layer, spinous layer, and basal layer (**Figure 2**). The spinous layer contains many of the intercellular anchors and proteins that form a barrier between the rumen and the bloodstream (Graham and Simmons, 2005, Baldwin et al., 2012). While rumen pH can decrease to as low as 5.2 without clinical ruminal acidosis (Aschenbach et al., 2011), live epithelial cells in the granular, spinous, and basal layers must maintain an intracellular pH of 7.4. Facing the rumen contents, the corneal layer offers protection to the underlying layers, avoiding contact with the low pH.

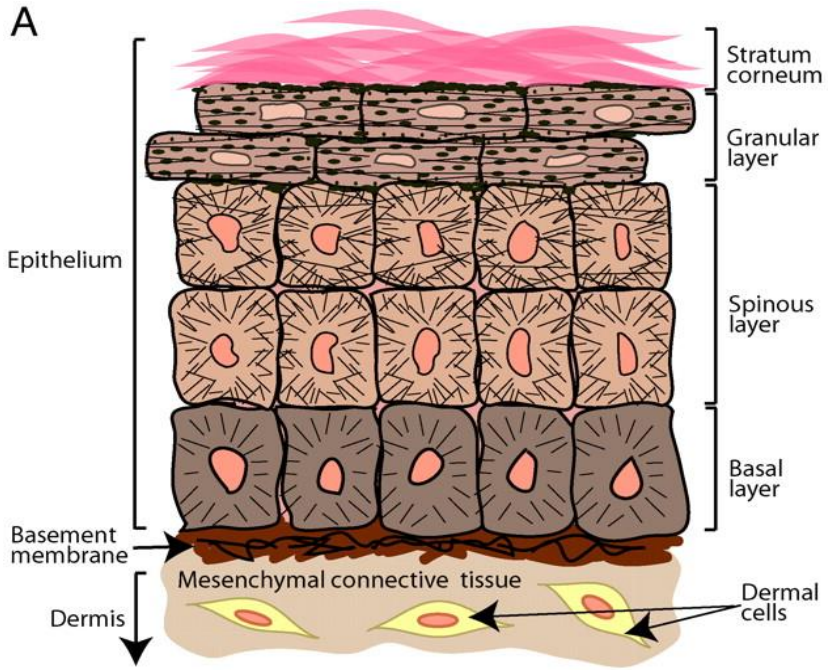

FIGURE 2. LINING THE RUMEN IS A 4-LAYER EPITHELIUM THAT PREVENTS BACTERIA FROM ENTERING THE BLOODSTREAM, LEADING TO LIVER ABSCESSES AND LAMINITIS (ALONSO AND FUCHS, 2003; ©NATIONAL ACADEMY OF SCIENCE. USED FOR NON-COMMERCIAL PURPOSES)

Sudden transitions from a low fermentability diet to a high fermentability diet, such as those in the transition period, comprise a considerable strain on the rumen. When the rumen is insufficiently adapted to the fermentability of the diet, it cannot remove VFA and protons fast enough, resulting in subacute ruminal acidosis (**SARA**).

The impact of SARA on ruminal health is profound. The corneal layer begins to slough (**Figure 3**), exposing the granular and spinous layers to ruminal microbes and pH, resulting in increased permeability of the rumen epithelium (Steele et al., 2011). Indeed, in another study, low rumen pH increased permeability of the rumen epithelium to *E. coli* translocation (Emmanuel et al., 2007). Translocation of rumen microbes into the bloodstream is associated with adverse effects on animal health and productivity, including laminitis, ruminitis, and reduced milk production (Plaizier et al., 2008; Stone, 2004). Maintaining rumen pH during the transition period is of paramount importance.

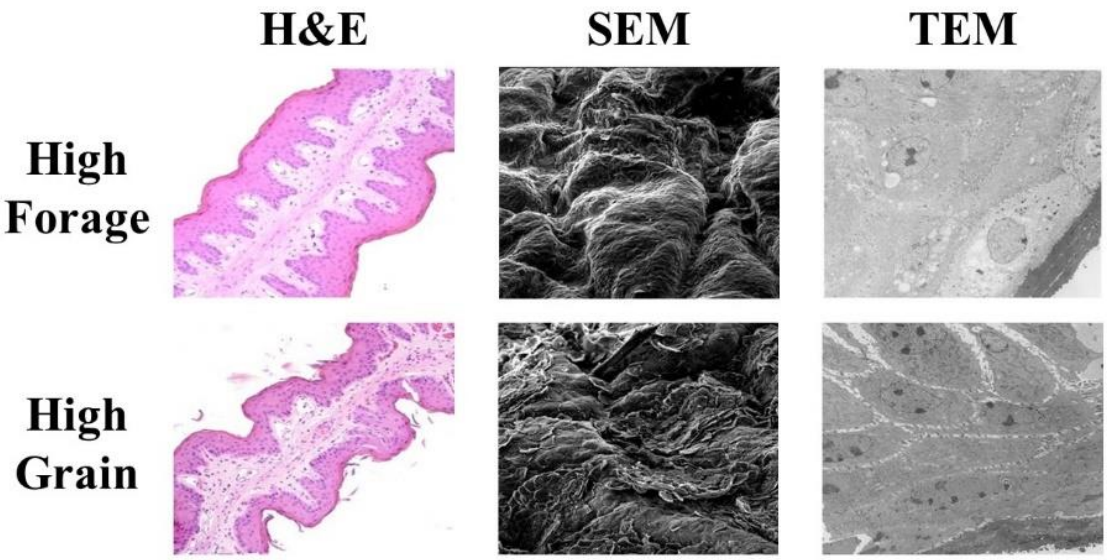

FIGURE 3. IMPACT OF SWITCHING DAIRY COWS FROM A HIGH FORAGE TO A HIGH GRAIN DIET. H&E STAIN SHOW EXTENSIVE SLOUGHING OF THE CORNEAL LAYER FACING THE RUMEN (LEFT). SCANNING ELECTRON MICROGRAPH (SEM; MIDDLE) SHOW STRIPPING OF EPIMURAL MICROBES, WHILE TRANSMISSION ELECTRON MICROGRAPH (TEM; RIGHT) DEMONSTRATE INCREASED PERMEABILITY BETWEEN EPITHELIAL CELLS. STEELE ET AL., 2011. ©ELSEVIER PUBLISHING INC.

Rumen pH dynamics are largely dependent on the adapted state of the rumen. In a study in calves, pre-weaned calves were fed either milk replacer and hay only, or milk replacer, starter, and hay (Laarman and Oba, 2011; **Table 1**). Calves fed starter had 50% higher VFA concentrations in the rumen, but showed no difference in rumen pH, highlighting the adaptability of the rumen. Maintaining rumen pH at physiologically healthy levels prevents adverse animal health issues such as laminitis and ruminitis (Plaizier et al., 2008). Indeed, the ability to remove VFA from the rumen is a key factor in determining resistance to SARA (Penner et al., 2009).

TABLE 1. IMPACT OF FEEDING CALF STARTER ON RUMEN FERMENTATION DYNAMICS. DESPITE HIGHER FERMENTABILITY OF THE CALF STARTER TREATMENT, AS INDICATED BY GREATER TOTAL VFA, RUMEN PH EXHIBITED NO DIFFERENCE BETWEEN TREATMENTS, DEMONSTRATING ADAPTABILITY OF RUMEN TO CHANGES IN DIET FERMENTABILITY. (LAARMAN AND OBA, 2011) ©ELSEVIER PUBLISHING INC.

	Milk & Hay	Milk & Hay & Starter
Average pH	6.42 ± 0.10	6.27 ± 0.12
Duration pH < 5.8, min/d	101 ± 100	237 ± 126
Total VFA, mM	64.6 ± 8.6	99.1 ± 8.1*
Starter DMI, kg/d	N/A	0.76 ± 0.04
Hay DMI, kg/d	0.23 ± 0.07	0.34 ± 0.8

* $P < 0.05$

The rumen's ability to absorb VFA consists of 2 major components: cellular transport (through transporters and passive diffusion) and absorptive surface area, both of which adapt to dietary changes in the short-term and long-term. In the short term, the papillae can make use of cellular transport mechanisms to improve VFA transport, using both passive diffusion and transporters in the epithelial cells to increase VFA transport (Laarman et al., 2016). In the longer-term, the transport capacity is increased by increased absorptive surface area, as demonstrated by increases in papillae length and width (Dirksen et al., 1985). Morphological changes in rumen papillae begin in the first week after calving and persist for at least 6 weeks (Laarman et al., 2015). During this adaptation, passive diffusion of VFA is responsible for most of the changes in VFA transport (Schurmann et al., 2014). In the end, the rumen papillae have changed morphologically and cellularly, with the result of improving VFA transport capacity to more closely meet the energy demands of the lactating cow.

Setting Up the Rumen for Transition Success

During the lactation transition, the energy demands of lactation and energy intake and absorption through the rumen will ultimately dictate the extent of negative energy balance. Restricting feed intake during the dry period improves the metabolic transition of dairy cows to lactation. Simultaneously, rumen papillae must increase VFA transport capacity to eventually bring the cow out of negative energy balance. As a result, priming the rumen for transition requires improvements in rumen papillae function and/or surface area without overfeeding cows. The potential to prime the rumen without overfeeding the cow mostly lies in feed additives that stimulate rumen adaptation.

One such additive is butyrate, one of the principal VFA well known for its bioactivity. In dairy cows, supplementing a highly fermentable diet (45% NFC) with butyrate at 2.5% of DMI increases VFA transport capacity and improves barrier integrity (Laarman et al., 2013a,b; Baldwin et al., 2012). When fed to prepartum Holstein cows in the last week before parturition at 300 g/day (0.66 lb/day), butyrate improved DMI by 1.7 kg/day (3.7 lb/day) (Kowalski et al., 2015). When fed to goats, butyrate increased ruminal papillae surface area (Malhi et al., 2013). The ultimate success of this supplementation strategy will ultimately be dependent on dose and timing of supplementation.

