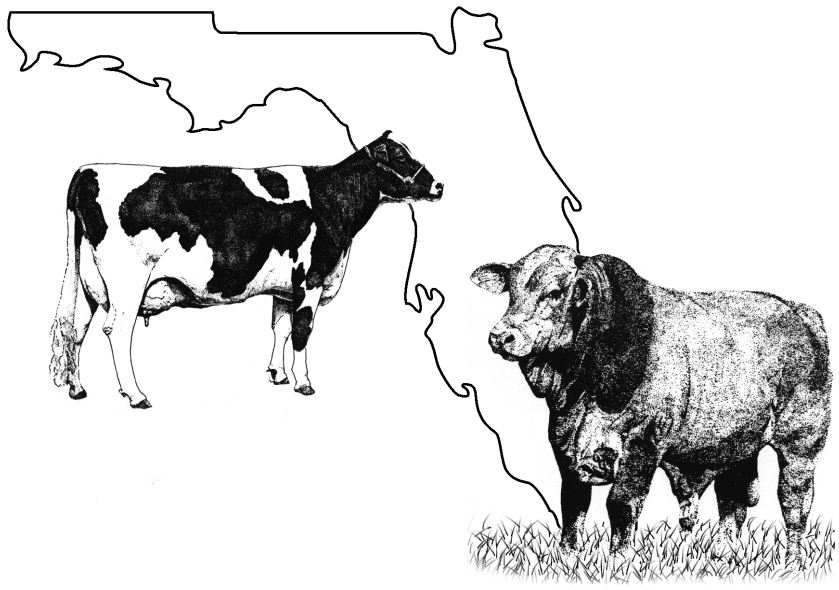
2020 Florida Ruminant Nutrition Symposium

31st Annual Meeting



February 3 - 5, 2020
Best Western Gateway Grand
Gainesville, Florida

PROCEEDINGS

UF UNIVERSITY of FLORIDA
IFAS
Department of Animal Sciences

2020

31st ANNUAL FLORIDA RUMINANT NUTRITION SYMPOSIUM

February 3 - 5, 2020
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 3 to 5, 2020

Monday, February 3, 2020 – Mini-Symposium sponsored by Balchem Corporation *"Methyl Donors and 1-C Metabolism in Dairy Cows"*

2:00 PM	**Dr. Ryan Ordway**, Balchem Corporation. *Welcome and introductions*
2:05 PM	**Dr. Joe McFadden**, Cornell University. *"Methyl donor nutrition in the transition dairy cow: contemporary health perspectives"*
2:50 PM	**Dr. Christiane Girard,** Agriculture and Agrifood Canada. *"Folic acid and vitamin B_{12} nutrition of dairy cow"*
3:35 PM	Refreshment Break
4:00 PM	**Dr. Peter Hansen**, University of Florida. *"Methyl donors and epigenetic regulation of the early embryo"*
4:45 PM	**Dr. Tom Overton**, Cornell University. *"Methyl donor nutrition in the transition dairy cow: impacts on performance"*
5:30 PM	**Dr. Ryan Ordway**. *Summary and wrap-up*
5:40 PM	Poolside barbeque

Tuesday, February 4, 2020 - Pre-Conference *Sponsored* by Virtus Nutrition *"Novel Aspects of Fatty Acid Nutrition in Dairy Cows*"

8:15 AM	**Dr. Kevin Murphy**, Virtus Nutrition. *Welcome and introductions*
8:30 AM	**Dr. Barry Bradford**, Michigan State University. *"Transition cows – How fatty acids affect immunity, production and health"*
9:20 AM	**Dr. Joe McFadden**, Cornell University. *"Fatty acid nutrition and biology to optimize health and production"*
10:10 AM	Refreshment Break
10:40 AM	**Dr. Kevin Harvatine**, Pennsylvania State University. *"The intersection of dietary and milk fatty acids - How photoperiod and variability affect production"*
11:30 AM	Buffet Lunch

Tuesday, February 4, 2020 – Symposium

1:00 PM	**Dr. José E. P. Santos**, University of Florida. *Welcome*
1:05 PM	**Dr. Saqib Mukhtar**, University of Florida. *IFAS update*
1:10 PM	**Dr. William Thatcher**, University of Florida *"Fatty acids and fertility – the contributions of Dr. Charlie Staples"*

1:50 PM **Dr. Tom Jenkins**, Clemson University "*Factors that modify rumen fatty acid outflow versus feed input"*

2:30 PM **Dr. Jacquelyn Boerman**, Purdue University. *"What have we learned about fatty acid digestibility in dairy cattle?"*

3:10 PM Refreshment Break

3:40 PM **Dr. Rodrigo Bicalho**, Cornell University. *"The role of the calf microbiota on performance and health"*

4:20 PM **Dr. Michael Steele**, University of Guelph. *"Pre-weaning calf nutrition and rumen epithelium development, metabolism and health"*

5:00 PM Welcome reception

Wednesday, February 5, 2020 – Symposium

6:30 AM Continental Breakfast

8:00 AM **Dr. Pedro Veiga**, Cargill Animal Nutrition. *"Dietary strategies for the cow calf herd – the experience of the Brazilian beef industry"*

8:40 AM **Dr. Philipe Moriel**, University of Florida. *"Nutritional strategies for developing replacement Bos Indicus-influenced beef heifers"*

9:20 AM **Dr. Rodolfo Cardoso**, Texas A&M University. *"Nutritional control of puberty in beef heifers, mechanisms and application"*

10:00 AM Refreshment Break

10:30 AM **Dr. Diwakar Vyas**, University of Florida. *"The role of N recycling in improving efficiency of N utilization in dairy cattle"*

11:10 AM **Dr. Antonio Faciola**, University of Florida. *"Digestion and nutrient flow in continuous culture system and animal responses. Do they match?"*

11:50 AM Ruminant Nutrition Symposium Adjourns

2020 Symposium Speakers

Guests

Dr. Rodrigo Bicalho, Cornell University
Dr. Jacquelyn Boerman, Purdue University
Dr. Barry Bradford, Michigan State University
Dr. Rodolfo Cardoso, Texas A&M University
Dr. Christiane Girard, Agriculture and Agrifood Canada
Dr. Kevin Harvatine, Pennsylvania State University
Dr. Tom Jenkins, Clemson University
Dr. Joe McFadden, Cornell University
Dr. Tom Overton, Cornell University
Dr. Michael Steele, University of Guelph
Dr. Pedro Veiga, Cargill Animal Nutrition

University of Florida

Department of Animal Sciences

Dr. Antonio Faciola
Dr. Peter Hansen
Dr. Philipe Moriel
Dr. William Thatcher
Dr. Diwakar Vyas

31st Annual Florida Ruminant Nutrition Symposium

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BIOGRAPHIES

Dr. Rodrigo C. Bicalho is an Associate Professor of Dairy Production Medicine in the Department of Population and Diagnostic Sciences. He received his D.V.M. degree from the Federal University of Goiás, Brazil. He then completed a clinical residency and the Ph.D. degree at Cornell University. His research interests are diverse and include areas of microbiology, molecular biology, vaccinology, infectious diseases, and immunology. The unifying objective of his research program is to increase the health and the productivity of farm animals, with a particular focus on the dairy cow. His current efforts include the study of the normal and pathogenic microbiota of domestic animals using high-throughput sequencing, using reverse vaccinology approach to develop new vaccines against bacterial diseases of humans and animals, using recombinant cytokines to manipulate the immune system and metabolism of animals to maximize health and productivity.

Dr. Jackie Boerman is an Assistant Professor in the Department of Animal Sciences at Purdue University. She is originally from a dairy farm in western New York and received her B.Sc. from Cornell University. Jackie received her M.Sc. from the University of Illinois and her Ph.D. degree from Michigan State University focusing on lipid metabolism in dairy cattle. In 2017, Dr. Boerman began an extension/applied research and teaching appointment at Purdue University after working for 2.5 years in industry as a dairy specialist with Cargill Animal Nutrition. Her research and extension programs focus on nutrition and management strategies that promote the production and health of dairy cattle. Specifically, Jackie is interested in understanding variation in tissue mobilization around calving, identifying nutritional, and management changes that optimize both protein and fat mobilization. Additionally, Dr. Boerman has research projects focused on improving on-farm decision making by utilizing data generated on farms to increase our understanding of animal behavior, growth, health, and production.

Dr. Barry Bradford is a Professor and the Clint Meadows Chair in Dairy Management in the Department of Animals Sciences at Michigan State University. He completed dual B.Sc. degrees at Iowa State University and a doctorate in animal nutrition at Michigan State University. He served on the faculty at Kansas State University from 2006 to 2019, and in 2020 he returned to Michigan State University. Dr. Bradford's research focuses on dairy cattle nutrition and metabolism, with a particular emphasis on attempting to translate novel findings in fundamental metabolic physiology to practical applications in animal agriculture. Contributions by his group have largely focused on dietary utilization of byproducts in lactation diets, the physiological impacts of systemic postpartum inflammation, and the roles of nutrients as signals.

Dr. Rodolfo C. Cardoso is an Assistant Professor in the Department of Animal Science at Texas A&M University. Dr. Cardoso received his D.V.M. and M.Sc. degrees from São Paulo State University in Brazil, and completed his Ph.D. at Texas A&M University. Dr. Cardoso completed a postdoctoral fellowship in Reproductive Endocrinology at the University of Michigan before joining the Texas A&M University faculty in 2016. His research efforts focus on the impact of prenatal and early postnatal nutrition on reproductive neuroendocrine function in female ruminants. His laboratory integrates whole animal physiology with cellular and molecular biology to elucidate the neuroendocrine mechanisms controlling puberty and to develop managerial strategies for optimal development of replacement heifers.

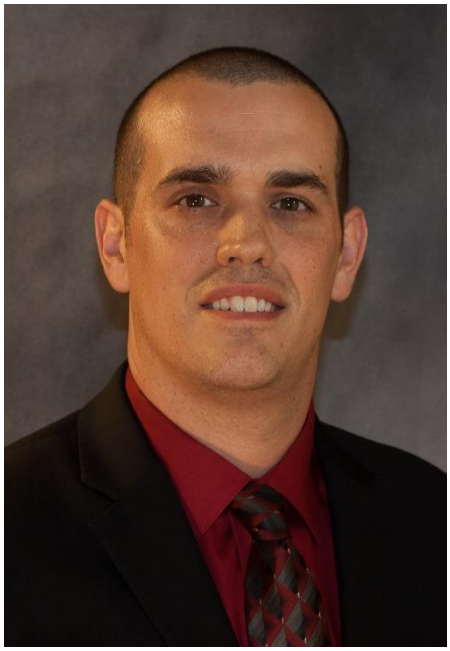
Dr. Antonio Faciola is an Assistant Professor in the Department of Animal Sciences at the University of Florida. Prior to joining UF in the summer of 2017, Dr. Faciola served on the faculty at the University of Nevada for 4 years. Antonio received his B.Sc. and M.Sc. degrees in Animal Sciences from the Federal University of Viçosa, Brazil, the Ph.D. degree in Dairy Science from the University of Wisconsin-Madison, and completed a postdoctoral fellowship at the ARS-USDA U.S. Dairy Forage Research Center in Wisconsin. The goal of his laboratory is to further our understanding of ruminant nutrition to improve the efficiency of nutrient utilization in order to enhance production and minimize environmental impact of livestock. Projects include evaluating canola meal as a protein supplement for dairy cows, evaluation of feedstuffs and determining the nutritional value of different additives for dairy cows. Methodological approaches include the dual-flow continuous culture system and the omasal sampling technique.

Dr. Christiane Girard is a research scientist with Agriculture and Agri-Food Canada at the Sherbrooke Research and Development Centre in Sherbrooke, Quebec. She received her M.Sc. and Ph.D. degrees from the University of Laval in Quebec. She completed a postdoctoral fellowship at the National Institute for Research in Dairying (NIRD), Shinfield, Reading, Berkshire, England and worked at the INRA in Clermont-Ferrand-Theix, France before returning to Canada. Dr. Girard research focuses on defining B-vitamin requirements of high producing dairy cows to optimize their well-being and metabolic efficiency and defining dietary conditions affecting ruminal synthesis and supply of B-vitamins to dairy cows. More recently, her work has been focusing on the metabolic interactions between folic acid and vitamin B_{12} and characterizing the effects of dairy cow nutrition on milk nutritional quality, especially milk concentrations of vitamin B_{12}.

Dr. Kevin Harvatine is an Associate Professor of Nutritional Physiology at Penn State University. He grew up on a dairy farm in Pennsylvania and received his B.Sc. in Animal Science from Penn State. He earned an M.Sc. from Michigan State University and the Ph.D. degree from Cornell University. He was appointed as an Assistant Professor at Penn State in 2009 and was promoted to Associate Professor in 2015. Dr. Harvatine's research is focused on the nutritional regulation of milk synthesis. Harvatine's goal is to identify bioactive factors and nutritional strategies to improve animal production, efficiency, and health. His research spans from applied nutrition to basic biology and provides both real-world applications to the dairy industry and a basic understanding of biological mechanisms. His current research program focuses on investigating the nutritional regulation of milk fat synthesis, fatty acid metabolism, and circadian regulation of intake and mammary metabolism.

Dr. Peter J. Hansen is a Distinguished Professor and L.E. "Red" Larson Professor of Animal Sciences at the University of Florida. He received the B.Sc. in Agricultural Sciences from the University of Illinois and the M.Sc. and Ph.D. degrees from the University of Wisconsin. He did a postdoctoral fellowship at the University of Florida from 1983-1984 before joining the faculty at Florida as an Assistant Professor in 1984. Hansen's research focuses on the biology of pregnancy and embryonic survival and development of methods to improve fertility and assisted reproductive technologies in livestock, particularly dairy cattle. Particular emphasis is placed on elucidating effects of elevated temperature on pregnancy, characterizing the nature of maternal control of early embryonic development and identifying genes controlling embryonic survival and fertility. In addition, work is underway to develop methods to improve dairy cow fertility during heat stress and to increase profitable uses of embryo transfer.

Dr. Tom Jenkins attended Penn State University for his B.Sc. and M.Sc. degrees, and received the Ph.D. degree at Cornell University. After a postdoctorate at The Ohio State University, he moved to Clemson University where he continued to work on dairy cattle nutrition for over 30 years. Dr. Jenkins taught undergraduate and graduate courses in nutrition and coordinated a research program on use of fat in diets for dairy cattle including basic work on rumen lipid metabolism. He has published extensively in scientific journals and conference proceedings, and has given numerous invited presentations across more than a dozen countries on lipid metabolism in dairy cattle and the practical aspects of fat feeding. Dr. Jenkins has received numerous awards from Clemson University and The American Dairy Association for his research accomplishments in rumen lipid metabolism.



Dr. Joseph W. McFadden is an Assistant Professor and the Northeast Agribusiness and Feed Alliance Faculty Fellow in Dairy Cattle Biology in the Department of Animal Science at Cornell University. He received his B.Sc. degree in Animal Science from Cornell University, the M.Sc. degree in Animal Science from the University of Illinois and the Ph.D. degree in Dairy Science from Virginia Tech. He completed a postdoctoral fellowship in the Department of Neuroscience and the Center for Metabolism and Obesity Research at Johns Hopkins University. In 2012, he joined the faculty in the Division of Animal and Nutritional Sciences at West Virginia University as an Assistant Professor of biochemistry. Dr. McFadden joined Cornell University in 2017. His scientific interests involve lipid biology in dairy cattle. His areas of interest include defining the role of sphingolipids in mediating insulin resistance and milk production efficiency, developing methyl donor and fatty acid feeding regimens that enhance postpartum liver health, exploring the role of complex lipids within the context of gut health and immune function, and identifying practical approaches to enhance fatty acid digestibility in dairy cattle.

Dr. Philipe Moriel is an Assistant Professor in the Range Cattle Research & Education Center at the University of Florida. He received his B.Sc. from São Paulo State University, Brazil, the M.Sc. degree in Animal and Veterinary Sciences from University of Wyoming, and the Ph.D. degree in Ruminant Nutrition from the University of Florida. Dr. Moriel worked as an Assistant Professor at North Carolina State University from 2013 to 2016. His research program focuses on developing and implementing nutritional strategies specifically tailored to enhance the productivity of beef cattle adapted to tropical and subtropical environments. More specifically, Dr. Moriel focuses on pre- and post-weaning nutritional strategies for stressed beef calves and replacement beef heifers, fetal-programming, and early-postnatal nutritional manipulations of calf metabolism to subsequently modulate the growth, health, and reproductive success of *Bos indicus*-influenced beef cattle.

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

Dr. Michael Steele is an Associate Professor and NSERC Industrial Research Chair at the University of Guelph and the Past-President of the Canadian Society of Animal Science (CSAS). He completed his Ph.D. at the University of Guelph and worked for Nutreco Canada Agresearch for 2 years prior to returning to academia at the University of Alberta and Guelph as an NSERC Industrial Research Chair. He was recently awarded the CSAS Young Scientist Award, the Cargill Young Animal Nutritionist Award, the Lallemand Award for Excellence in Dairy Nutrition Research and the American Society of Animal Science Early Researcher Award. His current research is focuses on the mechanisms that control gastrointestinal health and development in cattle

Dr. William (Bill) W. Thatcher is a Graduate Research Professor Emeritus in the Department of Animal Sciences at the University of Florida. Bill received his B.Sc. from the University of Maryland, the M.Sc. degree from a joint program between the University of Maryland and the USDA in Beltsville, and the Ph.D. degree in physiology of lactation and reproduction from Michigan State University. His research program in cattle involves ovarian follicular development, maternal-embryo interactions, and developmental approaches for regulating reproductive function to enhance production and health. Major focus has been dealing with effects of the postpartum period, nutrition, and heat stress on ovarian follicular and corpus luteum functions and embryo survival.

Dr. Pedro Veiga is a Global Technology Manager for Beef Cattle in Cargill Animal Nutrition. He earned his Ph.D. from the University of California, Davis and the Federal University of Viçosa in beef cattle nutrition. His Ph.D. work focused on nutrient requirements of *Bos indicus* cattle. Dr. Veiga acquired his postdoctoral experience at Iowa State University where he worked in the areas of animal growth physiology and meat science. He was a faculty member in the Department of Animal Sciences at the Federal University of Viçosa before joining Cargill Animal Nutrition. At Cargill, he provides technical services and works on research and development focusing on cow/calf production systems and supplementation strategies, and stocker and feedlot nutrition mainly in tropical areas.

Dr. Diwakar Vyas is an Assistant Professor of Ruminant Nutrition in the Department of Animal Sciences at the University of Florida. He earned his Ph.D. from the University of Maryland in Dairy Cattle Nutrition. His Ph.D. work was focused on the mammary lipid metabolism. Dr. Vyas completed a postdoctoral fellowship at Lethbridge Research and Education Center of Agriculture and Agri-Food Canada where he worked in areas of environmental sustainability and rumen physiology of beef production systems. At present, his research program is focused on optimizing the inclusion of feed additives for improving economic and environmental sustainability of dairy production systems.

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Dr. Charles Richard Staples was born April 7, 1951, in Greeley, Colorado to the late Raymond and Elise Staples. Charlie received his Bachelor's degree in Animal Sciences from New Mexico State University in 1973 and stayed at New Mexico State for his M.Sc. degree in ruminant nutrition that was completed in 1975. From Las Cruces, Charlie moved to Urbana-Champaign to pursue his Ph.D. at the University of Illinois. During his graduate studies at Illinois, Charlie worked under Dr. George C. Fahey Jr. in the area of forage and fiber quality for ruminants. The title of his dissertation was *"Evaluation of Factors Affecting Digestibility of the Fibrous Portion of the Ruminant Diets"*. Upon receiving his Ph.D. degree in 1983, Charlie completed a post-doctorate program under Dr. Jimmy Howard Clark also at Illinois in which he studied changes in energy balance in early lactation and restoration of ovarian activity in dairy cows.

Charlie moved with his family to Gainesville in 1984 to accept an Assistant Professor position in the Department of Dairy Sciences. He was an Assistant (1984-1989), Associate (1989-1995) and Professor (1995-2019) at the University of Florida. During his tenure at the University of Florida, Charlie was recognized in 2003 with the Research Foundation Professorship for outstanding research and scholarly achievements which contributed to the reputation of the University. In his almost 35 years as a faculty member, Charlie published 150 scientific peer-reviewed manuscripts and 14 book chapters, and presented and published 160 papers in proceedings of national and international conferences. Charlie mentored 23 graduate students and post-doctorates from the USA, Argentina, Brazil, Colombia, Costa Rica, Malawi, Mexico, Peru, Puerto Rico, and South Korea, in addition to the graduate students mentored by colleagues that he devoted endless time to help and assist during their programs. His scientific contributions spanned from forage quality, supplementation of cows under grazing, fatty acid nutrition of cows and calves, establishing choline as a required nutrient for dairy cows, among others. For his contributions to dairy sciences, the American Dairy Science Association awarded him the American Feed Industry Association Award and the Nutrition Professionals Applied Dairy Nutrition Award. In 2017, Charlie received the Fellow Award from the American Dairy Science Association for his services to the association and scientific discoveries impacting the dairy industry. In his last year before retirement, he served as interim Chair of the Department of Animal Sciences. Charlie is survived by his wife, Debbie and his two daughters, Charity and Spring. He was a devoted husband and father, a mentor to his students, a Civil War history buff, and a devout Christian. Above all, Charlie was an outstanding colleague and wonderful human being.

Transition Cows - How Fatty Acids Affect Immunity, Production and Health

Barry Bradford[1]
Michigan State University

Introduction

The transition to lactation remains one of the most challenging and important phases of the production cycle. We continue to gather more insight into the specific mechanisms underlying this transition, but the basic challenge is the cow at this time needs to rapidly shift gears while she's under many forms of stress – metabolic, immunological, and even social. The list of nutritional interventions that have been tested for aiding this transition is lengthy, with wide variation in the evidence supporting product use. The focus of this article is on the bioactivity of fatty acids that can be fed to transition cows, the processes in the cow that may be altered, and the functional impacts of such strategies on the transition cow. As we'll see, fatty acids have a wide variety of impacts that make this an exciting avenue to potentially improve transition cow health and performance.

The Transition Puzzle

Transition cows present something of a paradox. Despite moving into a physiological state where nutrient requirements generally increase by 2 to 5-fold in the course of a week or two, they often show poor feed intake. The cause of this predicament is not fully understood, but we do know that most transition cows experience at least a few days of systemic inflammation after calving, and inflammatory molecules inhibit appetite. Those cows who resolve the inflammatory state quickly are likely to be the ones who show a strong improvement in feed intake in the first week of lactation.

Likewise, calving and the onset of lactation introduce new avenues for microbial invasion, and infectious disease risk is greater during this time than the rest of lactation. Unfortunately, altered immune function at the same time seems to make it hard for cows to combat this increase in disease pressure. Circulating neutrophils (the key rapid-response immune cell type) decline after calving, and there is some evidence of reduced humoral immunity as well (impaired antibody production). Enhancing immune function may be especially important for subsequent fertility, as those cows with a strong uterine immune response in the first 2 weeks after calving generally avoid chronic endometritis and have increased odds of becoming pregnant at first service. Further complicating efforts to help the cow with dietary or pharmaceutical "nudges" is the issue that inflammation is a key component of the immune response. We thus have

[1] Contact at: Department of Animal Sciences, Anthony Hall, 474 S. Shaw Lane, East Lansing, MI 48824-1225; Tel: (517) 432-5400; E-mail: bjbrad@msu.edu.

the difficult task of trying to reduce inflammation (thereby, we hope, enhancing appetite) while promoting immune response to better resolve metritis and mastitis. These are not readily compatible goals! Nevertheless, dietary lipids may help us to strike the right balance between these competing objectives.

Fatty Acids- The Original Bioactive Nutrients?

The big shift that is driving much nutrition research today is the understanding of nutrients as signals rather than just fuels and building blocks (Bradford et al., 2016). This shift in thinking arguably started with research into mechanisms by which polyunsaturated fatty acids alter gene expression, which revealed nuclear receptors that bind fatty acids and then interact with DNA. Prior to these discoveries, only drugs and hormones were thought to work through such receptors. Since that time, cell-surface receptors have been discovered that also respond to fatty acids and their downstream metabolites. Therefore, fatty acids not only provide energy and serve as a structural component of cells, but they also have multiple avenues for changing the function of cells and organs.

To add another layer of complexity to this, dietary triglycerides are rapidly cleaved to free fatty acids in the rumen, and microbial biohydrogenation then substantially alters the composition of fatty acids leaving the rumen. Although the majority of dietary fatty acids are completely saturated through this process, there are intermediates that escape the rumen, and composition of these intermediates is influenced by dietary fatty acid profile, microbial populations, and rumen chemistry. Calcium salts of fatty acids are less available for microbial modification in the rumen compared to free oils, but they are not totally inert. In fact, less than 25% of a polyunsaturated fatty acid fed to a cow will be absorbed in that form (Lundy et al., 2004; Harvatine and Allen, 2006), so we have to keep that in mind when supplementing bioactive lipids. Despite this challenge, we have evidence that dietary lipids can alter the physiology of transition cows in important ways. Let’s dig into a few key strategies that have been attempted for aiding the transition to lactation.

Energy Balance

Many cows experience a month or more of negative energy balance postpartum, resulting in body condition loss. Rapid body fat mobilization is a risk factor for metabolic diseases in early lactation, and this weight loss also impairs fertility. Dietary fat sources provide a possible means to increase energy supply for cows in early lactation; however, increasing energy *density* may not increase energy *supply* if feed intake decreases. Intake responses are important to consider, because unsaturated fat sources in particular can substantially decrease intake (Bradford et al., 2008). One recent study with 48 cows explored responses to 2% saturated fatty acids fed during the first 30 days of lactation. Surprisingly, cows offered diets including supplemental fat actually consumed more feed than cows on the control diets (Piantoni et al., 2015). Although dietary fat clearly slowed the loss of body condition in early lactation, it did not improve milk production; in fact, the saturated fatty acid treatment decreased milk yield

during peak lactation (after treatments ended) by 8%. Therefore, in spite of nice improvements in early lactation energy intake, subsequent benefits were lacking.

More recent work at Michigan State has explored the impacts of feeding specific fatty acids to fresh cows. Supplementing palmitic acid in the first 24 days of lactation significantly increased milk fat yield compared to no supplemental fat (de Souza and Lock, 2019), similar to effects in mid-lactation cows. However, palmitic acid appeared to have an impact beyond just driving more palmitic acid content in milk; loss of both body condition and body weight was greater in cows on this treatment. A follow-up study then evaluated fresh diets (24 days) supplemented primarily with palmitic acid, but also including 10, 20, or 30% oleic acid. The dose-response to oleic acid was impressive – as its inclusion increased, dry matter intake increased, blood insulin increased, and body weight loss was slowed (de Souza et al., 2018). How can adding small amounts of such a common fatty acid impact transition cow energy balance like this? Follow-up research suggested that oleic acid administration significantly decreased the lipolytic response of bovine adipose tissue and increased insulin sensitivity, which would promote lipid storage rather than release (Laguna et al., 2019).

Conjugated Linoleic Acid

One intriguing approach to manipulating energy balance in early lactation is the dietary supplementation of conjugated linoleic acids (**CLA**). There are several forms of CLA produced within the rumen during the biohydrogenation of polyunsaturated fatty acids. One form, *trans*-10, *cis*-12 CLA, is produced in the rumen primarily when diets with excessive rumen-available unsaturated fatty acids and inadequate fiber substantially change ruminal biohydrogenation. *Trans*-10, *cis*-12 CLA is believed to be largely responsible for the suppression of milk fat synthesis in these scenarios, and it also promotes adipose tissue fat synthesis. Although this is typically avoided by dairy nutritionists, in early lactation, suppressing milk fat secretion could decrease milk energy content and help to partially restore energy balance.

A series of studies conducted in Germany has evaluated responses to feeding a product that serves as a potent source of mixed CLA, including the *trans*-10, *cis*-12 isomer. Unfortunately, most results have been disappointing. Although feeding CLA has consistently decreased milk fat concentration in transition cows as proposed, cows fed this product have simply responded by increasing milk yield (von Soosten et al., 2011) and/or decreasing dry matter intake (Schäfers et al., 2017). The net result in nearly every study has been a neutral effect on energy balance, with no clear evidence of a more rapid end to body weight loss.

Dietary Fat Can Influence Transition Weight Loss

Dietary fatty acids do appear to have the potential to modulate partitioning of energy between the mammary gland and other organs in early lactation, with the most promising responses to date being with oleic acid. Further research into specific mechanisms driving changes in adipose tissue metabolism with oleic acid may open up

opportunities to more dramatically change the trajectory of body weight loss in early lactation cows.

Inflammatory Signaling Pathways

Most endocrine signals in the body are protein hormones, but there are also important lipid hormones. Lipid hormones include cholesterol-derived steroids and the broad group known as eicosanoids or oxylipids. Eicosanoids include some relatively familiar signals like prostaglandin $F_{2\alpha}$, but there are dozens of other less-familiar compounds in this class, many with roles that are still emerging (Sordillo, 2018). From a nutritionist's point of view, these endocrine factors are of great interest because eicosanoid concentrations are heavily influenced by the availability of their fatty acid substrates. Eicosanoids are derived from omega-6, omega-3, and sometimes omega-9 unsaturated fatty acids, and to paint with a broad brush, the omega-6 derived eicosanoids promote inflammatory processes, whereas those downstream of omega-3 fatty acids tend to drive resolution of inflammation.

Studies have documented that transition cows experience a shift in the profile of eicosanoids in blood, with more inflammatory lipids generally increased during this period compared to established lactation (Kuhn et al., 2017). Furthermore, transition cows challenged with additional inflammatory insults had a delay in the recovery of anti-inflammatory eicosanoids in the first week after calving (Yuan et al., 2013). Eicosanoids clearly participate in the systemic inflammatory shift after calving, which may be important, given the associations between inflammatory markers, health, and productivity in transition cows (Bradford et al., 2015).

Dietary unsaturated fatty acids serve as substrates for these eicosanoids, and the fatty acids themselves can influence inflammatory tone as well (Oh et al., 2010). Is it possible to alter transition cow inflammation by feeding different sources of fat?

Altering Omega-6:Omega-3 Ratio

Omega-3 fatty acids include alpha-linolenic acid (**ALA**), eicosapentaenoic acid (**EPA**), and docosahexaenoic acid (**DHA**). Omega-6 fatty acids are more abundant in the diet and in the cow, with the primary form being linoleic acid. With diets typically fed to dairy cows, plasma omega-6:omega-3 ratios generally exceed 10:1. Given the impact of biohydrogenation on absorbed fatty acids, is it possible to impact this ratio? A review of dozens of studies shows that feeding unprotected fish or flaxseed oil (common sources of omega-3 fatty acids) have little impact on plasma omega-3 fatty acids, but that feeding products designed to limit ruminal availability of fatty acids can decrease the plasma omega-6:omega-3 ratio by up to 40% (Moallem, 2018). Therefore, although all of these polyunsaturated fatty acids are absorbed in small quantities in ruminants, some feed products can shift the profile of absorbed lipids.

Impacts on Immunity

Lessard et al. (2003) reported that feeding fresh cows whole flaxseed (a source of alpha-linolenic acid) increased serum omega-3 fatty acid concentration, resulting in a marked reduction in the omega-6:omega-3 ratio compared with those fed micronized soybeans or calcium salts of palm oil. Interestingly, on day 5 after calving, the lymphocyte proliferative response (a component of adaptive immunity) of cows fed flaxseed was reduced compared with other groups, suggesting an anti-inflammatory effect was achieved, albeit not necessarily a beneficial one.

Silvestre and colleagues (Silvestre et al., 2011) attempted to promote immune function in the transition period by supplementing omega-6 fatty acids compared to omega-3 fatty acids, supplied in the form of calcium salts of fatty acids. Increasing the ratio of omega-6:omega-3 fatty acids increased the production of hydrogen peroxide and phagocytosis of bacteria by neutrophils (Silvestre et al., 2011), which could be due to increased supply of omega-6 precursors of inflammatory eicosanoids and/or decreased supply of anti-inflammatory omega-3 fatty acids. This treatment also increased plasma concentrations of 2 acute phase proteins (Silvestre et al., 2011), indicating a more inflamed state of the liver during the transition period. The observed effects on neutrophil function and acute phase response would be expected to improve the ability of the immune system to ward off infection, but like the results of Lessard et al. (2003), it's not entirely clear whether this would be a net benefit for a transition cow. The potential benefits of either approach may depend on the incidence of metabolic vs. infectious diseases on a given farm, the metabolic state of the cows in question, and even the diet to which the fatty acid supplement is added.

Impacts on Productivity

Greco and colleagues (2015) used combinations of calcium salt products to offer cows diets with omega-6:omega-3 ratios of 6:1, 5:1, or 4:1 between 14 and 90 days in milk, while holding total unsaturated fat supply relatively constant. In avoiding the first 2 weeks postpartum, this study bypassed potential impacts on the typical transition inflammation window, but the results were insightful nonetheless. Decreasing the omega-6:omega-3 ratio (increasing omega-3 supply) resulted in significantly greater dry matter intake as well as increased yields of all milk components. An intramammary endotoxin challenge at 75 days in milk demonstrated that some systemic inflammatory responses were elevated for the treatments with greater omega-6 content compared to the 4:1 ratio (Greco et al., 2015). Positive productivity responses to increased omega-3 supply are consistent with impacts of supplying other types of anti-inflammatory agents in early lactation (Carpenter et al., 2016; Olagaray et al., 2019).

Altering Inflammatory Status with Dietary Lipids

Considered together, research to date suggests that diets with elevated concentrations of omega-3 fatty acids (protected in part from ruminal biohydrogenation) can be used to mildly decrease inflammatory status of postpartum cows, which may benefit productivity but potentially dampens immune response. Interpretation of most such studies, furthermore, is complicated by differences in not only inflammation, but

also in supply of multiple fatty acids that serve as precursors for eicosanoids, including prostaglandins and other reproductive hormones. One study demonstrated that supplementing *either* omega-3 or omega-6 fatty acids during early lactation increased peak progesterone concentrations during the estrus cycle (Dirandeh et al., 2013), indicating that essential fatty acid deficiency may be a separate issue worth considering.

Conclusions

Despite the challenges associated with sneaking bioactive polyunsaturated fatty acids past the rumen, products available today offer opportunities to alter the transition to lactation. Palmitic acid remains the most potent tool available for enhancing milk fat yield, but oleic acid appears to offer an important means to shift some fat to body stores in the critical first month of lactation. Additionally, the omega-3 and omega-6 fatty acids provide subtle but effective tools to nudge inflammatory tone of transition cows, with omega-3 supplements potentially suppressing inflammation to enhance productivity, whereas omega-6 supplements may enhance immune vigilance and could decrease infectious disease incidence. Dairy nutritionists should consider which fatty acid strategies best address the challenges on individual farms.

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SESSION NOTES

Fatty Acid Biology and Nutrition to Optimize Health and Production

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Introduction

The onset of the periparturient period is characterized by a series of coordinated metabolic adaptations to support fetal and neonatal development in dairy cattle (see review by (McFadden and Rico, 2019). These processes are controlled by endocrine signals including placental lactogen, somatotropin, and insulin. The consequence of their action is the fine-tuned control of metabolism to spare key nutrients such as glucose, fatty acids, and amino acids for growth of the fetus and milk production. The demand for glucose by the mammary gland for the synthesis of milk lactose, the osmotic regulator of milk volume, is supported by (i) increased hepatic gluconeogenesis, ketogenesis, and glycogen breakdown, (ii) increased blood flow to the mammary gland, (iii) decreased skeletal muscle protein synthesis and adipose tissue lipogenesis, (iv) elevated adipose tissue lipolysis and circulating fatty acid supply, and (v) increased utilization of fatty acids and amino acids for oxidative metabolism. These changes in nutrient metabolism are due in part to reductions in pancreatic insulin secretion and the effectiveness of insulin. Specifically, lactation is supported by a decrease in insulin sensitivity (i.e., enhanced insulin concentration to achieve half-maximal response) and responsiveness (i.e., a decrease in maximal response at a specific insulin concentration; Debras et al., 1989; Vernon et al., 1990; Baumgard et al., 2017). Early lactation is also characterized by the uncoupling of the somatotropic axis and low circulating concentrations of insulin-like growth factor-I, an insulin-sensitizer. Although the mechanisms of maternal insulin resistance are not completely defined, this review discusses the potential interplay of fatty acids and the implications nutrient partitioning may have for milk production and health.

Defining the role of fatty acids within the context of metabolism, nutrient partitioning and lactation has scientific merit for several reasons. Fatty acids derived from the diet or adipose tissue lipolysis constitute an important energy source for the dairy cow, especially during early lactation when the cow produces milk equivalent to 50 to 70% of total daily requirements for energy. Mitochondrial or peroxisomal fatty acid oxidation produce reducing equivalents (i.e., $FADH_2$ and NADH) and acetyl-CoA, which may be used to support electron transport and adenosine triphosphate synthesis. Acetyl-CoA may also be utilized to support the production of ketones, which when used as a fuel source spares glucose. Fatty acids from circulation or those synthesized *de novo* are also utilized to generate major constituents of milk including triacylglycerols, glycerophospholipids, and sphingolipids (e.g., ~30% of milk solids in a modern Holstein cow; Jensen, 2002). Fatty acids may also directly influence nutrient utilization in the mammary gland (Cant et al., 1993). Increased fatty acid availability holds potential to

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preserve acetate, a building block for mammary *de novo* fatty acid synthesis, for oxidation in extra-mammary and -hepatic tissues (Cant et al., 1993). One concern for the transition dairy cow is that exacerbated body fat mobilization with inadequate hepatic fatty acid oxidation and triacylglycerol secretion are predisposing factors for fatty liver disease, ketosis, and other metabolic maladies that may compromise milk production, fertility, and the cow's productive lifespan. This review focuses on the role of fatty acids as agonists and antagonists of insulin-stimulated glucose utilization and lipogenesis. The effects of nutrient restriction, and saturated and unsaturated fatty acid feeding are highlighted. To provide a contemporary perspective, the emerging role of the sphingolipid ceramide is considered within the framework of somatotropin action, nutrient partitioning, lactation, and health. Lastly, the potential interplay of somatotropin, adiponectin and fibroblast growth factor-21 (**FGF21**) are also discussed.

Fatty Acids as Modulators of Insulin Sensitivity

Fatty acids modulate insulin signaling and sensitivity in non-ruminants. In pregnant women for example, the increase in plasma fatty acid concentrations during late pregnancy is considered a potential cause of insulin resistance (Sivan and Boden, 2003). The increase in lipolysis is due in part to the actions of placental lactogen and growth hormone. Women with gestational diabetes experience defective insulin secretion and fatty acids play a key role in mediating skeletal muscle insulin resistance. The ability of specific fatty acids to modulate insulin-stimulated glucose utilization in the cow was characterized at the start of the 21st century. At the University of Wisconsin, Pires et al. (2007) demonstrated that the abomasal infusion of nicotinic acid, a potent suppressor of lipolysis, was able to lower circulating fatty acids and insulin following an intravenous glucose challenge. The investigators also confirmed that nicotinic acid administration increased glucose clearance rate following the administration of an intravenous glucose bolus. In non-pregnant and non-lactating Holstein cows fasted to trigger hepatic lipidosis, elevations in circulating fatty acids occurred with a decrease in insulin-stimulated glucose disposal (Oikawa and Oetzel, 2006). In late-gestation dairy cows, feed deprivation increased circulating fatty acids and reduced glucose clearance rate following a glucose challenge, relative to ad libitum fed cows (Schoenberg et al., 2012). It has also been established that overconditioning during gestation increases postpartum circulating fatty acids from lipolysis, which occurs concomitantly with reductions in insulin sensitivity (Rico et al., 2015, 2017b; Davis et al., 2019). Indeed, cows prone to high weight loss during the periparturient period develop adipose tissue insulin resistance (Zachut et al., 2013). Collectively, these studies support the conclusion that adipose-derived fatty acids reduce insulin-mediated glucose utilization in dairy cows.

The current scientific consensus is that the directional change in insulin sensitivity is specific for individual fatty acids in dairy cows. Saturated fatty acids deserve consideration because of their established ability to antagonize insulin action in non-ruminants (Kennedy et al., 2008). In transition dairy cows, the plasma fatty acid pool contains a greater proportion of saturated palmitic acid (C16:0), whereas the proportion of unsaturated linoleic acid (C18:2) and arachidonic acid (C20:4) are lower

(Douglas et al., 2007). Contreras et al. (2010) also demonstrated that palmitic acid is elevated within circulating non-esterified fatty acid and phospholipid fractions during the immediate postpartum. We can postulate that palmitic acid from adipose tissue lipolysis may be responsible for reductions in insulin-stimulated glucose disposal in the aforementioned feed-restricted cows. In support, the intravenous infusion of tallow was compared to linseed or fish oil infusion in non-pregnant and non-lactating Holstein cows (Mashek et al., 2005). The result was a respective increase in circulating concentrations of palmitic acid, linolenic acid (C18:3), and docosahexaenoic acid (**DHA**; C22:6 n-3). In addition, tallow infusion results in greater plasma glucose concentrations and total fatty acid concentrations, relative to fish oil or linseed oil infusion, respectively. Cows infused tallow also had higher circulating glycerol and insulin concentrations, relative to cows infused linseed or fish oil. The authors postulated that the intravenous saturated fat infusion may have increased insulin secretion and decreased insulin-stimulated glucose uptake and suppression of adipose tissue lipolysis. In support, Pires et al. (2008) concluded that the abomasal infusion of tallow impaired the ability of insulin to stimulate glucose uptake and prevent lipolysis in cows, relative to cows abomasally infused linolenic acid-rich linseed oil. These studies also suggested that unsaturated fatty acids may improve insulin action in cows. Such a hypothesis was considered by Gingras et al. (2007). The investigators demonstrated that the continuous abomasal infusion of long-chain omega-3 polyunsaturated fatty acids (**PUFA**) from fish oil increased insulin-stimulated whole-body disposal of amino acids and glucose, activated muscle insulin signaling intermediates protein kinase B and mammalian target of rapamycin, and increased muscle glucose transporter-4 protein abundance in growing steers. More recently, Laguna et al. (2019) abomasally infused oleic acid to postpartum cows and subcutaneous adipose tissue explants were collected, which were challenged with isoproterenol or insulin. The result was that oleic acid was able to enhance insulin sensitivity and reduce lipolysis. Because adipose tissue is enriched in palmitic acid and oleic acid, and both of these fatty acids contribute to the rise in circulating total fatty acids during the peripartum and feed restriction, the interactions between palmitic acid and oleic acid within the context of insulin sensitivity deserves further consideration. Regardless, transition dairy cows do experience decreases in the proportion of long- and very-long chain PUFA including arachidonic acid and DHA in plasma and tissues during the peripartum (Douglas et al., 2007). We postulate that periparturient increases in the ratio of available saturated fatty acids to unsaturated fatty acids may reduce insulin action, promote lipolysis and body weight loss, and increase a cow's risk for developing a metabolic disease; however, such outcomes would also enhance nutrient partitioning towards the mammary gland to support milk production.

Dietary Fatty Acid Feeding and Nutrient Partitioning

Supplemental dietary fat is a common dietary strategy to support the energy needs of high producing dairy cows. Given its high energy density per unit of weight, fat supplementation increases energy supply, and may modulate dry matter intake, and yields of milk and milk solids (i.e., fat and protein). The comprehensive reviews by Palmquist and Jenkins (1980), Grummer (1991), and Coppock and Wilks (1991), and a meta-analysis by Rabiee et al. (2012), summarize a large body of work and are

recommended for further reading. Although supplemental fat is typically included in lactating cow rations to increase energy density and support milk production, there is growing interest to utilize individual fatty acid feeding for non-caloric purposes. This is because not all fatty acids appear to induce the same biological response. In the meta-analysis using 68 comparisons described by Rabiee et al. (2012), responses to supplemental fat were highly heterogeneous with variation attributed to the type of dietary fat supplement (i.e., animal rendered fats, highly unsaturated oilseeds and calcium soaps of fatty acids, and highly saturated prilled fats). Relevant to this paper, we have come to understand that the degree of fatty acid saturation (e.g., saturated stearic vs. monounsaturated oleic, vs. polyunsaturated linoleic) influences milk production response (Relling and Reynolds, 2007), nutrient metabolism and body composition (Pires and Grummer, 2008; Zachut et al., 2010; de Souza et al., 2018), and immune system and cow health (Lessard et al., 2004; Contreras et al., 2012; Mavangira and Sordillo, 2018).

Although it is clear that fatty acid digestibility and absorption vary depending of fatty acid chain length and degree of saturation (Glasser et al., 2008; Boerman et al., 2015), less is known about the direct effects of specific fatty acids on energy metabolism and how specific fatty acids are preferentially partitioned across tissues throughout lactation. Such information may explain the underlying variation in production responses to fatty acid feeding. This question was recently considered in lactating dairy cows by de Souza et al. (2018), who fed fat supplements varying in the proportion of palmitic, stearic (C18:0), and oleic (C18:1 *cis*-9) acids, as follows: 1) PA (~80 C16:0), 2) PA+SA (40% C16:0 and 40% C18:0), and 3) PA+OA (46% C16:0 and 34% C18:1 *cis*-9). Although milk production was comparable across the fatty acid treatments, milk fat yield was highest in cows fed PA, as might be expected. However, body condition score and body weight change were positive and highest in cows fed PA+OA, relative to PA or PA+SA treatment. Further, the PA+OA diet resulted in body weight change 50% above that of PA+SA. These results indicate a preferential partitioning of palmitic acid towards milk energy output, while oleic acid feeding seemed to favor energy partitioning towards body weight storage. In support, palmitic acid feeding has been shown to increase energy partitioning towards milk without a change in energy partitioned towards body reserves in multiparous cows, relative to a no-added fat control (de Souza and Lock, 2018). In addition, palmitic acid feeding accelerated body weight and body condition loss in fresh cows when compared to cows fed a no-added fat control diet (de Souza et al., 2019). While the reasons for these unique responses need to be elucidated, the mechanisms may involve suppressed and enhanced insulin sensitivity with palmitic acid and oleic acid feeding, respectively. In support, we have reported that palmitic acid feeding results in increased milk energy output and plasma fatty acids, and reduced estimated insulin sensitivity and glucose-stimulated fatty acid disappearance, the latter being an indicator of increased lipolytic activity in adipose tissue (Mathews et al., 2016). As described above, the abomasal infusion of oleic acid to postpartum cows was shown to enhance adipose tissue insulin sensitivity and reduce lipolysis (Laguna et al., 2019).

Although the dairy cow's diet is rich in PUFA, mostly in the form of omega-6 linoleic (C18:2 *cis*-9, *cis*-12) and omega-3 linolenic (C18:3 *cis*-9, *cis*-12, *cis*-15) acids,

their ruminal biohydrogenation is so extensive (70 to 95% and 85 to 100% for linoleic and linolenic acids, respectively) that the duodenal fatty acid outflow is mostly saturated stearic acid (Lock et al., 2006). The consequence is that dairy cows typically have limited amounts of essential n-6 and n-3 PUFA available for absorption. Over the past few decades, attention has centered on n-3 PUFA supplementation (i.e., linolenic acid and DHA), because of their ability to modulate prostaglandin synthesis (e.g., prostaglandin F2α; Staples et al., 1998; Mattos et al., 2004) to improve fertility and embryo survival (Santos et al., 2008; Sinedino et al., 2017), and modulate immunity (Lessard et al., 2004). The ability of PUFA to modulate nutrient partitioning during lactation is less clear. Omega-3 fatty acid feeding has been shown to improve insulin sensitivity in rodents (Andersen et al., 2008; Capel et al., 2015); however, a meta-analysis of randomized controlled trials was unable to demonstrate this relationship in humans (Akinkuolie et al., 2011). Cartiff et al. (2013) was able to show an improvement in insulin sensitivity in growing steers fed calcium salts of n-3 fatty acids. Feeding extruded flaxseed enriched in omega-3 linolenic acid to transition dairy cows reduced circulating palmitic acid concentrations, improved postpartum energy balance, and increased early lactation body weight (Zachut et al., 2010). Decreasing the ratio of n-6 to n-3 fatty acids in the diet by feeding calcium salts of fish oil enhanced milk yield and 3.5% FCM in Holstein cows (Greco et al., 2015). Approximately 28% could not be accounted for by increases in caloric intake. The authors explain that nutrient partitioning towards the mammary gland could have favored lactation. Future studies comparing saturated and omega-3 fatty acid feeding on nutrient partitioning, health, and milk production are needed.

The Role of the Sphingolipid Ceramide

The synthesis and accumulation of ceramide is a dominant feature that defines the mechanisms of insulin resistance in rodent models of type 2 diabetes mellitus and non-alcoholic fatty liver disease caused by surplus saturated fatty acids (Summers, 2006; Pagadala et al., 2012). Specifically, ceramide is a bioactive sphingolipid and mediator of insulin resistance (Summers, 2006). The modes by which ceramide inhibits insulin-stimulated glucose appears to involve the activation of protein kinase C-ζ, phosphatase and tensin homolog, and protein phosphatase 2A (Hajduch et al., 2008; Blouin et al., 2010; Chavez and Summers, 2012). Moreover, ceramide-dependent caveolin-enriched microdomain-recruitment of protein kinase C-ζ inhibits protein kinase B activation and glucose transporter translocation to the plasma membrane (Powell et al., 2003). The emerging role of ceramide in the dairy cow experiencing insulin antagonism was recently reviewed by McFadden and Rico (2019). **Table 1** summarizes the current body of work. Several studies have defined ceramide status in the transition dairy cow (Rico et al., 2015; Rico et al., 2017b; Davis et al., 2019). We demonstrated that ceramide accumulates in circulation, and liver and skeletal muscle tissue during the transition from gestation to lactation, regardless of prepartum adiposity status. However, overconditioned prepartum cows experienced greater postpartum body weight loss, hepatic lipid deposition, and circulating fatty acid and ceramide concentrations, relative to cows with moderate body condition. We also demonstrated that plasma and skeletal muscle ceramides were negatively associated with glucose clearance rate and insulin-

stimulated reductions in glucose following an intravenous insulin challenge after calving, respectively. Moreover, plasma glycosylated ceramide concentrations were inversely related to glucose-stimulated reduction in total fatty acids following an intravenous glucose challenge. These studies suggest a role for ceramide in the transition cow.

Our data suggested that fatty acids from adipose tissue lipolysis are utilized to stimulate de novo ceramide synthesis in bovine liver and skeletal muscle. We explored this possibility in Holstein cows following a feed deprivation protocol (Davis et al., 2017a). The result was an increase in circulating fatty acids, liver lipid accrual, impaired glucose disposal following an intravenous insulin challenge, and the accumulation of ceramide in serum and liver. In a subsequent study, Holstein cows were intravenously infused with a soybean oil emulsion (Rico et al., 2018a). The infusion of triacylglycerol increased circulating fatty acids, hepatic triacylglycerol accumulation, and plasma and liver ceramide concentrations. We observed marked increases in hepatic dihydro-ceramide concentrations and ceramide synthase 2 mRNA expression, which suggest that de novo ceramide synthesis was upregulated by hyperlipidemia induction. We postulated that saturated palmitate would activate de novo ceramide synthesis because the pathway is controlled by serine palmitoyltransferase. Indeed, culturing bovine primary neonatal hepatocytes with sodium palmitate increased intracellular ceramide concentrations, which was prevented by co-treating hepatocytes with a serine palmitoyltransferase inhibitor called myriocin (McFadden et al., 2018). More recently, we confirmed that intravenous myriocin administration inhibits ceramide accumulation in feed-restricted adult ewes (unpublished). Collectively, these data confirm in ruminants that hepatic de novo ceramide synthesis is activated during negative energy balance and fatty acids from lipolysis are involved.

Fatty acid feeding regimens that modulate ceramide supply have potential to influence insulin sensitivity and nutrient partitioning. Initial research focused on the effects of palmitic acid feeding on ceramide status in cows. Rico et al. (2016) demonstrated that feeding mid-lactation Holstein cows palm fat enriched in palmitic acid increased circulating fatty acids, ceramide concentrations, and milk yield and milk production efficiency, relative to cows fed a diet without supplemental palm fat. In the same experiment, cows fed palm fat also experienced reductions in glucose-stimulated fatty acid disappearance following an intravenous glucose-challenge. These data suggest that palmitic acid feeding may decrease the ability of insulin to suppress adipose tissue lipolysis via ceramide-dependent mechanisms. In support, Rico et al. (2018b) demonstrated that ceramide inhibited insulin-stimulated 2-deoxyglucose uptake in bovine primary differentiated adipocytes. In contrast, treating bovine adipocyte cultures with myriocin effectively lowered intracellular ceramide concentrations, and enhanced insulin signaling (i.e., protein kinase B activation) and insulin-stimulated 2-deoxyglucose uptake (Rico et al., 2018b). These findings demonstrated that ceramide causes insulin resistance in bovine adipocytes. The ability of palmitic acid to increase circulating ceramide concentrations was also observed in early lactation cows that experienced heightened body weight loss during the fresh period (Davis et al., 2017b; de Souza et al., 2019). Subsequent studies were performed to evaluate the effects of other fatty acids on plasma ceramide concentrations in lactating cows and lambs. In

brief, abomasal palmitic acid infusion elevates circulating ceramides in cows, relative to stearic acid or medium-chain fatty acids (i.e., C8:0 and C10:0) in the form of triacylglycerol (Rico et al., 2017a). When compared to a palmitic acid treatment, only the abomasal infusion of behenic acid (C22:0) has been shown to increase ceramides (Myers et al., 2019). Feeding a fish oil enriched in palmitoleic acid (C16:1), an insulin-sensitizing fatty acid, was shown to increase circulating eicosapentaenoic acid and DHA, and reduce serum ceramide concentrations in lambs, relative to a no-added fat control (Duckett et al., 2019). Lastly, the abomasal infusion of a fish oil highly-enriched in DHA was able to lower circulating ceramides in cows, relative to cows infused with palmitic acid or behenic acid (Myers et al., 2019). Across these studies, we have consistently demonstrated that circulating ceramides are associated with enhanced milk production and the efficiency to produce milk (McFadden and Rico, 2019). In ruminants, our studies suggest that saturated fatty acids (i.e., palmitic and behenic acids) increase ceramide synthesis, whereas PUFA (i.e., palmitoleic acid or DHA) decrease ceramide synthesis. A major implication is that saturated fat feeding has potential to exacerbate insulin resistance and accelerate body weight loss during the fresh period via the actions of ceramide, which may be prevented by increasing bioavailable unsaturated fatty acids and inhibiting ceramide synthesis. Additionally, saturated fat feeding may be a means to maintain or restore insulin antagonism as a means to spare glucose and fatty acids for milk production later in lactation.

Endocrine Control of Ceramide Synthesis in Cows

McFadden and Rico (2019) describes the potential interplay between somatotropin action, ceramide synthesis, and insulin resistance. In brief, uncoupling of the somatotropic axis inhibits skeletal muscle glucose uptake by downregulating insulin signaling. Moreover, recombinant bovine somatotropin has the ability to increase adipose tissue lipolysis in dairy cows experiencing negative energy balance. Therefore, we posed the possibility that the ability of somatotropin to increase milk production during a catabolic state may involve the synthesis of ceramide from adipose-derived fatty acids, which would in turn downregulate skeletal muscle insulin signaling to spare glucose for milk production. In support, the McFadden lab confirmed that cows administered recombinant bovine somatotropin experience marked increases in circulating ceramides with the concomitant inhibition of insulin-stimulated glucose disposal (unpublished; in review).

Fibroblast growth factor-21 and adiponectin are endocrine signals involved in fatty acid metabolism (Steinberg and Kemp, 2007; Potthoff et al., 2009). The ability of FGF21 to improve glucose homeostasis during obesity involves the stimulation of adiponectin secretion (Holland et al., 2013). In diet-induced obese mice, the insulin-sensitizing effects of FGF21 is due in part to the ability of adiponectin to prevent ceramide accrual (Holland et al., 2011; Holland et al., 2013). This was not observed in ad libitum fed non-pregnant, non-lactating Holstein cows intravenously infused a soybean oil emulsion (Caixeta et al., 2017; Krumm et al., 2017; Rico et al., 2018a). In the postpartum cow experiencing negative energy balance, circulating FGF21 levels are elevated but adiponectin concentrations are low (Schoenberg et al., 2011; Giesy et al.,

2012). We hypothesize that suppressed adiponectin release from adipose tissue during early lactation may contribute to activation of ceramide synthesis from saturated fatty acids. If observed, these findings would suggest that FGF21 does not modulate ceramide supply or increase insulin sensitivity in postpartum cows because of unresponsive adiponectin secretion and enhanced ceramide production.

Conclusions

Our current understanding of lipid biology points to fatty acids, not merely as energy dense sources to sustain milk production, but as bioactive nutrients that influence metabolism and health. The current thought is that saturated fatty acid reduce insulin sensitivity and shift energy partitioning away from body fat reserves and towards milk production. Increasing unsaturated fatty acid availability is likely to increase insulin-stimulated glucose utilization and nutrient utilization for body weight gain. What is unclear is how changes in insulin action and nutrient partitioning influence liver health, inflammation and immune response. We could hypothesize that decreasing insulin sensitivity during early lactation has potential to accelerate body weight loss and increase a cow's risk for developing fatty liver, ketosis or associated disorder. Such challenges may reduce fertility success and productive lifespan. We can also argue that increasing insulin sensitivity during the immediate postpartum is likely to reduce body fat mobilization and inflammation to optimize health. Beyond peak milk production, reducing insulin sensitivity in the cow may enhance nutrient partitioning towards the mammary gland to boost milk production. Because energy balance is restored by this stage of lactation, any potential detriments on health are unlikely to be observed. Future research is likely to answer these questions and delineate the role of individual fatty acids.

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Table 1. Studies of ceramide biology in ruminants

Experiment	Approach	Main outcomes
Rico et al. (2015)	Lean vs. overweight cows during the transition period	Plasma ceramides increased during the transition period, more so for overweight cows. Ceramides were positively related to circulating fatty acids and estimated insulin resistance.
Mathews et al. (2016); Rico et al. (2016)	Palmitic acid feeding at 4% dry matter to mid-lactation cows, relative to no-added fat	Palmitic acid feeding increased milk yield, and plasma fatty acids and ceramide while reducing glucose-stimulated fatty acid disappearance. Milk yields were positively related to plasma ceramides.
Rico et al. (2017b)	Lean vs. overweight cows during the transition period	Postpartum ceramide status was elevated, more so for overweight cows. Muscle ceramides accumulate postpartum. Ceramides were associated with direct measures of impaired insulin sensitivity.
Rico et al. (2017a)	Abomasal infusions of medium-chain triglycerides (8:0+10:0), palmitic acid, or stearic acid to mid-lactation cows	Plasma ceramides were highest in cows infused palmitic acid, relative to other fatty acids tested. Milk yields were positively related to plasma ceramides.
Davis et al. (2017b); de Souza et al. (2019)	Palmitic acid feeding at 1.5% dry matter to early lactation cows, relative to no-added fat	Palmitic acid feeding increased plasma ceramides during early lactation with accelerated body weight and body condition loss. Milk yields were positively related to plasma ceramides.
Davis et al. (2017a)	Ad libitum fed vs. feed-restricted non-pregnant, non-lactating cows	Feed-restriction increased serum ceramides and impaired insulin-stimulated glucose disposal.
Phipps et al. (2017)	Characterization of ceramides in bovine lipoprotein fractions	Ceramides are concentrated in bovine low-density lipoproteins.
Rico et al. (2018a)	Intravenous triacylglycerol or saline infusion to non-pregnant, non-lactating cows	Plasma and hepatic ceramide concentrations increased with circulating fatty acids and hepatic triacylglycerol deposition. Hepatic ceramide synthase 2 mRNA expression increased with triacylglycerol infusion.
McFadden et al. (2018)	Bovine primary neonatal hepatocytes treated with bovine serum albumin (vehicle), palmitic acid, or palmitic acid plus serine palmitoyltransferase inhibitor (myriocin)	Palmitic acid increased hepatocyte de novo ceramide synthesis by activating serine palmitoyltransferase.
Rico et al. (2018b)	Bovine primary differentiated adipocytes treated with cell-permeable ceramide or serine palmitoyltransferase inhibitor (myriocin)	Ceramide inactivates insulin signaling and insulin-stimulated 2-deoxyglucose uptake in bovine adipocytes.
Davis et al. (2019)	Lean vs. overweight cows during the transition period	Circulating ceramides within low-density lipoproteins accumulate postpartum.
Duckett et al. (2019)	Lambs fed no-added fat (control), flaxseed oil, or fish oil enriched in palmitoleic acid	Palmitoleic acid reduced serum very-long chain ceramides, relative to control or flaxseed oil.
Myers et al. (2019)	Abomasal infusion of palmitic acid, behenic acid, or fish oil enriched in DHA	Plasma very-long chain ceramides were elevated, relative to palmitic acid or DHA. DHA lowered muscle ceramides (unpublished).
Unpublished	Subcutaneous injection of recombinant bovine somatotropin to mid-lactation cows	Recombinant bovine somatotropin increased milk yield, reduced insulin-stimulated glucose disposal, and enhanced plasma ceramide concentrations.

Unpublished	Intravenous infusion of serine palmitoyltransferase inhibitor (myriocin) in feed-restricted ewes.	Adipose tissue lipolysis enhances de novo ceramide synthesis.

SESSION NOTES

The Intersection of Dietary and Milk Fatty Acids - How Photoperiod and Variability Affect Production

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Introduction

Diet-induced milk fat depression explains large decreases in milk fat that occur during disrupted rumen fermentation and was the predominant focus of milk fat research for nearly twenty-five years. More recently, research has focused on other dietary and non-nutritional factors that impact milk fat yield. Importantly, these factors have broad application to allow small, but very economically significant increases in milk fat yield and profitability. We have recently characterized the seasonal variation in milk component concentration and yield. It appears that two seasonal timekeepers are present in the cow with changes in milk fat and protein concentration driven by lengthening and shortening days (aligns with solstices) and changes in milk yield driven with the change in day length (aligns with equinoxes). Milk fat is a highly heritable trait and large variation exists between cows within a herd, although there does not appear to be much variation between herds. A number of other factors impact diet-induced milk fat depression including variation in fatty acid profile between corn silage hybrids and interactions with production level of cows. Lastly, variation in patterns of feed intake influence milk component levels and also variation in components between milkings. It is important to consider season, cow, diet, and time of day factors while setting goals, evaluating herd milk production, and designing diets and strategies to maximize milk fat yield.

Annual Rhythms in the Dairy Cow

Rather than simply *responding* to a change in the environment after it occurs, time keeping mechanisms in the hypothalamus allow the animal to *anticipate* yearly environmental changes before they occur. Annual rhythms are present in nearly all studied organisms as a mechanism to perceive and adapt to seasonal environmental changes. For example, migrating birds undergo astonishing changes in metabolism prior to spring and fall migration, including initiation of nocturnal activity and accretion of body fat reserves.

Yearly patterns of milk production have been recognized for over 40 years (Wood, 1970). Producers are familiar with summer declines in milk production, and recovery during the fall. When examining average monthly bulk tank records from the United States Federal Milk Marketing Orders, the presence of an annual rhythm is apparent. These yearly patterns fit a robust cosine function, suggesting that they

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represent a biological rhythm (Salfer et al., 2019). The variation in milk fat concentration due to the annual rhythm is between 0.15% and 0.30%, depending on the region with a lower amplitude in southern regions of the United States.

The presence of yearly production rhythms was confirmed using ten years of dairy herd improvement association (**DHIA**) data from individual herds in Minnesota, Pennsylvania, Texas and Florida (Salfer et al., 2017). Similar to the U.S. milk markets, milk fat and protein concentration peak around January 1 and reach a nadir on July 1 in Minnesota, Pennsylvania, and Texas. Florida, on the other hand, had the greatest fat concentration in November and greatest protein concentration in October. States in the northern U.S. have markedly greater amplitude rhythms of fat and protein concentration. For example, in Pennsylvania and Minnesota the difference between peak and trough for fat concentration was 0.32% and 0.28%, respectively, while Texas was 0.16% and Florida's was 0.08%.

Although fat and protein concentration both peak near the first of the year, the annual rhythm of milk yield peaks between late March and early April, right around the vernal equinox (Salfer et al., 2017). Fat and protein yield peak between late February and early March. Contrary to the rhythms of fat and protein concentration, amplitudes of annual milk yield rhythms are greater in the southern U.S. compared to the north. Fat and protein yield also oscillated more in the southern U.S. than the northern U.S.

Environmental temperature is often blamed for causing the seasonal changes in milk production. Granted it is certainly a factor, our results suggest that an annual rhythm exists independent of temperature (Salfer et al., 2017), with temperature expected to have a separate effect. It is important to note that when cows are placed in heat chambers milk yield decreases and milk fat percent increases, which is not consistent with the decrease in milk fat observed during the summer.

Potential Mechanisms of Seasonality

As discussed above, a primary role of annual rhythms is to coordinate reproduction with resource availability to maximize the likelihood of survival of the offspring. As an important component of reproduction, it is reasonable to expect that lactation is controlled through similar mechanisms. Producing more energy-dense milk with greater concentrations of fat and protein in the winter when energetic demands are greater may increase the likelihood of calf survival.

A consistent environmental factor that impacts the system is day length. It appears that two mechanism may have an impact on lactation with lengthening and shorting days regulating milk fat and protein concentration and the change in day length, that aligns with the equinoxes, regulating milk yield. Management of seasonal rhythms has not been specifically investigated and is complicated by the fact that the endogenous rhythms will be maintained in the absence of controlled lighting. Management of photoperiod for constant long-days is a well-established method to

increase milk and milk component yield (Dahl et al., 2012) and its impact on seasonality needs further exploration.

Other Factors Interacting with Milk Fat Yield

Variation in Milk Fat Between and Within Herds

Milk fat is variable between farms because of differences in diet, management practices, and herd genetics among other factors. Significant variation in milk fat composition exists within herds because of differences in stage of lactation, genetics, physiological state, feeding and ruminating behavior, and the interaction of these and other factors.

Interaction of Milk Production Level and Response to Diet

The relationship between milk fat concentration and milk yield is well demonstrated by a 905-cow example herd with low milk fat (herd average = 3.2%). The 25th and 75th percentiles of milk fat concentration were 2.6% and 3.6%, respectively. We have observed a negative relationship between milk yield and milk fat percent in multiple databases, although more work is required to understand the mechanism and implications. Decreased milk fat with increased milk yield may be due to dilution of milk fat in greater yields, but may also be due to some degree of diet-induced milk fat depression (**MFD**).

In several experiments we have observed variation in individual cow response to a MFD induction diet and that high-producing cows were more susceptible to MFD risk factors. For example, Harvatine and Allen (2006) compared saturated (highly saturated prilled free fatty acids [**FA**]; Energy Booster 100) and unsaturated (calcium salts of FA; Megalac R) FA supplements to a no supplemental fat control in low and high producing blocks of cows (milk yield in control treatment 39.4 vs. 47.0 kg/d, respectively). When fed the same control diet in the same barn, the low-producing cows averaged 3.45% milk fat whereas the high-producing cows averaged 3.05%. Additionally, the response to treatment differed with low-producing cows having a non-significant 6% decrease in milk fat when fed the calcium salts of unsaturated FA, whereas the high-producing cows decreased milk fat over 20%. This and more recent studies demonstrate that there is a strong correlation between the level of milk production and diet-induced MFD. The exact mechanism is unclear, but high-producing cows also have higher intakes. Increased intake is expected to increase rumen passage rate, which may modify the microbial population and increase ruminal outflow of *trans* intermediates before complete biohydrogenation has occurred. Additionally, high-producing cows may differ in feeding and ruminating behavior and increased meal size or higher amount of intake after feed delivery may result in rumen acidosis.

Genetics of Milk Fat and Milk Fatty Acid Profile

Milk fat concentration and yield are highly heritable [0.45 and 0.29, respectively; (Welper and Freeman, 1992)] and milk fat is unique in that the genetic variation is due to a limited number of single nucleotide polymorphisms (**SNPs**) with large individual effects (Hayes et al., 2010). The largest effect is a K232A SNP in diacylglycerol acyltransferase [**DGAT1**; (Grisart et al., 2002)] followed by the F279Y SNP in the growth hormone receptor [**GHR**; milk fat allele substitution effect 0.46 percentage units; (Signorelli et al., 2009)]. Wang et al. (2012) identified four quantitative trait loci that explained over 46% of the genetic variation in milk fat concentration including 34% explained by DGAT1 and 12% by GHR. We recently characterized the variation in predicted transmitting ability for fat production between nearly 6,000 herds available in the Dairy Records Management System database. Very little variation was observed between herds, although larger variation is observed between cows within a herd.

Variation in Corn Silage Fatty Acid Profile

Rumen available unsaturated FA are one of the largest risk factors for diet-induced milk fat depression. Nutritionists commonly select feeds based on expected FA concentration and profile, but unexpected variation can lead to issues. Although corn silage is low in dietary fat, its high feeding rate results in it contributing a large amount of unsaturated FA to the diet. We characterized the variation in corn silage FA concentration and profile in test plots in Pennsylvania and South Dakota. Varieties from the 10^{th} to the 90^{th} percentile differed in C18:2 by ~0.6% of dry matter. Fatty acid concentration is the larger contributor, but differences in FA profile also exist. In the future we may select corn silage hybrids that are low in C18:2 by selecting for higher C18:1. High oleic soybeans have similarly been shown to have a lower risk for diet-induced milk fat depression. Genetics are the largest contributor to corn silage FA profile and it is recommended that FA profile is determined for each crop and when diagnosing low milk fat.

Circadian Patterns

Circadian rhythms are daily patterns and the dairy cow has a daily pattern of feed intake and milk synthesis. Dairy producers commonly recognize that morning and evening milking differ in milk yield and composition. Gilbert et al. (1972) reported 1.4 lbs (0.64 kg) greater milk yield at the morning milking, but 0.32 and 0.09 percentage-unit greater milk fat and protein, respectively, at the evening milking in cows milked at 12-h intervals. More recently, Quist et al. (2008) conducted a large survey of the milking-to-milking variation in milk yield and composition on 16 dairy farms. Milk yield and milk fat concentration showed a clear repeated daily pattern over the 5 d sampled in herds that milked twice and thrice daily. We have also observed milk yield and milk composition across the day while milking every 6 h in multiple experiments. Feeding cows in four equal feedings every 6 h increased milk fat and decreased the amplitude of milk fat concentration and yield across the day (Rottman et al., 2014). More recently we have observed that fasting cows for 6 h during the day versus during the night shifts the daily pattern of milk synthesis. These experiments demonstrate that the daily rhythm of milk

synthesis is dependent on the timing of intake and highlight the importance of selecting feeding times and frequency on milk synthesis.

Conclusions

Milk fat yield is impacted by many nutritional and non-nutritional factors and their interactions. Diet-induced milk fat depression explains large decreases in milk fat, but does not explain every change in milk fat yield. The season of the year should be considered when setting goals and evaluating herd performance, but it is unclear if we can overcome this pattern through management. We should also consider genetic potential, stage of lactation, and milk yield when evaluating individual cow performance. Lastly, appreciating the impact of feeding behavior on rumen fermentation and milk fat provides additional opportunities to increase milk fat yield through management. The advances in our understanding of the biology and mechanism regulating milk fat synthesis over the past twenty-five years provide many insights, but the interaction of factors makes predictions difficult and requires careful development of strategies to optimize milk fat yield on farm.

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SESSION NOTES

Fatty Acids and Fertility – The Contributions of Dr. Charlie Staples

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Introduction

Dr. Charles (Charlie) Richard Staples accepted an Assistant Professor position at the University of Florida UF) in 1985. However, he began his industrious and interdisciplinary career during his postdoctoral program with Dr. Jimmy Clarke at the University of Illinois. We discussed the dynamics and importance of the postpartum period relative to restoration of reproductive competence in association with changes in energy balance and restoration of ovarian activity. It was a time at Florida when we were well on our way of being able to control the time of artificial insemination and had methodologies for hormonal and metabolic assays and the use of ultrasound to monitor ovarian activity and presence of the fetus. Furthermore, we realized that many cows presented for controlled breeding postpartum were anovulatory that contributed to poor herd fertility. Dr. Clarke commented that they were doing an extensive postpartum nutrition experiment at Illinois dealing with energy balance, and Charlie suggested measuring progesterone to establish postpartum patterns of ovarian activity. He collected samples throughout the experiment and progesterone measurements were analyzed at Florida. Thus, his inquisitive mind, willingness to share with others, and the value of interdisciplinary programs began early and was sustained throughout his academic career as a research scientist, teacher, mentor and sought-after speaker in the area of extension as well.

Postpartum Responses to Fat Supplementation

This first experiment introduced the importance of nutrition in utilization of dietary fats. Progesterone profiles in plasma were categorized such that anovular cows were compared with two cycling groups of 25 cows undergoing corpus luteum (**CL**) activity within 40 d after parturition and a second group of 14 cows expressing CL activity between 40 and 60 d postpartum. *Anovular cows ate less feed, produced less milk, and lost more body weight, resulting in a more negative energy status than cycling cows.* Differences in energy balance among cow groups were greatest the first 3 weeks. postpartum. Anovular cows obtained more energy from body reserves for milk production the first 2 weeks of lactation (i.e., greatest negative energy balance), than cows cycling prior to d 40. This focused our attention to the importance of potential nutritional strategies to be targeted in the peripartum and postpartum periods (Staples et al., 1990).

Charlie partnered with Matt Lucy a PhD student in my laboratory in those early years focusing on ovarian follicular dynamics in response to dietary supplementation

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with Ca salts of long-chain fatty acids (**Ca-LCFA**) for the first 60 days postpartum (Lucy et al., 1991). On d 25 postpartum, cows were injected with 25 mg of prostaglandin $F_{2\alpha}$ and treated for 15 d with an intravaginal controlled-internal drug release (**CIDR**) insert containing 1.9 g progesterone. During the first estrous cycle after CIDR removal, the average size of the largest (18.2 vs. 12.4 mm) and second largest (10.9 vs. 7.4 mm) follicles were greater in cows fed Ca-LCFA compared with control cows. Cows fed Ca-LCFA had more class 1 and 4 and fewer class 2 follicles during the estrous cycle after CIDR removal (**Figure 1**). The ability of larger follicles to influence the growth of smaller follicles is a phenomenon known as follicular dominance. Lactating cows fed Ca-LCFA had a reduction in the number of Class 2 medium-sized follicles indicative of the presence of large healthy dominant preovulatory follicles. Within this experimental model feeding of CA-LCFA stimulated follicular growth in restoration of estrous cycles in the postpartum period.

Charlie and his student Carlos Garcia-Bolalil demonstrated clearly that postpartum reproductive and metabolic responses were altered when multiparous Holstein cows were assigned randomly at calving to one of four treatment diets arranged in a 2 by 2 factorial (Garcia-Bolalil et al.,1998a;1998b). Factors were two dietary concentrations of ruminally degradable protein, 11.1 or 15.7% of dry matter (**DM**), and supplemental fat as Ca-LCFA from palm fatty acids distillated fed at 0 or 2.2% of dietary DM. Cows fed excess ruminally degradable protein had less ovarian follicular development, delayed first postpartum luteal activity (25.2 vs. 38.6 d), accumulated less luteal tissue, and had lower plasma progesterone concentrations. These changes were likely mediated by the greater body weight loss, increased plasma concentrations of nonesterified fatty acids and reduced concentrations of plasma insulin. In dairy cows fed a 15.7% degradable protein diet, supplementing Ca-LCFA doubled the number of CL, reduced the interval to first rise in progesterone by 6 d, doubled the number of normal luteal phases, and restored the pattern of accumulated plasma progesterone concentrations similar to that induced by lower ruminally degradable protein diets (**Figure 2**). Accumulated percent of cows pregnant by 120 d postpartum increased from 52.3 to 86.4% by supplemental fat. Therefore, in cows fed excess ruminally degradable protein, feeding Ca-LCFA diminished adverse changes in body weight, attenuation in ovarian activity, and pregnancy rate.