Other strategies to improve rumen adaptation to lactation diets have mixed results. Dieho et al. (2016) fed supplemental concentrate to cows in the dry period. While DMI remained similar to cows not fed supplemental concentrate, rumen papillae surface area increased. The increase in papillae surface area did not correspond to an increase in VFA transport rates, suggesting papillae surface area and VFA transport rates may behave independently. In another study focusing on cellular changes in the transition period from 3 weeks prepartum to 9 weeks post-partum, cows exhibited morphological changes in rumen papillae but no differences in VFA transport capacity (Laarman et al., 2015).

Together, these strategies aim to capitalize on the adaptability of the rumen to prepare it for the energy demands of early lactation. Successful adaptation of the rumen requires nutritional strategies that stimulate papillae adaptation without providing excess energy to the cow. Within that targeted window lie opportunities to improve the VFA absorption capacity of cows as they enter lactation. The more VFA absorption capacity is improved at calving, the more energy can be taken in by the cow, and the more diminished the negative energy balance will be. Diminishing negative energy balance will ultimately improve cow productivity and health, and the rumen can play an important role in accomplishing that goal.

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SESSION NOTES

Ruminal Acidosis – Much More Than pH

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Introduction

Studies show that acidosis is a very significant disorder of cattle. Studies in Wisconsin found that 20.1 and 23% of cows had subacute acidosis as defined by rumen pH < 5.5 (Oetzel et al., 1999, Oetzel, 2004) and others in Ireland had 11% (O'Grady et al., 2008). A large Australian study found that 10% of dairy cows less than 100 days in milk had acidosis, as defined by assessment of ruminal VFA, ammonia, lactic acid, and pH (Bramley et al., 2008). Therefore, it is likely that many cows will experience some level of acidosis during lactation and, indeed, some may be affected many times. It can be estimated that if the prevalence of subacute acidosis is 10% (Bramley et al., 2008) and the duration of a case is 2 days based on data by Golder et al. (2014b), then there would be an incidence of approximately 1500 cases over a 300 d lactation per 100 cows. Understanding and controlling acidosis is therefore critical to ensuring animal well-being and production.

There is now considerable debate about the definition of acidosis with papers providing varying definitions, many based on ruminal pH, others referring to conditions not solely based on ruminal changes (Plaizier et al., In Press), and some based on a series of different rumen measures (Bramley et al., 2008; Golder et al., 2014; Lean et al., 2013a; Morgante et al., 2007). Providing thoroughly defensible definitions of the condition is critical to management of acidosis, because a failure to properly define the condition in scientific experiments can lead to a failure to adequately control the condition. In this paper, we discuss definitions of acidosis, provide some suggestions for definitions, and examine recent findings on rumen function that may help prevent acidosis.

What is Acidosis?

Researchers, primarily based in the EU, state that “The classification of and terminology used in relation to dietary-induced disorders of the ruminant digestive system are confused and not fit for purpose. The problem is most apparent in relation to the condition referred to as sub-acute rumen acidosis (**SARA**), for which there are no adequate, accepted criteria for definition. Sub-acute is a poorly defined descriptor of the time-course of a disease and is often misinterpreted to refer to either subclinical disease or disease in which clinical signs are mild.” We agree with their synopsis and provide the following supported thoughts to provide definitions of these conditions that may help with diagnosis and prevention of the disorder.

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Acidosis is a continuum of conditions of varying severity that reflect the challenge of safely sequestering hydrogen that accumulates from carbohydrate fermentation. Safe pools to 'hide hydrogen' include starch engulfment by protozoa, bacterial glycogen formation, growth of bacteria, methane, and weak organic acids (**VFA**). Less safe pools include lactic acid, because that acid is 10 times stronger than the VFA. Decreasing the hydrogen supply by increasing the more slowly fermenting fiber content of the diet and enhancing rumination can reduce the risk of acidosis. It is important to recognize that the effects, and possibly even pathogenesis of acidosis, may not be solely ruminal and other parts of the gastro-intestinal tract play a role.

Acute acidosis

Acute acidosis is defined by the generation of significant amounts of lactic acid in the rumen. Nagaraja and Titgemeyer (2007) characterize acute acidosis as being present when rumen pH is < 5.0, there is > 50 mM/L of lactic acid and ruminal VFA are less than 100 mM/L. Other studies support these criteria (Golder et al., 2014a, Golder et al., 2014b). There is a general consistency of definition and understanding of this condition in the literature. Acute acidosis is caused by the sudden access of cattle to rapidly fermentable carbohydrates (**RFCHO**) or changed processing of the same RFCHO. Fructose (Golder et al., 2012b, Golder et al., 2014b) appears to have greater potential to cause acute acidosis than starches and glucose has been used to create lactic acidosis (Nagaraja et al., 1981). Acute acidosis is characterized by fatal or serious disorders.

Definition: Acute acidosis is a serious condition of cattle characterized by death, dehydration, ruminal distension, diarrhea (often with grain in the feces and a sickly, sweet smell), abdominal pain, tachycardia, tachypnea, staggering, recumbency, coma, a marked decline in milk yield, and sequalae including ruminitis, liver abscess, pulmonary infections, epistaxis, and poor production that arises subsequent to the ingestion of large amounts of RFCHO.

Findings include milky white rumen fluid often containing grain. The rumen fluid has a rumen pH of < 5.0, > 50 mM/L of lactic acid, and ruminal VFA of < 100 mM/L when rumen fluid is examined.

Acidosis

The definition 'subacute' does not sit easily in definitions that apply to metabolic diseases. It is simpler and more correct to ignore the term 'subacute'. Lean et al. (2009) provided a series of conditions that define metabolic disease based on the postulates of Evans (1976). It is clear that increasing dietary starch (Li et al., 2012), sugars (Nagaraja et al., 1981, Golder et al., 2012b), changing the forage fed (Khafipour et al., 2009), and changing the particle size of the feed (Zebeli et al., 2012) can create acidosis and meet the postulates proposed (Lean et al., 2009). However, there is very considerable variation in the responses of individual cattle to the increase in RFCHO and rumen pH is not the most consistent and easily measured change in rumen outcomes.

Plaizier et al. (In Press) highlighted a large number of studies that estimate the prevalence of low rumen pH, but cows with low pH did not have significantly different clinical outcomes compared to other cows, apart from low body condition score. By way of contrast, Bramley et al. (2008) who used both rumenocentesis and stomach tube measures of ruminal pH, but also ruminal VFA and ammonia concentrations found that the rumen pH measures were not highly predictive for a group of cows that were characterized by being in herds where dietary NFC were higher, NDF lower, and that had a markedly (> 100%) higher incidence of lameness (Bramley et al., 2013) than other herds. The best predictors for these cows, that also had a low milk fat to milk protein content and ratio, was a combination of rumen VFA concentrations, particularly valerate and propionate and rumen ammonia. The least predictive, albeit significant, variables for classifying cows as acidotic were rumen pH and lactic acid. In this paper, we explore the implications of these findings and support for them. Further, it is important to recognize that there is the potential for hindgut changes to influence outcomes of a RFCHO challenge (Gressley et al., 2011).

We consider that the following factors, some of which we explore in this paper are likely to influence the expression of acidosis: i) production of toxic substances and clearance of these from the rumen. The generation of toxins and clearance of toxins will be influenced by ruminal populations of micro-organisms; ii) compromised epithelia, through chemical action, conditions such as pestivirus that damage epithelial integrity and ability to appropriately process toxins; and iii) rate of passage and differential clearance and exposure of different parts of the gastrointestinal tract. All of the above functions may be influenced by genetics and understanding the interactions of these with the metabolome (physiological responses) and metagenome (the population of rumen microbes) is an important new frontier.

Consequently, we propose the following definition. *Definition:* Acidosis is a serious condition of cattle characterized by cyclic inappetence, increased risk of lameness, diarrhea (often with grain and/or gas in the feces), increased risk of low milk fat percentage, and sequalae including ruminitis, liver abscess, pulmonary infections, abomasal displacement, epistaxis, and poor production that arises subsequent to the ingestion of large or moderate amounts of rapidly fermentable carbohydrates.

On a herd basis, findings include variable individual production, high prevalence (> 40%) of lameness (Bramley et al., 2013), high prevalence of milk fat: milk protein ratio of < 1.02, and diets that are high in NFC (> 40%), but low in NDF (< 31%). Findings based on Bramley et al. (2008), include rumen fluid that is high in total VFA > 100 mM/L, of moderately low pH (< 5.8 rumenocentesis or 6.2 stomach tube), with concentrations of propionate > 30 mM/L and low ammonia < 3 mM/L.

Other observations likely to be pertinent to increasing the risk of acidosis include evidence of sorting of diets, overstocking of corrals, mixing of heifers and cows, and mixing of new cattle (Lean et al., 2014).

Limitations of pH as a Diagnostic Measure

The series of changes caused by the increase in RFCHO extends well beyond a decrease in pH and includes changes in a vast number of metabolic pathways involved in acidosis including the generation of potentially toxic metabolites (Ametaj et al., 2010, Zhang et al., 2017). Zhang et al. (2017) found ruminal increases in amino acids, bacterial degradation products including amines, and sugars with increased concentrates fed. There is considerable speculation in regards to the agents that might be implicated in causing some of the clinical signs of acidosis and Lean et al. (2013b) summarized some of the evidence supporting potential roles for histamine, endotoxin, and lactic acid to cause laminitis (**Table 1**). Given 1) the known agents capable of causing inflammation and clinical signs and 2) that less well-known metabolites may be involved in clinical signs of acidosis, it is unsurprising that rumen pH *per se* is largely unrelated to the clinical signs of acidosis. Given the large number of potential toxins, often produced simultaneously in the rumen, a singular focus on any particular toxin is not appropriate.