Meta-analysis was used to integrate a cross-section of smaller experiments and increase the statistical power over any single study to test the effects of dietary fats and diet composition on fertility using meta-regression method (Rodney et al., 2015). A total of 17 experiments (*i.e., 4 experiments contributed from Charlie Staples' program*) containing 26 comparisons were suitable for inclusion in statistical evaluations. Reproductive variables evaluated were risk of pregnancy (proportion pregnant), primarily to first service. A 27% overall increase in pregnancy to service was observed (relative risk = 1.27; 95% confidence interval = 1.09 to 1.45), and results were relatively consistent (small heterogeneity, I^2 = 19.9%). A strong indication of a reduction in calving to pregnancy interval was also identified, which was consistent across studies, supporting a conclusion that, overall, the inclusion of fats does improve fertility.

Fat and Fatty Acid Effects on Uterine Secretion of Prostaglandins

Prostaglandins (**PG**) are secreted profusely during two reproductive windows of the dairy cow: the peripartum period for 2 weeks after parturition and during subsequent estrous cycles associated with either regression or maintenance of the corpus luteum in cyclic or pregnant cows, respectively. One intensive area of investigation was whether fat feeding and specific fatty acids would modulate secretion of prostaglandins and other endocrine modulators in a manner that may subsequently benefit lactating cow lactational and reproductive performance.

A series of *in vitro* experiments were performed with uterine bovine endometrial (**BEND**) cells incubated for 24 h with fatty acids and then treated with phorbol ester to stimulate $PGF_{2\alpha}$ secretion (Mattos et al., 2003). The BEND cells were incubated with no fatty acid (control) and a variety of fatty acids that included C18:1n9, C18:2n-6, C18:3n-3, C20:4n-6, C20:5n-3, and C22:6n-3 at a concentration of 100 μ*M*. Only the omega-3 fatty acids (C18:3n-3, C20:5n-3, and C22:6n-3) suppressed synthesis of $PGF_{2\alpha}$, with C20:5n-3 and C22:6n-3 being the most suppressive (**Figure 3**). An additional experiment compared the inhibitory effects of the C20:5n-3 fatty acid with the bovine interferon-tau protein produced by the 17 to 25-day old embryo. Both suppressed $PGF_{2\alpha}$ secretion in this *in vitro* BEND cell system. These results raise the possibility that feeding diets enriched with omega 3-fatty acids that alter tissue composition may have an additive effect to improve pregnancy per insemination and reduce pregnancy losses. This concept is largely substantiated by *Charlie and his team of collaborators* in subsequent nutrition-reproduction experiments with lactating dairy cows.

One of the initial *"Charlie proofs"* of a fatty acid nutraceutical concept with lactating dairy cows was abomasum infusions of emulsions and solutions of either water, glucose (1 kg/d), tallow (0.45 kg/d) or yellow grease (0.45 kg/d) continuously (11.5 mL/minute) over a 16-h period each day. This was carried out as a 4 by 4 Latin square design with each period lasting 35 days: 14 d allowed for adjustment of treatments and 21 days allowed for data collection during a synchronized estrous cycle (Oldick et al., 1997). Plasma progesterone concentration peaked higher during the estrous cycle for cows infused with fat than for those infused with glucose. Mean growth rate and maximum size of the first wave dominant follicle were greater with tallow than with yellow grease. During the period of infusion of yellow grease and afterward, release of the metabolite of $PGF_{2\alpha}$, 13,14-dihydro-15-keto-PGF2α (**PGFM**) in response to an injection of oxytocin on d 15 of the estrous cycle was attenuated.

An additional study fed cycling multiparous cows (n = 32) diets containing menhaden fish meal: 0, 2.6, 5.2 or 7.8% fish meal for 56 days (Mattos et al., 2002). At day 15 of a synchronized estrous cycle (i.e., at 56 days of feeding the treatment diets) cows were injected with estradiol-17*β* (3 mg, i.v.) at 0900 h and oxytocin (100 IU, i.v.) at 1300 h. Plasma PGFM concentrations after oxytocin injection were reduced in cows fed diets containing fish meal compared with those fed no fish meal. Milk production (39.1 kg/d) and concentrations of fat, protein, or urea nitrogen in milk were not affected by diet. Feeding fish meal and fish oil containing C20:5n-3 and C22:6n-3 fatty acids

reduced the proportion of n-6 fatty acids and increased that of n-3 fatty acids in milk in a dose-responsive manner.

Whether feeding of omega-3 fatty acids would suppress synthesis of $PGF_{2\alpha}$ at a time when dairy cows naturally produce copious amounts of $PGF_{2\alpha}$, at the time of parturition was evaluated (Mattos et al., 2004). Holstein cows were fed diets containing either olive oil or fish oil (1.8 to 2% of dietary DM) from 21 days before expected calving date through 21 days postpartum. Blood samples were collected daily and analyzed for PGFM. Concentrations of C20:5n-3 and C22:6n-3 in caruncular tissue collected within 12 h of parturition were increased 5 to 6-fold in cows fed fish oil. Cows fed fish oil had reduced concentrations of PGFM in plasma during the period of maximum secretion of uterine $PGF_{2\alpha}$ in the early postpartum period compared with cows fed olive oil. Differences were significant ($P < 0.05$) at 0, 0.5, 2, and 2.5 days postpartum (**Figure 4**). The increased concentrations of C20:5n-3 and C22:6n-3 in caruncular tissue of cows fed fish oil suggest that these fatty acids may be the active compounds reducing secretion of uterine $PGF_{2\alpha}$.

Fats and their Nutraceutical Roles to Improve Reproductive Performance

Several characteristics and components of fats possibly contribute to inherent effects on reproduction. From an energy perspective, fat sources contain 2.5 times the caloric density of carbohydrate sources. Also, the polyunsaturated fatty acids such as linoleic acid (C18:2n-6), α-linolenic acid (C18:3n-3), eicosapentaenoic acid (**EPA**; C20:5n-3) and docosahexaenoic acid (**DHA**; C22:6n-3), and *trans* fatty acids partially escape rumen microbial biohydrogenation and then are absorbed in the small intestine into the blood stream. These absorbed long chain fatty acids are deposited into cells such as leucocytes or tissues such as endometrium, liver, and adipose depots (Bilby et al., 2006; Silvestre et al., 2011a).

In a Florida experiment, Silvestre et al. (2011a; 2011b) randomly allocated cows (n = 1,083) into two experimental transition diets beginning at approximately 30 days before the expected day of parturition and continued until 30 days postpartum. After 30 days, cows within each transition diet were allocated randomly into the experimental breeding diets that were fed until 160 days postpartum. Experimental transition and breeding diets differed only in the source of supplemental fatty acids. Transition diets consisted of Ca-LCFA containing palm oil (**PO**; EnerGII; 47% C16:0) or safflower oil (**SO**; Prequel 21; 64% C18:2n-6). The breeding diets consisted of PO or Ca-LCFA containing fish oil (**FO**, StrataG; 11% of C20:5n-3 + C22:6n-3). All Ca-LCFA were supplemented at 1.5% of the dietary DM. The combinational experimental diets fed during the transition and breeding periods were PO-PO, PO-FO, SO-PO, and SO-FO, respectively. Reproductive performance was evaluated extensively for first and second postpartum artificial inseminations after synchronizing the estrous cycles of cows. Neutrophil ratios of n-6:n-3 fatty acids were greater at 35 days postpartum in the SO diet and less at 85 days postpartum in FO compared with PO diets. Cows supplemented with Ca-LCFA containing SO had improved innate measures of immunity during the transition period (i.e., acute phase response, neutrophil function, and

cytokine production) to better cope with the bacterial challenges at calving. Conversely, cows fed Ca-LCFA containing FO had attenuated neutrophil cytokine production at 85 days postpartum, at the time of potential fertilization following a timed artificial insemination.

Transition and breeding diets did not affect pregnancies per artificial insemination at 32 and 60 days after the first insemination (**Table 1**). Pregnancy loss from days 32 to 60 after first timed insemination was less ($P < 0.05$) for cows fed FO compared with that of cows fed PO. At second service (**Table 1**), breeding diet altered ($P < 0.05$) pregnancy per artificial insemination on day 32 and a tendency ($P = 0.10$) for a dietary interaction was detected between transition and breeding diets. The increase in pregnancy per insemination on day 32 caused by feeding FO was greater in cows fed the transition diet containing SO, whereas no increase in pregnancy per insemination in cows fed the PO breeding diet was found, regardless of transition diet. Likewise, a similar transition by breeding diet interaction ($P < 0.05$) was detected for the 60-day pregnancy per insemination. Accumulated proportion of cows pregnant (i.e., all artificial inseminations; **Table 1**) on day 32 after timed artificial insemination was not affected by transition or breeding diets, but tended to be significant ($P = 0.10$) for the interaction of diets. Accumulated proportion pregnant on day 60 after timed artificial insemination was not affected by transition diets, but it was greater ($P < 0.01$) for cows supplemented with FO compared with those supplemented with PO during the breeding period, and a tendency ($P = 0.07$) was observed for interaction between transition and breeding diets. Accumulated pregnancy losses were not affected by transition diets, but were less ($P < 0.01$) in cows supplemented with FO compared with those supplemented with PO). The greater pregnancy per artificial insemination at 32 and 60 d after the second service for FO-fed cows was detected partially during the warm season of the year and appeared to be preferentially beneficial when FO-supplemented cows were fed SO during the transition period before FO feeding (i.e., transition and breeding diet by season interaction). One possibility contributing to the interaction with season was that fewer cows received their second artificial insemination during the cool season (n = 193) than during the warm season (n = 411) of the year.

Sinedino et al., (2017) evaluated the effects of supplementing docosahexaenoic acid (C22:6n-3)-rich algae on reproduction of dairy cows. Holstein cows were assigned randomly to either a control (n = 373) or the same diet supplemented daily with 100 g/cow of an algae product containing 10% C22:6n-3 (algae, n = 366) from 27 to 147 days postpartum. Feeding algae increased resumption of estrous cyclicity (77.6 vs 65.9%) and pregnancy at first artificial insemination (47.6 vs 32.8%) in primiparous cows. Algae increased pregnancy per artificial insemination for all inseminations in both primiparous and multiparous cows (41.6 vs 30.7%), which reduced days to pregnancy by 22 days (102 vs 124 days) compared with control cows. Pregnant cows fed algae had greater expression of *RTP4* in blood leukocytes on day 19 after insemination compared with those in pregnant control cows. The change in expression of *RTP4,* a gene stimulated by conceptus interferon-tau, in cows fed C22:6n-3 rich supports an advancement of day 19 conceptuses in those cows. More advanced conceptuses would produce greater amounts of interferon-tau that would in turn stimulate interferon induced

genes such as *RTP4*. This would be emblematic of an overarching mechanism of cross-talk between the conceptus and maternal unit for embryo development and maintenance of pregnancy. The lack of an algae feeding effect on pregnancy per artificial insemination at the beginning of the breeding period in multiparous cows may be due to the amount of supplemental fatty acids fed was insufficient and longer feeding is necessary in cows with a larger body size and greater milk yield. Indeed, after 120 days of treatments, feeding algae increased pregnancy per artificial insemination and reduced days to pregnancy in both multiparous and primiparous cows. Feeding algae increased the incorporation of C20:5n-3, C22:6n-3, conjugated linoleic acid isomers *cis*-9 *trans*-11, *trans*-10 *cis*-12 and total n-3 fatty acids in phospholipids in plasma and milk fat. Yields of milk and true protein increased by 1.1 kg/day and 30 g/day respectively, whereas fat yield decreased 40 g/day in algae compared with control cows. Supplementing C22:6n-3 rich algae altered the fatty acid composition of lipid fractions and improved reproduction in dairy cows. The benefits on reproduction might be mediated by enhanced embryo development based on changes in interferon-stimulated gene expression.

Zenobi et al (2018) hypothesized that supplementation of ruminally protected choline (**RPC**) in the periparturient period would preferentially benefit those multiparous dairy cows that were more prone to develop a fatty liver postpartum. Experimental objectives were to evaluate the effect of prepartum energy intake on performance of dairy cows supplemented with or without ruminally protected choline (RPC; 0 or 12.9 g/d of choline ion in the form of choline chloride in a rumen-protected form; 0 or 60 g/d of ReaShure, Balchem Corp., New Hampton, NY). At 47 ± 6 days before the expected calving date, 93 parous Holstein cows were assigned randomly to 1 of 4 dietary treatments utilizing a 2 × 2 factorial arrangement of treatments. Cows were fed energy to excess (EXE; 1.63 Mcal of net energy for lactation/kg of diet DM) or to maintenance (MNE; 1.40 Mcal of net energy for lactation/kg of die DM) in *ad libitum* amounts throughout the dry period. The RPC was top-dressed from 17 ± 4.6 d prepartum through 21 d postpartum. After calving, cows were fed the same postpartum diet for the first 15 weeks of lactation that was supplemented with a blood-meal product enriched with lysine and methionine. Liver tissue was sampled at −14, 7, 14, and 21 d relative to parturition. Cows fed EXE or MNE diets, respectively, consumed 40 or 10% more Mcal/d than required at 15 d before parturition. Cows fed the MNE compared with the EXE diet prepartum consumed 1.2 kg/d more DM postpartum but did not produce more milk (41.6 vs.43.1 kg/d). Thus, postpartum cows fed the EXE diet prepartum were in greater mean negative energy balance, tended to have greater mean concentrations of circulating insulin, fatty acids, and β-hydroxybutyrate, and had greater triacylglycerol in liver tissue (8.3 vs. 10.7% of DM) compared with cows fed the MNE diet prepartum.

Cows fed RPC in transition tended to produce more milk (43.5 vs. 41.3 kg/d) and energy-corrected milk (44.2 vs. 42.0 kg/d) without increasing DM intake (23.8 vs. 23.2 kg/d) during the first 15 weeks postpartum. Supplementing RPC to cows resulted in a tendency for increased milk yield over the first 40 weeks postpartum (37.1 vs. 35.0 kg/d), although RPC supplementation stopped at 21 d postpartum. Also, response to RPC was observed regardless of amount of energy consumed during the dry period.

Energy balance of cows fed RPC was more negative at weeks 2, 3, and 6 postpartum, but mean circulating concentrations of fatty acids and β-hydroxybutyrate did not differ from those of cows not fed RPC. Despite differences in energy balance at 2 and 3 weeks postpartum, mean concentration of hepatic triacylglycerol did not differ between RPC treatments. Feeding RPC reduced the daily prevalence of subclinical hypocalcemia from 25.5 to 10.5%, as defined by concentrations of total Ca of <8.0 mg/dL in serum in the first 7 d postpartum. RPC treated cows tended to produce more milk over the first 40 weeks postpartum (37.1 vs. 35.0 kg/d) regardless of amount of energy consumed during the pregnant, nonlactating period.

Supplementation with RPC did not influence the proportion of cows cycling by 40 ±3 DIM (80.5 vs. 80.9% for −RPC and +RPC treatments, respectively). Feeding RPC tended to increase the proportion of cows pregnant at first artificial insemination (P = 0.09; 41.3 vs. 23.6%) but not at 40 weeks postpartum (69.8 vs. 62.5%). The hazard of pregnancy for +RPC and −RPC cows was 1.29 (95% CI = 0.44 to 1.35). The number of days open were 143 versus 123 d for −RPC and +RPC groups, respectively. Cox's proportional analysis of average days to pregnancy from the end of the voluntary waiting period until 210 DIM indicated that treatments did not affect (P = 0.14) rate at which cows became pregnant (**Figure 5**). Collectively, if these coordinated effects programed by RPC in the transition period were confirmed in subsequent experiments by Charlie's group at Florida (Arshad et al., 2020; Bollatti et al., 2020). His work reinforces the case for choline as an essential nutrient during the transition period for high-producing dairy cows.

Programming Effect of Dietary Fatty Acids on Performance of Holstein Heifers from Birth Through First Lactation: "Fatty Dancing: Mom and the Kids"

The concept that strategic feeding dairy cows' specific classes of fatty acids during late pregnancy would have potential programming effects on the newborn calf manifested during subsequent first lactation is a rather novel and insightful concept put forth by *Charlie* and his talented PhD student Dr. Miriam Garcia. This hypothesis was tested with supplementation of essential fatty acids, primarily linoleic acid (C18:2n-6), to prepartum cows during the last 2 months of pregnancy and to their newborn heifer calves. Productive and reproductive responses of heifers were followed for their first 3 years of life (Garcia et al., 2016).

During the last 8 weeks of pregnancy, Holstein cattle (n = 96) were fed either no fat supplement (**CTL**), a saturated fatty acid (**SFA**) supplement enriched in C16:0 and C18:0, or an unsaturated fatty acid supplement enriched in linoleic acid (**EFA**). Newly born heifers (n = 56) from these dams were blocked by dam diet and fed a milk replacer for 60 d with either a low linoleic acid (**LLA**) or high linoleic acid (**HLA**) concentration. The milk replacer was the sole feedstuff during the first 30 days of age. A grain mix with minimal linoleic acid was offered between 31 and 60 days of life. Profile of fatty acids in colostrum reflected that of dam diets. Profiles of fatty acids in plasma of heifers at 30 and 60 days of age reflected those of milk replacers consumed. Heifers fed HLA compared with LLA milk replacer had increased proportions (g/100 g of fatty acids) of

linoleic acid (45.8 vs. 40.7, $P < 0.01$), α-linolenic acid (0.83 vs. 0.66, $P < 0.01$) and lowered proportions ($P < 0.01$) of C12:0 and C14:0 in plasma. The maternal diet fed prepartum also did not change the plasma metabolic profile and productive performance of heifers, but heifers fed HLA compared with LLA milk replacer had or tended to have increased concentrations of anabolic metabolites and hormones coupled with a better body weight gain and greater conversion of dry matter intake into body weight. Heifers born from dams supplemented with fat prepartum tended to have a greater number of artificial inseminations at first pregnancy (2.53 vs. 1.85, P = 0.08). After correcting for predicting transmitting ability of the parents and body weight at calving, heifers born from dams supplemented with fat tended to produce more milk (9,100 vs. 8,415 kg, $P = 0.10$) and more protein (277 vs. 256 kg, $P = 0.09$) in a 305-day standardized lactation. Heifers fed HLA instead of LLA milk replacer produced more milk protein (283 vs. 258 kg, $P = 0.04$) and tended to produce more fat (350 vs. 319 kg, $P = 0.10$), coupled with a numerical increase in total milk yield (9161 vs. 8582 kg, $P = 0.14$). The increased number of artificial inseminations of primiparous diagnosed as pregnant by 300 DIM that were born from dams supplemented with fat (1.61 vs. 2.43) was not significant ($P = 0.14$). **Figure 6** shows the lactation curves for the effect of dam diet. Major effects ($P < 0.10$) due to dam diet were observed in early (weeks 7 to 11) and late lactation (weeks 33 to 43). These weekly milk yields were greater for heifers born from dams fed EFA compared to CTL. The SFA response was intermediate between CTL and EFA. The intermediate differences between SFA with control or EFA were only numerical. Strategic feeding of fatty acids during late uterine life and preweaning appears to enhance milk production of heifers in their first lactation. Future studies evaluating the impact of fat and fatty acid supplementation at different physiological stages of dairy animals on future productive and reproductive efficiency are warranted.

Conclusion

Dr. Charles Richard Staples developed and provided leadership to a basic and applied program in ruminant nutrition that has bridged the disciplines of applied ruminant nutrition, nutritional physiology, statistics, endocrinology and molecular biology. His integrated research efforts have focused primarily on strategic management of dietary nutrients to positively impact reproductive, mammary, digestive, and metabolic tissues to coordinate health, well-being and production performance of lactating dairy cows. He has accomplished this through his leadership as a nutritionist, strong functional participation in interdisciplinary programs, mentorship of graduate students in ruminant nutrition and associated programs at the University of Florida. Furthermore, he has team taught in 3 undergraduate and 2 graduate courses. His breadth of vision, understanding and application to dairy cattle production and management is evident in this presentation today dealing solely with *fatty acids and fertility*. Improved fertility has been achieved through feeding dietary fatty acids leading to recrudescence of ovarian follicle development, ovulation, and programing of the corpus luteum/uterine tissues to support fetal development and programing of both the calf and lactating maternal unit. A presentation on *"Fats and Fertility"* is only one

cornerstone of many in describing Dr. Charles Staples contributions in ruminant nutrition. His humble and giving personality combined with scientific excellence has contributed to his priorities in life: Love of Family, Faith in God and Mankind, and Science.

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Table 1. First, second, and accumulated pregnancy (% and number of cows) per AI at 32 and 60 d after AI and pregnancy loss of cows fed fat supplements in 4 different sequences (Silvestre et al., 2011a)

	Diet[1]				Diet contrast[2] (*P*-value)		
AI	PO-PO	SO-PO	PO-FO	SO-FO	C1	C2	C3
First AI							
d 32	38.7 (107/276)	35.8 (96/268)	39.1 (103/263)	35.8 (89/248)	NS	NS	NS
d 60	33.7 (92/273)	29.7 (79/266)	37.0 (97/262)	32.8 (81/247)	NS	NS	NS
Loss	11.5 (12/104)	15.9 (15/94)	4.9 (5/102)	7.9 (7/88)	NS	<0.05	NS
Second AI							
d 32	27.7 (43/155)	26.7 (41/154)	30.3 (44/154)	43.3 (65/150)	NS	<0.05	0.10
d 60	21.0 (38/152)	22.5 (34/151)	27.3 (39/143)	41.3 (62/150)	NS	<0.01	<0.05
Loss	5.0 (2/40)	10.0 (4/38)	7.1 (3/42)	4.6 (3/65)	NS	NS	NS
All AI							
d 32	54.4 (152/279)	50.5 (138/273)	53.8 (147/273)	59.5 (154/259)	NS	NS	0.10
d 60	48.3 (132/273)	42.5 (114/268)	50.3 (136/270)	55.4 (143/258)	NS	<0.01	0.07
Loss	9.6 (14/146)	14.3 (19/133)	5.5 (8/144)	6.5 (10/153)	NS	<0.01	NS

[1]PO (palm oil; EnerGII); SO (safflower oil; Prequel 21); FO (fish oil; StrataG). All fat supplements were manufactured as calcium salts by Virtus Nutrition, LLC, Corcoran, CA.

[2]Contrasts are C1 (transition diets; PO-PO + PO-FO vs. SO-PO + SO-FO); C2 (breeding diets; PO-PO + SO-PO vs. PO-FO + SO-FO); and C3 (interaction of diets; PO-PO + SO-FO vs. PO-FO + SO-PO). NS = nonsignificant.

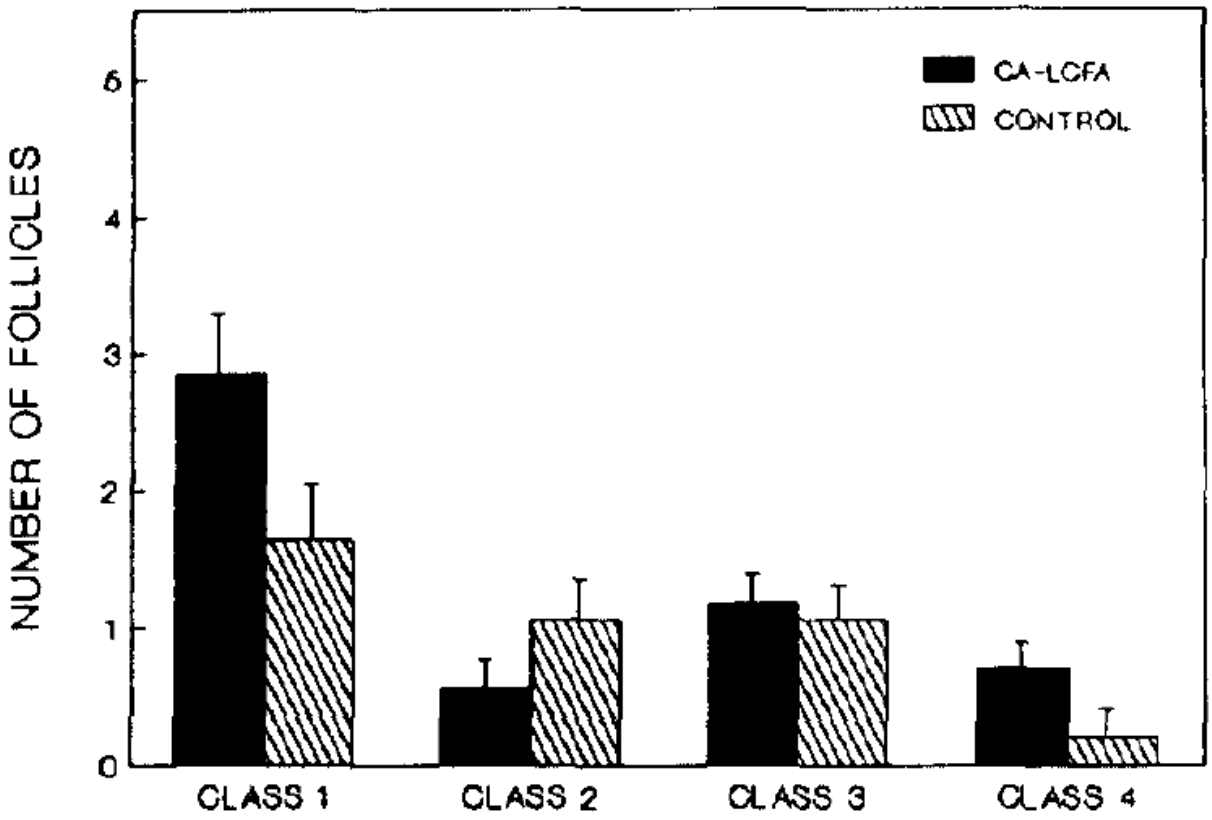

Figure 1. Average number of follicles within different follicle *size* classes (class 1,3 to 5 mm; class 2,6 to 9 mm; class 3, 10 to 15 *mm;* class 4, >15 mm) during an estrous cycle (d 6, 12, and 18) after removal of the CIDR insert for lactating dairy cows fed diets with (CA-LCFA) and without (CONTROL) calcium salts of long-chain fatty acids. Adapted from Lucy et al. (1991).

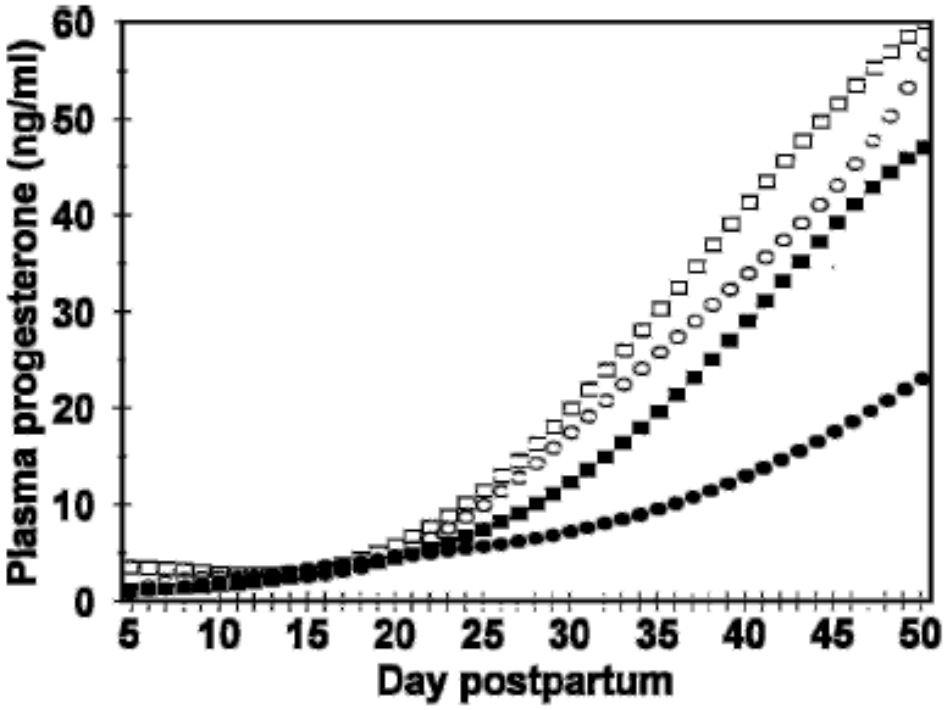

Figure 2. Fifth-order polynomial regression curves of accumulated plasma progesterone concentrations (ng/mL) from lactating Holstein cows fed diets containing 11% degradable intake protein (DIP) and 0% Ca salts of long-chain fatty acids (□), 15.7% DIP and 0% Ca salts of long-chain fatty acids (●), 11.1% DIP and 2.2% Ca salts of long chain fatty acids (○), and 15.7% DIP and 2.2% Ca salts of long chain fatty acids (■). An interaction (*P* = 0.001) between DIP and Ca salts of long chain fatty acids was detected. The standard error of the mean was 0.9. Adapted from Garcia-Bojalil et al. (1998b).

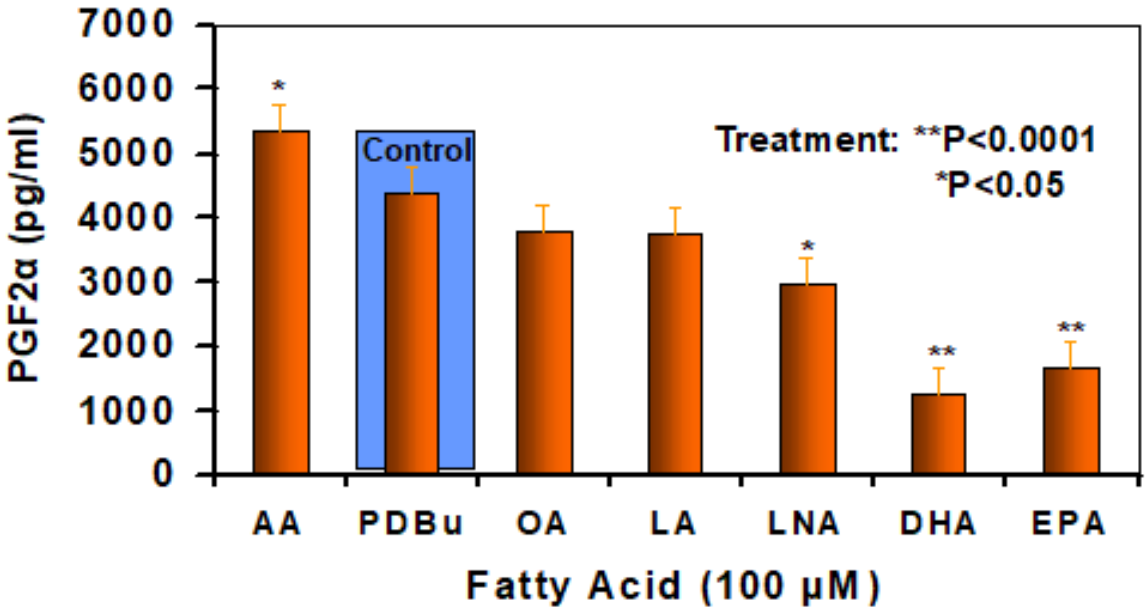

Figure 3. Synthesis of prostaglandin (PG) $F_{2\alpha}$ by bovine endometrial cells incubated with a variety of fatty acids. AA = arachidonic acid; OA = oleic acid; LA = linoleic acid; LNA = linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid. Difference between each fatty acid and control: *P < 0.05; **P <0.01. Adapted from Mattos et al. (2003).

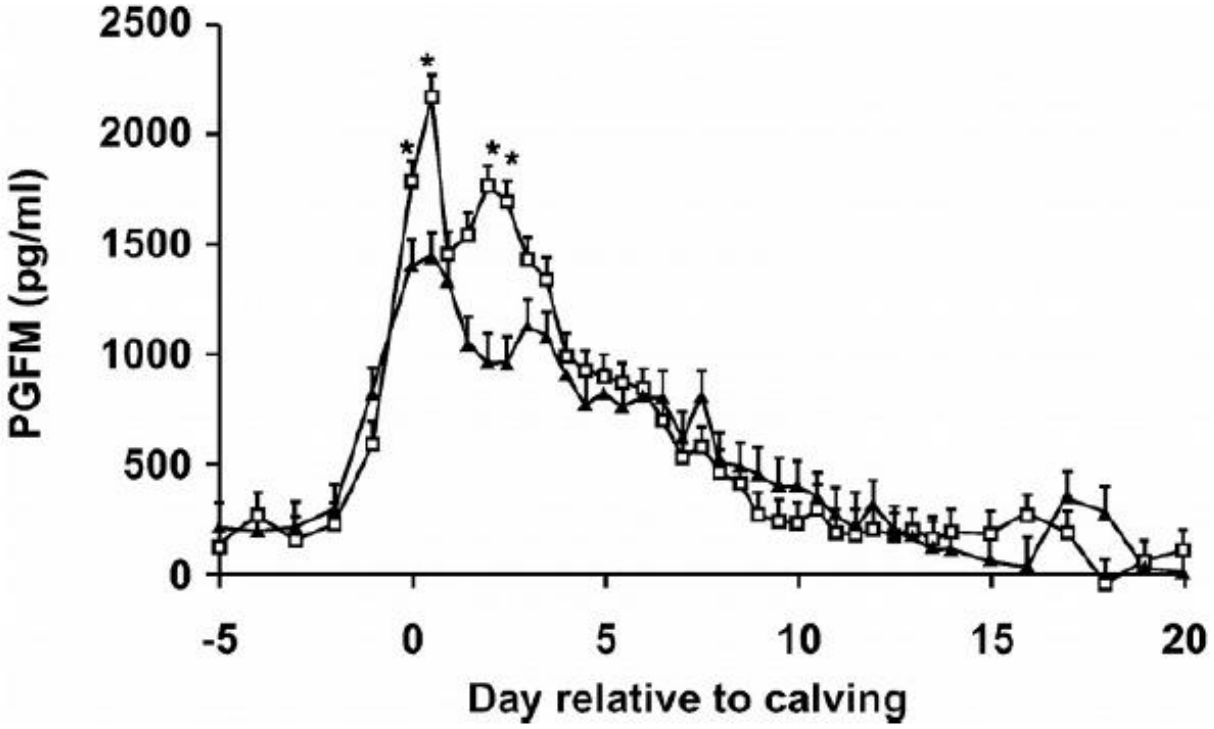

Figure 4. Pre- and postpartum plasma concentrations of prostaglandin$F_{2\alpha}$ metabolite (PGFM) of cows fed fish oil (▲, n = 6) or olive FM concentrations were lower in fed fish oil at 0, 0.5, 2, and 2.5 d after parturition (*, $P < 0.05$). Adapted from Mattos et al. (2004).

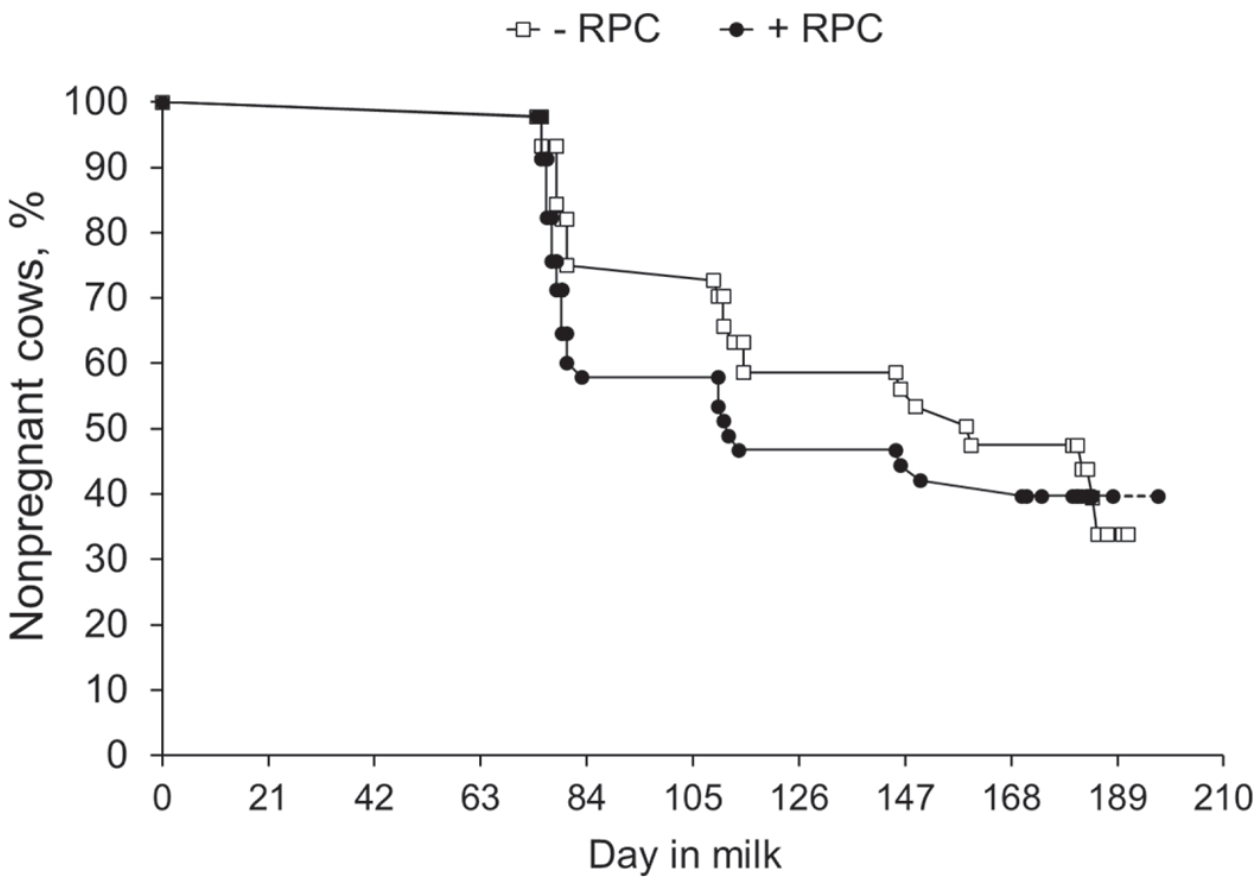

Figure 5. Survival curves for days to pregnancy up to 210 DIM for multiparous Holstein cows supplemented with or without ruminally protected choline (+RPC or –RPC, respectively). Only cow's synchronized and artificial insemination were included in the analysis (n = 91). Effect of RPC (P= 0.14) was not detected. Average (143 vs. 123) and median (160 vs. 112) days to pregnancy for −RPC and +RPC treatments, respectively. Hazard ratio was 1.29 (95% CI = 0.74–2.26). Pregnancy by 210 DIM was 55.4 vs. 59.7% for –RPC vs +RPC, respectively. Adapted from Zenobi et al. (2018).

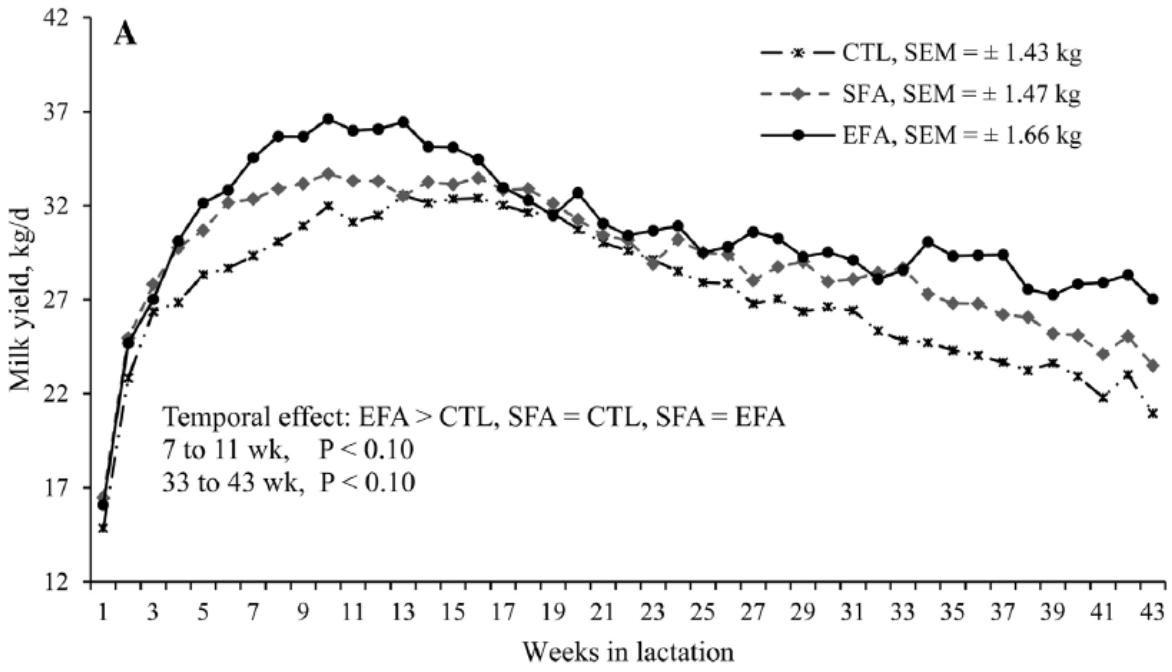

Figure 6. Effect of prepartum dam diets (A) and milk replacer (B) on lactation curves of primiparous Holstein cows fed milk replacer containing low linoleic acid (LLA) or high linoleic acid (HLA) when baby calves from 1 to 60 days of age. Primiparous cows were born from dams fed diets supplemented with no fat (CTL), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 weeks before expected calving date. Adapted from Garcia et al. (2014).

Factors that Modify Rumen Fatty Acid Flow Versus Feed Input

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Introduction

Dietary fatty acids undergo significant structural changes via a process called biohydrogenation as they pass through ruminal contents and are delivered to the intestines for absorption. A significant portion of milk fat yield, which is a primary driver of milk income, is guided by the direction of these biohydrogenation pathways. Changes in nutritional composition of the feed, brought about either by design or inadvertently because of nutritional variation in feed ingredients, can shift biohydrogenation pathways causing changes in rumen fatty acid outflow of bioactive lipids that adversely affect milk fat synthesis. Therefore, identifying the trigger that shifts fatty acid biohydrogenation in the rumen from “milkfat friendly” to “milkfat unfriendly” is of upmost importance. \

The intention of this paper is to offer a possible trigger mechanism that initiates the rumen microbial population to shift its pathways of biohydrogenation toward a direction unfavorable for milk fat synthesis. Much of the direct evidence for the trigger is revealed from recent studies of isolated ruminal bacteria, in vitro rumen cultures, and cow data. Data across these studies suggests that when dietary fatty acids, coming from both the basal diet and from added fat, reaches a level sufficient to cause antibacterial effects in the rumen the result is a shift from normal biohydrogenation to an alternate pathway. The alternate pathway produces lipid bioactive intermediates that lower milk fat. The data summarized below also shows that the type and concentration of fatty acid required to reach antibacterial effects is subject to modification by other dietary nutrient considerations.

What Are Fatty Acids?

Before beginning a discussion about the fate of fatty acids as they pass through the rumen, it seems appropriate to start with a brief refresher on defining fatty acids. Put simply, fatty acids are the basic building blocks of fats just as amino acids are the building blocks of protein. Amino acids are chained together with peptide bonds in different lengths to form everything from dipeptides (2 amino acids) to polypeptides (> 10 amino acids). Fats, unlike protein, consist of no more than three fatty acids grouped together as attachments on a glycerol backbone. Fats and oils primarily consist of three fatty acids attached to glycerol referred to as triglycerides (or more correctly triacylglycerols). Forage lipids contain primarily galactolipids, where the glycerol backbone has two bound fatty acids along with a bound sugar molecule.

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Fatty acids, and not the glycerol backbone, provide the benefits to animal performance, including high energy, tissue benefits, and rumen effects. For this reason, it is important to have a basic understanding of the differences among fatty acids. Fatty acids are chains of carbons that end in an acid group, or carboxyl group as it is referred to in biochemistry. An example of a common fatty acid is stearic acid with 18 carbons and no double bonds (**Figure 1**). Stearic acid is low in plant oils, but present in higher amounts in animal fats, particularly in fats obtained from ruminant species such as beef tallow.

Oleic, linoleic, and linolenic acids are examples of unsaturated fatty acids containing one or more double bonds (**Figure 2**). Oleic acid has a single double bond between carbons 9 and 10, and is referred to as a monounsaturated fatty acid. Linoleic acid is a polyunsaturated fatty acid containing two double bonds between carbons 9 and 10, and between carbons 12 and 13. Oleic acid is the predominant fatty acid in animal fats and some plant oils, such as canola oil. Linoleic acid is the predominant fatty acid in many plant oils, including cottonseed oil, soybean oil, and corn oil. Linolenic acid, with three double bonds, is the primary fatty acid in most pasture species and in linseed oil from flax.

Fatty Acid Input into The Rumen

Typical daily intakes of unsaturated fatty acids for diets with and without added fat are shown in **Table 1**. Total fatty acid concentration in feed mixes can range from less than 20 mg/g of dry matter (**DM**) for basal ingredients to more than 80 mg/g DM when fat is added. Linoleic acid is the predominant unsaturated fatty acid consumed in most cases with upper intakes exceeding 700 g/day in published studies (**Table 1**), or even exceeding 1000 g/day under field conditions (Chase, 2019).

Fatty acid concentration in ruminal contents reflects their concentration and variability in feed. Using results from three published studies as an example (**Table 2**), fatty acids varied in ruminal contents from < 10 mg/g DM for a basal diet containing 50% bermudagrass hay (Bateman and Jenkins, 1998) to 29 mg/g DM for an alfalfa/corn silage diet (Loor et al., 2002), and to just under 50 mg/g DM for diets with added plant oils. Cows grazing high quality ryegrass and clover pasture (Sun and Gibbs, 2012), interestingly, had ruminal fatty acid concentrations approaching levels observed for totally mixed rations (**TMR**) with added fat. The implication of maintaining fatty acids in ruminal contents is that many microbial species are sensitive to high fatty acid concentrations and respond with reduced growth and metabolic activity. More specifically, antibacterial activity is greatest for unsaturated fatty acids and is not a characteristic of saturated fatty acids.

Select ruminal bacteria have an inherent protective mechanism in place designed to reduce unsaturated fatty acid concentration in the rumen and lessen the chances of antibacterial activity. This protective mechanism is referred to as biohydrogenation, where unsaturated fatty acids are enzymatically converted to saturated fatty acids (Jenkins et al., 2008). The efficiency of biohydrogenation can be seen from the results

of the three studies in **Table 2** where ruminal unsaturated fatty acid concentrations are much lower and less variable compared with feed or ruminal total fatty acid concentrations. Biohydrogenation, while assisting the microbial population in controlling antibacterial effects of unsaturated fatty acids, also greatly transforms the nature of fatty acid outflow from the rumen compared to its inflow from feed. This fatty acid transformation process has both positive and negative consequences on animal production and acceptability of animal based food products.

Changes in Rumen Fatty Acid Concentration Over Time

The pathways of biohydrogenation are highly complex and yield a wide variety of intermediates. The three main unsaturated fatty acids consumed (oleic, linoleic, and linolenic acids) are all subjected to enzymatic transformations that yield a multitude of unique intermediates. As an example, the pathways and intermediates of linoleic and linolenic acid biohydrogenation to stearic acid are shown in **Figure 3** (Ferlay et al., 2017). As knowledge increases about the pathways of biohydrogenation the identity of intermediates expands. Input into the rumen of just three unsaturated fatty acids (oleic, linoleic, and linolenic acids) as raw materials leads to the production of dozens, if not hundreds, of complex fatty acid isomers in rumen outflow. Yet, this complexity of biohydrogenation is largely ignored in most discussions of biohydrogenation. Most of the attention is directed at a more simplistic version of linoleic acid biohydrogenation (**Figure 4**) that emphasizes only the few intermediates that were shown previously to inhibit fat synthesis in the mammary gland.

Very briefly, biohydrogenation of linoleic acid in the rumen begins with its conversion to conjugated linoleic acid (**CLA**). In this initial step, the number of double bonds remains the same but one of the double bonds is shifted to a new position by microbial enzymes. Normally, the double bonds in linoleic acid are separated by two single bonds, but in CLA, the double bonds are only separated by one single bond. Many types of CLA are produced in the rumen of dairy cows, but a common CLA produced from biohydrogenation of linoleic acid is *cis*-9, *trans*-11 C18:2.

As biohydrogenation progresses, double bonds in the CLA intermediates are then hydrogenated further to *trans* fatty acids having only one double bond. A final hydrogenation step by the ruminal microbes eliminates the last double bond yielding stearic acid as the final end product. *Trans* double bonds only differ from *cis* double bonds in the placement of the hydrogens. The hydrogens are shown on opposite sides of the double bond for *trans* fatty acids, but on the same side of the double bond for *cis* fatty acids. Although the difference in structure between *trans* and *cis* fatty acids appears small, it causes significant differences in their physical and metabolic properties. The *trans*-11 route (abbreviated as t11) of linoleic acid biohydrogenation in **Figure 4** involves intermediates, including *cis*-9, *trans*-11 CLA, proven to have little effect on milk fat. The *trans*-10 route (abbreviated as *t*10) involves intermediates, including *trans*-10, *cis*-12 CLA, proven to reduce milk fat synthesis (Baumgard et al., 2000).

With biohydrogenation in place, it might be argued that unsaturated fatty acid concentration remains low enough to avoid antibacterial effects. However, when the time course of biohydrogenation is examined immediately after feeding, it is common to see a large spike in unsaturated fatty acids that quickly declines over time. An example of the spike in unsaturated fatty acids immediately after feeding is shown in **Figure 5**. In a continuous culture study done at Clemson University, suddenly switching from a basal diet to a 3% soybean oil diet after 5 days of fermentation increased linoleic acid concentration from < 1 mg/10 ml culture contents to over 8 mg/10 culture contents by 1 hour after feeding. Sampling times earlier than 1 hour after feeding might have revealed a linoleic acid spike that was even higher. Declines in linoleic acid concentration occurred by hour 2 and then steadily declined with each advancing hour after feeding. The spike in unsaturated fatty acids immediately after feeding might induce antibacterial effects, even though biohydrogenation maintains much lower concentrations of unsaturated fatty acids at most other times.

An in vivo example of this unsaturated fatty acid spike in ruminal contents was seen in the data of Baldin et al. (2018). They reported the highest concentration of unsaturated fatty acids in ruminal contents of cows within 5 minutes of an intraruminal dose of 200 g unsaturated oil. Ruminal concentrations of unsaturated fatty acids then returned to basal values by 4 hours after dosing. This suggests that cows fed under field conditions experience a spike in ruminal unsaturated fatty acid concentration immediately after feeding that might be sufficient to cause antibacterial effects in the rumen. The amplitude of the spike would likely be a function of fatty acid input from the diet, percentage unsaturation of diet fatty acids, feeding frequency, and rate of feed consumption.

Antibacterial Effects of Unsaturated Fatty Acids

There is an extensive literature describing the antibacterial activity of various fatty acids (Desbois and Smith, 2010). Two factors that affect the antibacterial activity of lipids are fatty acid structure and concentration. Free fatty acids generally disrupt fermentation more than triglycerides and antibacterial activity of free fatty acids can be enhanced by increasing the number of double bonds (Desbois and Smith, 2010). Growth of some bacterial species is stimulated by low concentrations of fatty acids, but inhibited at higher concentrations (Maczulak et al., 1981). In attempting to predict ruminal fermentation changes caused by dietary lipid, it is often assumed that the fat load is contributed only by the fat supplement and that free fatty acids (**FFA**) concentration is low. Both assumptions can be wrong. Fatty acids from the TMR and forage can significantly contribute to total rumen fat load, for example when animals are consuming immature pasture. Also, FFA concentration may be elevated in some feed ingredients such as whole cottonseed stored in warm, humid conditions (Cooke et al., 2007), or in forages resulting from hydrolytic cleavage of esterified lipids during hay-making (Yang and Fujita, 1997).

The mechanisms of fatty acid antibacterial effects are primarily directed at their intrusion into the bacterial cell membrane. The details of the mechanistic processes

(**Figure 6**) can be classified based on the relationship between the following three aspects: (i) increased membrane permeability and cell lysis, (ii) disruption of electron transport chain and uncoupling oxidative phosphorylation, and (iii) inhibition of membrane enzymatic activities and nutrient uptake (Yoon et al., 2018). For anaerobes that inhabit the rumen, fatty acids exert antibacterial properties through disorganization of the cytoplasmic outer membrane that can lead to increased membrane permeability and even cell lysis, inhibition of membrane enzymatic activity, and impaired nutrient uptake.

Several properties of antibacterial effects in the rumen directly impact the *t*10 versus *t*11 pathways of biohydrogenation and the eventual impact on milk fat.

I. One important factor is that fatty acids appear to exhibit antibacterial effects quickly. Maia et al. (2010) reported a > 96% reduction in metabolic activity in the ruminal bacterium *B. fibrisolvens* within 20 minutes following the addition of 0.2 mg/mL of linoleic acid to cultures. A recent continuous culture trial done at Clemson University examined how quickly soybean oil shifted biohydrogenation pathways from the normally predominant *t*11 pathway to the minor *t*10 pathway. Cultures were maintained on a basal diet for 4 days with *t*10-18:1 and *t*11-18:1intermediates analyzed just before the morning feeding (8 am) and then again at 2 and 4 hours after the morning feeding. On day 5 the cultures were suddenly switched to a diet containing 3% added soybean oil with samples analyzed every hour for 12 hours. The results (**Figure 5**) revealed an escalation of the *t*10 pathway within a few hours after introducing soybean oil into the cultures. This could mean that unsaturated fatty acid concentration may not need to be sustained at high levels at all times to cause antibacterial effects. Perhaps just the transient peak in ruminal unsaturated fatty acid concentration that occurs immediately after feeding is sufficient to trigger antibacterial effects.

II. A second critical point of antibacterial activity in the rumen is that not all bacterial species are equally susceptible. Disruption of membrane integrity following the addition of linoleic acid to cultures ranged widely across 17 species of ruminal bacteria (Maia et al., 2007) monitored by fluorescence techniques. Generally, the bacterial species following the *t*11 route of biohydrogenation showed greater disruption of membrane function, including > 50% disruption for *Butyrivibrio spp.* and > 90% disruption for *Pseudobutyrivibrio*. Membranes of *M. elsdenii* that follows the *t*10 route of linoleic acid biohydrogenation were < 5% disrupted by the same dosage of linoleic acid. Thus, fatty acid concentrations above the antibacterial threshold cause selective damage in the rumen depending on bacterial species, with less damage seen for *t*10 microorganisms.

III. Third, not all unsaturated fatty acids have equal propensity to cause antibacterial effects at the same concentration. For instance, relative inhibitory effects of individual fatty acids on growth of *B. fibrisolvens* was linolenic>linoleic>oleic>stearic according to Maia et al. (2010). The general trend was greater inhibition with increasing number of double bonds in the acyl chain. Similar trends have been

reported in vivo. Dorea and Armentano (2017) reported feeding saturated fatty acids to cows, such as palmitic acid, increased total milk fatty acids, mainly by increasing milk C16 yield. However, feeding unsaturated fatty acids decreased total milk fatty acid by inhibiting secretion of milk fatty acids shorter than C18, with linoleic acid being more inhibitory than oleic.

IV. A fourth and perhaps most significant property of antibacterial effects is that the threshold to cause a shift in the pathway of biohydrogenation is modified by environmental and chemical conditions in the rumen. If the threshold was a constant concentration of unsaturated fatty acids in ruminal contents, then feeding recommendations for fat could be modelled much easier. Instead, low pH and lactic acid accumulation were both shown to accentuate antibacterial effects of unsaturated fatty acids, specifically targeting *t*11 microorganisms (Maia, personal communication and Maia et al., 2010). Both of these conditions implicate high starch levels as a negative predictor of milk fat synthesis.

Conclusions

Using the antibacterial switch described in this paper, a sequence of events can be suggested whereby the pathways of biohydrogenation change course moving the rumen from milkfat “friendly” to milkfat “unfriendly”. The initial step is for unsaturated fatty acid concentration in the rumen to exceed the threshold for antibacterial effects. This could happen in one of two ways; 1) increase dietary concentration of unsaturated fatty acids that could arise from variation in basal fatty acids or from added fat to the diet, or 2) lower the antimicrobial threshold. The threshold that ruminal microorganisms can tolerate is lowered by increasing starch, lowering rumen pH, increasing lactate, or a combination of these. Once the antibacterial threshold is reached, *t*11 microorganisms respond within hours by shutting down metabolic activity including rates of biohydrogenation. This reduces the flux of linoleic acid flow through the normal *t*11 pathway of biohydrogenation. Consequently, because *t*10 microorganisms are less sensitive to antibacterial effects, more linoleic acid is now available for biohydrogenation through the alternate *t*10 pathway. With each subsequent feeding of the same diet the accumulation of CLA (specifically *trans*-10, *cis*-12) in the rumen continues providing a steady CLA flow to the mammary gland where *de novo* fatty acid synthesis is inhibited.

Some high producing herds consume in excess of 1000 g unsaturated fatty acids per day but still maintain milk fat around 4% (Chase, L. E., 2019). Other herds experience milk fat depression with <500 g of unsaturated fatty acids. McCarthy et al. (2018) failed to detect a significant relationship between milk fat yield and intake of unsaturated fatty acids across 79 herds in the northeast and upper Midwest. This variation clearly shows that unsaturated fatty acid intake alone is not a good predictor of milk fat. Models predicting milk fat should include all factors known to affect their antibacterial effects including amount and type of starch, rumen pH (effective fiber, type and amount of buffers, TMR mixing, etc), and fatty acid release rates from plant structure.

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Table 1. Average intakes of major unsaturated fatty acids by dairy cattle fed a TMR without and with added fat averaged across five published studies[1]

	DMI[2] kg/d	Diet FA[3] mg/g DM	18:1 g/d	18:2 g/d	18:3 g/d	RUFAL[4] g/d
Control (n=5)						
Mean	19.4	37.3	139	299	44	473
Min	12.0	18.6	53	133	26	220
Max	27.3	55.4	242	690	74	973
Fat Diets (n=21)						
Mean	19.4	59.5	280	371	56	696
Min	12.3	28.2	111	164	26	362
Max	25.7	83.5	571	710	88	1118

[1] Taken from Jenkins and Bridges (2007).
[2] DMI = Dry matter intake.
[3] FA = Fatty acid
[4] RUFAL = rumen unsaturated fatty acid load (g/day) = C18:1 + C18:2 + C18:3.

Table 2. Fatty acid concentrations (mg/g DM) reported in feed and rumen samples from three studies when cows were fed a basal diet with and without added oil

Reference/Diet	Feed	Rumen total	Rumen unsaturated
Loor et al. (2002)			
Basal (61% alfalfa/corn silage)	35.2	28.8	1.33
3.5% canola oil	61.4	48.2	2.42
3.5% soybean oil	63.8	48.9	1.69
Bateman and Jenkins (1998) nonlactating cows			
Basal (50% bermudagrass hay)	14.7	8.1	1.40
4% soybean oil	49.7	25.0	1.75
8% soybean oil	83.5	32.4	2.14
Sun and Gibbs (2012)			
High quality pasture	42.4	46.9	20.5

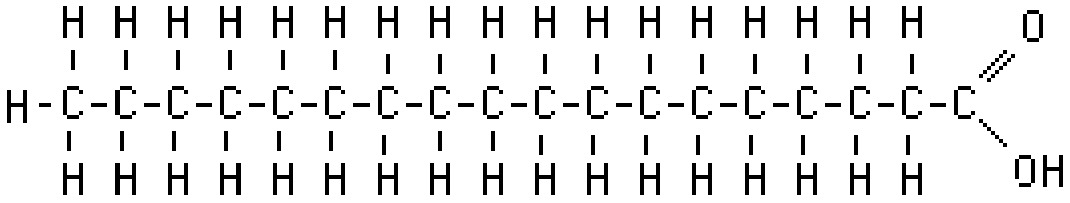

Figure 1. The structure of stearic acid; a saturated long-chain fatty acid.

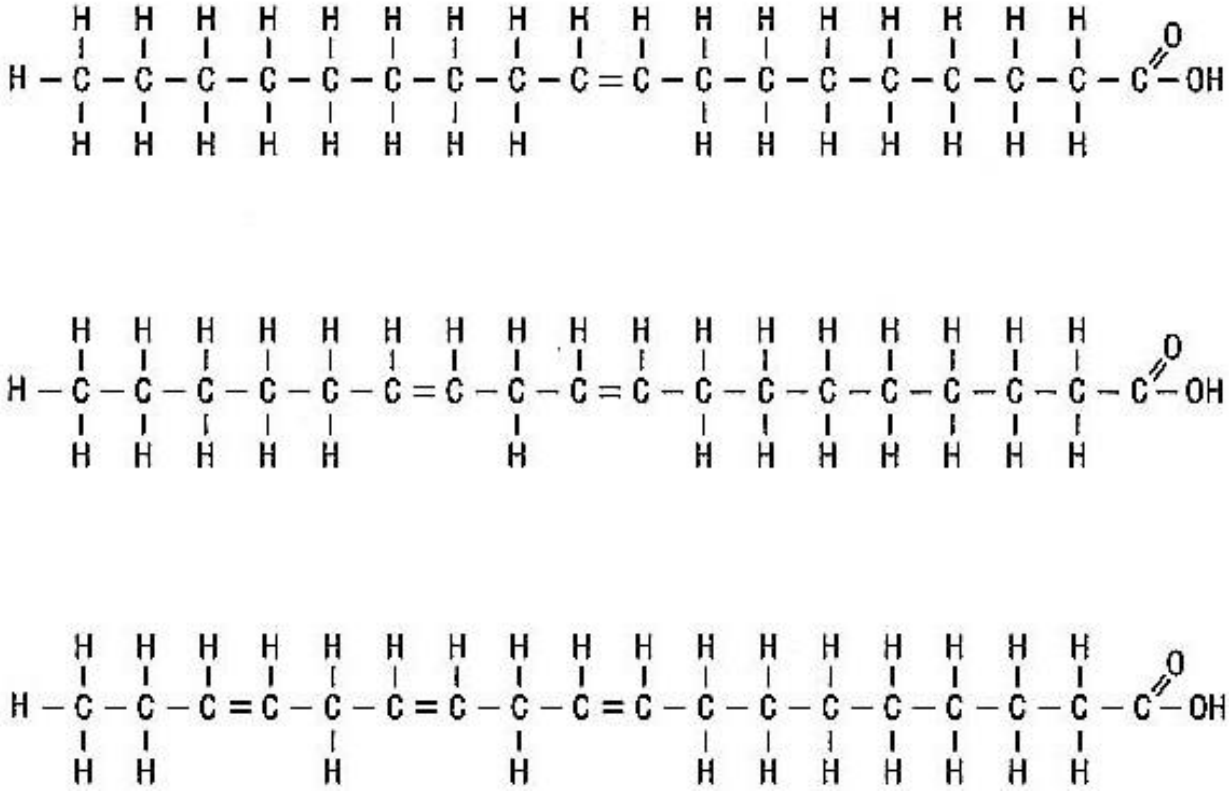

Figure 2. Structures of the three primary unsaturated fatty acids consumed by cattle, oleic acid (top), linoleic acid (middle), and α-linolenic acid (bottom).

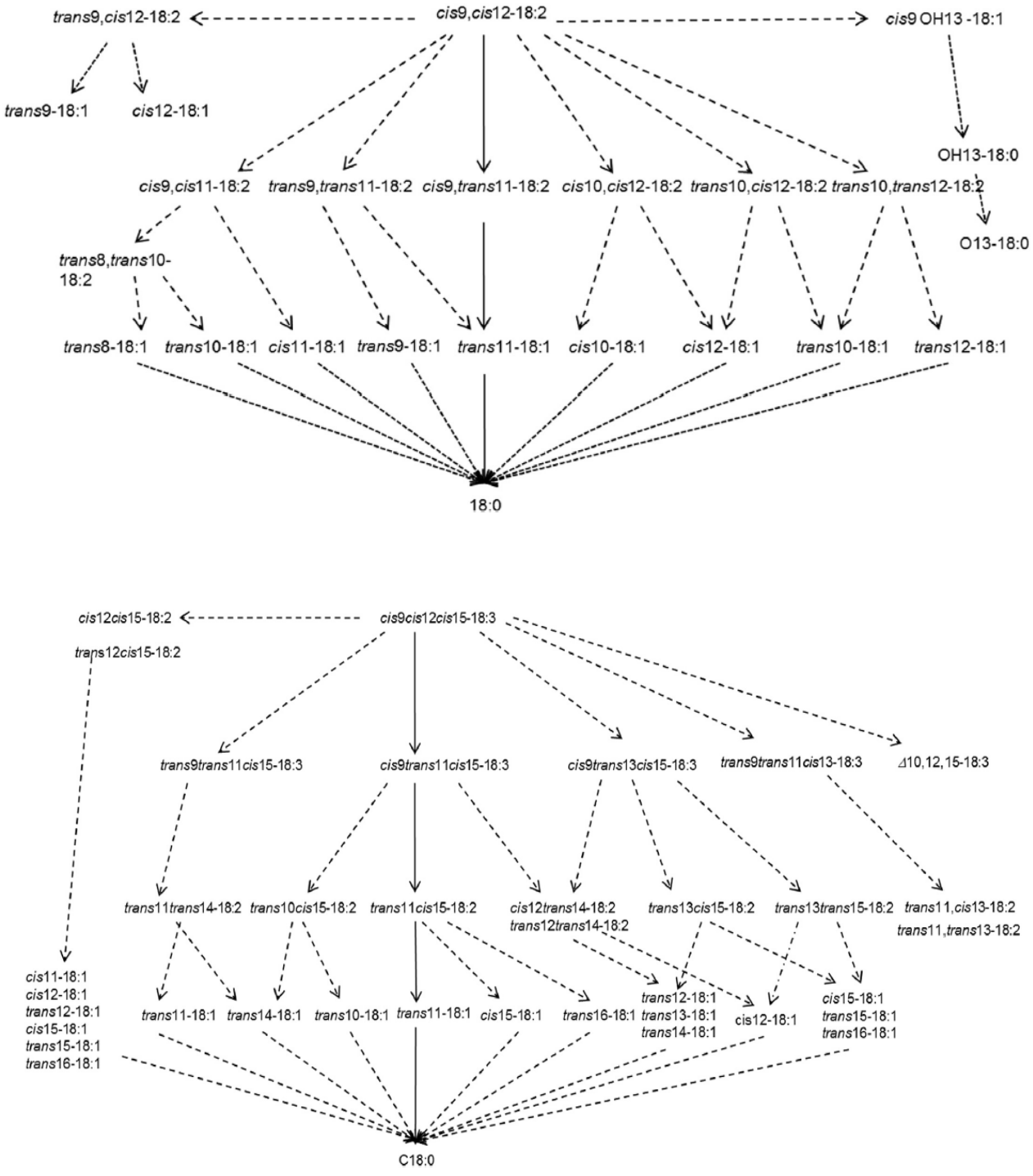

Figure 3. Proposed pathways of linoleic (top) and α-linolenic acid (bottom) biohydrogenation to stearic acid in ruminal contents proposed by Ferlay et al. (2017) illustrating the complexity and abundance of intermediates.

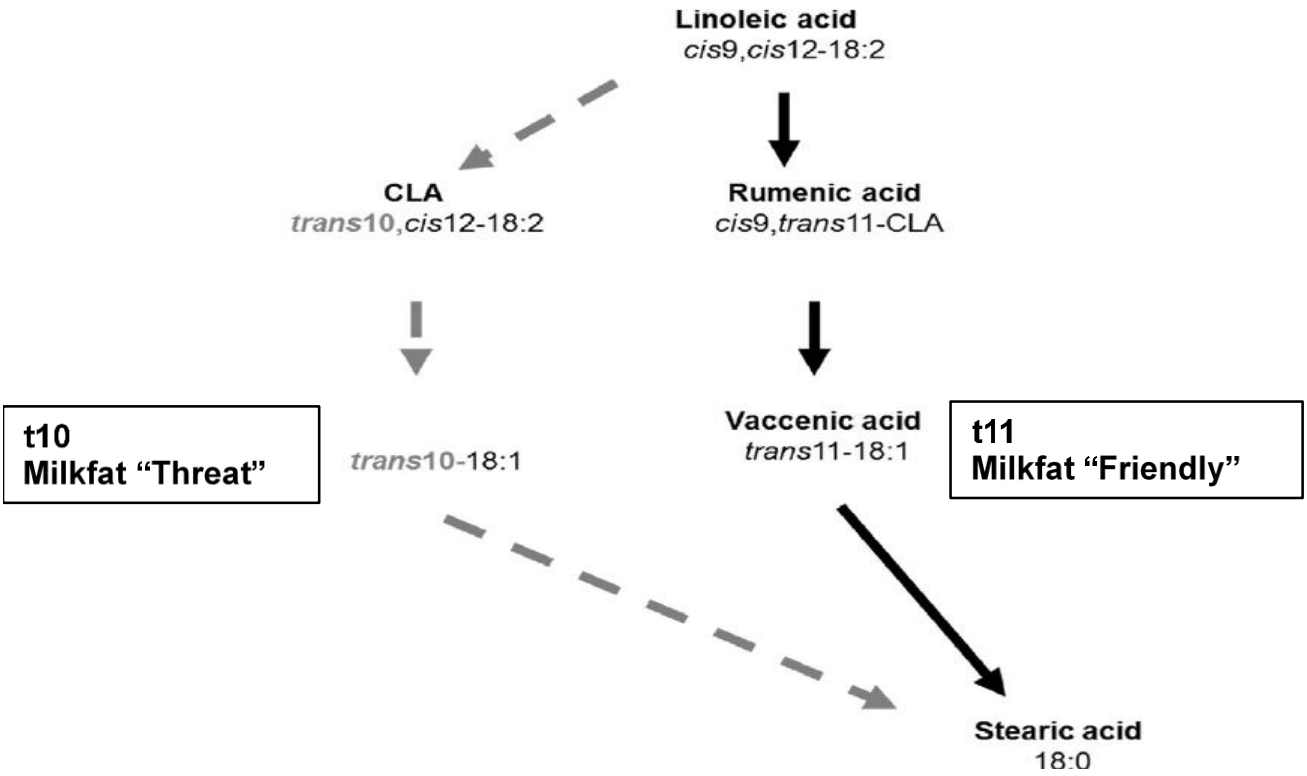

Figure 4. Simplified pathways of linoleic acid biohydrogenation emphasizing the major ***t*11 route** involving milkfat friendly intermediates and the minor ***t*10 route** involving intermediates known to inhibit milk fat synthesis by the mammary gland. Adapted from Ferlay et al. (2017).

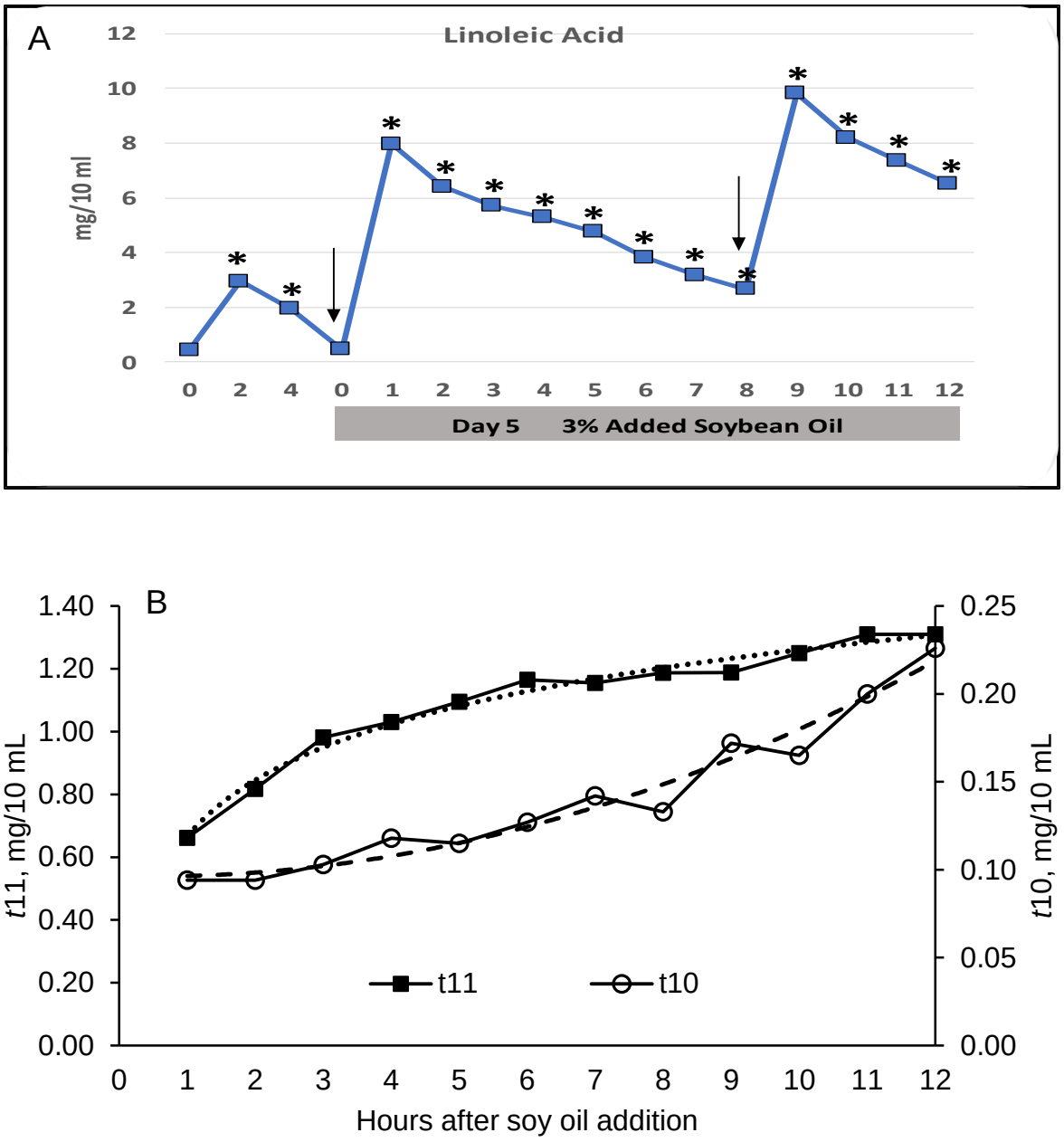

Figure 5. Concentrations of linoleic acid (A, mg/10 mL) and *trans* 18:1 isomers (B) in contents taken from continuous cultures of mixed ruminal bacteria. Cultures were fed a basal diet without soybean oil for 4 days then immediately switched to a diet containing 3% soybean oil on day 5. Top graph (A) shows linoleic acid concentrations on day 4 taken at 0, 2, and 4 hours after the morning feeding, and on day 5 taken hourly after soybean oil addition. Arrows on top graph (A) indicate when the diet containing soybean oil was introduced into cultures. Bottom graph (B) shows *t*11-18:1 and *t*10-18:1 concentrations taken hourly on day 5 after feeding soybean oil. Unpublished results from Clemson University.