More critically perhaps, in terms of diagnostic potential, a highly accurate measurement of rumen pH is nearly impossible. Simply, the rumen is dynamic and not homogenous and any measure whether continuous and indwelling, or static, regional and singular has limitations. Similar observations can be made in regard to most rumen measures, as rumen function varies within the rumen mat, liquid phase, and near the rumen wall and papillae (Penner, 2014). **Table 2** from Golder (2014) shows the differences and correlations between different measures of rumen pH. **Figure 1** derived from Bramley et al. (2008) shows the correlations between rumen samples drawn by stomach tube and rumenocentesis in 660 cows ($R^2 = 0.2$). **Table 3** shows the value of different tests for acidosis and highlights that rumen pH provided very similar results whether obtained by stomach tube or rumenocentesis. An extensive series of studies in the United Kingdom with indwelling pH meters demonstrated that these could detect changes in the diet of cattle, but variability in individuals in their baseline pH and responses to diet did not provide adequate diagnostic outcomes for predicting differences among individual cattle without careful use of complex statistics (Denwood et al., 2018). It is, however, this large variability among cattle that provides the most interesting directions for research and prevention of acidosis in the future.

Is There a Good Test for Acidosis?

For a test to be effective, it needs to be able to be both sensitive (i.e. detect true cases of the condition and be specific, that is, have few false positive detections) and be applicable across a wide range of conditions. Bramley et al. (2008) conducted their study on a wide range of herds that fed only pasture, though to different levels of grain and supplement feeding including total mixed ration herds. Herd was not a significant factor in the study in the prediction of acidosis. Subsequent, tightly-controlled challenge studies using 1.2% of body weight fed as grain, showed that propionate, ammonia, and valerate concentrations were the most sensitive indicators of the potential for different

grains to cause acidosis (Lean et al., 2013a), and that the Bramley model was sensitive to ruminal change consistent with acidosis.

Further, a study performed using gradated steps of 2 kg of additional supplement, primarily wheat grain but also canola meal, demonstrated that as supplement increased, so did acidosis as measured using the Bramley model and that at 16 kg of supplement all cattle were acidotic most of the day (**Figure 2**). The cattle with acidosis had decreased milk production and milk fat percentage; however, feeding the supplement as a part of a mixed ration or substituting some of the wheat with canola deceased the prevalence of acidosis. There were very few cattle with acidosis in the low supplement groups and a high prevalence in the high supplement groups. It appears that the model for evaluating acidosis is fit for purpose, but requires a method to simply apply in the field. While it is likely that this model will be refined, the critical value in the model is that it demonstrates that acidosis is much more than pH and that performance of cattle is much more closely aligned to a model that considers more than pH.

Ruminal Ecology and Risk

The rumen is central to our understandings of cattle nutrition but is still largely unexplored, which is not too surprising given the large number of organisms present. Only a minority of the bacteria, archaea, viruses, fungi, and protozoa present in the rumen have been named or are able to be cultured, let alone their functions fully characterized. However, this field is rapidly changing with rapid sequencing of the DNA and rRNA of rumen organisms, termed metagenomics, allowing investigations of the rumen environment to become more detailed (Jami et al., 2013). Recently, the effects of perturbing the rumen have been evaluated (Weimer et al., 2010, Golder et al., 2014b, Plaizier et al., 2017). Goldansaz et al. (2017) reviewed the opportunity for metabolomics, that is analytical techniques that can quantify small molecular weight products of metabolism, to be utilized in the investigation of production disease. Examples of this include (Loor et al., 2007) and Hailemariam et al. (2014). Metabolomics may be particularly powerful when used to evaluate responses to rumen perturbation (Zhang et al., 2017). These new techniques are offering insights to the function and control of the rumen.

The *Bovidae,* including cattle, are among the most widely disseminated of the mammals. An important perspective can be obtained from Ley et al.(2008). This paper examined similarities and differences in the fecal biota of a very diverse selection of mammals in the context of co-evolution of meta-genomic communities. A key finding was that bacteria appear to be fairly promiscuous between hosts, a factor the authors speculated could account for the spectacular success of herbivores in general. The observations of Ley et al. (2008) are important to consider in the context of the way in which a species manages risk. In the case of cattle, times of abundance, for example lush legumes or abundant sugars or starches, or even toxic plants pose a risk to the animal and even a herd. This leads to a key understanding of the concept of a core rumen microbiome and a group of non-core organisms (Jami and Mizrahi, 2012, Lettat and Benchaar, 2013, Firkins and Yu, 2015). The core organisms appear to be common

to most cattle in a group, however, there is very considerable diversity in the non-core. Perhaps the best example to consider is the protozoa that cattle maintain despite a high cost of predation of bacteria, leading to loss of approximately 20% in microbial protein outflow and lower average daily gain than defaunated cattle. However, these physiological responses are less for cattle on concentrate diets, suggesting an important role for protozoa in slowing the rate of starch degradation (Eugène et al., 2004) and a potentially valuable role in reducing the risk of acidosis. The adaptive responses of the rumen to severe dietary challenge therefore, might be an expected variable based on the concept that maintaining populations of organisms that may be less efficient but vital for survival, under particular challenge conditions, is a function of managing risk in a population.

Perturbing the Rumen

The primary methods used to perturb the rumen are feeding or administering single or multiple doses of RFCHO in the form of starches, sugars, or their combinations. Studies have noted considerable variation in responses among cattle fed a common diet designed to induce ruminal acidosis (Brown et al., 2000, Bevans et al., 2005, Penner et al., 2009, Golder et al., 2014b). Perturbation differences appear to be affected by genetic and environmental factors and likely their interactions. Substrate and other factors such as length of challenge and prior exposure to RFCHO etc. affect rumen perturbation. Golder et al. (2012b) fed non-pregnant Holstein heifers no grain or combinations of grain (1.2% of bodyweight), fructose (0.4% of bodyweight with 0.8% of bodyweight grain), and histidine (6 g) in a single challenge feeding. It was evident that the rumen altered in response to the different substrates and substrate combinations. Heifers that had fructose included in their challenge ration had bacterial populations associated with increased total lactic acid and butyrate concentrations and decreased pH, while those that were not fed fructose had bacterial populations associated with the amount of grain consumed and ruminal ammonia, valerate, and histamine concentrations (**Figure 3**).

In a longer-term challenge study, rumen perturbation increased with an increase in the amount of supplementary feeding and when isoenergetic diets included grain supplements fed in the milking parlor as opposed to supplements primarily fed in a mixed ration as shown by acidosis eigenvalues in **Figure 2** for late lactation dairy cattle (Golder et al., 2014c). Differences in associations between microbial populations and rumen metabolites between different groups of cattle fed differing amounts of supplement with these different feeding strategies are shown in Figure 4. Importantly, these findings show that substrate types (**Figure 3**) and amounts (**Figure 4**) determine the rumen populations and functions. Subsequently work (Golder et al., 2017) has found associations between the genome of cattle, the metagenome (rumen microbes) and function.

Further, Golder et al (2014b) fed grain and fructose to pregnant heifers with a target DMI of 1.0% and 0.2% of BW of wheat and fructose, respectively with a non-fiber carbohydrate (**NFC**) content of 76.3% if 100% of the ration was consumed. These

heifers had a 20-day exposure to total mixed rations including 10 days with an NFC content of 47.7% and a NFC of 46.1% prior to challenge. In contrast with the shorter challenge study with very similar amounts of grain and/or fructose (Golder et al., 2012b), there were very large intra-group differences in rumen metabolites on the challenge day. Similarly, Firkins and Yu (2015) noted that differences in the microbiome structure among animals within the same diet group often exceeded those among different diet groups. These differences have been explained by different host genetics and interactions with the rumen microbiome (Weimer et al., 2010, King et al., 2011, Hernandez-Sanabria et al., 2013). Weimer et al. (2010) showed the ability of the rumen to revert to pre-exchange VFA concentration and rumen pH and nearly return to pre-exchange bacterial community composition within 24-hours of a 95% exchange of ruminal content with a cow on a similar diet. A second cow took a longer period to revert indicating the potential for variability in this response (Weimer et al., 2010). More work is required to elucidate host-microbe-metabolome interactions and how they can be optimized.

Controlling the Rumen

The studies outlined above, when combined with other literature, provide the following clear guidelines for controlling rumen fermentation.

Diet form, formulation, and function

Consistency of supply of feed is important as many studies have withheld feed as part of a protocol to create acidosis (Nagaraja and Titgemeyer, 2007). Providing adequate fiber and particle length (Zebeli et al., 2012) and greater than 30% NDF, based on Bramley et al. (2008), is appropriate for lactating dairy cattle. Diets formulated as partial mixed rations were safer, despite a higher NFC content, than diets that were component-fed (Golder et al., 2014c).

Sugars in the diet should be controlled based on Nagaraja et al. (1981) and Golder et al. (2012; 2014 b). We suggest the following guidelines for TMR based on Bramley et al. (2008) and Golder et al. (2014c): a maximum total NFC of 40 to 42%, 22 to 24% starch, 8% sugar based on not exceeding approximately 0.35% of bodyweight for sugars intake. It is very likely that not all sugars will have the same effect on the rumen (Plazier et al., 2018 in press), and it is very evident that not all grains (Lean et al., 2013) or starches have the same effect on rumen function. Further, form of processing the concentrate components in the diet will influence function.