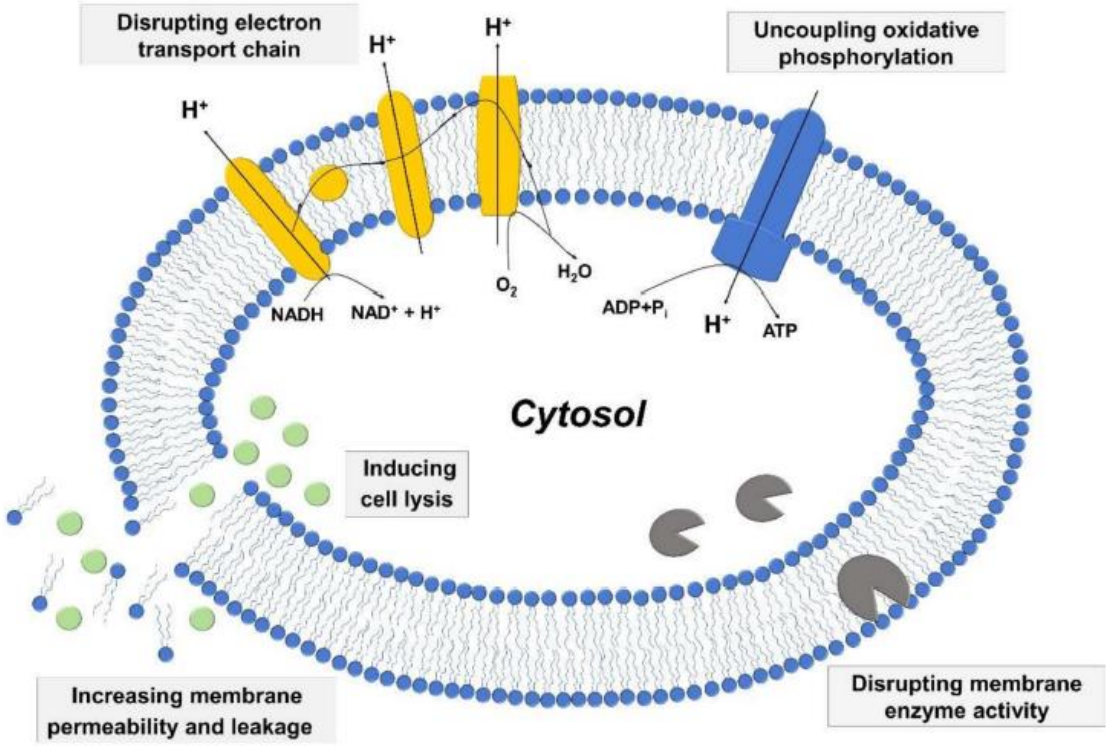

Figure 6. Mechanisms of antibacterial activity of fatty acids (Yoon et al., 2018). For ruminal microorganisms, fatty acids exert antibacterial properties through disorganization of the cytoplasmic outer membrane that can lead to increased membrane permeability and even cell lysis, inhibition of membrane enzymatic activity, and impaired nutrient uptake.

SESSION NOTES

What Have We Learned About Fatty Acid Digestibility in Dairy Cattle?

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Introduction

Fats may be supplemented to cows to increase energy density of the diet, improve milk components, improve milk yield, and/or maintain or gain body condition. In order for a fat supplement to be able to accomplish all of those things in a dairy cow, the fat has to be digested. There is increased recognition that individual fatty acids (**FA**) have different bioactive properties, and not all FA or fat supplements are going to respond similarly in a cow. For instance, some FA more often increase milk fat production while others are a better FA to use for gaining body condition. A considerable amount of research has been conducted that increases our understanding of individual FA digestibility because of the specific roles that those FA play in the dairy cow. Understanding what factors impact FA digestibility will allow models to more accurately predict the energy and specific outcomes from fats. Currently, most ration balancing software treats FA as energy sources but does not necessarily discount their energy value based on differences in FA composition and differences in FA digestibility. We will discuss some of the fundamental information regarding FA digestibility as well as more recent research studies reporting individual FA digestibility. Work in this area has increased our understanding of how individual FA digestibility differs and also how individual FA interact with one another to impact digestibility.

How FA Are Altered in a Ruminant

The fat that ruminants consume is mostly unsaturated esterified fat (i.e. triglycerides or glycolipids) with a wide variety of fat supplements commercially available that include both saturated and unsaturated FA in multiple forms. The rumen converts esterified fats to FA and glycerol through hydrolysis, breaking the ester bond between the glycerol and FA. Hydrolysis of fats occurs quickly in the rumen from bacterial enzymes (Palmquist and Jenkins, 1980). These now un-esterified FA have a free carboxyl end, a requirement for biohydrogenation to occur. Due to unsaturated FA being toxic to certain rumen microbes, the rumen has developed an adaptation to hydrogenate the unsaturated FA into saturated FA. Saturated FA are considered mostly rumen inert and are therefore have little effect on rumen fermentation. An excess of H are present in the rumen due to anaerobic fermentation, therefore biohydrogenation occurs relatively rapidly. The FA leaving the rumen are mostly saturated FA, unlike in a monogastric where a considerable amount of monoglycerides and unsaturated FA are present leaving the stomach. Because of these changes in FA profile from intake FA to

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what is digested (or undigested), feed to feces estimates of FA are not accurate to estimate individual FA digestibility in ruminants. Although there are changes in the form of the fat in the rumen, FA that are commonly fed to dairy cattle are not extensively metabolized in the rumen.

Digestion of FA in The Small Intestine

The digesta leaving the rumen is at near neutral pH. At this pH, the free carboxyl end of the FA binds with salts (potassium, sodium, or calcium) to form salts of FA. However, the low pH in the abomasum causes dissociation of these salts from the FA. As digesta enters the duodenum, it now has a pH of < 3 (Moore and Christie, 1984). Any esterified fat that reach the duodenum are quickly hydrolyzed (Doreau and Ferlay, 1994) leaving FA with a free carboxyl end. However, there is evidence that triglycerides of saturated FA have reduced digestibility compared to nonesterified FA (de Souza and Lock, 2019) with hydrogenated fat supplements especially having reduced digestibility (Pantoja et al., 1996). Indicating that even with what is thought of as rapid hydrolysis both in the rumen and in the small intestine, triglycerides of saturated FA may have reduced digestibility. Bile salts and pancreatic phospholipase act on the FA to form micelles with increased surface area. The secretion of bile and pancreatic secretions is relatively constant regardless of flow rates of digesta (Noble, 1981). Bile salts in ruminants are mostly taurine-conjugated which are more effective at a low pH compared to monogastrics that are more glycine-conjugated and not as effective at a low pH.

In the duodenum, the low pH and bile containing increasing amounts of taurocholic acid increase the solubility of the FA. Pancreatic phospholipase acts upon lecithin to produce lysolecithin, a potent amphiphile. Amphiphiles contain both hydrophilic and lipophilic properties and help with micelle formation that is required to diffuse the FA across the enterocyte membrane. Lysolecithin is a potent emulsifier for stearic acid (Freeman 1969 and 1984) and contains a polar head group and a hydrocarbon tail generally in the form of lysophosphatidylcholine. However, feeding 10g/d of lysolecithin had modest to negative effects on production responses when feeding a higher fiber and lower unsaturated fat diet or a lower fiber and higher unsaturated fat diet, respectively (Rico et al., 2017a). Feeding de-oiled soy lecithin up to 0.36% of diet DM had no effect on 16 or 18-carbon FA digestibility (Fontoura et al., 2019; Rico et al., 2019). Feeding unprotected lysolecithin or lecithin may not be the solution to increase FA digestibility in the small intestine.

Oleic acid also acts as an amphiphile to increase stearic acid digestibility (Freeman 1969 and Freeman 1984). Typically, due to extensive biohydrogenation of unsaturated FA the amount of oleic acid reaching the duodenum may not be sufficient to improve stearic acid digestibility. However, feeding protected oleic acid may improve digestibility of saturated FA. de Souza et al. (2018) reported increased 16 and 18-carbon digestibility when a fat supplement blend containing 35% oleic acid mostly as a calcium salt compared to a blend of palmitic and stearic acid. Calcium salts of palm FA containing 38% oleic acid increased digestibility of 16-carbon FA compared to a palmitic acid triglyceride supplement (de Souza and Lock, 2018). A calcium salt of FA containing

oleic acid appears to have the ability to improve both 16 and 18-carbon digestibility, likely due to its amphiphilic properties, which increases emulsification and helps with micelle formation required for FA absorption.

Fat Sub-Model

Moate (2004) developed a fat sub-model to describe intestinal digestibility of long chain fatty acids in dairy cattle. To quantify the amount of FA available at the small intestine available for digestion, the model considers intake of dietary FA, ruminal lipolysis of dietary FA, biohydrogenation of long chain FA, de novo production of long-chain FA in the rumen, ruminal passage of FA, and ultimately intestinal absorption of those FA. All of those steps have inherent error associated with them. However, if our ultimate goal is to provide accurate FA digestibility measurements, we need accurate estimates of all of these factors that will change the composition and amount of FA available for absorption. Moate (2004) reported a linear relationship between duodenal flow of long-chain FA and absorbed total long-chain FA, with an increase in the amount of FA reaching the duodenum resulting in more FA being absorbed. The form of the fat was also a consideration for digestibility and provided a foundation for understanding individual FA digestibility.

Measuring Individual FA Digestibility

Due to the change in FA from intake to feces, duodenally cannulated animals are required for individual FA digestibility estimates. The large intestine can also hydrogenate unsaturated FA (Pantoja et al., 1996) and most FA absorption occurs in the jejunum, therefore samples of the contents of the ilium would be optimal to measure small intestinal digestibility of FA. Alternatively, difference from duodenal to fecal FA can be used to estimate individual FA digestibility, with a potential bias against stearic acid digestibility, as 18-carbon unsaturated FA could be biohydrogenated to stearic acid, increasing the amount of stearic acid in feces. There are a limited number of published studies reporting individual FA digestibility from measurements in the duodenum and ileum. Therefore, we combined both methods and found negligible differences between methods for individual FA digestibility. To better understand both dietary factors and FA profile factors that impact individual FA digestibility, we combined 15 research trials, including 61 treatment means, that measured individual FA digestibility and conducted a meta-analysis to increase our scope and increase our number of observations (Boerman et al., 2015).

As mentioned above, due to extensive biohydrogenation in the rumen, more unsaturated FA are consumed and more saturated FA are present at the duodenum (**Figure 1**). As there is minimal digestion of FA in the rumen, a positive relationship exists between duodenal flow of total FA and total FA intake. When duodenal flow of total FA increased, the total FA digestibility is reduced (**Figure 2**). We compared all individual FA to stearic acid (C18:0), because palmitic acid (C16:0) is a saturated FA and oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) are all unsaturated 18-carbon FA, making stearic acid a logical comparison. Mean apparent intestinal digestibility is

not different between palmitic and stearic acid. Whereas, oleic and linolenic acid had increased apparent intestinal digestibility compared to stearic acid. However, making those same comparisons for diets with no added fat in the diets, stearic acid digestibility was not different from any FA and stearic acid digestibility was significantly greater than when fat supplements were included in the diet. At lower levels of stearic acid reaching the duodenum, digestibility of stearic acid is high, this increases the mean stearic acid digestibility. However, as duodenal flow of stearic acid increases, intestinal digestibility of stearic acid is reduced. Reductions in stearic acid digestibility with increasing amounts of stearic acid reach the duodenum are consistent with other studies that reported reduced stearic acid digestibility in fat supplemented diets compared to control diets with no added fat (e.g. Pantoja et al., 1996).

At the amount of individual FA reaching the small intestines reported in the studies included in the meta-analysis, stearic acid is the only FA that had reduced digestibility as the amount reaching the duodenum increased (**Figure 3**). Comparatively, unsaturated FA appear to have a neutral to positive effect as more reaches the duodenum and palmitic acid digestibility is changed to smaller extent than stearic acid. It is important to point out that stearic acid is by far the greatest FA reaching the duodenum and that some of the unsaturated FA represent < 10 grams of FA. The reduction in total FA digestibility observed in **Figure 2** is primarily from the reduction in C18:0 digestibility as more FA reaches the small intestine.

A FA digestibility meta-analysis utilizing individual cow observations rather than treatment means, reported similar reductions in total FA digestibility as flow of FA leaving the rumen increased (de Souza et al., 2018). Stearic acid was the major FA leaving the rumen and was the FA that had the largest reduction in digestibility as the amount increased. Palmitic acid digestibility remained unchanged across the range FA leaving the rumen and entering the rest of the digestive tract. Therefore, comparing both treatment means and individual cow data points showed similar reductions in stearic acid digestibility as duodenal flow of stearic acid increased.

A dosing study of a highly pure stearic acid supplement reported reduced digestibility of total FA when increasing amounts of stearic acid was supplemented (Boerman et al., 2017). Increasing amounts of stearic acid reduced the digestibility of both 16 and 18-carabon FA, however it reduced 18-carbon digestibility to a greater extent. However, still more 18-carbon FA were absorbed when intake of stearic acid was increased. The more stearic acid that was fed, the more was absorbed but the efficiency of absorption is greatly reduced (Boerman et al. 2017). In this instance, if digestibility of this supplement was considered constant, there would be a reduction in energy available to the cow because of the reduction in 18-carbon FA digestibility.

A dosing study of a highly pure palmitic acid supplement utilized two different basal diets, one with lower total FA content and one with added cottonseed and higher total FA content (Rico et al., 2017b). Overall, increasing palmitic acid reduced 16-carbon FA digestibility, however there was a difference between the lower and higher fat diets. The higher fat diet had less of a reduction in 16-carbon FA digestibility and

total FA digestibility. These results may be surprising, as we would likely see more stearic acid reaching the duodenum with a higher fat diet, which from research discussed above reduces total FA digestibility. However, the additional fat in the form of cottonseed may have offered some protection from biohydrogenation leading to more unsaturated FA that may improve digestibility compared to more saturated FA at the duodenum. Compared to the stearic acid supplemented study, dosing a highly pure source of palmitic acid reduced total FA digestibility less. The differences between digestibility of FA based on basal diet FA content indicates that not just amount of FA but, also profile likely influences FA digestibility.

Form of FA Impacts Digestibility

Daley et al. (2018) analyzed 31 studies and 142 treatments means for total tract FA digestibility across 11 different categories of fat supplements. Total tract digestibility coefficients of FA are influenced by the source of the dietary fat. Fat supplements with high levels of palmitic, stearic or hydrogenated triglycerides have lower FA digestibility than those with higher amounts of unsaturated FA. Results from Daley et al. (2018) provide further evidence that the form of the fat supplement impacts digestibility. We utilized meta-regression to determine which dietary factors impact individual FA digestibility (Boerman et al., 2015). Fat type impacted the individual digestibility of palmitic acid, oleic acid and linoleic acid, with calcium salts and vegetable oils having numerically higher digestibilities. Specifically, whole seeds had lower FA digestibility, while whole seeds may offer some protection from biohydrogenation, they also have reduced intestinal digestibility. For saturated FA, increasing dry matter intake (**DMI**) negatively impacted palmitic and stearic acid digestibility. Potentially, increasing the passage rate of feedstuffs would negatively impact saturated FA digestibility due passage of FA too fast to allow for absorption in the small intestine.

Profile of FA Impacts Digestibility

Duodenal flows of FA influence individual FA digestibility (Boerman et al., 2015). For stearic acid, total flow of FA as well as the proportion of stearic acid and oleic acid negatively impacted digestibility. The proportion of stearic acid reaching the duodenum also negatively impacted the digestibility of all unsaturated 18-carbon FA. These results indicate that the profile that reaches the duodenum in addition to the amount of individual FA likely influences digestibility. Because of difference in digestibility between individual FA, the amount of digestible energy available to the cow differs based on fatty acid flow and profile. These changes in digestible energy between individual FA influences production responses observed when supplementing fat.

The amount of FA included in diets is relatively small compared to other nutrients; however, as cows are genetically capable of making more milk, we are looking for every opportunity to increase milk production and/or milk components. Another important consideration is the effect of fat supplements on DMI and digestibility of other nutrients. Reducing DMI and reducing fiber digestibility may have more negative effects on digestible energy rather than modest reductions in FA digestibility. In

order to accurately predict digestibility of FA and digestible energy from FA, we need to not only treat individual FA as unique when it comes to predicting their digestibility but also consider their interactions with other FA and their effect on digestibility of other nutrients. Cows consume blends of FA and because of the difficulty in predicting biohydrogenation rates and passage rates of FA, accuracy of individual FA reaching the duodenum will be challenging. However, a first step is accounting for differences in digestibility as flow of FA increases to better account for individual FA available for the cow.

Conclusions

Stearic acid is the FA in the greatest quantity reaching the small intestine and available for absorption. Increasing the amount of stearic acid, reduces the digestibility of stearic acid as well as reducing the digestibility of other 18-carbon FA. Improving the ability of FA to emulsify and form a micelle is a logical place to try to improve stearic acid digestibility. Thus far, rumen protected oleic acid appears to have the most positive effect on increasing stearic acid digestibility. The form of the fat, the profile FA reaching the duodenum, and the amount of fat all impact digestibility of individual FA. Although relatively little fat is supplemented to dairy cows, maximizing the amount of FA digested increases digestible energy as well as the increasing the specific FA available for bioactive properties.

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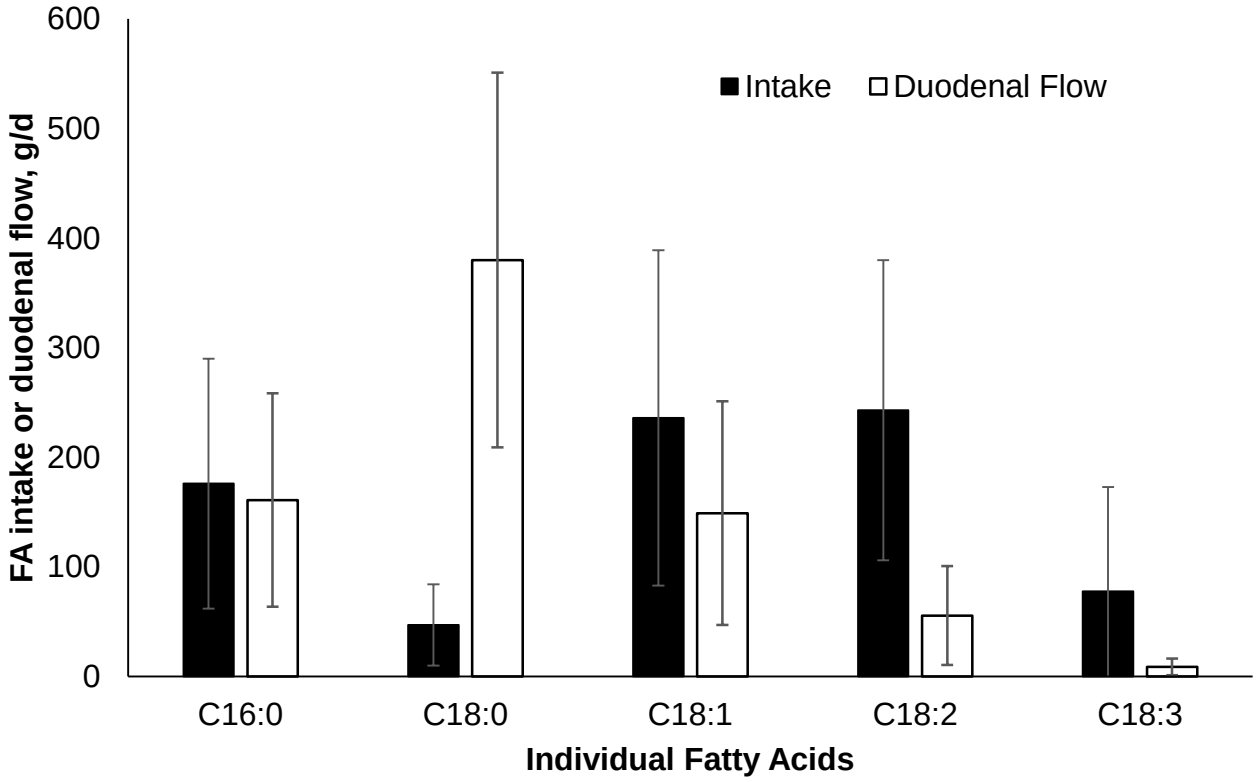

Figure 1. Mean and standard deviation reported from 15 studies of individual FA intake and duodenal flow. Adapted from Boerman et al. (2017). Due to extensive biohydrogenation, there are a reduction in unsaturated FA reaching the duodenum and an increase in stearic acid.

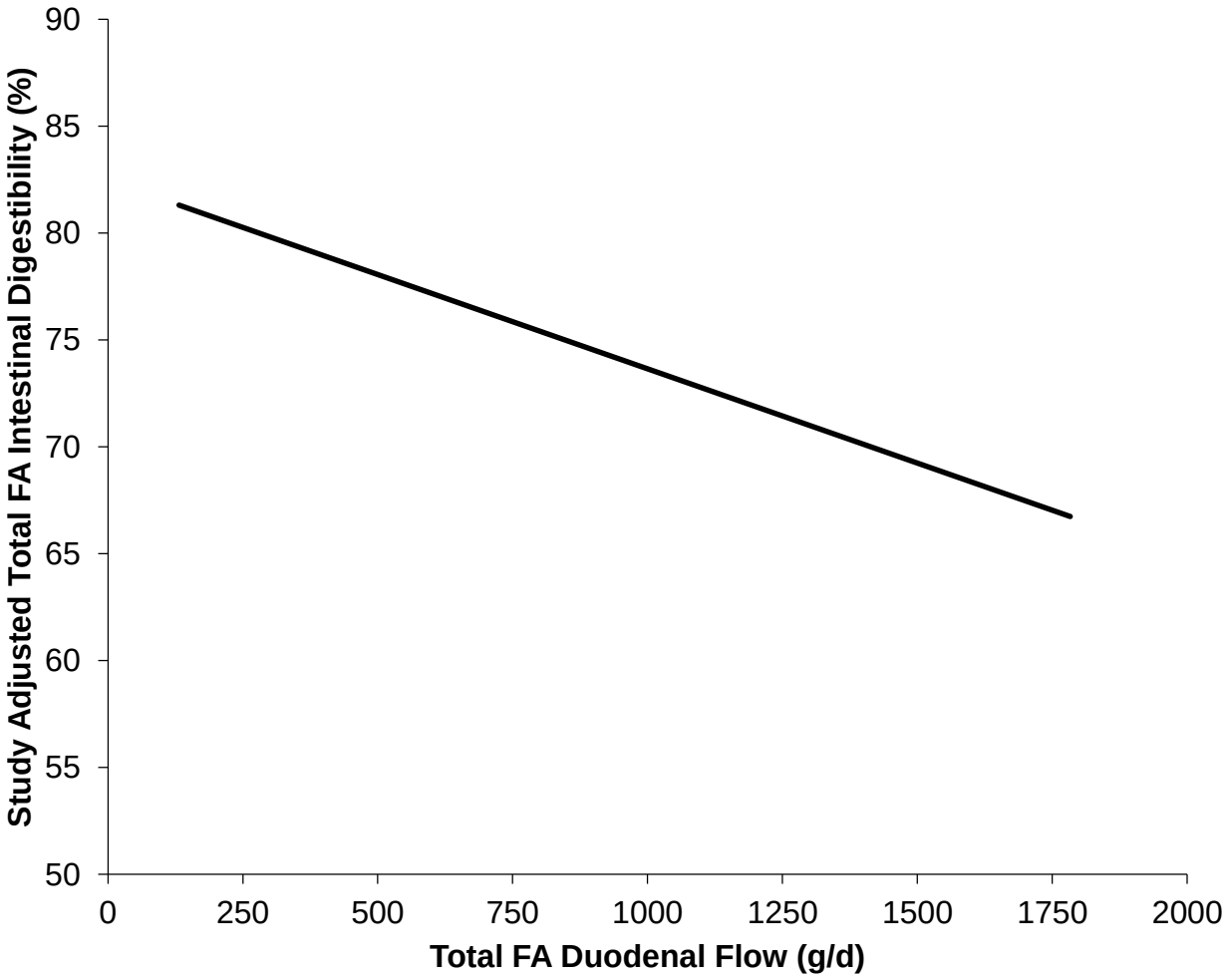

Figure 2. Best-fit line for study adjusted total FA digestibility from 61 observations from 15 studies reporting individual and total FA digestibility measured from duodenal to ileal or fecal disappearance of FA. Adapted from Boerman et al. (2015).

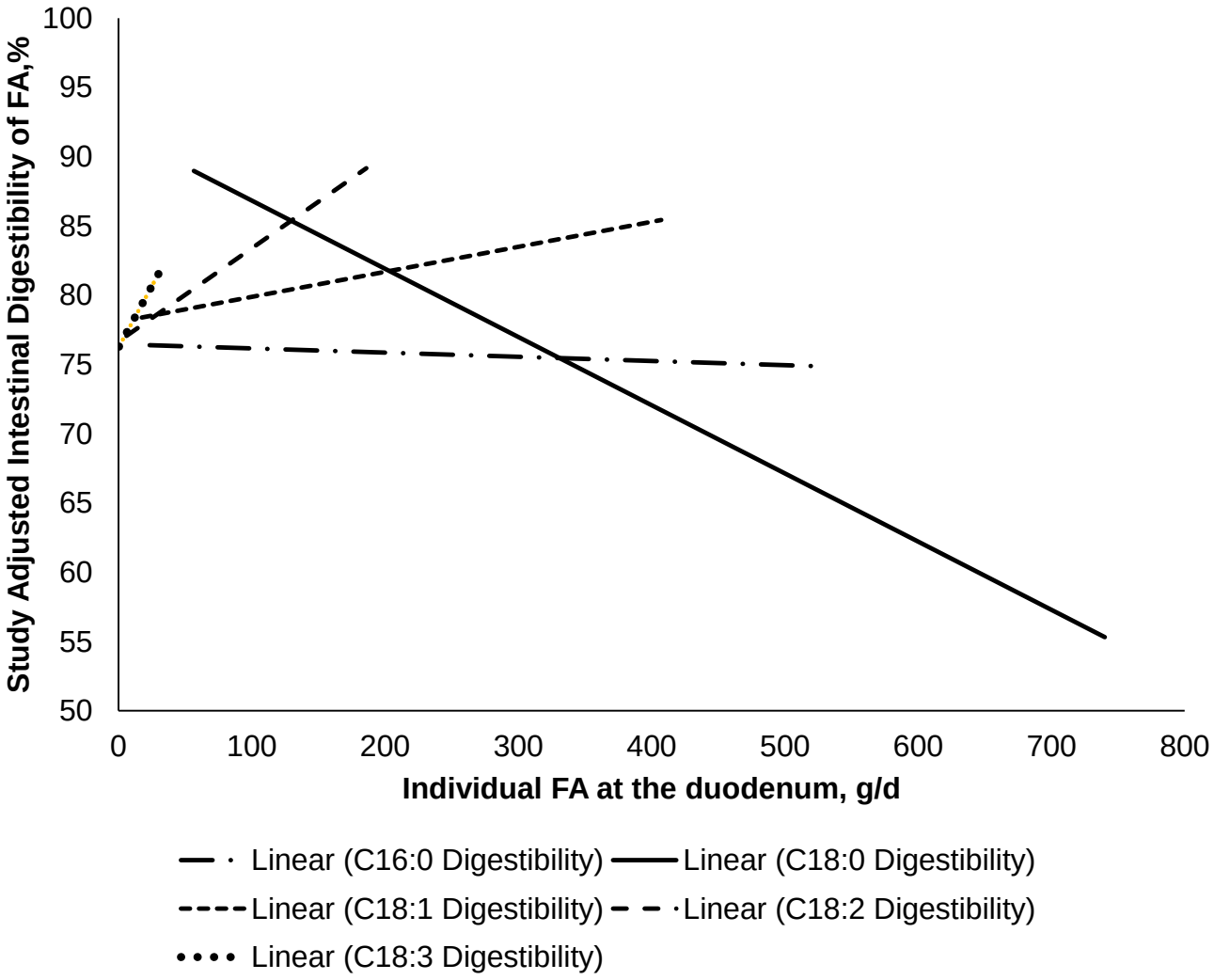

Figure 3. Best-fit lines for study adjusted intestinal digestibility of individual FA by grams of individual FA at the duodenum reported from 61 treatment means from 15 studies. Adapted from Boerman et al. (2015).

SESSION NOTES

The Role of the Calf Microbiota on Performance and Health

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Introduction

Human population expansion, higher living standards in many countries, and economic growth have resulted in increased consumption of animal protein globally (Boland et al., 2013). For example, in Asia, animal protein consumption rose by 225% from 1961 to 2007 (FOASTAT). By 2050, protein consumption is expected to increase by a further 29% over the current level (Herrevo, 2013). Yet, approximately 843 million people worldwide continue to experience chronic hunger and nutrient deficiencies (Food and Agriculture Organization), and almost 1 billion receive insufficient amounts of protein (United Nations; Grover et al., 2009; Ghosh et al., 2012). Therefore, there is a pressing need to increase animal numbers, animal productivity and ultimately animal protein production without intensifying the environmental footprint of agriculture.

Antibiotic Resistance

In animal agriculture, antimicrobial compounds are widely administered (Chee-Sanford et al., 2001, Smith et al., 2004, Sawant et al., 2007, McKinney et al., 2010). In 2012 alone, >32.2 million pounds of antimicrobial drug active ingredients were sold for animal use (Center for Veterinary Medicine, Food and Drug Administration, 2015). Antibiotics are used in food animals to treat disease, promote growth, and prevent disease by metaphylaxis (McEwen and Fedorka-Cray, 2002, Viola and DeVincent, 2006). In dairy cows, they are used to treat diseases such as mastitis, diarrhea and respiratory infection as well as to improve feed efficiency of pre-weaned calves. Sub-therapeutic levels of antimicrobials (e.g. tetracycline and neomycin) are routinely added to milk or milk replacer for disease prophylaxis and growth promotion (McEwen and Fedorka-Cray, 2002). Because pre-weaned dairy calves are prone to disease, there are advantages to adding antibiotics to calf feed (Morrill et al., 1995)), including higher feed consumption, average daily gain, and phagocytic efficiency, and lower mortality, incidence of scours, and protein requirements (Morrill, 1977).

However, ever-increasing numbers of antibiotic-resistant pathogens are emerging; for example, 44% of coagulase-negative Staphylococci isolated from intra-mammary infections in dairy cows were found to be resistant to one or more antibiotics (Rajala-Schultz et al., 2004). Addition of antibiotics to livestock feed for growth stimulation and other non-therapeutic applications is believed to be the principal route by which antibiotic-resistant strains arise in food animals (McEwen and Fedorka-Cray, 2002). However, the rise in antimicrobial resistance is not necessarily evident among the pathogenic bacteria for which the antibiotics were applied, but is more likely to occur

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in the ‘innocent’ bystanders, i.e. bacteria making up the normal microbial flora of the intestinal tract (Mollenkopf et al., 2012), the respiratory tract, and possibly the mammary gland.

The emergence of antibiotic-resistant bacteria is a mounting concern, since many of the antibiotics used in animal agriculture can also be used in human treatment, including tetracyclines, penicillins and sulfonamides (Silbergeld et al., 2008). There is potential for antimicrobial resistance to spread to humans (Smith, 2015) through food products/animal protein (Price et al., 2005), the environment (Graham et al., 2009), and by direct contact via agricultural workers (Smith et al., 2013). Moreover, commensal bacteria in livestock may potentially serve as hosts for resistance genes; in turn, these strains could be transmitted to people via fresh meat products, ultimately leading to transfer of the resistance genes to pathogenic organisms in humans (Diarrassouba et al., 2007, Mena et al., 2008). Disease associated with antimicrobial-resistant pathogens is expensive to treat, time consuming, and results in increased morbidity and mortality rates (Cosgrove, 2006, Maragakis et al., 2008, Mauldin et al., 2010).

Due to the emergence of antibiotic resistant microbes, the use of antibiotics as growth promoters is now banned in the European Union (**EU**) and limited in the United States. The use of select antibiotics in animal feed was first sanctioned in Sweden in 1986 (Castanon, 2007), since then all antibiotics used as growth promoters or for metaphylaxis have been prohibited by EU countries (European Parliament and Council Regulation EC No. 1831/2003). Consequently, a reduction in animal productivity and a rise in animal infection, morbidity and mortality occurred (Cheng et al., 2014). In 2015, the US Food and Drug Administration published new directives for the use of antimicrobials in the feed of livestock in the United States, creating a new category of products called veterinary feed directive (**VFD**) drugs. Under this directive, a VFD drug must be used under the professional supervision of a licensed veterinarian (FDA, 2015). Treatment of animal diseases such as diarrhea with antibiotics and subsequent supportive care is expensive and leads to increased antimicrobial resistance; therefore, development of new strategies to prevent diarrhea and other diseases will improve overall productivity, animal welfare, profitability, and mitigate the emergence of resistance to antibiotics. Considering this, there is a growing demand for a substitute to antibiotic use (Seal et al., 2013); potential alternatives include antibacterial vaccines, immunomodulatory agents as well as prebiotics and probiotics (Millet and Maertens, 2011).

Alternative to Antibiotics

The World Health Organization (**WHO**) and the Food and Agriculture Organization of the United Nations (**FAO**) define probiotics as “microorganisms that when administered live and in adequate amounts, confer a benefit to the health of the host.” A common application of probiotics is to improve gastrointestinal health, presumably by producing a gut environment that disfavors pathogenic microbes. Lactic acid bacteria and bifidobacteria are the most widely administered probiotic microbes (Didari et al., 2014). The biological impact of probiotics depends on the strain type;

therefore, strain identification and molecular analysis is critical (Azais-Braesco et al., 2010).

The gut microbiota has an essential role in determining many aspects of postnatal life, such as contributing to the development of the immune system (Round and Mazmanian, 2009, Peterson and Cardona, 2010) and influencing host physiology, including energy balance by affecting energy expenditure and storage (Ridaura et al., 2013). Gut microbes serve their host by functioning as a key interface with the environment; for example, they can protect the host organism from pathogens that cause infectious diarrhea (O'Hara and Shanahan, 2006). In several studies of food animals, treatment with probiotics reduced the need for antibiotics, thereby potentially reducing human exposure to antimicrobial compounds and lowering the incidence of multidrug resistant microbes (Pedroso et al., 2013, Liang et al., 2014, Punaro et al., 2014). Whereas probiotics are a promising alternative to improve food-animal productivity and health, scientific evidence supporting the use of specific microbes to benefit animal health and performance is limited.

Altering The Gut Microbiome of Neonates Have Lasting Metabolic Consequences

Acquisition of the intestinal microbiota begins at birth, and a stable microbial community develops from a succession of key organisms. Disruption of the microbiota during this important maturation can alter host metabolism and adiposity. Early life is a critical period for metabolic development (Dietz, 1994, Cunningham et al., 2014) and microbiota disruption during this window could change weight gain and body composition. In humans, early-life microbiota disruption, either due to delivery by Caesarian section (Dominguez-Bello et al., 2010) or antibiotics, is associated with increased risk of overweight status later in childhood (Ajslev et al., 2011). For decades, farmers have been making use of sub-therapeutic doses of antibiotics to promote the growth of farm animals. A landmark study by Cox et al. (2014), in which an in depth investigation of the mechanisms of action of growth promoting antibiotics using a mice model was performed, showed strong evidence that early antibiotic use disrupted the gut microbiome leading to increased weight gain, fat mass, and dramatic changes in liver metabolism. (Cox et al., 2014). Interestingly, it was reported that microbial communities recovered after the cessation of antibiotics, yet the metabolic phenotypes persisted, highlighting the importance of early-life microbiota in growth and development.

Faecalibacterium prausnitzii

Faecalibacterium prausnitzii (**FP**) is part of the normal intestinal microbiota of many animal species and one of the most abundant bacteria present in human feces, comprising 2-20% of the human gut microbiota (Suau et al., 2001, Hold et al., 2003, Eckburg et al., 2005, Schwiertz et al., 2010, Arumugam et al., 2011, Walker et al., 2011). FP is also found in the feces of healthy non-human animals such as swine (Haenen et al., 2013), mice (Nava and Stappenbeck, 2011), and poultry (Lund et al., 2010). It is a strict anaerobe, a member of the phylum *Firmicutes* (Duncan et al., 2002),

and can produce large quantities of butyrate, D-lactate and formate through fermentation and utilization of acetate.

A lower abundance of *Firmicutes*, especially FP, characterizes the fecal and mucosa-associated microbiota of Crohn's disease patients (Sokol et al., 2008, Swidsinski et al., 2008, Sokol et al., 2009, Willing et al., 2009, Lopez-Siles et al., 2014). Moreover, lower levels of ileal mucosa-associated FP were correlated with postoperative relapse of ileal Crohn's disease 6 months after surgical resection (Sokol et al., 2008). Similarly, reduced levels of *Firmicutes* were detected in ulcerative colitis (Frank et al., 2007, Nagalingam and Lynch, 2012, Machiels et al., 2014), and low levels of FP specifically were identified in association with alternating-type irritable bowel syndrome (Rajilic-Stojanovic et al., 2011, Miquel et al., 2013), colorectal cancer (Balamurugan et al., 2008), ulcerative colitis (Machiels et al., 2014), and even type II diabetes (Qin et al., 2012, Karlsson et al., 2013). These studies highlight the beneficial role of FP in the human gut. However, higher levels of fecal FP have been linked to obesity in children (Balamurugan et al., 2010), suggesting that FP is related to the energy-harvesting capacity of the intestinal microbiota.

Faecalibacterium prausnitzii as an Anti-Inflammatory Microbe

A large body of scientific evidence exists to support the beneficial effects of FP on the prevention and treatment of inflammatory disorders. A lower abundance of *Firmicutes*, especially FP, characterizes the fecal and mucosa-associated microbiota of patients with Crohn's disease (Sokol et al., 2008, Swidsinski et al., 2008, Sokol et al., 2009, Willing et al., 2009, Lopez-Siles et al., 2014), ulcerative colitis (Frank et al., 2007, Nagalingam and Lynch, 2012, Machiels et al., 2014), irritable bowel syndrome (Rajilic-Stojanovic et al., 2011, Miquel et al., 2013), colorectal cancer (Balamurugan et al., 2008), and more importantly type-2 diabetes (Qin et al., 2012, Karlsson et al., 2013). The anti-inflammatory properties of FP have been well described. In a pivotal study by Sokol et al (2008), both *in vitro* and *in vivo* studies were performed to evaluate the immunomodulatory properties of FP (Sokol et al., 2008). It was demonstrated that the FP supernatant abolished interleukin (**IL**) 1β-induced nuclear factor (**NF**) κB activity using Caco-2 cells and led to significantly lower secretion of the pro-inflammatory cytokines such as tumor necrosis factor (**TNF**) α and IL-12, and higher secretion of IL-10 by peripheral blood mononuclear cells. More importantly, oral administration of either live FP or its supernatant markedly reduced the severity of experimentally induced colitis and tended to correct the dysbiosis associated with it (Sokol et al., 2008). Others have reported that both FP and its supernatant can also inhibit IL-8 secretion (Quevrain et al., 2016a, Quevrain et al., 2016b, Martin et al., 2017). The ability of FP to induce IL-10 expression by dendritic cells was well described by Rossi et al. (2015), and the anti-inflammatory characteristic of FP has been demonstrated by several others (Quevrain et al., 2016a, Rossi et al., 2016, Martin et al., 2017, Munukka et al., 2017). The mechanism of action associated with the anti-inflammatory characteristics of FP are not fully understood but involves the synthesis of butyrate and other factors. Butyrate regulates proliferation, differentiation, and apoptosis of gastrointestinal tract cells while stimulating the production of mucus and decreasing cell permeability, hence preventing leakage of bacterial endotoxins and inflammation (von

Engelhardt et al., 1998, Augenlicht et al., 2002). Additionally, butyrate is also known to directly inhibit NF-κB activity (Inan et al., 2000, Segain et al., 2000, Yin et al., 2001, Luhrs et al., 2002a, Luhrs et al., 2002b). Quévrain et al (2016), identified a novel protein from an FP isolate, which they named microbial anti-inflammatory molecule (**MAM**). Cloning and overexpression of the MAM in human intestinal epithelial cells revealed an inhibitory effect on NF-κB activity. Importantly, oral administration of MAM-expressing recombinant *Lactococcus lactis* but not the wild-type strain reduced severity of experimentally induced colitis. Animals that received MAM-expressing *L. lactis* showed improved histopathology, less severe weight loss, and reduced interferon (**IFN**) γ and IL-17 expression compared with controls (Quevrain et al., 2016b).

Oral administration of FP reduced the incidence of severe diarrhea and related mortality rate and increased weight gain in pre-weaned dairy Heifers (Foditsch et al., 2015). This research evaluated the effect of administering a live culture of FP to newborn dairy calves on their subsequent growth, health, and fecal microbiome. *Faecalibacterium prausnitzii* was cultured in VTR2RF medium as previously described (Foditsch et al., 2014). Initially, a safety trial was conducted using 30 newborn bull calves to assess potential adverse effects of oral and rectal administration of live FP to neonatal calves, compared to controls. No adverse reactions (e.g. increased body temperature or heart and respiratory rates) were observed. All bull calves survived the experimental period, and there was no difference in attitude, appetite, dehydration or fecal consistency score between the treatment and control groups. The rectal route was not practical, whereas the oral route ensured that the full dose could be administered to the treated calves.

Subsequently, a randomized field trial was completed in a commercial farm with pre-weaned calves. In total, 554 Holstein heifers were assigned to one of two treatment groups: treated calves (**FPTRT**) and non-treated calves (control). Treated calves received two oral doses of live culture of FP, the first dose at treatment assignment (1st week) and the second a week later. The FPTRT group experienced a significantly lower incidence of severe diarrhea (3.1%) compared to the control group (6.8%) (**Figure 1C**). Treated calves also had a lower mortality rate associated with severe diarrhea (1.5%) compared to control calves (4.4%) (**Figure 1A and B**). Furthermore, FPTRT calves gained significantly more weight (4.4 kg) over the pre-weaning period than controls calves. The relative abundance of FP in the fecal microbiota was significantly higher in the 3rd and 5th weeks of life of FPTRT calves compared to control calves, as revealed by sequencing of the 16S rRNA gene. These findings demonstrated that oral administration of live culture of FP improves gastrointestinal health and growth of pre-weaned calves, supporting its use as a potential probiotic.

Faecalibacterium prausnitzii and Insulin Sensitivity

Recent studies highlight the importance of the gut microbiota as an environmental factor linked to type-2 diabetes (Furet et al., 2010, Qin et al., 2012, Karlsson et al., 2013). A consistent finding from these studies was a decrease in the relative abundance of butyrate producing bacteria such as FP and *Roseburia spp*. in

individuals with type-2 diabetes. Furthermore, butyrate producers, and more specifically FP, have been linked to improved insulin sensitivity and diabetes amelioration in studies of the human fecal microbiota (Furet et al., 2010, Vrieze et al., 2012).

There is a great body of knowledge both supporting the strong anti-inflammatory characteristic of FP and the link between inflammation and type-2 diabetes, but ironically, to the best of our knowledge, no published studies have evaluated the direct effect of FP treatment on glucose metabolism. Recently, our group demonstrated that treatment of pre-diabetic, obese mice [10-week-old Male DIO C57BL/6J mice (strain 380050)] with viable FP culture cells and supernatant resulted in a dramatic improvement of insulin sensitivity (**Figure 2**). Briefly, C57BL/6J wild type mice were fed a high fat diet and treated daily for 20 days with a cocktail of four high butyrate producing FP strains (isolated from bovine and porcine (Foditsch et al., 2014)) or placebo; treatment was performed by oral gavage. Before sacrifice an oral glucose tolerance test was performed, and blood samples collected for insulin serum concentrations. Daily administration of FP significantly increased insulin sensitivity, showing its utility in protecting against insulin resistance in diet induced obese mice (**Figure 2**).

The work by Munukka et al. (2017), provides further support that oral FP treatment improved insulin sensitivity in high-fat-fed mice (Munukka et al., 2017). FP-treated mice had increased insulin receptor β, increased hormone-sensitive lipase phosphorylation in in the adipose tissue, decreased leukocyte infiltration into adipose tissue, lower hepatic fat content, and improved liver function compared with control mice. They concluded that FP treatment improved insulin sensitivity, decreased adipose tissue inflammation, and improved hepatic health (Munukka et al., 2017). Interestingly, FP-treatment increases weight gain because of improved insulin sensitivity, FP-treated mice significantly increased muscle mass and subcutaneous fat when compared with placebo treated mice (Munukka et al., 2017).

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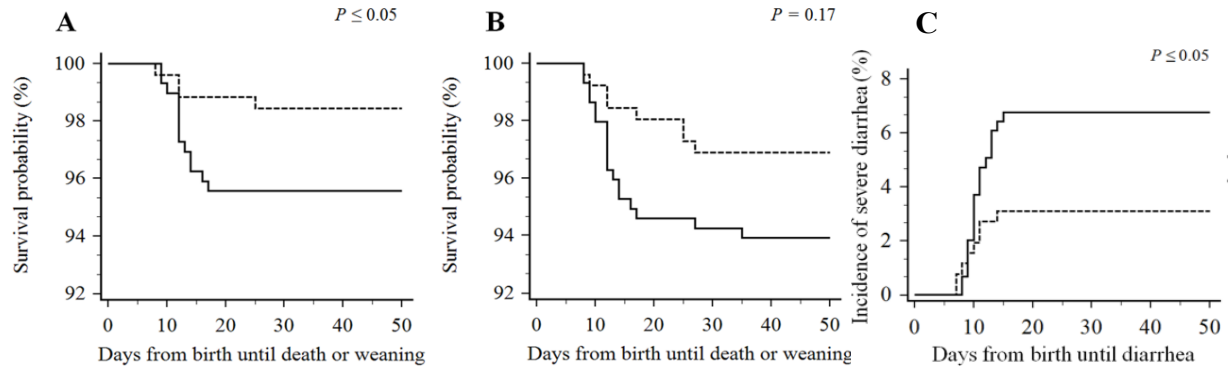

Figure 1. Effect of oral administration of *F. prausnitzii* on calves. A) Effect of *F. prausnitzii* on mortality rate related to severe diarrhea. B) Effect of *F. prausnitzii* on overall mortality. C) Incidence of severe diarrhea. Solid and dashed lines represent controls and treated calves (FPTRT), respectively.(Foditsch et al., 2015)

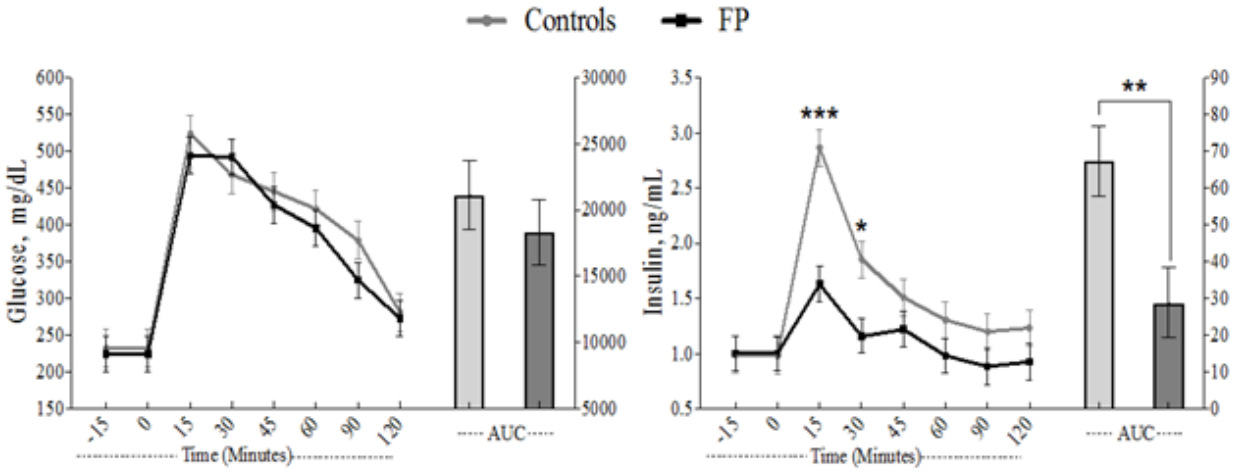

Figure 2. Glucose and Insulin responses to the glucose tolerance test of FP and control mice. Light gray bars and dark gray bars represent the area under the curve [AUC; mg/dL (glucose) and ng/mL (insulin) per 120 min] of control and FP-treated mice, respectively. ***$P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, Ţ $P \leq 0.1$. Error bars indicate SEM. “data not published”

SESSION NOTES

Nutritional Regulation of Gut Function and Development During the Pre-Weaning and Weaning Period of the Dairy Calf

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Introduction

Raising healthy and productive calves is the key to the long-term success of the dairy industry. Unfortunately, amongst all animals on the dairy farm, calves suffer from the highest rates or morbidity and mortality, reaching up to 34% and 5%, respectively (Urie et al., 2018a). In 2010, the Dairy Calf and Heifer Association (**DCHA**) reported that the target morbidity rate for calves 24 to 60 d of age is less than 25% (DCHA, 2010; Urie et al., 2018a), demonstrating that there is still a significant need to reduce the high prevalence of morbidity. Digestive disorders are the most common cause of morbidity and mortality and can often be mitigated through sound early life nutrition and health management programs. As such, knowledge centered on nutritional strategies that promote optimal gut health in young calves is fundamental to ensuring the sustainability and profitability of the dairy industry. Therefore, this paper will focus on how gut function and development are regulated by differing nutritional strategies during the colostrum, transition milk, and whole milk or milk replacer feeding periods, as well as during the weaning transition.

Colostrum Feeding

Ensuring Passive Transfer

The bovine placenta prevents the passive transfer of immunoglobulins (**Ig**) to the calf in utero, and as a result, the neonatal calf is born immune-deficient. Thus, the calf relies on the timely feeding of adequate volumes of high-quality colostrum during early life to ensure that passive transfer of IgG is achieved. Specifically, it is recommended that calves are fed 3 to 4 L of colostrum containing > 50 g of IgG/L and a total bacterial count < 100,000 cfu/mL (McGuirk and Collins, 2004) before 6 hours of life (Stott et al., 1979; Fischer et al., 2018a). Unfortunately, failure of passive transfer (**FPT**, serum IgG < 10 mg/mL) still occurs in 12.1% of heifer calves (Shivley et al., 2018), which appears to be an improvement from recent FPT rates of 19.1% (Beam et al., 2009) and 40% (USDA, 1993). However, there has recently been a push to increase the FPT threshold from the dated recommendation of 10 mg/mL (Tyler et al., 1996; BAMN, 2001) to 15 mg/mL (Furman-Fratczak et al., 2011) or even 20 mg/mL (Chigerwe et al., 2015), as these concentrations better favour the absence of morbidity and mortality. Although this would result in on-farm FPT rates concurrently rising, this can be considered a positive push for dairy producers to improve colostrum and newborn calf management.

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As previously mentioned, one of the most critical factors in achieving successful passive transfer of immunity is the timely feeding of colostrum. It is widely accepted that the gut is no longer permeable to IgG after approximately 24 h of life; however, 41% and 23% of dairy producers reported only occasionally or never feeding colostrum overnight, respectively, which is when the majority of calvings occur (Winder et al., 2018). These practices are concerning, as research from the 1970s and 1980s has shown that serum IgG decreases in a linear trend as calves age (Stott et al., 1979; Bush and Staley, 1980). To our knowledge, Fischer et al. (2018a) was the first study to determine the effect of delaying colostrum feeding after birth using current colostrum recommendations, feeding an average of 3.2 L and 198 g of IgG, and standardizing colostrum intake by feeding pooled colostrum at 7.5% of birth body weight (**BW**). Calves fed within 1 h after birth had higher serum IgG concentrations (22.3 mg/mL) at 24 h compared to calves fed at 6 and 12 h after birth, which did not differ (17.0 mg/mL). This suggests that serum IgG concentrations may decrease in a non-linear trend with colostrum feeding time, and that the closure of the intestine may progress to a finite degree between 1 and 6 h of life (Fischer et al., 2018a). Previous work has shown that the ability to non-selectively absorb macromolecules during early life is unique to fetal intestinal cells (El-Nageh, 1967), which are completely absent by the third day of life (Smeaton and Simpson-Morgan, 1985). However, little is known about the factors that control the turnover of permeable fetal intestinal cells and future research is needed to determine the mechanisms by which gut closure occurs in the absence of colostrum feeding.

In addition to the quickness, quality, quantity, and cleanliness of colostrum feeding, additional factors can influence serum IgG concentrations of the neonatal calf. For instance, Hare et al. (unpublished data) demonstrated that feeding multiple meals of colostrum resulted in a greater maximum serum concentration of IgG (30.4 mg/mL) compared to calves only fed one meal of colostrum followed by whole milk (23.9 mg/mL). In addition, serum IgG concentrations were more persistent – i.e., remained at a greater proportion of the maximum IgG concentration reached – in calves fed multiple colostrum meals compared to those only fed one meal. Feeding multiple meals of colostrum, and thus improving IgG persistency, is likely to assist in preventing early life morbidity by reducing the high prevalence of digestive disorders. In addition, although previous work has found that tube feeding can reduce passive transfer (Godden et al., 2009), recent work by Desjardins-Morrissette et al. (2018) found no differences in IgG concentrations between calves fed 3 L of colostrum via tube or bottle. These conflicting findings likely arise from tube feeding small volumes of colostrum (e.g. 1.5 L) resulting in a large proportion of the meal remaining in the rumen compared to when 3 L is fed. Therefore, tube-feeding can be used as an efficient method to deliver colostrum and achieve passive transfer in neonatal calves, permitting that $\geq$ 3 L is fed.

Establishing a Healthy Gut

Although colostrum is well known for promoting the acquisition of passive immunity, it also plays a fundamental role in establishing the calf gut bacterial community, known as the microbiome. The gut microbiome plays a key role in shaping

early life gut development and maturation. As such, dysbiosis of the microbiome can lead to increased risk of digestive disorders and bacterial infections, which are the main cause of morbidity, mortality and economic loss in the dairy industry. Feeding colostrum during the first hour of life accelerates the bacterial colonization of the calf small intestine, with calves fed fresh colostrum reaching a total bacteria density of 10^{10} 16S rRNA gene/g at 12 h of life compared to calves not fed colostrum, who only achieve 10^{8} 16S rRNA gene/g (Malmuthuge et al., 2015) at 12 h of life – total bacterial levels similar to newborn calves. Similarly, calves fed colostrum immediately after birth tended to have an increased prevalence of *Bifidobacteria* and *Lactobacillus* - which are well-known for their beneficial roles in the newborn gut - associated with the colon at 48 h compared with calves fed colostrum at 12 h of life (Fischer et al., 2018a). Furthermore, Malmuthuge et al. (2015) found that not feeding colostrum increased the prevalence of *Escherchia coli* in the small intestine compared with calves fed colostrum, and recent research has demonstrated an association between late colostrum feeding and the abundance of opportunistic pathogens, namely *Enterococcus* and *Streptococcus* (Ma et al., 2019). Together, these results suggest that bacterial colonization occurs at a slower rate in the absence of colostrum feeding and delaying the first colostrum meal may increase the risk of pathogen colonization and subsequent infection and disease.

In addition to fresh colostrum promoting an optimal gut bacterial community, feeding heat-treated colostrum may have even greater benefits. Specifically, feeding heat-treated colostrum to calves results in a greater prevalence of *Bifidobacteria* in the small intestine compared to calves fed fresh colostrum (Malmuthuge et al., 2015), which may explain the reported reduction in enteric infections with heat-treated colostrum feeding (Godden et al., 2012). Further work in the study by Malmuthuge et al. (2015) revealed that heat-treated colostrum contained 3 times more 3'sialyllactose (**3'SL**), the primary sialylated oligosaccharide (**OS**) in bovine colostrum, compared to fresh colostrum (Fischer et al., 2018b). Bovine colostrum OS can act as the primary energy substrate for beneficial gut bacteria (Yu et al., 2013), suggesting high concentrations of OS may mediate the early establishment *Bifidobacteria* in the calf intestine. To date, over 50 bovine OS have been detected (Aldredge et al., 2013; Albrecht et al., 2014), with concentrations of the primary sialylated bovine OS, 3'SL, 6'sialyllactosamine (**6'SLN**), disialyllactose (**DSL**) and 6'sialyllactose (**6'SL**), present at 15, 72, 22, and 5 times greater concentrations in colostrum compared to whole milk, respectively (Fischer-Tlustos et al., 2020). In addition to promoting beneficial bacterial colonization in the gut, bovine colostrum OS can inhibit adhesion of pathogens to the intestinal surface (Martin et al., 2002), indirectly support intestinal barrier and immune function (Chiclowski et al., 2012), and may enhance the binding of IgG to the intestinal epithelium and its subsequent uptake (Gill et al., 1999). Large quantities of bovine OS can be extracted during cheese whey processing (Barile et al., 2009; Aldredge et al., 2013), which may provide the opportunity for dairy producers to supplement these compounds in colostrum or colostrum replacer to promote optimal calf gut health.

In addition to OS, colostrum contains high levels of growth factors, namely insulin-like growth factor (**IGF**)-I, which is the most abundant growth factor and is thought to have a positive indirect effect on the growth of the intestine (Baumrucker et

al., 1994; Blum and Hammon, 2000). Colostrum also has elevated levels of antimicrobial compounds, such as lactoferrin, lysozyme and lactoperoxidase, which help to maintain a healthy gut (Pakkanen and Aalto, 1997). Furthermore, high levels of nutrients in colostrum stimulate the production of the beneficial gut hormones glucagon like peptide (**GLP**)-1 and -2 (Desjardins-Morrissette et al., 2018; Inabu et al., 2018), which is not observed with milk feeding. Delaying colostrum feeding up to 12 h after birth can suppress the amount of GLP-1 and -2 produced compared to calves fed immediately after birth (Inabu et al., 2018). This may compromise early life growth and gut maturation, as GLP-2 is known to directly stimulate gut development, while GLP-1 promotes insulin release resulting in increased uptake of glucose for energy use by peripheral tissues. Therefore, although the majority of these compounds have been largely overlooked, it is clear that colostrum has a greater role in calf health and development than simply providing IgG.

Transition Milk

After 1 to 2 colostrum feedings during the first day of life, calves are often transitioned directly to whole milk or milk replacer (**MR**). This is a stark contrast to nature, in which calves would consume transition milk (**TM,** milkings 2 to 6) from suckling the dam (Blum and Hammon, 2000). Not feeding TM to calves may be a missed opportunity to improve gut health, as TM contains elevated levels of growth hormones, IGF-1, insulin (Blum and Hammon, 2000), nucleotides (Gill et al., 2011), and OS (Fischer-Tlustos et al., 2020) compared to whole milk. Furthermore, TM contains elevated proportions of omega-3 fatty acids (**FA**) (Hare et al., 2019), which have been shown to benefit antioxidant status and long-term immune response when supplemented to neonatal calves (Opgenorth et al., 2019). Although a previous study showed no benefit of TM feeding on serum IgG concentrations (Conneely et al., 2014), recent work by Hare et al. (unpublished data) demonstrated that serum IgG was more persistent (91% of maximum concentration, Cmax) in calves fed a colostrum:whole milk mixture (**MIX**, 1:1 ratio to simulate TM) compared to calves fed whole milk (75% of Cmax). Furthermore, calves fed MIX tended to have increased production of GLP-1 (Inabu et al., 2019) and displayed increased small intestinal surface area and cell proliferation in certain intestinal segments at 3 days of life compared to calves fed whole milk (Pyo et al., 2020). Although this work is promising, further research to determine the specific role each bioactive molecule may play in promoting optimal gut development is needed.

Milk Nutrition

Plane of Nutrition

Pre-weaning milk or MR feeding programs typically consist of feeding a conventional or elevated plane of nutrition. A conventional milk feeding program aims to encourage starter intake by limiting milk consumption to 10% of birth BW (4-5 L of milk/d or 750 g of MR powder/d). By decreasing milk intake, these programs can result in increased rumen development due to increased starter intake during early life

(Tamate et al., 1962), which may result in less susceptibility to health and production challenges during weaning. Furthermore, conventional feeding programs are often associated with a reduced feeding cost compared to feeding greater volumes of milk. However, lower BW gains (300-500 g/d) are often observed during the first month of life and calves often suffer from hunger, leading to animal welfare concerns (Jasper and Weary, 2002). In contrast, elevated programs feed milk or MR *ad libitum* or at approximately 20% of birth BW (> 8 L of milk/d or 1.2 kg of MR powder/d), which improves animal welfare as it reduces hunger-associated behaviours. A recent study by Haisan et al. (2019) showed that all calves (n = 26) offered large volumes of milk were able to consume over 8 L of milk/d and up to 10 L/d using an automated calf rail during the first week of life, demonstrating that this type of feeding program is synergistic with the calf's natural ability to consume large volumes of milk during this time. Furthermore, calves fed > 8 L/d achieved an average daily gain (**ADG**) of 750 g/d during the first three weeks of life, while calves restricted to 5 L/d only gained 350 g/d. This result is likely due to starter intake being negligible during early life and thus the majority of metabolizable energy is consumed directly from milk, regardless of the feeding program. In addition to increased BW gain and animal welfare, elevated feeding programs have the potential to result in more milk during lactation, improved mammary development, and reduction in age at first calving (Khan et al., 2007a; Soberon et al., 2012).

Despite the well-known benefits of feeding elevated levels of milk, Canadian (Vasseur et al., 2010) and United States (Urie et al., 2018b) dairy producers continue to feed an average of 5.5 and 5.7 L of milk/d, respectively. It is typically thought that feeding elevated planes of nutrition is only feasible through automated feeding, given concerns around feeding large volumes of milk in only 2 meals/d. However, a recent study (Ellingsen et al., 2016) demonstrated that 2-week old calves were able to consume 5 to 9 L of milk/meal without any overflow into the rumen, suggesting that we have largely underestimated how much calves are able to consume in a single feeding. Furthermore, work by MacPherson et al. (2018), demonstrated that there are no differences in insulin sensitivity in calves fed 8 L of milk over 2 meals from the first week of life compared to calves fed 8 L over 4 meals. Calves fed only 2 meals/d had a slower abomasal emptying rate, indicating that glucose delivery was slowed, which may have regulated the insulin response. Therefore, it may be important to begin feeding elevated planes of milk during the first week of life, as this may be a critical metabolic developmental window in which the calf adapts to consuming high volumes of milk.

Whole Milk vs. Milk Replacer Composition

In the United States, solely MR or a combination of MR and whole milk is fed on 60% of dairy operations (Urie et al., 2018b). Furthermore, the majority of dairy producers feed MR containing an average of 20.2% fat (Urie et al., 2018b). This is a stark contrast to whole milk, which contains approximately 30% fat. High fat consumption is essential for calves during early life because it is crucial in meeting energy demands and assisting with thermoregulation. Moreover, the odds of mortality are 3 times higher for calves fed < 0.15 kg of fat/d compared with calves fed > 0.22 kg

of fat/d (Urie et al., 2018a). In addition to containing low amounts of fat, MR contains significantly more lactose than whole milk (45% vs. 35%). With the recent shift in calves being progressively fed larger volumes of MR, there is concern around how feeding large volumes of MR containing a high amount of lactose and a low amount of fat may impact gut development and health. Specifically, high lactose inclusion in MR increases the osmolality of MR (~400-600 mOsm/L) compared to whole milk (~300 mOsm/L), which has been shown to increase intestinal permeability and potentially disturb gut function in calves (Wilms et al., 2019). Moreover, feeding high amounts of lactose could negatively affect glucose homeostasis, resulting in high concentrations of blood glucose and insulin that may eventually lead to insulin resistance. Recent work by Welboren et al. (2019a) showed that calves fed 6 L of MR with high lactose and low fat (**HL**) content twice daily during the first week of life experienced a greater rise in blood glucose and insulin concentrations compared to calves fed a high fat and low lactose (**HF**) MR. Calves fed HF-MR tended to have slower abomasal emptying compared to HL-fed calves, which may have delayed the digestion of nutrients, namely fat and glucose, resulting in the positive effects observed on glucose regulation. To date, research investigating how current MR macronutrient compositions affect calves fed elevated planes of nutrition, especially long-term, is lacking. Moreover, future research is needed to evaluate the specific mechanisms by which MR compositions directly affect calf gut barrier function, development and overall metabolism and health.

Weaning Transition

Current Weaning Strategies

In nature, calves can consume over 10 L of milk/d in 8 to 12 small meals and will be fully weaned between 7 and 14 months of age (Reinhardt and Reinhardt, 1982). This is in contrast to current weaning practices, in which the average weaning age is approximately 9 weeks (Urie et al., 2018b). Furthermore, many operations often practice early weaning programs, with weaning occurring from 4-6 weeks of life, in order to limit milk feeding costs, encourage early intake of starter and thus stimulate rumen development. However, calves fed elevated levels of milk experience a challenge at weaning, especially if weaning occurs before 6 weeks of life, because of low pre-weaning solid feed intake (Jasper and Weary, 2002). This has been shown to result in decreased digestibility of dry and organic matter, neutral detergent fiber, crude protein, and gross energy after weaning (Terre et al., 2007; Hill et al., 2016; Dennis et al., 2018), suggesting that the digestive tract of calves fed elevated planes of nutrition are not equipped to digest solid feed following weaning. Previous research has demonstrated that calves fed an elevated plane of nutrition had greater starter feed intake and weight gain when the weaning transition was extended from 6 to 8 weeks (Eckert et al., 2015) and extending weaning can decrease the reduction of weight gain at weaning (Meale et al., 2015).

In addition to delaying the age of weaning, the “step-down” weaning method can be used to mitigate potential negative outcomes when weaning from elevated levels of milk. Khan et al. (2007a,b) was the first to report on the step-down protocol, in which

calves either received elevated levels of milk at 20% of BW until d 23, followed by a step-down to 10% of BW from d 23 to d 44, or were fed at 10% of BW until d 44. The results showed that intake of solid feed and weight gain increased in calves fed according to the step-down protocol, suggesting that this is a feasible and efficient strategy to maximize weight gain while simultaneously achieving early weaning. When using automation, linear declines in milk intake can be implemented without increasing labor costs. For instance, reducing MR allowance by 2.5% daily from 6 L/d at d 36 to 2 L/d until d 63, results in increased performance compared to reducing milk intake by abrupt 2 L intervals (Welboren et al., 2019b). In contrast to these current protocols, it has long been recommended that calves should be weaned based on starter intake - specifically, calves should consume at least 700 to 900 g of starter for 2 to 3 consecutive days prior to weaning (BAMN, 2003). Despite this, only 31% of calves are currently weaned based off starter intake (Urie et al., 2018b), which is a practice that needs to be further investigated on dairy farms.

Weaning and Gut Function

Prior to weaning, calf gut function is similar to that of a monogastric, with glucose from milk providing the primary energy source. As starter intake increases, the rumen will gradually become the main site of fermentation and short chain fatty acids (**SCFA**) will account for 80% of the calf's energy source after weaning. As such, the weaning transition is a period in which the calf gut undergoes drastic physical changes, with total volume of the rumen increasing from 30% to 70% of the entire forestomach (Warner et al., 1956). The rumen transcriptome and microbiome also undergo rapid maturation, with enhanced expression of metabolic (Connor et al., 2013) and gut barrier (Malmuthuge et al., 2013) genes and altered microbial populations (Meale et al., 2017), resulting from exposure to substrates in the form of calf starter. Interestingly, the lower gut also undergoes significant changes, with microbial diversity increasing (Li et al., 2012); however, there is currently a lack of research characterizing the functional changes that occur to initiate these changes in the lower gut during weaning.

Calves are often fed high starch (>30%) in the form of calf starter in an effort to initiate rapid rumen development. However, this can result in an accumulation of SCFA and reduced ruminal pH, which may lead to ruminal acidosis. Recent work by Van Niekerk et al. (2017) demonstrated that it can take up to 5 weeks after weaning for the rumen environment of calves fed elevated levels of milk to be in a state that is not considered ruminal acidosis. If acidosis is severe, excessive amounts of starch can reach the hindgut (Li et al., 2012), resulting in high levels of fecal starch during weaning for calves fed elevated levels of milk (Eckert et al., 2015; Van Niekerk et al., 2018). This is likely a result of the calf gut lacking the necessary gut adaptations required to digest high amounts of starch at this time. These outcomes are unfavourable, as acidosis of both the rumen and hindgut can lead to systemic inflammatory responses. In addition, the site of fermentation – i.e. the rumen or hindgut – may be shifted depending on the processing of the source of starch in starter. For instance, feeding processed grain, such as steam-flaked corn, increased the risk of ruminal acidosis (Krause and Oetzel, 2006), while whole corn shifted fermentation to the lower gut (Gressley et al., 2011).

Van Niekerk et al. (2018) found that feeding an elevated level of milk combined with whole corn in calf starter resulted in decreased fecal pH 2 weeks after weaning compared to calves fed starter containing flaked corn, indicating that whole corn may shift the site of fermentation to the lower gut, possibly resulting in hindgut acidosis. However, there is currently a paucity of information regarding the influence of preweaning feeding regimes and weaning on the intestinal function of calves.

Conclusions

From the multitude of aforementioned research, it is clear that nutritional management can have a large impact on growth performance, health, and gut function and development. While ensuring passive transfer of newborn calves is of great importance, colostrum and transition milk also contain additional bioactive molecules beyond IgG that can have beneficial effects on gut function and development. In addition, maximizing whole milk or MR intake during the pre-weaning period is essential to animal welfare and growth when starter intake is negligible. Calves are often susceptible to health and production challenges during weaning when fed elevated levels of milk during the pre-weaning period, but this can be mitigated through the use of a step-down weaning program or weaning based on starter intake. Overall, further research is needed to determine the long-term effects that differing nutritional strategies during the first days, weeks and months of life may have on calf gut development and function. This will allow industry representatives and producers to make confident decisions that promote calf health, welfare, and productivity in order ensure the long-term success of the dairy industry.

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SESSION NOTES

Dietary Strategies for The Cow Calf Herd – The Experience of the Brazilian Beef Industry

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Introduction

Brazil is the fourth largest country in the world area wise and has an important beef cattle industry. It is estimated that the Brazilian cattle herd is around 213.5 million head, being 75 million breeding age beef cows. Beef production in Brazil has grown substantially in the last decade, and most of it is due to intensification of the production systems. The feedlot industry has experienced a large increase in the number of cattle fed and more cattle is correctly supplemented throughout the year according to season and forage quality. So, slaughter age has decreased and carcass weight has increased in the last decade around 20 to 25%. However, grass-fed beef is responsible for more than 90% of all beef produced in the country, as only 4.5 to 5 million animals are fed in feedlots, compared to 35 million finished on pasture (including cull cows).

On the beef cow side, less improvement has happened in the same period. Brazil is a large country, has very heterogenous production systems, and almost 100% of the beef cows are managed extensively on tropical pastures and rangelands. Even though the average herd size in the country is around 60 cows/operation, there are numerous ranches running over 10,000 to 30,000 cows, making their overall management an import challenge. It is important to note that the majority of the beef cattle herd is concentrated in the Midwest and north part of the county, encompassing the states of Mato Grosso, Goiás, Mato Grosso do Sul, Pará, and Rondônia. The states of Minas Gerais and São Paulo, in the southeast, also are two important states in terms of cattle number. Despite the large size, the average yield of beef per head is still very low. Having twice the number of cattle, Brazil produces less beef than the United States.

There are a lot of opportunities in the country to intensify beef production and the cow-calf segment in particular, which is the main target nowadays. The production of good quality calves has become the bottle neck of the Brazilian beef industry. Cattle prices sky rocketed in 2019 because of the high demand, mainly by the export market. China has been the main driver, and the availability of finished cattle ready for slaughter has not increased concurrently with demand. Thus, there is an increasing demand for high quality calves in order to fulfill the pressure for producing more beef.

The average weaning rate in Brazil as a whole is around 55 to 60%, and the main reason for this low number is poor nutritional management of the cows. Genetics, health, are other factors as well, but nutrition is by far the main hurdle. Professional ranches achieve weaning rates as high as 82 to 88%, and invest time and effort in

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better nutrition, genetics, people training, and overall management of the cows. The adoption of timed artificial insemination (**TAI**) has allowed an advancement in the use of better genetics, which in turn leads to investment in better nutrition as well. It is estimated that 15% of beef cows are under some type of TAI program in the country today. Just to give a better perspective, the sale of Angus semen has overtaken the sales of Nellore in the last 5 years. And that is an important change. Nellore, a breed originated from India, comprises over 80% of the Brazilian beef herd. Brazil imported Nellore dams/bulls in the early 50's and 60's from India to form the basis of the national herd and has implemented a serious genetic improvement program since then. Nowadays, the country has a very rich germplasm of *Bos indicus* Nellore breed that is very well adapted to the environmental conditions prevalent in Brazil. It's important to note that successful cattle operations in the tropics should comprise cattle that are physiologically and behaviorally adapted to the high ambient temperatures and humidity and low forage quality (Bell et al., 2017).

Nutritional Management of the Cow-Calf Herd

Similar to the USA, Brazil is a large country and the beef cattle production systems are very heterogeneous. There are markedly differences across the country in terms of precipitation, soil fertility, forage type, breed composition, management, herd size, etc., which impair the adoption of a common nutritional strategy to the cow herd. Depending on the resources available, the season and, as a consequence, forage quality and availability, the dietary strategies vary. However, considering the middle west region of the country, that concentrate around 60% of the national beef production, there is a clear division of the year into 2 main seasons: rainy/wet and dry season. The rainy season goes from October/November until February/March, and then the dry season follows until the rains start again in October / November of the following year. Beyond differences in precipitation between the rainy/dry season (1,200 to 3,000 mm vs. close to 0 mm), there are important variations in temperature, day length and sunlight, which impact forage availability and quality. It has been known from a while already that the fluctuations in forage quality along the year do happen markedly. Crude protein (**CP**) and neutral detergent fiber (**NDF**) contents and organic matter (**OM**) digestibility change markedly from season to season. Taking into account that around 80% of the pastureland in Brazil is covered by C4 grasses of the *Brachiaria/Urochloa* and *Panicum* genera (both of them originally from Africa), one might expect poor cattle performance during the dry season if no supplementation program is considered and implemented. Protein levels in the grass drops to around 4 to 6% in the dry season. The limited CP availability has been recognized as the critical threshold for adequate microbial growth on the fibrous carbohydrates in basal forage which results in decreased intake and animal performance (Detmann et al., 2014). Under these circumstances, the supplementation with nitrogenous compounds is the primary nutritional tool to improve the utilization of low quality forage by grazing cattle.

In a recent meta-analysis on protein supplementation of cattle grazing tropical pastures, Detmann et al. (2014) showed that the maximum digestible OM intake would be obtained with a ruminal ammonia nitrogen concentration close to 13 mg/Dl. Low CP

forages (< 7 %) show a marked increase in intake to a protein meal supplement up to a supplement level of 5 g/kg of body weight (**BW**) per day (Poppi et al., 2018), allowing the animal to deal better with a deficient N situation. Based on this concept, most of the beef industry in Brazil adopts a protein supplementation strategy to the cow herd during the dry season.

For any supplementation program to be effective we need to take into account the forage quality / availability, the feed resources availability / price and the animal production level expected. There is a quite wide availability of different protein feedstuffs in the country, mainly from the soybean and cotton industry, i.e., soybean meal, soy hulls, cottonseed cake, cottonseed meal, whole cottonseed. The corn-based ethanol industry has expanded remarkably in the last few years, mainly in the states of Mato Grosso and Goiás, making ethanol by products such as wet and dry distiller' grains (**WDG** and **DDG**) available to producers.