Lastly, observations that acidotic cattle have low rumen concentrations of ammonia (Bramley et al., 2008) and a reduction in the incidence and prevalence of acidosis with increased nitrogen in the diet (Golder et al., 2014c) support the observation that microbial protein is a significant sink for hydrogen in the rumen and that energy spilling (i.e. an inability of bacteria to reproduce, hence produce more VFA) may be an important part of the pathogenesis of acidosis.

Feed additives

Buffers and Neutralizing Agents. These have been well reviewed and a buffer, by definition, reduces the decrease in pH without causing an increase in pH (Staples and Lough, 1989). Questions remain, however, in regard to the function of sodium bicarbonate, potassium carbonate, potassium bicarbonate, sodium sesquicarbonate, and the skeletal remains of the seaweed *Lithothamnium calcareum.* In the case of sodium bicarbonate, there are questions whether the effects are mediated through buffering the accumulated acid or increases in DM and water intakes caused by sodium, facilitated through an increased ruminal fluid dilution rate and reduced starch digestion rate (Russell and Chow, 1993, Valentine et al., 2000). Similarly, potassium-based products, including potassium carbonate sesquihydrate, may be contributing to production increases through increased dietary cation-anion difference or potassium requirements rather than through buffering actions. There are positive interactions for sodium bicarbonate with magnesium oxide and combination of sodium bicarbonate and magnesium oxide had similar effects as virginiamycin in controlling cyclic eating behaviour in cattle during adaptation to a diet high in grain and containing fructose (Golder et al., 2014b).

Antibiotics: While these are subject to regulatory change, there is strong evidence that some antibiotics can control the risk of acidosis (Lean et al., 2014). Tylosin has been widely used in finishing diets for the US beef industry. Virginiamycin is effective in controlling acidosis and tylosin, in combination with monensin, is also effective. It appears that combinations of monensin and bambermycin are also effective in favourably modifying rumen function.

Ionophores: Ionophores, particularly monensin and lasalocid are widely used in beef and dairy production. There is evidence of more sustained appetite (Lunn et al., 2005) and of increased production of propionate from lactate, which is a ruminal adaptation that sequesters hydrogen ions in safer ruminal pools, when monensin is fed in diets that may cause acidosis. Monensin appears to be very effective in controlling acidosis risk when fed with tylosin or virginiamycin. Nagaraja et al. (1981) investigated the use of lasalocid to control lactic acidosis induced using finely ground corn or glucose. Use of lasalocid equalled or exceeded the reduction in lactic acid production observed for monensin (Nagaraja et al., 1981). Both monensin and lasalocid prevented acute lactic acidosis in the study of Nagaraja et al. (1981); however, both products were included in the diet at concentrations of 1.30 ppm of diet, and above concentrations recommended. Nagaraja et al. (1982) found that 0.33, 0.65, and 1.30 ppm of lasalocid were effective in reducing lactic acid concentrations and increasing pH compared to control cattle with lactic acidosis induced using glucose 12.5 g/kg of BW. More studies would be useful to evaluate the effect of lasalocid on rumen acidosis.

Other agents: There is increasing evidence that yeasts and yeast cultures may have a role in stabilizing rumen function. Actions that have been identified with live yeasts include small increases in rumen pH, reductions in lactic acid, enhanced fiber digestion, and small increases in VFA production. These actions are modest in

magnitude but may synergize with other strategies to control the risk of acidosis. Li et al. (2016) found that a *Saccharomyces cerevisiae* fermentation product stabilized rumen pH. There is also some evidence that probiotics may provide benefits in terms of acidosis control; however, there are challenges in this area as candidate agents such as *Megasphera elsdenii* has not provided clear and consistent benefit in studies to date. It seems likely that more studies will investigate the roles of other agents in acidosis control in the future.

Conclusions

Acidosis is a much more complex condition than simply reflected in a drop in pH. Acidosis is increased by diets higher in starch and sugars and lower in fiber and is reflected in increases in propionate and valerate concentrations and reduced ammonia concentrations and rumen pH. While the clinical expression of acidosis may be influenced by the interactions of the gastrointestinal tract and immune system, we consider that prevention will depend on control of substrate and form and delivery of the diet. Better tests for acidosis will help identify, research, and manage the condition. These better tests, resulting in the more accurate identification of cattle with acidosis, will be critical to produce new interventions to assist in the control of acidosis in a higher percentage of the population. Recent developments in evaluating and understanding the rumen and gastrointestinal tract function will provide new methods for controlling rumen function.

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Table 1. Summary of the evidence supporting the potential for histamine, endotoxin, and lactic acid to cause laminitis in cows fed diets rich in rapidly fermentable carbohydrates. Sourced from Lean et al. (2013b).

	Histamine	Endotoxin	Lactic acid
Generated in the rumen	√	√	√
Absorbed by healthy rumen	√	√[a]	√
Absorbed by damaged rumen	√	?	?[b]
Induced laminitis when injected	√	√	√

[a] Evidence is inconsistent.
[b] Appears to be probable.

Table 2. Difference and relationship between ruminal pH measurements in ruminal fluid collected using stomach tubing, rumenocentesis, and rumen fistula methods in cattle.

Methods compared	No. of cows sampled	Difference in ruminal pH values between methods[1]	Relationship between methods (r^2)	Reference
Stomach tube and rumenocentesis				
	6	+0.04		Shen et al. (2012)
	58	+0.76	0.11	Enemark et al. (2004)
	5	+1.1		Nordlund et al. (1995)
	660	+0.54	0.20	Bramley unpublished
	16	+0.35	0.25	Duffield et al. (2004)
Rumenocentesis and fistula				
	30	+0.28	0.52	Garrett et al. (1999)
	16	+0.33	0.42	Duffield et al. (2004)
	30	+0.34	0.73	Garrett et al. (1995)
Stomach tube and fistula				
	16	+0.34	0.58	Duffield et al. (2004)
Continuous ruminal pH measurement system and fistula			Correlation coefficient (r)	
Mean over 1 min	14	Mean of 1 and 5 min -0.03	0.98	Penner et al. (2006)
Mean over 5 min	14		0.97	Penner et al. (2006)
	4	-0.04	0.99	Sato et al. (2012)
	4	+0.39	0.93	Phillips et al. (2010)
	12	+0.11	0.85	Dado and Allen (1993)
	6		0.65	Graf et al. (2005)
	1	-0.07	0.88	AlZahal et al. (2007)
	16	cranial-ventral site	0.68	Duffield et al. (2004)
	16	caudal-ventral site	0.61	Duffield et al. (2004)
	16	central site	0.35	Duffield et al. (2004)
	16	cranial-dorsal site	0.50	Duffield et al. (2004)
Continuous ruminal pH measurement system and stomach tube				
	16	First sample	0.15	Duffield et al. (2004)
	16	Second sample	0.31	Duffield et al. (2004)
Continuous ruminal pH measurement system and rumenocentesis				
	16		0.43	Duffield et al. (2004)
	6		0.56	Marchesini et al. (2013)

[1]Difference in ruminal pH values were calculated by subtracting the mean ruminal pH value for the second named ruminal collection method from the first named collection method (i.e. Mean ruminal pH of stomach tube ruminal sample - Mean ruminal pH of rumenocentesis ruminal sample).

Table 3. Sensitivity, specificity, area under the curve, and cut-off points from receiver operator curves for the acidosis diagnostic value of rumen and milk measure from samples obtained by Bramley et al. (2008). Sourced from (Golder et al., 2012a).

Measure	Sensitivity	Specificity	Area under the curve	Cut-points
Acetate (mM)	0.94	0.27	0.627	36.7
Butyrate (mM)	0.94	0.20	0.530	5.28
Propionate (mM)	0.93	0.87	0.955	23.10
Valerate (mM)	0.90	0.90	0.954	1.62
pH (Stomach tube)	0.68	0.84	0.801	6.54
pH (Rumenocentesis)	0.74	0.79	0.822	5.96
Milk Fat:Protein	0.54	0.81	0.716	1.02

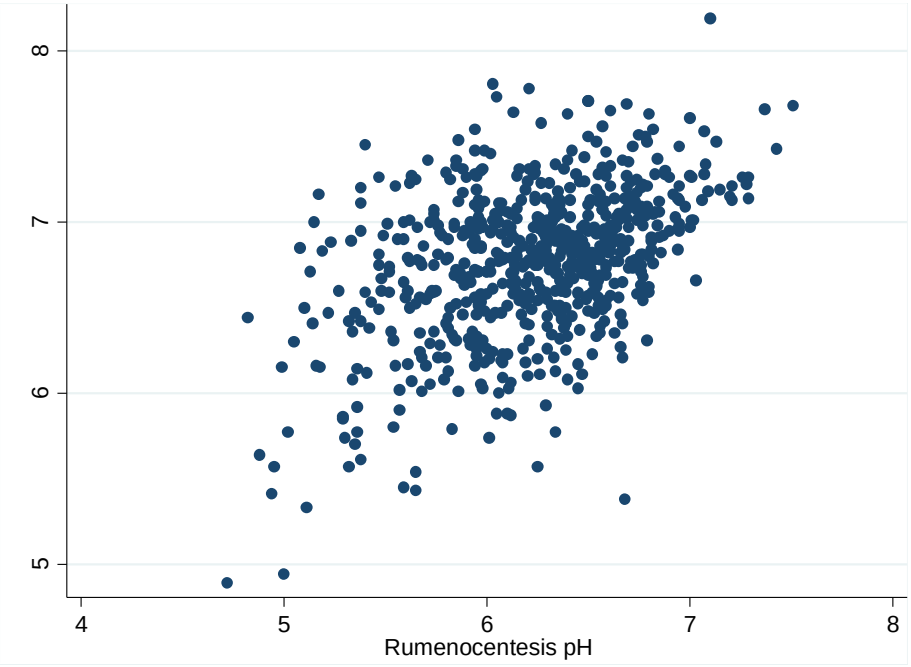

Figure 1. Scatter plot comparing rumen pH measured by rumenocentesis vs. stomach tube (R^2 = 0.20). Sourced from Bramley et al. (2008)