Although protein byproducts are available, urea is by far the main nitrogen source used in supplementation programs for beef cows during the dry season. Most producers use a urea-salt based products to supplement cows during the dry season, and these products contain non-protein nitrogen varying from 28.2 to 56.4%, according to the severity of the dry season and forage quality. Urea and salt are used as intake limiters and in most situations, and the supplement is provided two to three 3 times a week. The target intake is around 40 to 50 g/kg of cow BW. Thus, for a cow with average BW of 440 kg (~ 970 lb), the supplement intake would be around 180 to 200 g/day (0.40 to 0.45 lb/d). Under this level of intake and considering the limitation of protein that the supplement is intended to alleviate, most producers would expect the cows to maintain body condition score during the dry season. Obviously, the availability of stockpiled forage is critical for the success of this supplementation program and we have used a target to try to provide ideally 4 to 5% of the cow's BW of potentially digestible dry matter coming from pasture. The main idea is to allow optimal utilization of energy from forage fiber, which will be reached by increasing the microbial utilization of potentially degradable fraction of NDF, that represents 60 to 70 % of tropical forages dry matter (Sampaio et al., 2009).

Recently, some producers are evaluating different supplementation strategies for pregnant cows in order to obtain a calf with better growth potential. Fetal programming has been a hot topic in the Brazilian beef industry in the last 3 to 4 years and more and more cattle ranchers are considering using this concept to produce more kg of calf weaned per cow exposed. One of the concerns by cattle producers relative to using strategies to influence fetal programming itself is the cost-benefit of such management strategies. A question often posed is: Does supplementation of cows during mid to late gestation with larger amounts of supplements pay off? Beyond better reproduction status, will such strategies result in a heavier calf all the way from weaning to the rail that pays the additional supplementation costs? What we have seen in the industry is an adoption of more intensive supplementation programs for heifers and primiparous cows, with protein supplementation levels up to 0.2 to 0.3% of their BW during the dry season. Data from Marquez et al. (2017) revealed that maternal supplementation (1 to

1.5 kg of a 28.3% CP supplement) during mid-gestation in Nellore cows grazing low quality tropical pastures increased the number of myofibers in skeletal muscle of the offspring when compared with calves born from dams that were not supplemented during gestation.

The response of beef cows to supplementation with the goal of having heavier calves at weaning depends on a number of factors, including when supplementation takes place (mid vs. late gestation), the nutritional challenge the cow is facing (forage quality and availability), the duration of the breeding season, and the level of supplementation that is dictated by costs. With the growing adoption of TAI in the last decade, it was possible to concentrate pregnancy in the first 21 days of the breeding season. In 2002, only 5.8% of the beef cows in Brazil were artificially inseminated, being only 1% under a TAI protocol, whereas in 2019, 13.1% of beef cow herd received AI with 86% of them under a TAI protocol (Baruselli et al., 2019). Under those management conditions, the cows bred early in the season, will encounter low quality forage only in the final stages of gestation. Conversely, the cows that are bred in the middle and end of a 90 to 120 days breeding season will face nutritional challenges all the way from mid to late gestation.

Therefore, the dietary model must vary according to each ranch reality and objective. Results from Marquez et al. (2017) indicate that supplementation at late gestation may not substantially contribute to increase myogenesis in fetal skeletal muscle. On the other hand, supplementation at mid-gestation may be more effective to increase the commitment of mesenchymal stem cells into myogenesis as well the proliferation of myogenic cells, allowing the formation of more secondary muscle fibers, leading to a greater number of myofibers at birth.

Recent research, on the other hand, has shown that energy restriction during late pregnancy may trigger a more pronounced stress response in the offspring that may impair the muscle tissue and immune system development (Sanglard et al. 2018). Low protein intake under grazing conditions in the tropics during the dry season lead to energy restriction due to low intake, poor diet digestibility, an, as a consequence, low volatile fatty acids production in the rumen. Thus, the dietary strategy needs of the beef cow herd has to be flexible enough to accommodate the variations on breeding season length and the distribution of pregnancy throughout the breeding season. Research on fetal programming in Brazil, with Nellore cows grazing tropical grasses, has shown positive results of implementing a better dietary strategy to the cow herd. Lopes et al. (2019) concluded that protein supplementation for grazing late pregnant beef cows changed the profile of plasma circulating amino acids and synthesis of skeletal muscle tissue in the offspring. Gomes et al. (2018) showed a 14 kg difference in calves weaned from Zebu cows supplemented during mid gestation compared to control counterparts.

During the rainy season, in most situations, there is plenty of rain and moisture in the soil to support forage growth. Along with that, good pasture management allows the production of high quality forage, which in turn maximizes the production of kg of calves weaned per hectare. However, soils that support the vast Cerrado vegetation (also known as Brazilian savanna that concentrates great part of the beef herd) in Brazil can

correctly be considered as some of the most chemically infertile in the word. These soils in the Brazilian tropical regions have high concentrations of aluminum, low pH, and low concentration of Ca, P and the majority of trace minerals, especially those important for animal nutrition. Such conditions result in deficiencies of most necessary minerals in native plants and even on cultivated pastures needed by beef cows and calves. As a result, without a correct mineral supplementation program, poor animal performance would be expected.

There is a vast and broad body of literature supporting the role of trace minerals in proper physiological function, including reproduction. There is an argument, though, about the reproduction response to organic vs inorganic minerals. Recently, Dantas et al. (2019) concluded that the complete replacement or inorganic with a complexed source of trace minerals might be necessary in order to achieve reproductive benefit. It is important to note that under most Brazilian beef cow production scenario, providing mineral supplement consistently can be a challenge. Vast extensive areas, large herds, lack of labor, heavy rain, mud, among other factor, make it complicated to provide minerals frequently and achieve a consistent mineral intake. As a result, weatherized minerals have become a reality in Brazil in the last couple of years. Few animal nutrition companies have used different weatherization technologies, with the same claim: proper mineral supplement intake during the rainy season, when the cows are bred and need the macro and trace minerals the most; and the capacity to provide the minerals more infrequently (for instance, once a week). Brummer et al. (2019) suggested that newer forms of mineral delivery such as organically based and chelated minerals for beef cattle may provide additional long-term supplementation effect. Thus, combining weatherized technology with organic or chelated minerals seems to make sense during the rainy season. We have seen consistent intake and better performance of beef cows supplemented with this type of product and following a standard procedure when it comes to frequency of mineral provision in challenging environment such as in Pantanal and in the border of the Amazon.

The main nutritional approach used during the rainy season for beef cows under grazing in Brazil is to provide a mineral supplement aiming at an intake around 25 g/100 kg of BW, which would allow the provision of 8 to 9 g of P/cow/day and most of the key trace minerals such as Zn, Cu, Mn, Co, I, and Se. In most situations, the trace minerals intake from grass is not taken into account when formulating the mineral supplement, and most animal nutrition companies would consider the mineral requirements determined either by Nasem (2016), by BR CORTE (2016) or a combination of both to establish the concentration of trace minerals in the supplement. It has been recommended to feed around 120 to 130% of trace minerals in free-choice minerals year round, thereby ignoring the amount being supplied from pasture. Even though it is well established that a proper level and balance of minerals and vitamins are essential to the health, growth and reproduction of beef cows (Rasby et al., 1998), most free-choice minerals would be devoid of vitamins when used during the rainy season. Vitamins are in substantial concentration in green, leafy forages. Therefore, most forages during the rainy season will meet cow requirements for fat-soluble vitamins.

Another technology that has been used in fee-choice minerals for beef cows is incorporation of ionophores. These compounds have been used for a long time in other countries. Mature beef cattle grazing medium to high quality forages have been observed to have increased weight gain and feed efficiency when provided an ionophore supplemented compared with nonsupplemented control cows (Sprott et al., 1988). Webb et al. (2001) concluded that the addition of lasalocid to the diet would be beneficial in improving postpartum reproductive performance in Brahman cows. One concern that most producers have when feeding an ionophore in the free-choice mineral is related to is impact on supplement intake. Some studies have proven that mineral intake decreases with monensin addition (Beck et al., 2014; Maciel et al., 2019). Due to this effect, lasalocid has been the ionophore of choice for beef cows under grazing and usually supplemented incorporated with free-choice minerals. The main reason to utilize ionophores is to promote an increase in propionate synthesis in the rumen and, consequently increase supply of gluconeogenic substrate for glucose synthesis. A range cow must synthesize nearly all her glucose needs through gluconeogenesis, and tropical forage provides few precursors from which she can produce glucose. Most rumen fermentation in grazing cattle leads to acetate production and limited propionate. Glucose requirements increase with the onset of lactation and glucose is used first for milk production, which can create a deficit of glucose for other metabolic needs. Thus, in order to improve the glucose status of a cow, she must be fed a product that can promote increased glucose synthesis (Petersen et al., 2010).

The nutritional flushing prior to ovarian super stimulation may increase follicular population and super ovulatory response in cows, which may be associated with increased insulin and insulin-like growth factor-I concentrations in response to increased propionate concentrations in the rumen (Sartori et al., 2013). Thus, mineral supplements designed to beef cows in Brazil used during the rainy season and before the onset of the breeding season usually contain lasalocid. Furthermore, these supplements also contain chromium, as chromium alters glucose metabolism and elicit improvements in body condition and reproduction in beef cows (Stahlhut et al., 2006). A final detail on the dietary strategy for cows during the rainy season is the strategy used to change the sodium level in the supplement with the objective to attain consumption of the mineral supplement. When supplements include ionophores, the aimed level of intake is of 50 g/100 kg of BW, which is double the amount of regular free-choice mineral supplement. The reason for that is to try to guarantee the daily dose of the additive and the minerals, increasing the opportunity for improved reproductive performance.

Conclusions

The Brazilian beef cattle industry has evolved markedly in the last decade and the high demand in both domestic and international markets for beef has allowed the adoption of more technology in the different production systems. The cow-calf sector has become the bottle neck of beef production in the country. The technology implementation across the beef production chain has not been evenly balanced, as the feedlot and stocker industry are early adopters compared with the cow-calf. So, there is

a need for research and extension forces to support the cow-calf sector to advance and produce more kg of calves weaned per hectare, and calves with better muscle growth potential. Implemented strategically, technology can be used to improve beef operations across the board for a more integrated approach that saves time, improves processes and leads to increased profitability. The Brazilian beef industry, in particular the beef-cow herd is expected to experience, in the short term, a large increase in technology adoption that will improve productivity.

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SESSION NOTES

Nutritional Strategies for Developing Replacement *Bos Indicus*-Influenced Beef Heifers

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Introduction

Approximately 45% of U.S. beef cows are located in southern and southeastern states where *Bos indicus*-influenced cattle and extreme heat conditions predominate (NASS, 2017). Despite their wide importance, *Bos indicus*-influenced cattle are typically managed using practices developed for *Bos taurus* breeds reared in temperate zones. *Bos taurus* and *B. indicus* are different subspecies that diverge in social and biological functions (Cooke et al., 2020). Under the same environmental and nutritional conditions, *Bos taurus* and *Bos indicus* cattle exhibit differences in feed digestion (Habib et al., 2008; Bell et al., 2017) and physiology (Sartori et al., 2016).

A major limiting factor for reproductive success of *Bos indicus*-influenced beef heifers is the late attainment of puberty due to genetics, environment (i.e. heat stress), and nutrition. Heat stress is detrimental to cattle metabolism, growth, reproduction, health, and welfare (Mader, 2003; Key et al., 2014) and will become a greater challenge in the future due to the potential impact of global climate change (IPCC, 2007). Environmental conditions are considered thermoneutral when thermal-humidity index (**THI**) ≤ 70, mild heat stress when 70 ≤ THI < 74, heat stress when 74 ≤ THI < 77, and severe heat stress when THI ≥ 77 (Davis et al., 2003). **Figure 1** shows the average, minimum and maximum daily THI values obtained at the University of Florida - Range Cattle Research & Education Center (Ona, FL). From June to October 2019, average THI values were within or above the threshold considered as heat stress. Also, maximum THI values often reached severe heat stress levels. These challenging conditions during summer partially explain the poorer average daily gain (**ADG**; **Table 1**) of heifers, despite the greater nutritional composition of forage during Summer vs. Fall.

The cow-calf industry in Florida relies on warm-season forages as the main source of feed for beef cattle. This forage type often does not meet the requirements of growing heifers, even if herbage mass is not a limiting factor. Moore et al. (1991) compiled the nutritional analysis of 637 samples of forages commonly grown in Florida (bahiagrass, bermudagrass, digitgrass, stargrass, and limpograss) and reported that most of these grasses contained between 5 to 7% crude protein (**CP**) and 48 to 51% total digestible nutrients (**TDN)**, on the basis of dry matter (**DM)**. Developing heifers require diets with at least 55% TDN and 8.5% CP on a DM basis to achieve adequate growth rates (≥ 0.50 kg/d; NRC, 1996). Nevertheless, successful reproductive

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performance can still be obtained if heifers become pubertal before the initiation of breeding season (Moriel et al., 2017). For instance, our previous study funded by the Florida Cattlemen Enhancement Board demonstrated that average final pregnancy rates were 82% for heifers that achieved puberty BEFORE the start of the breeding season compared to 36% for heifers that achieved puberty DURING the breeding season. In this article, we will provide a summary of our previous and on-going studies to optimize growth and reproduction of *Bos indicus*-influenced beef heifers in tropical/subtropical environments.

Post-Weaning Energy Intake

Increasing the post-weaning energy intake led to positive impacts on the reproductive physiology of heifers (Moriel et al., 2017). Our group has observed that heifers supplemented with concentrate DM at 1.75% of their body weight for the entire development period (September to March) had greater overall ADG, puberty attainment before the start of the breeding season, and pregnancy rates compared to heifers supplemented at 1.25% of their body weight (**Table 2**). Lifetime productivity is significantly improved when heifers calve early in their first calving season (Cushman et al., 2013). Supplementation at 1.75% also increased the percentage of heifers calving during the first 4 weeks of the calving season. Total amount of supplement consumed during the study was 1,350 lb and 1,888 lb for heifers supplemented at 1.25% and 1.75% of body weight, respectively. Concentrate supplement cost was $0.15/lb. Total feed cost per heifer was $203 and $283 for heifers supplemented at 1.25% and 1.75%, respectively. Assuming all calves from these heifers are weaned with 550 lb at $1.40/lb, the net return per heifer after discounting feed costs would be $87 greater for heifers supplemented at 1.75% vs. 1.25% of body weight**,** despite their greater supplementation cost.

Frequency of Concentrate Supplementation

Up to 63% of annual production costs in cow/calf operations are associated with cattle feeding (Miller et al., 2001). Decreasing the frequency of concentrate supplementation from daily to 3 times weekly, for example, can help to reduce these costs by more than half. When supplementation frequency is reduced, the amount of supplement fed weekly remains the same (i.e., 21 kg/week), but the amount of concentrate fed at each feeding event is increased compared to daily supplementation (3 kg/day for 7 days vs. 7 kg/day on Mondays, Wednesday, and Fridays).

Previous studies reported that reducing the frequency of energy supplementation from daily to 3 times weekly had no impact (Drewnoski et al., 2011; Moriel et al., 2016) or decreased ADG of beef calves by 10 to 21% (Cooke et al., 2008; Artioli et al., 2015). Discrepancies among these results can be associated to differences in supplement composition, animal breed and sex, location of the study, forage species and quality, and the potential interactions among those factors (Artioli et al., 2015). However, differences in daily forage DM intake between cattle offered frequent or infrequent energy supplementation is the primary factor explaining the variable growth

performance among these studies. When supplementation frequency is reduced, cattle consume a large portion of concentrate in a single day and receive no concentrate supplementation on the next day. This less frequent schedule of supplementation leads to fluctuations in forage and nutrient intake.

In terms of performance of beef heifers, reducing the frequency of supplementation may be detrimental to reproduction. Moriel et al. (2012) evaluated the impact of similar weekly energy supplementation that was offered either daily (**S7 heifers**) or 3 times weekly (**S3 heifers**; Monday, Wednesday, and Friday) on growth and reproductive performance of developing beef heifers fed stargrass. Supplements were offered at weekly rates of 16 kg of DM/heifer. On days that both S3 and S7 heifers were supplemented, S3 heifers had lower hay DM intake compared with S7 heifers (2.55 vs. 3.36 kg/day, respectively). On days that only S7 heifers were supplemented, S3 heifers also had lower hay DM intake (3.15 vs. 3.38 kg/day for S3 and S7 heifers, respectively). Consequently, overall mean hay DM intake was 15.4% lower for S3 vs. S7 heifers (2.85 vs. 3.37 kg/day, respectively). Estimated net energy of gain (**NEg**) intake followed the same pattern observed on total DM intake, and overall estimated NEg intake was slightly greater for S7 vs. S3 heifers (2.75 vs. 2.59 Mcal/day, respectively). However, the magnitude of differences on estimated overall NEg intake between S7 and S3 heifers was not sufficient to impact ADG (0.28 vs. 0.27 kg/day for S3 and S7, respectively). Despite the similar ADG, attainment of puberty and pregnancy were delayed by decreasing the frequency of energy supplementation (**Figure 2**). At the end of the breeding season, approximately 38% of S7 heifers were pubertal, whereas only 17% of S3 heifers were pubertal. Final pregnancy rates did not differ between treatments but S7 heifers became pregnant earlier in the breeding season (**Figure 2**).

Enhanced reproductive performance have been associated with increased blood concentrations of glucose, insulin, and insulin-like growth factor 1 (**IGF-1**; Hess et al., 2005). In cattle, GnRH secretion is impaired when glucose availability is inadequate, but resumed when glucose levels are adequate (Hess et al., 2005). Cows with low plasma insulin concentrations have impaired LH surge, reduced numbers of LH receptors in the dominant follicle, and fail to ovulate (Diskin et al., 2003). Insulin-like growth factor 1 is a major metabolic signal regulating reproduction in cattle (Wettemann and Bossis, 2000; Thatcher et al., 2001). Plasma glucose, insulin and IGF-1 are positively affected by nutrient intake (Vizcarra et al., 1998; Bossis et al., 1999) and supplementation frequency (Cooke et al., 2007). For instance, plasma glucose and insulin concentrations were greater for S3 vs. S7 heifers on the days that only S7 heifers received supplementation, but not on days that both treatment groups were supplemented. More importantly, heifers supplemented every day had less daily variation in plasma concentrations of glucose and IGF-I than heifers supplemented 3 times weekly (**Figure 3**; Moriel et al., 2012). The differences in plasma concentrations of glucose and insulin were attributed to the pattern of nutrient intake of each treatment, and this lower fluctuation in blood parameters with a more frequent supplementation schedule likely collaborated for the improved puberty achievement compared to infrequent supplementation (Moriel et al., 2012).

Recently, we attempted to overcome the negative effects of frequency of supplementation by increasing the amount of supplement offered to heifers. In this 2-year study, heifers were supplemented with concentrate DM at: 1.25% of body weight offered 3 times weekly (**1.25-3X**); 1.25% of body weight offered 7 times weekly (**1.25-7X**); 1.75% of body weight offered 3 times weekly (**1.75-3X**); or 1.75% of body weight offered 7 times weekly (**1.75-7X**). The hypothesis was that by increasing the concentrate supplementation amount, heifers offered reduced frequency of supplementation would achieve similar puberty attainment and pregnancy percentage compared to heifers supplemented daily. Contrary to our hypothesis, effects of supplementation frequency × amount were not detected ($P \geq 0.71$) for any variable. Growth and reproductive performance of heifers supplemented at 1.25% or 1.75% were discussed previously (**Table 2**). Similar to our previous studies, growth and reproductive performance of heifers supplemented 3 times weekly were reduced compared to heifers supplemented daily (**Table 3**). Although pregnancy rates did not differ, heifers supplemented 3 times weekly calved later during their first calving season compared to heifers supplemented daily (**Table 3**). Therefore, despite including greater supplementation amounts and a puberty induction protocol, heifer reproductive performance was significantly jeopardized when supplementation frequency was reduced from daily to 3 times weekly.

Growth Pattern (Stair-Step Strategy)

Modifying the growth pattern during the post-weaning phase has been used to promote reproductive success of *Bos taurus* heifers. Lynch et al. (1997) developed beef heifers to achieve an even weight gain from weaning until breeding (EVENGAIN) or achieve a low weight gain from weaning until 45 days before breeding followed by a high weight gain in the final 45 days before breeding (LOW-HIGH). Both groups were fed enough nutrients to achieve 65% of the expected mature body weight by the start of the breeding season. The strategy of low weight gain followed by high weight gain is called Stair-Step strategy and is usually implemented to explore compensatory gains that occur when nutrition level is increased immediately after a period of nutrient restriction. In that study (Lynch et al., 1997), LOW-HIGH heifers had greater first-service conception rate compared to EVENGAIN heifers (71% vs. 56%). Although final pregnancy rates did not differ between these two treatments (88% vs. 88%), the greater first conception rates of LOW-HIGH heifers led to increased percentage of heifers calving early in their first calving season, which has been associated with greater lifetime productivity and longevity. Another study also reported that heifers developed using a Stair-Step strategy had approximately twice as many primordial follicles (an indicator of ovarian reserves) at 14 months of age compared to heifers developed on an even gain program (Freetly et al., 2014). This response is important because primordial follicles found within the ovary serve the needs of the entire reproductive lifespan. Also, larger ovarian reserves might be associated with increased fertility in cattle (Cushman et al., 2014). Hence, the Stair-Step strategy may allow producers to further improve the reproductive performance of their heifers without increasing feed costs. It is important to highlight that the studies described above used *Bos taurus* heifers. It is unknown if this strategy would generate similar results in heifers developed in the Florida, particularly

due the *Bos indicus* genetic contribution and the hot and humid Summer/early-Fall delaying puberty attainment. Our on-going study will explore the Stair-Step strategy for developing Brangus heifers to determine if such nutritional strategy may or may not be applied in FL production system.

The experiment will be conducted at the Range Cattle REC (Ona, FL) from September 2019 to June 2020 (Year 1) and replicated from September 2020 to June 2021 (Year 2). In September of each year, 64 Brangus heifers will be allocated into 1 of 16 bahiagrass pastures (4 heifers/pasture). Treatments will be assigned to pastures (8 pastures/treatment) and will consist of: control heifers supplemented with concentrate DM at 1.50% of body weight from September until the start of the breeding season in December (day 0 to 100 of the study; **CON**); or stair-step heifers initially offered concentrate DM at 1.05% of body weight from September to October (day 0 to 50 of the study), and then, concentrate DM at 1.95% of body weight (DM basis) from October until the start of the breeding season in December (**SST**; day 50 to 100 of the study). In average, both treatments will be supplemented with concentrate DM at 1.50% of body weight from September to December (22% CP and 73% TDN; DM basis).

In year 1, total supplement DM offered to heifers did not differ between treatments (410 vs. 405 ± 3.5 kg/heifer for SST and CON, respectively; $P = 0.26$). In terms of growth, ADG from day 0 to 50 did not differ between treatments (0.63 vs. 0.62 ± 0.040 kg/day; $P = 0.87$) but was greater for SST vs. CON heifers from day 50 to 100 (0.73 vs. 0.56 ± 0.044 kg/day; $P = 0.01$), leading to a tendency for greater overall ADG (0.68 vs. 0.59 ± 0.031 kg/day; $P = 0.07$) and greater body weight at start of estrus synchronization protocol for SST vs. CON heifers (311 vs. 302 ± 2.1 kg; $P = 0.009$).

Intravaginal thermometers were inserted into heifers to determine the intravaginal temperatures every 30 min from day 25 to 31 (Sep 7th to 12th) and day 85 to 91 of the study (Nov 6th to 12th; see **Figure 1** for THI values). From day 25 to 31, SST heifers had significantly lower intravaginal temperatures from 0930 h to 1800 h compared to CON heifers (nearly 0.25 to 0.32^{o}C lower for SST vs. CON), which is likely a result of lower heat increment and partially explains the lack of treatment effects on heifer ADG from day 0 to 50 despite the drastic differences in supplement DM offered (1.05 vs. 1.50% of body weight for SST and CON, respectively). From day 85 to 91, supplement DM amount did not ($P = 0.39$) affect intravaginal temperature of heifers, which likely prevented energy waste to cope with heat stress and allowed the greater ADG of SST vs. CON heifers.

Although overall ADG tended to differ, reproductive tract scores (4.52 vs. 4.37 ± 0.173 for SST and CON, respectively; $P = 0.58$) and percentage of pubertal heifers at the start of the synchronization protocol (79.3 vs. 71.9 ± 8.23 % of total for SST and CON, respectively; $P = 0.54$) did not differ between treatments. We will repeat this study for another year to confirm these results, but based on data from year 1, the SST strategy offered an opportunity to harvest greater growth performance before the start of the breeding season without increasing feed costs. This enhanced growth performance did not lead to any advantage on heifer puberty attainment before breeding in year 1 of

our study but might be important in situations when heifer post-weaning body weight are lighter than those reported herein.

Early-Weaning

Metabolic imprinting is the process by which nutrition during early-stages of a calf's life may permanently change its development and subsequent performance (Lucas, 1991). This concept has substantial economic implications for agriculture and should be explored to improve the performance of animals destined for food production. Early-weaning is a management practice consisting of permanent calf removal at ages often less than 5 months. Conversely, normal weaning traditionally occurs when calves are between 7 to 9 months of age. Early-weaning has been shown to improve calf growth (Moriel et al., 2014) and feed efficiency and reproductive performance of cows (Arthington and Kalmbacher, 2003). Despite the positive effects of early-weaning on cattle performance, few beef producers are willing to adopt the early-weaning practice because of the limited amount of information on how to manage early-weaned calves and increased labor associated with feeding calves daily. Thus, our group conducted a 2-year study at the UF/IFAS Range Cattle Research and Education Center to evaluate different calf management systems for early-weaned beef calves and their long-term consequences to heifer growth and reproduction (Moriel et al., 2014).

In January of each year (day 0 of the study), Brangus calves (70 days of age) were assigned to remain with their dams and be normally weaned at 250 days of age (day 180 of the study; **NW**), or early-weaned at 70 days of age and randomly assigned to 1 of 3 early-weaning management systems from day 0 to 180 of the study: 1) ryegrass and bahiagrass grazing for 180 days (**EWPAST**); 2) high-concentrate diet in drylot for 180 days (**EW180**); and 3) high-concentrate diet in drylot for 90 days, then bahiagrass grazing for additional 90 days (**EW90**). When early-weaned calves were in drylot, they were limit-fed the high-concentrate diet at 3.5% of body weight (as-fed). When early-weaned calves were on pasture, they were supplemented with the same high-concentrate diet at 1.0% of body weight (as-fed). Calves that were kept with the mothers until weaning (250 days of age) did not receive supplementation from 70 to 250 days of age.

We observed that EW90, EW180, and EWPAST heifers had similar or greater growth performance from day 0 to 180 than NW heifers (**Table 4**). From day 180 of the study until the end of the breeding season (day 395), all heifers were supplemented with concentrate DM at 1.5% of body weight (as-fed). During this period, no differences were detected for ADG among treatments (in average = 0.68 kg/day). Interestingly, limit-feeding a high-concentrate diet in drylot, for at least 90 days, increased the percentage of heifers cycling at the start of the breeding season compared to normally weaned heifers (**Table 4**). More specifically, a greater percentage of early-weaned heifers fed high-concentrate diet in drylot for only 90 days achieved puberty at the start of the breeding season, despite having similar body weight and ADG compared NW heifers. This response indicates that we can successfully hasten puberty achievement if *Bos*

indicus-influenced beef heifers by temporarily exposing young calves to high-concentrate diets and high-growth rates starting at approximately 70 days of age.

Pre-Weaning Injections of Bovine Somatotropin

The exact nutrition-mediated mechanisms involved in this early activation of the reproductive axis in beef heifers are unknown. However, circulating IGF-I can affect gonadotropin secretion and activity required for the first ovulation and subsequent puberty achievement in beef heifers by influencing hypothalamic–pituitary secretory activity (Schillo et al., 1992) and augmenting the effects of gonadotropins in ovarian follicular cells (Spicer and Echternkamp, 1995). Thus, metabolic imprinting may be explored by identifying strategies to increase heifer ADG and plasma IGF-1 during the developmental phase leading to optimized future reproductive performance. In agreement, heifer ADG and plasma IGF-1 concentrations from 70 to 160 days of age explained approximately 34% of the variability on age at puberty (Moriel et al., 2014). Although postweaning injections of bovine somatotropin (**bST**) hastened puberty attainment of *Bos taurus* heifers (Cooke et al., 2013), less emphasis has been placed on preweaning management strategies despite their greater impact on heifer puberty attainment compared with postweaning management practices.

Bos taurus and *Bos indicus* are different subspecies that diverge in social and biological functions (Cooke et al., 2020). Under the same environmental and nutritional conditions, *Bos taurus* and *Bos indicus* cattle not only exhibit diet-dependent differences in intake, digestion and ruminal fermentation (Habib et al., 2008; Bell et al., 2017), but also different ovarian function, circulating hormones and metabolites (Sartori et al., 2016). These differences may determine the direction and magnitude of performance responses to similar management applied to *Bos taurus* or *Bos indicus* breeds. Thus, we conducted 2 studies to evaluate the impacts of preweaning injections of bST on growth and reproductive performance of Brangus (*Bos indicus* × *taurus*; Experiment 1; Piccolo et al., 2018) and Nellore beef heifers (*Bos indicus*; Experiment 2; Moriel et al., 2019).

In Experiment 1, suckling Brangus heifers were stratified by body weight (147 ± 20 kg) and age (134 ± 11 days) on day 0, and randomly assigned to receive an s.c. injection of saline (**SAL**; 5 mL; 0.9% NaCl) or 250 mg of sometribove zinc (**BST**; Posilac, Elanco, Greenfield, IN) on days 0, 14, and 28. Heifers and respective dams were managed as a single group on bahiagrass pastures from day 0 until weaning (day 127), and provided the same diet during the entire post-weaning phase. In Experiment 2, suckling Nellore heifers were stratified by body weight (97 ± 16 kg) and age (80 ± 10 days), and randomly assigned to receive s.c. injections of saline (5 mL 0.9% NaCl) or 250 mg of sometribove zinc (**BST**) on days 0 and 10 of the study. Then, all Nellore heifers were managed as a single group in *Brachiaria decumbens* pastures, weaned on day 177, and provided a corn silage–based TMR from weaning until the end of the study (day 380).

In Experiment 1, Brangus-crossbred heifers administered preweaning bST injections had an 8.6 ng/mL increase in plasma IGF-1 concentrations (103 vs. 95 ± 3.2 ng/mL; $P = 0.05$) and 7.2% increase on ADG from days 0 to 42 (1.15 vs. 1.07 ± 0.03 kg; $P = 0.07$), but no differences on overall pre-weaning ADG (0.88 and 0.89 ± 0.02 kg/day; $P = 0.50$) and post-weaning ADG (0.28 and 0.30 ± 0.02 kg/day; $P = 0.61$) compared to saline heifers. Also, heifers assigned to BST tended to achieve puberty 26 days earlier (388 vs. 414 ± 13 days; $P = 0.10$), had greater percentage of pubertal heifers on days 244, 263, 284, and 296 of the study ($P \leq 0.04$; Fig. 4), and tended to have greater overall pregnancy percentage (82 vs. 69 ± 6.1%; $P = 0.10$) compared to saline heifers.

In Experiment 2, preweaning bST injections increased plasma IGF-1 concentrations by 52 ng/mL (211 vs. 159 ± 9.3 ng/mL; $P = 0.0001$) and ADG from days 0 to 10 by 35% (0.65 vs. 0.48 ± 0.061 kg/day; $P = 0.03$), but did not affect overall pre-weaning ADG (0.45 vs. 0.47 ± 0.009 kg/day; $P = 0.24$), tended to decrease post-weaning ADG by 3.6% (0.80 vs. 0.83 ± 0.014 kg/day; $P = 0.07$) and decreased puberty attainment on days 349, 359, and 380 ($P \leq 0.05$; Fig. 4) compared to saline injections.

Sartori et al. (2016) reported that *B. indicus* cattle naturally have greater circulating IGF-I concentrations compared with *B. taurus* cohorts. Moreover, Mendonça et al. (2013) demonstrated that even under the same environment and diet, *Bos taurus*–influenced dairy cows have less circulating concentrations of IGF-I compared to *Bos indicus* cows, which might be related to the different organ sensitivity to IGF-1. It is possible that the greater increment on plasma IGF-1 concentrations following bST injection in Experiment 2 vs. 1, in combination with the interval between bST injections, was detrimental to the development of the reproductive axis of Nellore heifers. Further studies investigating the effects of breed on ovarian activity and gene expression in reproductive tissue organs and brain, following bST injections, are warranted to confirm this hypothesis.

Conclusions

Despite the challenges encountered by *Bos indicus*-influenced beef heifers including extreme heat and humid conditions in combination with forages of relatively poor nutritional composition, acceptable reproductive performance may still be achieved. Some of these successful nutritional management practices to enhance growth and reproduction included: increasing the concentrate DM offered to heifers from 1.25% to 1.75% of body weight; daily rather than infrequent (3X/week) concentrate supplementation; stair-step strategy to boost growth (reproductive performance to be tested in 2019/2020); and early-exposure to high-concentrate diets. Although preweaning injections of bST are currently not allowed for beef cattle, our results indicated that early manipulation of the somatotrophic axis may benefit the reproductive performance of Brangus but not Nellore beef heifers. Identifying additional strategies that can enhance calf performance during early postnatal life may provide unique opportunities to optimize feed resources and increase the profitability of beef cattle operations.

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Table 1. Growth performance of beef heifers assigned to a low, medium, or high post-weaning growth rates during a 168-day development period (3 years; Moriel et al., 2017)

	Supplementation amount				
	Low	Medium	High	SEM	*P*
Average daily gain kg/day					
Sep to Oct	0.11[a]	0.14[a]	0.32[b]	0.050	0.02
Oct to Nov	0.19[a]	0.45[b]	0.54[b]	0.046	<0.0001
Nov to Dec	0.30[a]	0.59[b]	0.66[b]	0.042	<0.0001
Dec to Jan	0.27[a]	0.42[b]	0.58[c]	0.031	0.001
Jan to Feb	0.22[a]	0.33[b]	0.35[b]	0.037	0.10
Feb to Mar	0.35[a]	0.51[b]	0.62[c]	0.031	0.002
Overall ADG, kg/d	0.25[a]	0.41[b]	0.51[c]	0.020	<0.0001
Target ADG, kg/d	0.45	0.73	1.00		

Within a row, means without common superscript differ ($P \leq 0.05$).

Table 2. Reproductive performance of heifers supplemented at 1.25% or 1.75% of their body weight (BW, dry matter basis) for 167 days (September to March). Two-year study funded by Florida Cattlemen Enhancement Board (Moriel et al., in preparation)

Item	1.25% of BW	1.75% of BW	SEM	*P*
Average daily gain, kg/day	0.65	0.71	0.015	0.02
% of total				
Pubertal heifers at start of the breeding season	81	92	4.0	0.05
Final pregnancy rate	65	83	5.3	0.02
Heifers calving within the first 4 weeks of the calving season	68	51	7.7	0.05

[1]Heifers were offered concentrate at 1.25% or 1.75% of their body weight (DM basis). Effects of frequency of supplementation x concentrate amount were not detected ($P \geq 0.71$) for any variable in this study.

Table 3. Reproductive performance of heifers supplemented daily (7X) or 3 times weekly (3X) for 167 days (September to March). Two-year study funded by Florida Cattlemen Enhancement Board (Moriel et al., *in preparation*)

Item[1]	7X	3X	SEM	*P*
Average daily gain, kg/day	0.65	0.71	0.014	0.007
	% of total			
Pubertal heifers at start of the breeding season	86	80	4.0	0.03
Final pregnancy rate	75	72	5.2	0.70
Heifers calving within the first 4 weeks of the calving season	76	43	7.7	<0.01

[1]Heifers were offered concentrate at 1.25% or 1.75% of their body weight (DM basis). Effects of frequency of supplementation × concentrate amount were not detected ($P \geq 0.71$) for any variable in this study.

Table 4. Growth and reproductive performance of beef heifers developed on different management systems from the time of early weaning (EW; day 0 of the study) until the time of normal weaning (NW; day 180 of the study; Moriel et al., 2014)

	Treatments					
Item	NW	EWPAST	EW180	EW90	SEM	*P*
Body weight[1], kg						
day 90 (Early-weaning)	139[a]	135[a]	164[b]	171[b]	3.7	<0.001
day 180 (Normal weaning)	212[a]	178[b]	262[c]	216[a]	6.4	<0.001
day 335 (Breeding season)	323[a]	292[b]	363[c]	327[a]	7.9	<0.001
Age at puberty, days	429[a]	418[a]	298[b]	358[c]	14.9	<0.001
Body weight at puberty, kg	342[a]	306[b]	286[b]	292[b]	11.9	0.09
Pubertal heifers at start of breeding season, % of total	30[a]	40[a]	100[b]	80[b]	13.2	0.002
Pregnant heifers, % of total	60	50	78	70	15.6	0.64

[a,b] Within a row, means without common superscript differ ($P \leq 0.05$).

[1] Calves (70 days of age) were assigned to remain with their dams and be normally weaned at 250 days of age (day 180 of the study; NW), or early-weaned at 70 days of age and randomly assigned to: ryegrass and bahiagrass grazing for 180 days (EWPAST); high-concentrate diet in drylot for 180 days (EW180); or high-concentrate diet in drylot for 90 days, then bahiagrass grazing for additional 90 days (EW90). From the time of normal weaning to the end of the breeding season, all heifers were provided concentrate DM supplementation at 1.5% of body weight.