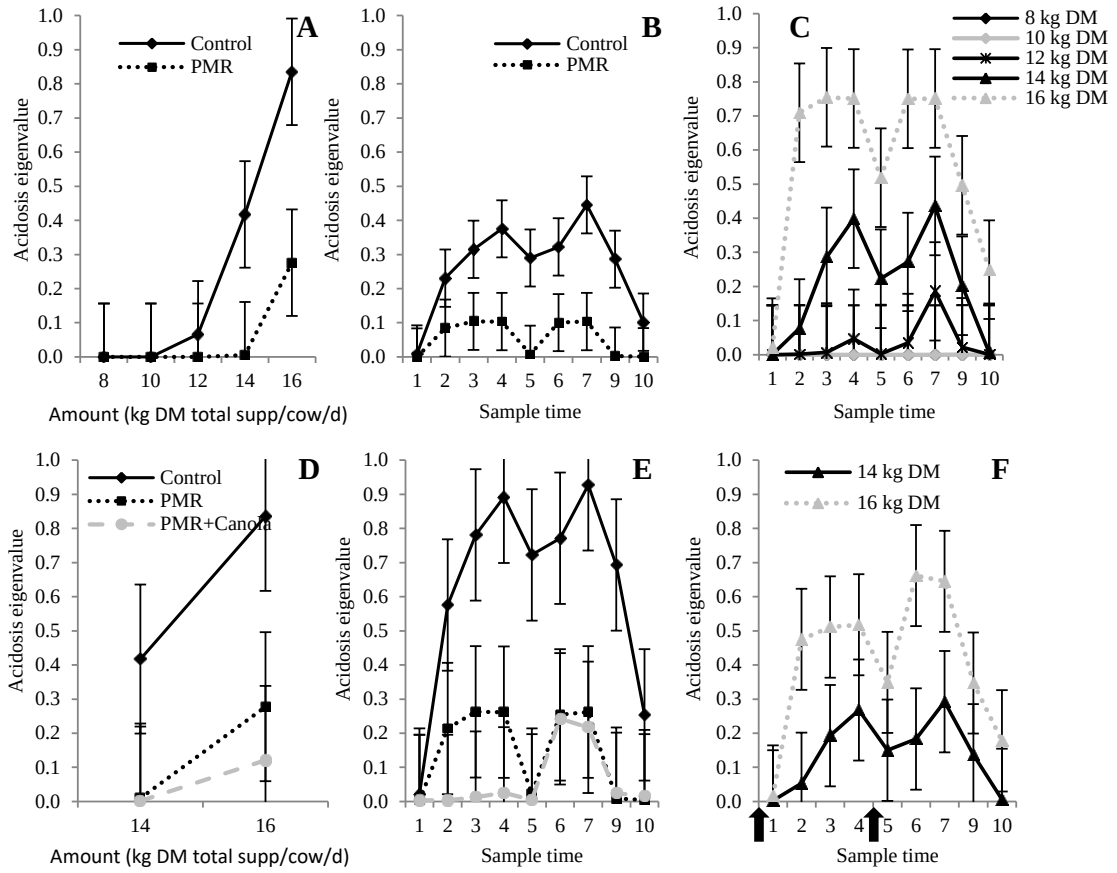

Figure 2. Mean (± SEM) acidosis eigenvalues for dairy cows from all feeding groups showing interactions between (A) feeding strategy and supplement feeding amount, (B) feeding strategy and sample time, and (C) supplement feeding amount and sample time. Mean (± SEM) acidosis eigenvalues for dairy cows from the high supplement feeding amount groups only (14 and 16 kg of DM of total supplement/cow per day) showing interactions between (D) feeding strategy and supplement feeding amount, (E) feeding strategy and sample time, and (F) supplement feeding amount and sample time. An eigenvalue of 0 corresponds to healthy, non-acidotic rumen sample and 1.0 represents an acidotic sample. Sample times were approximately 2.4 h apart over a 24-h period. Sample time 1 was approximately 8:20 h and milking was at 7:00 and 15:00 h (black arrows). PMR = partial mixed ration; PMR+Canola = partial mixed ration + canola meal; Amount = kg of DM of total supplement/cow per day.

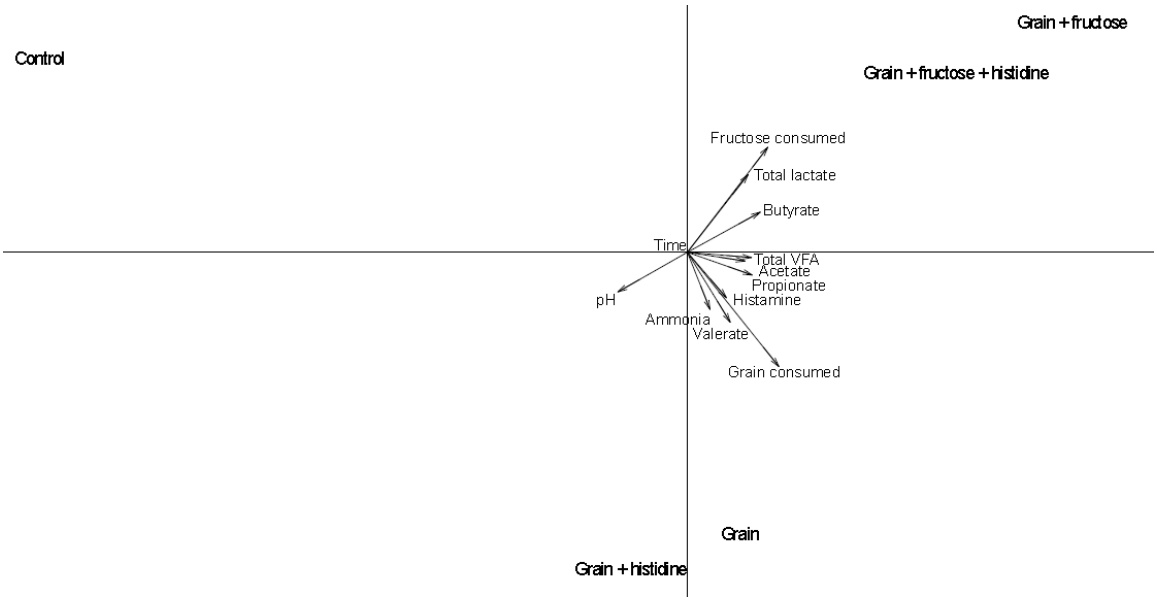

Figure 3. Duality diagram of co-inertia analysis of ruminal bacterial communities from 16S rDNA 454 pyrosequences, measures of ruminal fermentation, and percentages of offered grain and fructose from heifers that consumed the following single challenge rations: (1) control (no grain); (2) grain (1.2% of BW DM); (3) grain (1.2% of BW DM) + histidine (6 g/head); (4) grain (0.8% of BW DM) + fructose (0.4% of BW DM) or; (5) grain (0.8% of BW DM) + fructose (0.4% of BW DM) + histidine (6 g/head) (number of heifers = 6/group). Ruminal fluid was collected over approximately a 3.6-h period after (number of samples = 18/group). On the bi-plot the ruminal fermentation measures are represented as arrows. The direction of the arrow of each ruminal fermentation measure indicates an increasing concentration of that measure. The angle between the arrows indicates their degree of correlation. The magnitude of the arrows indicates the importance of the measure on the bacterial community composition. Measures with long arrows are more strongly correlated with the ordination axes than short arrows and have a greater influence on the pattern of variation (Carberry et al., 2012).

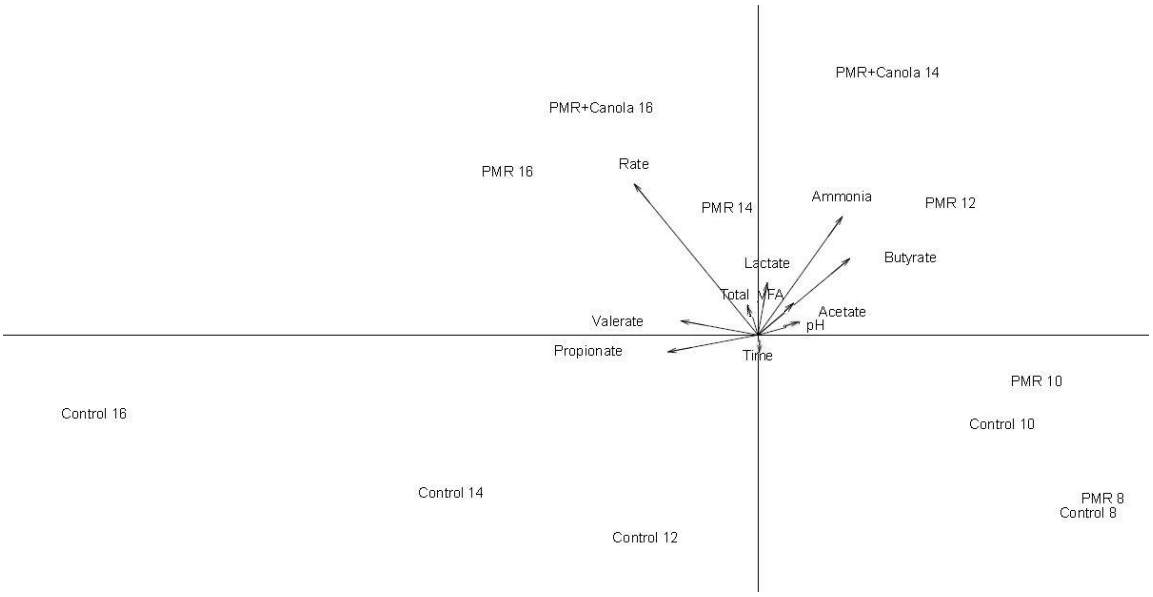

Figure 4. Duality diagram of co-inertia analysis of ruminal bacterial communities from 16S rDNA 454 pyrosequences, measures of ruminal fermentation, sample time, and amount of total supplements fed in dairy cattle fed 1 of 3 feeding strategies: control (n = 10 cows), partial mixed ration (PMR; n = 10 cows), or PMR+Canola (PMR+Canola meal n = 4 cows) at amounts of 8, 10, 12, 14, or 16 kg of DM of total supplement/cow per day (2 cows per supplement feeding amount at 3 times from each feeding strategy). On the bi-plot the ruminal fermentation measures are represented as arrows. The direction of the arrow of each ruminal fermentation measure indicates an increasing magnitude of that measure. The angle between the arrows indicates their degree of correlation. The magnitude of the arrows indicates the importance of a measure on bacterial community composition. Measures with long arrows are more strongly correlated with the ordination axes than short arrows and have a greater influence on the pattern of variation (Carberry et al., 2012).

SESSION NOTES

Use of Novel Feed Additives in Beef Cattle Production

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Introduction

Feed costs represent the largest input cost to produce beef (estimated to be 40-60%). When used strategically, feed additives have the potential to enhance feed efficiency, improve animal health, and/or add value by improving beef quality. Feed additives are defined as dietary ingredients that produce a desirable response in animals in a non-nutritive role. Several feed additives contain nutrients, however, they are not fed to meet a nutritional requirement, rather, they are fed to alter ruminal or post-ruminal metabolism in order to enhance nutrient utilization, animal productivity, or meat quality. Although many feed additives are effective, their practical implementation has been hindered by the variability in animal responses under experimental conditions, increased dosage, handling requirements, and increased cost. Producers should evaluate potential strategies for use of feed additives under specific feeding and economic conditions.

Updates on Selected Feed Additives

Microalgae

Microalgae are microscopic algal bodies that are rich in lipid (> 30% of DM) and omega-3 fatty acids (> 10% of DM). New technology allows for heterotrophic production of microalgae in bulk fermenters that do not require the lighting and electricity previously needed for phototrophic microalgae growth (Harel et al., 2002). Heterotrophic microalgae use organic carbon as an energy source and are easily grown, harvested daily, and can be cultivated using less space relative to typical animal feed ingredients. Algal lipids are used for a myriad of purposes including biofuel production and as a natural omega-3 fatty acid supplements for human food. The microalgae used in animal feed can be the de-oiled by-product of algae oil production (microalgae meal) or can be the high oil microalgae itself.

De-oiled microalgae meal provides protein, carbohydrate, vitamins, and minerals. Stokes et al. (2016) reported that a de-oiled microalgae meal + soybean hull product (43:57) fed up to 42% of the diet DM linearly increased DMI, did not impact ADG, and decreased fat thickness, KPH%, and yield grade in feedlot steers fed a 10% hay diet. At Purdue University, we have investigated feeding 100 g/d of high oil microalgae (ForPLUS; DHA-rich microalgae *Aurantiochytrium limacinum*; 63.6% fat; 17.9% DHA; 30 mg/kg Sel-Plex; Alltech Inc.) in order to increase the healthy omega-3 (**n-3**) long-chain polyunsaturated fatty acid (**PUFA**) content of beef. Feeding 100 g/d of ForPLUS

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more than doubled n-3 fatty acids in beef, produced a 4-fold increase in eicosapentaenoic acid (**EPA**; 20:5n-3) and over a 6-fold increase docosahexaenoic acid (**DHA**; 22:6n-3) (Carvalho et al., 2015). Research at Kansas State University has similarly demonstrated that high-oil microalgae will increase the omega-3 fatty acid content of beef (Phelps et al., 2016). In our study, ForPLUS also increased circulating glucose and decreased circulating insulin after a glucose tolerance test, indicating that whole body glucose metabolism was altered. High-oil microalgae decreased intake but did not statistically impact daily gain. Supplemental microalgae effectively increased the omega-3 content of beef and may be economical if producers are able to produce for a value-added market. Further research on health and metabolic impacts are needed.

Prebiotics

Prebiotics have been described as non-digestible food substances that selectively stimulate the growth of favorable species of bacteria in the gut, thereby benefitting the host (Gibson and Roberfroid, 1995). Examples of prebiotics include oligosaccharides found in yeast cell wall (**YCW**) components and clay minerals.

Yeast Cell Wall

Mannoproteins and β-glucans are the primary components derived from the YCW and have shown promise in keeping receiving cattle healthier (Spring et al., 2000; Volman et al., 2008). Yeast cell wall components adhere to bacteria and prevent their colonization in the gastrointestinal tract (Davis et al., 2004), leading to enhancement of the immune system (Ganter et al., 2003). In general, YCW products are thought to have greater efficacy than live yeast products due to the concentration of cellular components (Burdick Sanchez et al., 2014).

At Purdue University, we fed a proprietary blend of specialized mannan-rich fractions and glucan-rich fractions of yeast (Select TC™, Alltech Inc.). Cattle were fed a high-forage receiving diet and the proprietary blend at a rate of 13 g/d for the first 56 d of the feedlot period, and we observed an improvement in their immune status during a bacterial endotoxin challenge (Pukrop et al., 2017). Interleukin 6 (**IL-6**) is a cytokine associated with tissue degradation, energy mobilization, fever, and a decrease in voluntary feed intake (Johnson, 1997). We noted that receiving cattle fed Select-TC had lower circulating concentrations of IL-6, lower concentrations of non-esterified fatty acids (**NEFA**), and lower rectal temperatures following a bacterial endotoxin challenge. Interferon-γ is an important mediator of macrophage activation and contributes to the resistance of intracellular pathogens (Flynn et al., 1993). We observed an increase in serum IFN-γ concentrations after an endotoxin challenge in steers fed Select-TC compared to control suggesting that steers fed Select-TC had a stronger pro-inflammatory response relative to controls, which may allow for a greater ability to fight disease through identification and elimination of pathogens. Select-TC did not change serum cortisol, glucose, insulin, or blood urea nitrogen relative to controls during an endotoxin challenge.

Others have reported similar responses in beef cattle fed YCW products. Burdick Sanchez et al. (2013) observed that supplementing with YCW products decreased serum IL-6 concentrations, but had no effect on serum IFN-γ. Burdick Sanchez et al. (2014) observed that one YCW product did not affect serum NEFA, but increased serum BUN concentrations, while a second YCW product decreased serum NEFA and did not affect BUN. Buntyn et al. (2016b) found no differences in serum IL-6, an increase in serum IFN-γ, and a decrease in serum NEFA and BUN during an endotoxin challenge in steers supplemented with an active dried yeast compared with non-supplemented steers. Buntyn et al. (2016a) reported that serum concentrations of both IL-6 and IFN-γ were lower in steers receiving 5.0 g/d of a live yeast compared with steers that were not fed yeast.

Although immunologically, cattle fed a YCW product appear to be able to handle possible pathogenic challenges better than controls, the 46% drop in morbidity that we observed was not statistically different during the 56 d study and animal performance was not altered. However, animal health cost savings could be significant. Others (Burdick-Sanchez et al., 2014; Finck et al., 2014; Buntyn et al., 2016b) have similarly reported little statistical response of YCW on morbidity and performance. Source of yeast and YCW products, as well as the condition of calves when they received these products, likely influence efficacy. It has been reported that beneficial effects of yeast product supplementation are more pronounced under stress versus normal conditions (Arambel and Kent, 1990; Cole et al., 1992).

Clay mineral

Because of their physical and chemical structures, clay minerals are frequently used in the livestock industry as binding agents in the production of pelleted feeds, adsorbents for many toxins, and storage and release of microelements (Slamova et al., 2011). Clays are the products of silicate rocks that have been subjected to weathering processes for thousands of years (Buckman and Brady, 1969). The specific term “silicate” is used to describe these clays and the main classification is phyllosilicates. Phyllosilicates consist of many subcategories which are based on chemical composition: kaolinite, smectites, chlorites, and micas (Adamis and Williams, 2005). Smectite clays are 2:1 hydrated sodium calcium aluminosilicate (**HSCAS**) minerals organized in a sheet structure (phyllosilicate) that because of their physical and chemical structures can absorb mycotoxins, tannins, heavy metals, bacteria, and viruses and expel them from the body (Williams and Haydel, 2010). Bentonite is a common smectite clay mineral fed to livestock for this purpose. Zeolites, which are also used in livestock nutrition have properties similar to smectite clays, but form tubes or cage-like structures that can incorporate a variety of molecules and ions. Major advantages of clay mineral adsorbents are that they are relatively inexpensive, generally recognized as safe (**GRAS**), and can be easily added to animal feeds.

Our results with smectite clay (Antonelo et al., 2017) and results of others (Chestnut et al., 1992; Phillips et al., 1988; Moschini et al., 2008) indicate that smectite clays can adsorb mycotoxins in the normal physiological range of ruminal pH. In vitro

toxin binding assays don't always translate to in vivo conditions (Phillips et al., 1988), however, studies have demonstrated that the addition of smectite clays to dairy cattle diets resulted in a 40 to 48% reduction in milk aflatoxin M_1 concentration and a 43 to 46% decrease in aflatoxin M_1 excretion in cows fed diets containing significant aflatoxin concentrations (Harvey et al., 1991; Stroud, 2006; Kutz et al., 2009).

Antonelo et al. (2017) observed that the addition of 1% of the diet DM as clay mineral to a 10% roughage feedlot cattle diet had a positive effect on feedlot performance during the first 30 d, but this advantage was not sustained throughout the feeding period. Huntington et al. (1977a,b) similarly observed an improvement in performance during the initial 21 to 30-d of the feeding period when sodium bentonite was added to the diet of lambs fed a high concentrate diet. In contrast, some have observed improvements over the entire feeding period for ADG and DMI (Walz et al., 1998) or just DMI (Huntington et al., 1977b) in lambs fed bentonite. Others have found little improvement (Chestnut et al., 1992) or possible negative effects (Jacques et al., 1986; Rindsig and Schultz, 1970) of smectite clay on growth and intake.

Clay minerals appear to be effective in dietary transition periods or when mycotoxin content of the diet is elevated.

Alkalizers and buffers

Numerous physical treatments have been applied to roughages, including crop residues, in an attempt to increase digestibility. The bonds between lignin and structural carbohydrates are alkaline soluble, thus alkaline treatments can partially break these bonds and increase microbial fermentation (Jung and Deetz, 1993). Chemically treating crop residues with sodium hydroxide, urea, ammonia, calcium oxide (**CaO**), or calcium hydroxide (**$Ca(OH)_2$**) has been shown to increase nutritive value of these feeds and improve animal performance (Berger et al., 1994; Russell et al., 2011). However, none of these methods is widely used because of the capital and energy intensive nature of these methods as well as the cost and corrosive and/or hazardous nature of chemicals (FAO, 2011).

Our approach at Purdue University has been to investigate the addition of alkalizing agents ($Ca(OH)_2$ or CaO) or buffers such as potassium carbonate (**K_2CO_3**) to the total mixed ration (**TMR**) just prior to feeding as a so-called feed additive. This strategy decreases labor and time commitments, and simplifies implementation compared to chemical pre-treatment. The alkali and buffers serve to increase ruminal pH to a value that facilitates increased fiber digestion. Our research indicates that CaO is the most effective alkaline compound for improving cattle performance, increasing ADG by nearly 20% relative to cattle not fed an alkali or buffer (Lancaster, 2017). Calcium hydroxide, K_2CO_3, and a combination of $Ca(OH)_2$ and K_2CO_3 produced gains intermediate to control and CaO-fed cattle. Our initial research into this area suggests that 0.8 to 1.6% of the diet (DM basis) is optimal for improved fiber digestibility and animal gain (Nunez et al., 2014). Further, some of our recent data suggests that a CaO feed additive is most effective when dry, low quality forage is utilized (20% of diet DM as

stover) in corn-based diets containing 30% dry distillers grains plus solubles (DDGS; Muegge et al., 2015; Lancaster, 2017). We observed a 13.9% increase in NDF apparent digestibility, a 15% increase in daily gain, no change in dry matter intake, and a 10% increase in gain:feed (Lancaster, 2017). However, replacement of a portion of the corn with soybean hulls, a highly fermentable fiber source, also improved fiber digestibility and performance of cattle, and the inclusion of CaO combined with soybean hulls provided no benefit (Lancaster, 2017).

Addition of strong alkaline substances directly to the TMR is an effective strategy to improve fiber digestibility and performance of beef cattle, thus eliminating the need for labor intensive processing and handling methods of low quality forage pre-treatment.

Summary

Overall, there is evidence of the beneficial effects of feed additives on performance of cattle. However, inconsistent responses may arise from a variety of inherent factors such as interaction with dietary ingredients, ruminal environment of host animal, intake, fiber and/or starch content, length of feeding period and cattle management. Further, in a recent review, Kenney-Rambo et al. (2016) pointed out that characteristics of a feed additive beyond its efficacy may be the greatest obstacle limiting its application in the future. Shelf life (in storage and mixed in a TMR), regulatory oversight, particle size, ability to deliver, and dose size are examples of obstacles that may need to be overcome. Dose size in particular could have serious implications because at present the most widely used feed additives are based on low inclusion (400 mg/hd daily) rates (Kenney-Rambo et al., 2016). Most of the feed additives mentioned in this review and ones that are heavily considered for use in the industry require greater doses. Larger inclusion doses may require 3- to 90-fold greater production, storage, transfer and delivery capacity by the industry (Kenney-Rambo et al., 2016).

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SESSION NOTES

Canola Meal as a Protein Source for Lactating Dairy Cows

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Introduction

Canola is an offspring of rapeseed (*Brassica napus* and *Brassica campestris/rapa*), which was bred through standard plant breeding techniques to have low levels of erucic acid and glucosinolates. Canola seed is rich in oil, and after oil extraction, the remaining "canola meal" (**CM**), is a rich protein source used as feedstock to different animal species, mainly dairy cows in North America and in Europe (Canola Meal Feed Guide, 2015). Glucosinolates and erucic acid were reduced in rapeseed due to their toxicity, which may negatively affect digestion and health of most animals when fed in high levels (Kramer et al., 1990; Mawson et al., 1994). Over the past 25 years canola production in Canada has grown from approximately 3 million tons to about 17 million tons (Cliff Jamieson, 2015). Due to increased availability of canola oil, its by-product, CM, has become a viable protein source to dairy cow diets (Martineau et al., 2013).

Recent studies published in peer-review journals have shown that CM is a valuable protein source for lactating dairy cows. Results from these studies have indicated that CM can partially or completely replace the most common protein sources (e.g., soybean meal (**SBM**), cottonseed meal, dried-distiller's grains) without comprising dairy cows' performance and, in some cases, can improve performance and nitrogen (**N**) utilization of dairy cows. The objective of the present paper is to summarize and discuss the results from our studies comparing CM with SBM as a protein source for dairy cows with other recent studies published in peer-review journals comparing SBM with common protein sources fed to dairy cows.

Effects of Canola Meal on Performance of Dairy Cows

Recently, studies evaluating the replacement of CM with SBM or other common protein supplements fed to dairy cows have shown an increase in cows' performance and an overall improvement in N utilization by cows fed CM (**Table 1**).

Broderick et al. (2015) observed an increase in DMI (+ 0.4 kg/d), yields of milk (+ 1.0 kg/d) and milk true protein (+ 50 g/d), and improved efficiency of dietary N for milk N by replacing SBM with CM in isonitrogenous diets formulated with corn and/or alfalfa silage as forages. Two meta-analyses based on results of published peer-reviewed journals reported an increase of yields of milk and milk components, a reduction in milk urea N (**MUN**), and an increase in plasma concentration of branched-chain amino acids (**BCAA**) for cows fed CM compared to other protein supplements (Martineau et al., 2013, 2014). Furthermore, Huhtanen et al. (2011) in another meta-analysis evaluated

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the replacement of SBM with CM in isonitrogenous diets formulated with grass silage-based diets and observed an increase in DMI and yields of milk by cows fed CM compared to those fed SBM.

Paula et al. (2018) observed a significant reduction in MUN (8%), and a numerical increase in yields of milk, 3.5% FCM, and ECM of 1.3, 1.2, and 0.9 kg/d, respectively, by cows fed CM compared with those fed SBM. In this study the basal diets contained alfalfa and corn silage plus high-moisture corn and about 16% CP concentration. In a study with a similar basal diet as Broderick et al. (2015) and Paula et al. (2018), Brito and Broderick (2007) evaluated the performance of lactating dairy cows supplemented with equal CP concentration from urea, cottonseed meal (**CSM**), SBM, or CM. The authors observed a significant increase in DMI for cows fed CM compared to those fed SBM and intermediate values for cows supplemented with CSM of 24.9, 24.2, and 24.7 kg/d, respectively. Other findings were numerical differences in milk yield, 41.1, 40.5, and 40 kg/d, for CM, CSM, and SBM, respectively. Milk protein yield was significantly increased for cows fed CM or SBM compared to cows fed CSM.

Mulrooney et al. (2009) conducted a study evaluating the effects of replacing dried distillers grains with solubles (**DDGS**) with CM in different proportions (100, 66, 33, and 0%) on milk production of lactating dairy cows. They observed no differences in yields of milk and milk components. However, they concluded that despite no differences in yields of milk and milk components, diets with higher proportions of CM tended to be more desirable due to a reduction in MUN and a better concentration of blood amino acids (**AA**). Contrarily, Chibisa et al. (2012) evaluated the effects of replacing CM with wheat-DDGS (0, 10, 15, and 20% of DM) and observed an increase in DMI and milk yield by 1.2 to 1.8 kg/d by cows fed wheat-DDGS compare to cows fed CM. Also the authors observed a quadratic effect for milk protein yield when wheat-DDGS was fed.

Our results and the results in literature cited herein evaluating CM as protein source have indicated that CM is a valuable protein source for high-producing dairy cows. The overall improvement in cows' performance and N utilization when compared to SBM diets may be due to a greater contribution of methionine in the RUP, consequently improving the amino acid balance available for absorption when CM is fed.

Canola Meal: Ruminal Degradability and Metabolizable Protein

Overall, dairy farmers have a preference for SBM rather than CM in the diet (Huhtanen et al., 2011) possibly because SBM has a greater concentration of CP (53 vs. 42 % of DM) and of metabolizable energy (3.41 vs. 2.75 Mcal/kg) compared to CM (NRC, 2001). In addition, feed evaluation systems such as Agricultural and Food Research Council (AFRC 1993) and NRC (2001) estimate a lower amount of rumen undegraded protein (**RUP**) outflow and greater degradation rates of rumen degraded protein (**RDP**) for CM compared to SBM, consequently the estimated metabolizable protein is lower for CM.

Piepenbrink and Shingoethe (1998) observed greater ruminal CP degradability and lower intestinal digestibility for CM compared to blood meal, corn gluten meal, and menhaden fish meal. Nonetheless, the estimated AA profile reaching the small intestine of cows fed CM was closest to the milk AA profile. However, Brito et al. (2007) measured numerically greater RUP flows in vivo for CM compared to SBM diets, 34 vs. 29% (of CP), respectively. In addition, they observed similar yields of milk protein by cows fed CM or SBM, 1.27 and 1.23 kg/d, respectively, which may indicate an underestimation of MP for CM diets when using current nutritional models.

Recently, there have been speculations that RUP values and estimation of MP supply are underestimated for CM in current prediction models (Huhtanen et al. 2011; Martineau et al. 2013). Maxin et al. (2013) evaluated in situ ruminal degradation of CP from SBM and CM and reported lower CP degradability and greater RUP content for CM compared to SBM. Broderick et al. (2016) reported in a survey that included CM samples collected from 12 Canadian processing plants over 4 years that CM varied in RUP content from 43 to 51% (CP basis) with an overall average of 45% which is 26% greater than the RUP value for CM in the current NRC (2001) of 35.7% of CP with DMI at 4 times maintenance. Furthermore, studies comparing the effects of CM and SBM in the total diet in vitro (Paula et al., 2017) and in vivo (Rinne et al., 2015) on ruminal N metabolism did not observe significant differences between diets. In agreement, Paula et al. (2018) did not observe differences in microbial and non-microbial NAN flows at the omasal canal among cows fed SBM, CM, or heat-treated CM. In **Tables 2 and 3** we summarized the mean chemical composition of CM used in our recent studies and ruminal outflow of N fractions.

These results underscore the importance of revising MP, RDP, and RUP content of CM in nutrition models and feed tables to better reflect the MP supply of CM when fed to lactating dairy cows.

It is also worth mentioning that while a few attempts have been made to decrease CM ruminal degradability, for example by heat-treating CM, with the goal of increasing the RUP fraction, these have not resulted in better ruminal or post-ruminal N utilization and failed to improve milk production (Paula et al., 2018).

Interactions among Canola Meal and Forages Sources

It is well documented that the type of dietary forages may affect the limiting AA for yields of milk and protein (NRC, 2001; Schwab et al., 1976). For example, diets based on corn and corn silage are more likely to be limiting in lysine than alfalfa silage-based diets due to higher concentrations of lysine in alfalfa. On the other hand, studies have shown that histidine may be more limiting for cows fed grass silage and barley-based diets (Vanhatalo et al., 1999; Huhtanen et al., 2002).

In the meta-analysis by Huhtanen et al. (2011), authors concluded that CM value in diets based on grass silage is at least as equivalent as the value of SBM for lactating dairy cows. However, they did not include studies with diets based on corn and/or alfalfa silage (typical North American diets). Furthermore, Martineau et al. (2013) reported that type of forage (e.g., grass or legume forages vs. corn or barley silage) was one factor

that influenced the responses of replacing protein supplements with CM. They observed greater milk protein content for cows fed CM compared to other common protein sources only in studies based on grass and/or legume forages. Whereas, milk lactose content was lower for cows fed CM in studies based on corn or barley silage alone or in combination with grass or legume forage.

Rinne et al. (2015) evaluated lactation performance of dairy cows fed red clover/grass silage-based diets formulated to different concentrations of CP using expeller rapeseed meal or expeller soybean meal supplementation. The authors observed a tendency for increased DMI and a significant increase in milk production and N use efficiency by cows fed expeller rapeseed meal compared to cows fed expeller soybean meal. In addition, they observed an increase in plasma methionine concentration in cows fed rapeseed meal.

Faciola and Broderick (2013) evaluated the effects of replacing SBM with CM on performance of lactating dairy cows fed diets containing 3 different ratios of alfalfa to corn silage (1:5, 1:1, and 5:1; DM basis). Diets contained (DM basis) 60% forage, 8 to 15% high moisture corn, 2 to 5% soy hulls, 1.3% mineral-vitamin premix, 16.5% CP, and 31 to 33% NDF. Regardless of the forage ratio fed to the cows, replacing SBM with CM improved yields of milk (37.3 vs. 36.4 kg/d, respectively) and milk protein and decreased MUN concentration. However, cows' performance response to CM was smaller when corn silage was fed as the major forage source, possibly due to a greater portion of MP being supplied by microbial protein rather than from RUP.

Conclusions

Results from our recent studies and other published work indicate that, when replacing SBM, CM increases DMI, milk yield, and milk protein content while reducing ruminal ammonia and MUN concentrations. While less consistent, CM may also adequately replace other commonly used protein supplements such as DDGS. Responses to CM also vary depending upon forage sources. A greater response to CM feeding has been observed when alfalfa silage was fed. Variation in CM chemical composition has also been observed, notably with regards to RUP; however, differences in chemical composition did not affect in vitro ruminal digestion. Based on both in vitro and in vivo studies, replacing SBM with CM does not greatly change ruminal fermentation, suggesting that benefits of feeding CM may be related to increased DMI and/or better post-ruminal utilization (e.g., better AA profile). Lastly, a few studies that attempted at improving CM chemical composition (e.g., RUP content) did not improve CM ruminal or post-ruminal utilization.

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Table 1. Summary of recently published papers comparing CM and common protein sources used in North American diets on performance of lactating dairy cows.

	Item						
Reference	TRT[1]	DMI[2], kg/d	MY[2], kg/d	MP[2], %	MP[2], kg/d	MUN[2], mg/dL	Milk-N/N intake, %
Brito and Broderick (2007)	CM	24.9[a]	41.1[a]	3.12[a]	1.27[a]	11.6[a]	30.2[ab]
	SBM	24.2[b]	40.0[b]	3.15[a]	1.23[ab]	12.0[a]	30.4[a]
	CSM	24.7[ab]	40.5[a]	2.97[b]	1.18[b]	9.97[b]	28.5[b]
Mulrooney et al. (2009)	CM	25.2	35.2	3.1	1.1	7.1	-
	DDGS	25.1	34.3	3.0	1.0	7.25	-
Chibisa et al. (2012)	CM	29.7[b]	42.9[b]	3.32	1.4	-	24.1
	DDGS	31.8[a]	44.5[a]	3.30	1.4	-	24.5
Faciola and Broderick. (2013)	CM	23.8	37.3[a]	3.02	1.12[a]	12.9[a]	27.5
	SBM	23.5	36.4[b]	3.02	1.10[b]	14.0[b]	27.3
Broderick et al. (2015)	CM	25.2[a]	40.3[a]	3.06	1.22	10.3[b]	30.8[a]
	SBM	24.8[b]	39.3[b]	3.04	1.19	11.5[a]	30.0[b]
Paula et al. (2018)	CM	27.1	41.3	3.14	1.25	12.8[b]	29.2
	SBM	26.7	40.0	3.20	1.25	13.7[a]	30.7

[a,b] Means in the column within study with different superscripts differ ($P < 0.05$).

[1] Dietary treatments with the main protein source of CM = canola meal; SBM = soybean meal; CSM = cottonseed meal; DDGS = dried distillers grains.

[2] DMI = dry matter intake; MY = milk yield; MP = milk protein; MUN = milk urea nitrogen.

Table 2. Overall mean chemical composition of CM and SBM based on the chemical composition analyzed from our studies comparing CM vs. SBM on lactation performance of dairy cows.

	Canola meal		Soybean meal	
Item	Mean	Standard deviation	Mean	Standard deviation
Dry matter, % as fed	91.4	1.0	90.0	0.6
Organic matter, % DM	91.4	0.7	92.6	0.5
Crude protein, % DM	41.4	1.1	53.3	1.5
Rumen degraded protein, % of CP	55[1]	-	57[2]	-
Rumen undegraded protein, % of CP	45[1]	-	43[2]	-
NDF, % DM	27.8	2.2	8.1	1.3
ADF, % DM	19.3	2.8	4.7	0.8
NDIN, % of total N	17.9	7.3	3.7	3.3
ADIN, % of total N	5.4	1.0	0.7	0.7
B_3, % of total N	11.4	7.2	4.2	2.7

[1] Estimated according to Broderick et al. (2016).
[2] Estimated using the NRC (2001) model for a cow with DMI of 4% of BW.

Table 3. Summary of recently published papers comparing CM and common protein sources used in North American diets on ruminal outflow of nitrogen fractions.

		Item		
Reference	TRT[1]	Total NAN flow, g/d[2]	NMNAN flow, g/d[2]	Total microbial NAN flow, g/d[2]
Brito and Broderick (2007)	CM	616	172[b]	444
	SBM	639	159[b]	433
	CSM	587	206[a]	433
Chibisa et al. (2012)	CM	1,012	271[b]	743
	DDGS	1,042	311[a]	708
Paula et al. (2017; in vitro)	LCM	1.84	0.31	1.53
	HCM	1.91	0.40	1.51
	SBM	2.00	0.44	1.56
Paula et al. (2018)	CM	688	200	482
	TCM	671	183	488
	SBM	669	187	482

[a,b] Means in the column within study with different superscripts differ ($P < 0.05$).

[1] Dietary treatments with the main protein source of CM = canola meal, SBM = soybean meal, CSM = cottonseed meal, DDGS = dried distillers grains with solubles, LCM = low RUP solvent-extracted canola meal (38% RUP as a percentage of CP), HCM = high RUP solvent-extracted canola meal (50% RUP), and TCM = heat-treated canola meal.

[2] NAN = nonammonia-nitrogen; NMNAN = nonmicrobial NAN.

SESSION NOTES