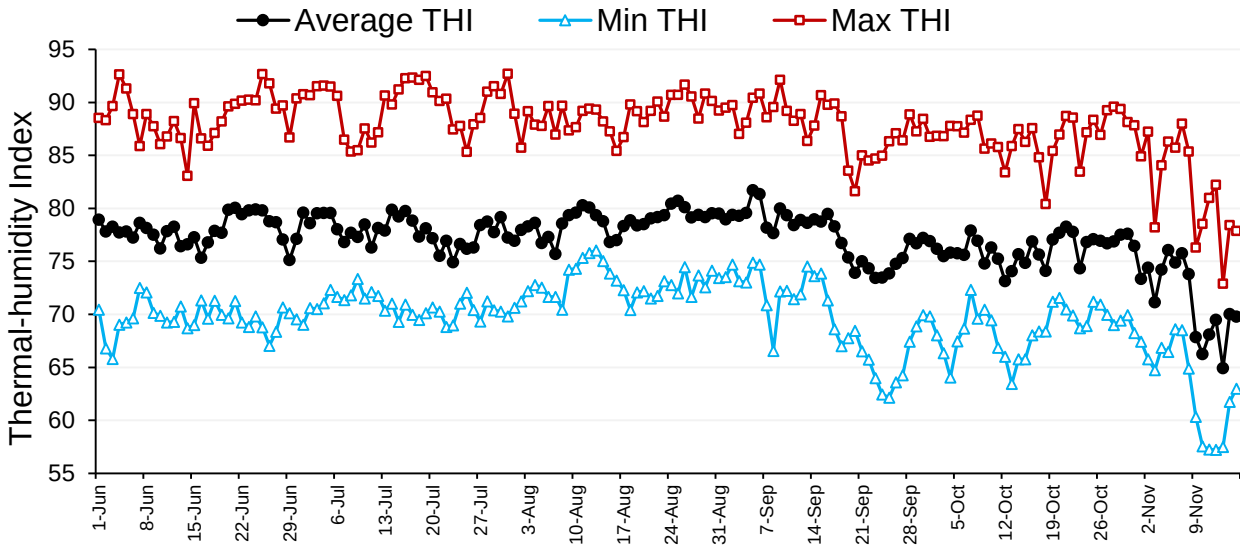

Figure 1. Daily average, minimum and maximum thermal-humidity index (THI) values observed from June to November 2019 at the Range Cattle Research and Education Center. THI = (1.8 × Temperature + 32) – [(0.55-0.0055 × Relative Humidity) × (1.8 × Temperature – 26)].

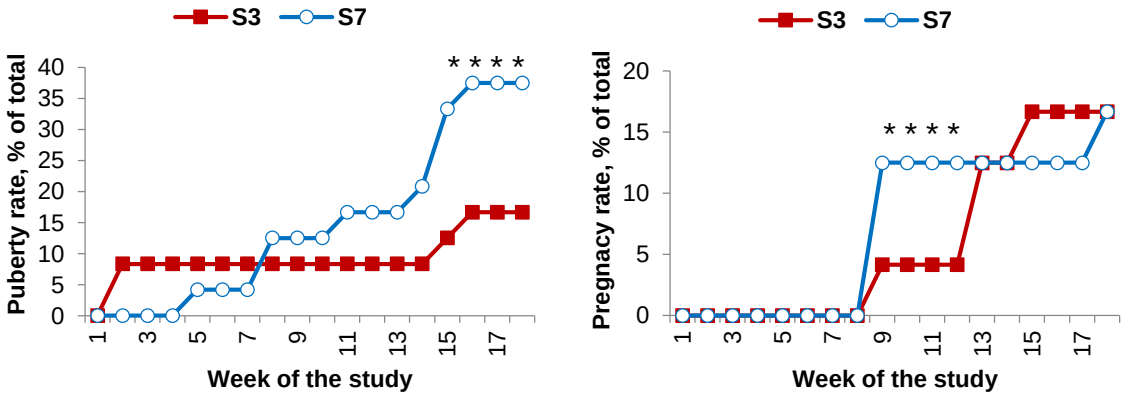

Figure 2. Weekly puberty (left) and pregnancy (right) attainment of beef heifers fed warm-season forages and supplemented with concentrate daily (S7) or 3 times weekly (S3; Moriel et al., 2012). *$P \leq 0.05$.

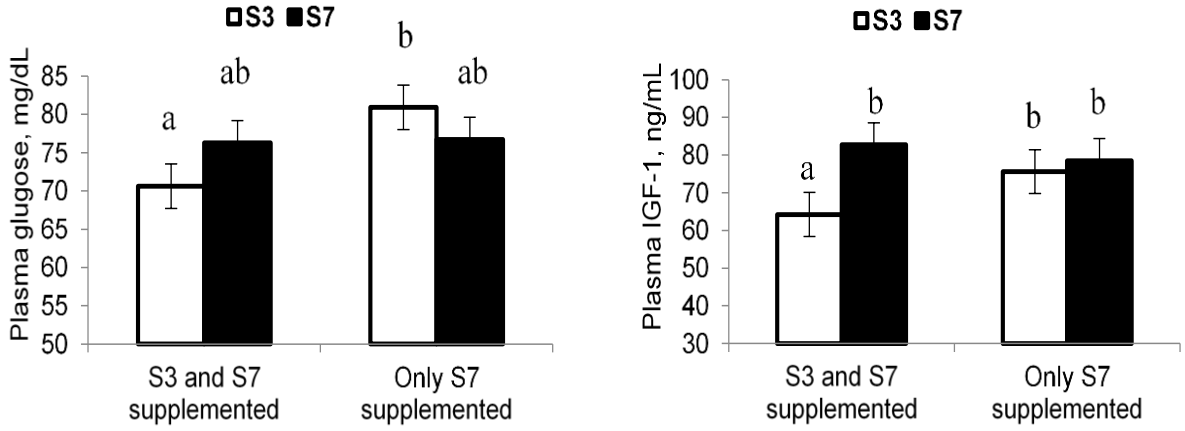

Figure 3. Plasma concentrations of glucose (left) and IGF-1 (right) of beef heifers supplemented with concentrate daily (S7) or 3 times weekly (S3; Moriel et al., 2012). The X-axis represent the days that both S3 and S7 heifers were supplemented (Monday, Wednesday, and Friday) and days that only S7 heifers were supplemented (Tuesday, Thursday, and Friday). [a,b] $P \leq 0.05$.

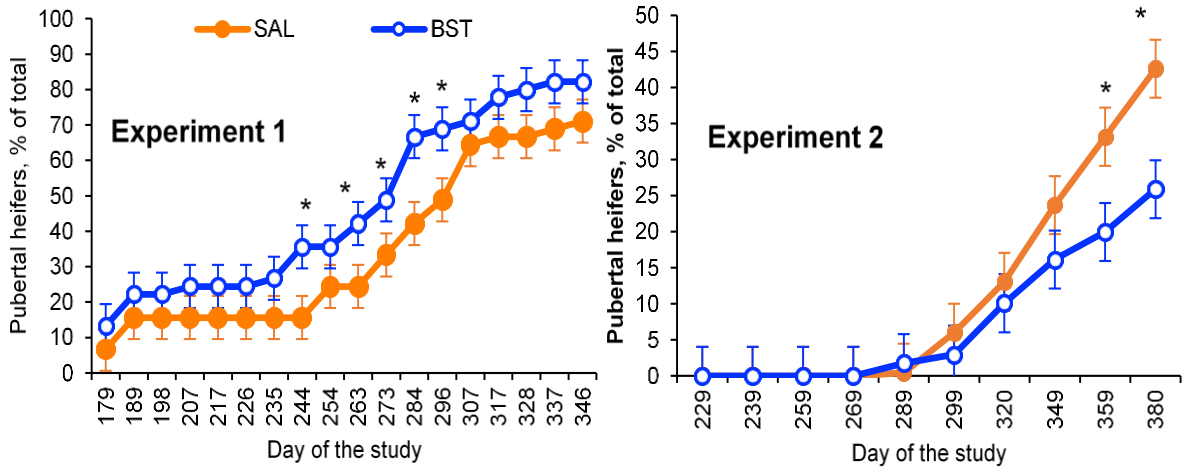

Figure 4. Puberty attainment of Brangus (Experiment 1) and Nellore (Experiment 2) beef heifers. In Experiment 1, heifers were stratified by age on day 0 and assigned to receive injection of saline (SAL) or 250 mg of sometribove zinc (BST) on days 0, 14, and 28. In Experiment 2, heifers were stratified by age on day 0 and assigned to receive injections of SAL or BST on days 0 and 10 of the study. *$P \leq 0.05$.

SESSION NOTES

Nutritional Control of Puberty in Beef Heifers

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Introduction

Management of beef heifers for reproductive success, both before and after puberty, plays a major role in determining the efficiency of any beef production system. A general understanding of the processes that underlie pubertal development in heifers can aid in understanding the basis of established management protocols. The prepubertal period is characterized, at least in part, by a state of anestrus or the absence of estrous cycles. The hormonal and physiological changes preceding first ovulation occur as a result of maturational changes within the central nervous system, which ultimately trigger normal ovarian function, the onset of regular estrous cycles, and the potential to become pregnant. The purpose of this communication is to review the physiological processes that control puberty in heifers and the genetic, nutritional, and managerial factors that influence it.

Physiology and Endocrinology of Puberty in the Heifer

Maturation of the Central Reproductive Axis

Puberty in heifers is defined as the attainment of a developmental state that supports normal ovarian cyclicity (follicular development and ovulation) and the ability to become pregnant. Activation of the central reproductive axis is a major event preceding the onset of ovarian cycles in all mammalian females, including the heifer. As puberty approaches, an increase in the release of a key hormone from the hypothalamus (lower brain) occurs. The hormone, gonadotropin-releasing hormone (**GnRH**), is the master regulator of reproductive function. It is secreted locally in discrete pulses into the portal circulation supplying the anterior pituitary. An increase in secretion of GnRH preceding puberty results in a concomitant increase in production and release of the pituitary hormone luteinizing hormone (**LH**) (Foster and Jackson, 2006). Each biologically significant GnRH pulse results in a pulse of LH and in an overall increase in the concentration of LH in the general circulation. This elevation in LH is the signal that drives final maturation of ovarian follicles and the production of steroid hormones within the follicle (e.g., estrogens). Thus, a major limiting factor for the onset of puberty is the lack of high-frequency pulses of GnRH and LH (Wildt et al., 1980). The relative inactivity of the central reproductive axis during prepuberty is created primarily by a negative feedback system involving estradiol-17β (**E2**), the most physiologically-relevant estrogen produced by the ovarian follicle. As puberty approaches, the hypothalamus becomes less sensitive to the negative feedback effect of E2 on GnRH secretion (Foster and Ryan 1979; Day et al., 1984). As a result, GnRH pulsatile release from the

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hypothalamus increases which in turn stimulates increased circulating LH (**Figure 1**). The development and maturation of a large, estrogen-active follicle that follows these events represents a “switch” from a negative to a positive feedback effect on both the hypothalamus and pituitary. Increased release of E2 by the maturing follicle also causes the expression of behavioral estrus (heat) and is responsible for triggering a surge release of LH. This results in the first ovulation and the formation of a corpus luteum (**CL**). The CL produces progesterone, which regulates the length of the estrous cycle. The duration of the estrous cycle of cattle is 17 to 21 days, with the average slightly less in virgin heifers than in mature cows. If the female does not become pregnant, the CL regresses near the end of the cycle, a new follicle matures, and a new cycle ensues. However, if the heifer is bred and becomes pregnant, the CL does not regress and high circulating concentrations of progesterone necessary for the maintenance of pregnancy persist.

Although it is known that developmental changes within the hypothalamus controlling negative feedback sensitivity to estradiol and secretion of GnRH drive the onset of puberty, changes within the brain that underlie the functional beginning of sexual maturation and the neural pathways controlling this phenomenon remain unclear. Recent studies in our laboratories indicate that changes in expression of specific signaling peptides (i.e., neuropeptide Y, **NPY**; and proopiomelanocortin, **POMC**) in a metabolic-sensing region of the hypothalamus, may serve as developmental focal points for modifications that precede activation of the GnRH pulse generator (Allen et al., 2012; Cardoso et al., 2018). In addition, the recent discovery of a new family of neuropeptides, the RF-amides, has revolutionized our understanding of the regulation of GnRH neurons. In particular, the hormone, kisspeptin has been shown to stimulate GnRH secretion (Messager et al., 2005), may communicate both the positive and negative effects of estradiol on GnRH/LH release (Smith et al., 2007), and is absolutely critical for pubertal development (Seminara et al., 2003). Mutations in the genes encoding kisspeptin (Lapatto et al., 2007) and kisspeptin receptor (Seminara et al., 2003) disrupt sexual maturation. Kiss1, the gene encoding kisspeptin, is expressed in specific areas of the hypothalamus (preoptic area and arcuate nucleus) that are critical for gonadal steroid hormone (estradiol and progesterone) control of reproduction (Pielecka-Fortuna et al., 2008). Kisspeptin is a potent stimulator of LH release in mammals, including cattle (Kadokawa et al., 2008) and this effect is believed to occur by direct actions on GnRH neurons (Smith et al., 2008). Our studies investigating the involvement of the kisspeptin system during pubertal development suggest that kisspeptin is a key neuropeptide mediating the nutritional acceleration of puberty in beef heifers (Cardoso et al., 2015). These results indicate that activation of the kisspeptin system is a major limiting factor, and perhaps the final neuroendocrine bridge, for the establishment of puberty.

Reproductive Tract Development

As the central reproductive axis becomes active nearing puberty, the production of ovarian hormones contributes to the development of secondary reproductive traits, including mammary and reproductive tract development. In the prepubertal heifer, the uterine horns tend to be rather flaccid and underdeveloped. However, as puberty

approaches, and in response to increased stimulation by ovarian estrogens, the uterus and cervix begin to grow larger and to exhibit more smooth muscle tone when palpated manually. Anderson et al., (1991) developed a procedure for estimating pubertal status using palpation of the reproductive tract. The bottom portion of **Figure 1** demonstrates the changes in reproductive tract score (**RTS**) that occur as the heifer develops from early prepubertal to pubertal. The complete reproductive tract scoring system is shown in **Table 1**. The scores are subjective estimates of sexual maturity, based on ovarian follicular development and palpable size of the uterus. A RTS of 1 represents an infantile or relatively undeveloped tract. In this case, the uterine horns are small and have very little tone, and the ovaries are small and lack significant structures. Heifers with a RTS of 1 are likely the furthest from puberty at the time of examination. As the heifer develops and approaches puberty, the RTS increases due to larger uterine horns and ovaries. Heifers given a RTS of 3 have significant uterine tone and large follicles, and are estimated to be very near the first pubertal ovulation. Heifers scoring a 4 are assumed to be pubertal and therefore exhibiting regular estrous cycles. These heifers will have greater size and tone of uterine horns compared to less developed heifers scored lower, the horns will exhibit coiling, and a large preovulatory size follicle (e.g., ≥ 10mm) may be present. Heifers assigned a score of 5 have a palpable CL indicating that ovulation has already occurred.

Interaction of Genetics and Nutrition in Regulating Age at Puberty

Lifetime productivity of beef heifers is heavily-dependent upon their ability to reach sexual maturity, to conceive early in the initial breeding season, and to calve the first time as 2 year-olds (Lesmeister et al., 1973). Importantly, early conception is positively influenced by the number of estrous cycles occurring before targeted first breeding (Byerly et al., 1987). However, a significant proportion of heifers either does not reach puberty, or become pubertal too late, to conceive during their first breeding season as yearlings. This is particularly true for later-maturing breeds, even those that have reached an apparent body size capable of delivering a calf safely. Nutritional management involving continuous, high rates of gain is one option for promoting the timely onset of sexual maturation using the concept of “targeted body weight”. This approach sets a target of 60 to 65% of mature body weight (**BW**) as a practical rule of thumb for individual heifers to have reached puberty. The physiological precept of this principle is that heifers are genetically programmed to reach puberty at a predetermined size (Lamond, 1970; Taylor and Fitzhugh, 1971).

More recent evidence indicates that such targets also represent a minimum level of adiposity and a threshold circulating level of the adipose-derived hormone, leptin (Williams et al., 2002). Therefore, it is clear that genetic composition will have a major impact on what 65% of mature BW represents. Heifers that ultimately will have a large frame size at maturity (e.g., frame score 9 and mature BW > 1400 lb or 420 kg) will need to reach a much heavier BW to reach puberty compared to those with an expected mature frame size of 1 and mature BW of < 900 lb (Fox et al., 1988). However, in all cases, puberty can be accelerated or delayed by nutrition both before and after weaning. Early work by Wiltbank et al. (1966) indicated that BW gain during the pre-

weaning period may have a greater influence on age at puberty than post-weaning BW gain. This falls in line with the concept of precocious puberty (puberty reached at 10 months of age or less) occurring as a result of early-age (4 to 7 months of age) exposure to high energy, high-gain diets (Gasser et al. 2006 a,b,c). However, although there is opportunity for manipulating early calfhood nutrition to accelerate puberty, this can result in excessive fattening, impairment of mammary development, and risk of unwanted, ill-timed pregnancies.

Therefore, our research team is currently exploring the development of novel strategies that address these issues. Our primary goal is to develop strategies to nutritionally program heifers in a manner that promotes sound development and consistent, timely onset (12-14 months of age) of puberty, while avoiding the negative effects that can occur with excessive fattening. In this regard, our recent studies (Cardoso et al., 2014) in *Bos indicus*-influenced beef heifers demonstrate that metabolic programming of processes underlying puberty can be shifted temporally through the use of a stair-step, compensatory growth model such that puberty is optimally-timed to occur at approximately 12 months of age (**Figure 2**). More specifically, our studies suggest that feeding heifers a high-concentrate diet during critical windows of development (4 to 9 months of age) results in changes in the metabolic endocrine status, characterized by elevated circulating concentrations of leptin, insulin, and IGF-1, which in turn can program the onset of puberty that occurs months later, allowing optimal timing of sexual maturation in replacement beef heifers. Importantly, we found that heifers that gained body weight at high rates between 4 and 6.5 months of age, and were subsequently subjected to a marked feed restriction between 6.5 and 9 months of age, still attained early puberty (puberty <12 months of age) at rates comparable to heifers fed a high-concentrate diet continuously (Cardoso et al., 2014). Similarly, *Bos taurus* heifers that were fed to gain body weight at high rates between 126 and 196 days of age exhibited a high incidence of precocious puberty (Gasser et al., 2006 a,b) However, puberty was not advanced to the same extent when heifers were fed a similar diet later during juvenile development. Collectively, these results indicate that during early development, plausibly between 4 and 9 months of age, heifers are more sensitive to the programming effects of nutrition in advancing puberty.

Conclusions

Approximately 4 million replacement beef heifers enter the U.S. cow herd annually. Very few of these heifers are “programmed” nutritionally or otherwise to optimize lifetime reproductive performance, even though pre-breeding growth and nutrition are major contributors to fertility and lifetime reproductive performance. Effective managerial and technological practices to program replacement heifers for optimal reproductive performance are available, yet adoption of these practices in the US beef production industry has been limited. Our studies in *Bos indicus*-influenced beef heifers clearly demonstrate that metabolic programming of processes underlying puberty can be shifted temporally through the use of a stair-step, compensatory growth model such that puberty is optimally-timed to occur at approximately 12 months of age.

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Table 1. Reproductive tract scores (RTS)[a]

RTS	Uterine horns	Ovarian length (mm)	Ovarian height (mm)	Ovarian width (mm)	Ovarian structures
1	Immature, < 20 mm diameter, no tone	15	10	8	No palpable follicles
2	20-25 mm diameter, no tone	18	12	10	8 mm follicles
3	20-25 mm diameter, slight tone	22	15	10	8-10 mm follicles
4	30 mm diameter, good tone	30	16	12	10 mm follicles, CL possible
5	> 30 mm diameter, coiled (?)	> 32	20	15	CL present

[a] From Anderson et al. (1991).

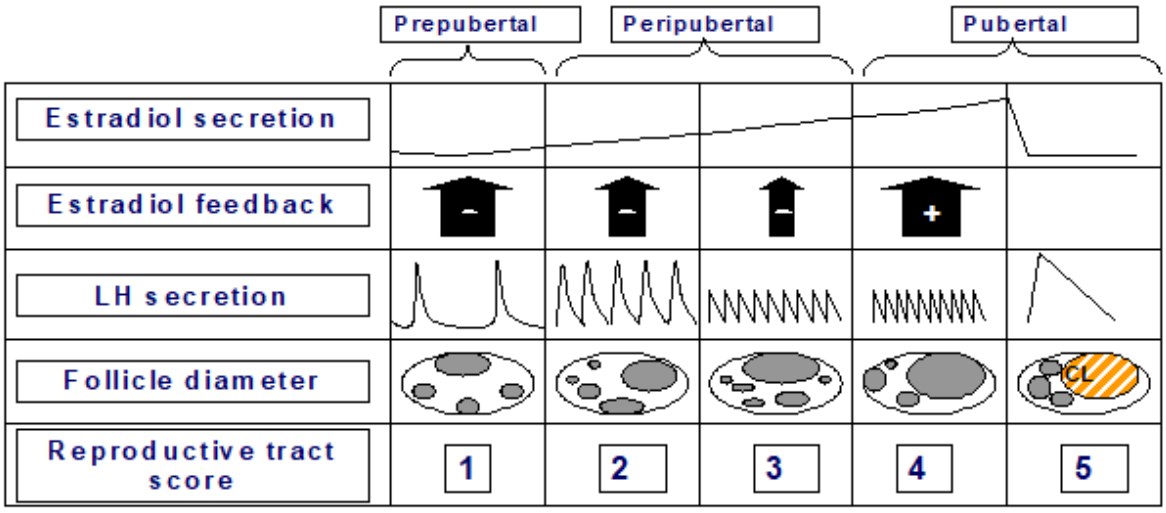

Figure 1. Endocrine and ovarian changes associated with puberty onset in the heifer and associated reproductive tract score (adapted from Day and Anderson, 1998, Anderson et al., 1991, and Patterson et al., 2005).

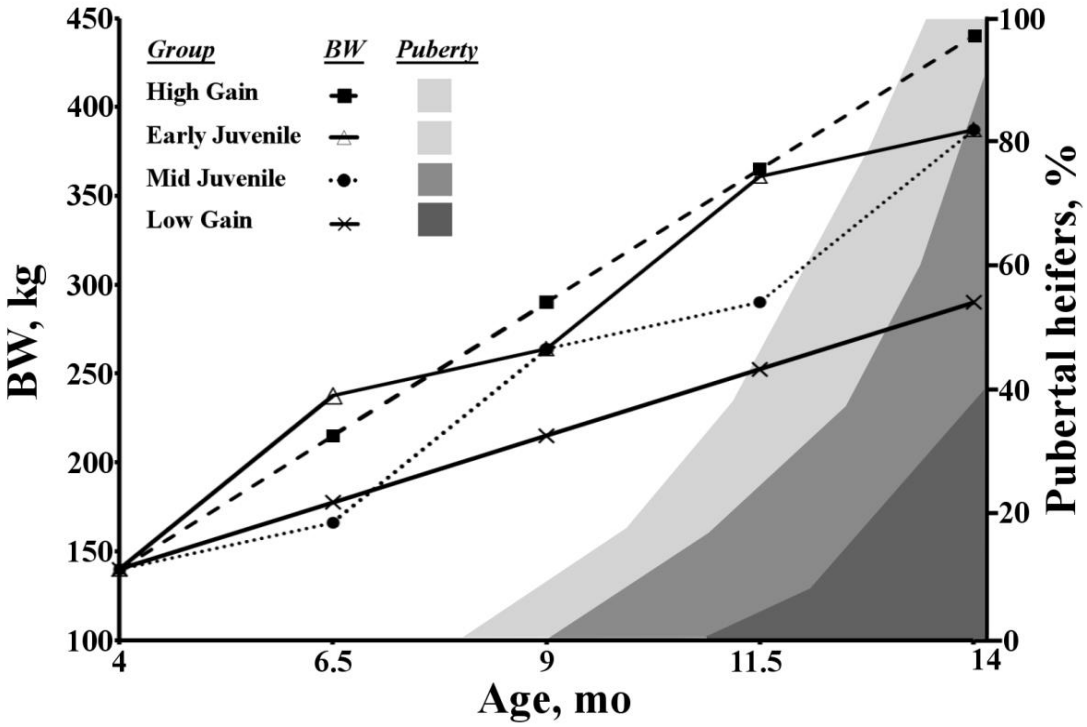

Figure 2. Model for a stair-step nutritional regimen applied to heifers during targeted periods of juvenile development to optimize growth, and promote genomic, biochemical and structural alterations in the hypothalamus that are permissive for early onset of puberty. Elevated body weight (BW) gain during the early- and mid-juvenile periods programs neuroendocrine functions and accelerates pubertal development in heifers. Heifers that are fed ad libitum a high-concentrate diet beginning at 4 months of age (Early Juvenile) become pubertal at a similar time as heifers gaining BW at high rates (High Gain) throughout the prepubertal period, even though feed restriction is applied during the mid-juvenile period. Most heifers that are restricted during the early juvenile period, but fed ad libitum between 6.5 and 9 months of age (Mid Juvenile) becomes pubertal by 14 months of age, whereas only as small proportion of heifers gaining BW at low rates (Low Gain) becomes pubertal by 14 months of age. Adapted from Cardoso et al. (2014).

SESSION NOTES

The Role of N Recycling in Improving Efficiency of N Utilization in Dairy Cattle

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Introduction

The growing environmental concerns from the US dairy industry has lead researchers to focus on nutrient management for more efficient utilization of available resources and reduce environmental emissions of greenhouse gases and volatile organic compounds (Place and Mitloehner, 2010). Milk nitrogen (**N)** efficiency, defined as conversion of dietary N into milk N, is typically low (20 to 35%; Chase et al., 2009) in lactating dairy cows. Most of the dietary N is lost in feces and urine and N is considered one of the major pollutants from dairy production systems (Noftsger et al., 2005). The most significant aspect of metabolism that contributes to N inefficiency is massive amount of N losses from gastrointestinal tract (**GIT**) as ammonia (**NH_3**; 46 to 47% of N available in the lumen of gut) and urinary urea excretion (30 to 70% of urea production). In addition, N losses also occur via intense metabolism of GIT where 0.25 to 0.6 of essential amino acids (**AA**) disappear from small intestines and recovered in portal vein. The potential for losses associated with these processes is significant and the magnitude of losses may increase depending on the type of diets fed or physiological stage of animals (Lapierre and Lobley, 2001). Lower N efficiency results in greater excretion of N in manure over milk resulting in reduced farm profitability and greater environmental N excretion (Noftsger et al., 2005; Hristov et al., 2011). The primary goal of ruminant nutritionists to improve N efficiency is to reduce urinary and fecal nitrogen losses and stimulate urea-N entry to rumen and provide ruminal conditions to enhance microbial uptake of recycled urea N. Better understanding of urea N recycling is important for improving N efficiency and reduce environmental impact of N emissions from dairy production systems (Recktenwald et al., 2014).

Urea is considered major end product of NH_3 and AA metabolism in ruminants and it plays important role in N economy for ruminants (Marini and Van Amburgh, 2003). The ability to recycle substantial amounts of urea into the GIT is a physiological mechanism in ruminants for the conservation of N (Lapierre and Lobley, 2001). It is estimated that 40 to 80% of urea synthesized in liver is recycled to different sections of the GIT; however, for recycled urea to contribute to microbial protein synthesis and absorbable microbial protein, urea must be recycled and captured in rumen. Typical values for the partition of urea between urine and GIT are 60:40 to 20:80, depending upon type of diet and level of feed and protein intake (Harmayer and Martens, 1980; Lapierre and Lobley, 2001).

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Urea Synthesis and Transporters

Urea synthesis via ornithine-urea cycle (**Figure 1**) in liver is the only pathway for NH_3 detoxification; however, Oba et al. (2004) showed urea synthesis in rumen epithelium and duodenal mucosal cells. The water-soluble property of urea makes it 10 times less toxic than NH_3. Ammonia produced in the GIT is transported to liver through portal vein and is converted to urea. The fractional extraction rate of 0.75 to 0.85 suggests that liver is very effective at removing NH_3 (Lobley and Milano, 1997).

We are still lacking mechanisms regulating urea partition to the GIT in ruminants. Recently, the presence of urea transporters in rumen tissues was confirmed suggesting that the process of urea recycling might be regulated and it may involve humoral factors (e.g. hormones, substrate concentration). This suggests that in addition to events occurring within the rumen environment, animal factors may have some control over urea recycling. Urea is transported across ruminal epithelium primarily via urea transporters (**UT**) located on the luminal and basolateral membrane of the epithelium (Lu et al., 2014; Patra and Aschenbach, 2018). In addition, aquaporins, family of membrane spanning proteins involved with movement of water, are also permeable to urea and helps with transporting urea across ruminal epithelium (Walpole et al., 2015). The expression of UT is dependent upon dietary protein and short-chain fatty acid (**SCFA**) concentration While ruminal NH_3 concentration is a negative regulator of UT-B mRNA and protein expression, SCFA upregulate UT-B expression at low pH (Lu et al., 2015). Both SCFA and NH_3 concentration regulates ruminal influx of urea such that urea influx is adjusted to the consumption capacity of NH_3 and activity of rumen microbiome. Urea influx as proportion of total hepatic urea output ranges from 29 to 99% and N transfer across ruminant GIT can be higher than N intake (Patra and Aschenbach, 2018).

Fates of Urea Entering GIT

Urea has potential to enter all compartment of GIT through GIT secretions and diffusion from blood because of its water soluble property (Kennedy and Milligan, 1980). Ruminants enhances transfer of urea to rumen via rumen wall and saliva so that urea-N can be captured by rumen microbes for synthesis of microbial protein. However, urea transfer to rumen is variable and depend upon ruminal environment resulting from the diets fed to animals. Nevertheless, urea that diffuses across the rumen wall is converted to NH_3 and carbon dioxide (**CO_2**) in presence of urease activity from bacteria in vicinity of rumen wall. Ammonia is either utilized the synthesis of microbial protein (~50%), reabsorbed as ammonia (~40%) or the remaining gets excreted in the feces (~10%; **Figure 2**). The NH_3 reabsorbed from GIT is removed by liver and again converted to urea. The amino acids of microbial origin and synthesized from recycled urea-N can also be reabsorbed and catabolized in liver to yield urea (Sarraseca et al., 1998). The fate of recycled urea-N depends upon dietary factors such as protein content, diet fermentability, forage-to-concentrate ratio etc. and ruminal condition including rumen pH, NH_3/NH_4^+, fermentation energy etc.

Factors Affecting Urea Recycling

Dietary Protein Content, Intake, and Solubility

Urea N recycling and microbial uptake of recycled urea is affected by dietary and ruminal factors with intake of crude protein (**CP**) and digestible organic matter being the major ones (Harmeyer and Martens, 1980; Kennedy and Milligan, 1980). Lapierre and Lobley (2001) reported correlation ($\mathbf{R^2}$) of 0.78 between N intake and hepatic urea production in cattle at low N intake and close of zero N balance; however, the correlation was reduced to 0.45 in cattle with high N intake. The correlation between portal drained viscera NH_3 absorption and hepatic urea production was 0.84 across all cattle (Lapierre and Lobley, 2001). Based on these observations, it can be assumed that in animals with low N requirements, more consistent proportion of N intake is directed towards urea synthesis; however, in high producing animals, N is absorbed as AA and utilized for anabolism rather than deaminated to NH_3. However, the proportion of urea-N entering the GIT (60 to 70%) and the proportion used for anabolic purposes (45 to 50%) is not affected in sheep fed high levels of feed intake with improved diet quality (Sarraseca et al., 1998; Lobley et al., 2000). In ruminants fed low quality hay or low protein diet, urea recycling to the GIT is 80 to 90% of urea entry rate and greater proportion of absorbed N is retained (Marini and Van Amburgh, 2003).

Inverse relationship was observed between dietary protein intake and urea-N entry into the rumen (Kennedy and Milligan, 1980) suggesting that low protein intake combined with low quality roughage diet will result in decreased hepatic urea synthesis, decreased urinary urea N excretion and lower transfer of urea to post stomach tissues (Marini and Van Amburgh, 2003). Archibeque et al. (2001) determined the effects of two forage species differing in N levels on urea kinetics and N metabolism and observed greater N efficiency at low N intakes. Recently, Batista et al. (2017) conducted meta-analysis compiling 25 studies with ruminants (beef, dairy cows, sheep) for evaluating urea kinetics and microbial uptake of recycled urea N. Based on this meta-analysis, hepatic synthesis of urea-N and gut entry rate (GER; N recycled to gut) linearly increased with increase in N intake; however, the ratio between GER and hepatic synthesis of urea-N decreased with increasing dietary CP concentration.

The major effects of changing CP and rumen degradable protein (**RDP**) on ruminal fermentation is observed on ruminal NH_3-N concentration. However, we are still lacking enough studies investigating the impacts of altering RDP concentration on urea-N transfer in rumen. Wickersham et al. (2008) observed greater urea-N transfer to the GIT with increasing dietary RDP; however, RDP had no impact on urea-N transfer to GIT was observed when it was expressed as proportion of endogenous urea-N production. We are still lacking studies showing T interactive effects of dietary CP and RDP concentrations on urea-N transfer and microbial protein production in high producing dairy cows with high requirement for metabolizable protein. Chibisa and Mutsvangwa (2013) observed lower ruminal NH_3-N as RDP was reduced; however, increases in urea entry rate was observed with lower CP and RDP diets resulting in

maintenance of microbial protein supply as observed by utilization for anabolic purposes.

Diet Fermentability

Increasing the fermentable carbohydrate fraction of the diet has potential to increase urea recycling to the rumen (Huntington, 1989) by decreasing urea transfer to post-gastric tissues. Improving fermentability of diets by adding grains, starch, and sucrose as energy source increase ruminal urea degradation probably due to lower NH_3 concentration or greater availability of energy due to greater rate of fermentation of dietary organic matter (Kennedy and Milligan, 1980). Previous studies have shown the potential of greater N utilization when starch is substituted with sugars and these effects may be attributed to increased urea recycling as greater carbohydrate digestion in rumen increase urea entry to rumen from blood and microbial protein synthesis (Huntington, 1989; Theurer et al., 2002). Seal and Parker (1996) observed greater propionate production along with lower ruminal NH_3 and plasma urea concentration and greater urea recycling to the GIT with intraruminal infusion of sucrose. Greater transfer of urea into the rumen with sugars may be attributed to lower ruminal NH_3 concentration. Lu et al. (2014) observed dose-dependent inhibitory effect of ammonia on urea transfer across rumen epithelia. Replacing starch with sugars may increase ruminal ATP supply (Russell et al., 1992) and lower rumen NH_3 concentration (Broderick et al., 2008) creating favorable conditions for urea entry into the rumen. Broderick and Radloff (2004) observed linear decrease in urinary excretion of urea with increasing sugar content of the diet with molasses in lactating dairy cows. Similarly, urinary excretion of urea-N was linearly reduced as sugars replaced corn starch in the diets (Broderick et al., 2008). These findings suggest repartitioning of urea toward recycling in GIT; however, we are still lacking direct evidence of greater urea recycling to GIT with sugars in the diets of lactating dairy cows. Recently, De Seram et al. (2019) observed effects of replacing barley starch with lactose and observed linear decrease in ruminal NH_3-N concentration; however, no changes were observed on anabolic utilization of recycled urea-N, or urea-N recycled to GIT as total sugar content increased in the diets of lactating dairy cows.

Nitrogen Gradient Across the Rumen Wall

Ruminal NH_3-N concentration regulate urea-N secretion into the rumen by permeability of ruminal epithelium thereby generating N gradient across rumen wall (Marini et al., 2005). Egan et al. (1986) proposed lower permeability of ruminal epithelium for urea-N with increased NH_3-N concentration (**Figure 3**). Greater NH_3-N concentration may limit ruminal entry of urea-N by inhibiting bacterial urease activity (Remond et al., 1996). Ammonia occurs in two forms as NH_3 or NH_4^+; however, NH_4^+ is the predominant form at ruminal pH of 6.4. Lu et al. (2014) speculated that luminal NH_4^+ enters the ruminal epithelium via a cation channel driven by potential difference of the apical membrane. A potential difference driven NH_4^+ transport into the cell alters urea transport in a dose dependent manner. Lu et al. (2014) demonstrated inhibitory effects

of NH_3 on urea transfer across ruminal epithelia using chamber and observed that inhibitory effects of NH_3 are concentration dependent with saturation at 5 mM/L.

Feed Processing

Feed processing can affect N efficiency, and transfer of urea-N either by modifying fermentability of diet or ruminal N status. Feed processing may synchronize ruminal starch and N supply thereby reducing N absorption and increasing N retention (Huntington, 1997). Theurer et al. (2002) observed greater urea-N recycling to the portal drained viscera along with lower urinary urea-N in growing beef steers fed steam-flaked sorghum compared to dry rolled sorghum. The effects on urea-N recycling might be attributed to greater starch and CP digestibility with steam-flaking compared to steam-rolling sorghum. Similarly, feeding steam-flaked corn compared to steam-rolled corn increased urea recycling to portal drained viscera by 140% (Delgado-Elorduy et al., 2002) while feeding dry rolled barley compared to pelleted barley to lactating dairy cows increased urea-N entry to GIT by 35% (Gozho et al., 2008). While various studies have shown greater urea-N recycling, no effects were observed on microbial yield suggesting inefficient microbial uptake of recycled urea-N (Gozho et al., 2008; Marini and Van Amburgh, 2003). Hence, such changes in feed characteristics should coincide with greater microbial N utilization.

Conclusions

The ability to recycle urea is a physiological mechanism in ruminants to ensure high rates of microbial protein synthesis, enhance N efficiency, and to supply high quality protein including meat, milk, and wool. Better understanding of urea N recycling is important for improving N efficiency; however, mechanisms regulating urea recycling and factors affecting urea partitioning in different sections of GIT still remains limited. Specifically, we are lacking information on ideal ruminal conditions that favor urea transfer and uptake by rumen microbes. With better understanding of the ruminal conditions and mechanisms underlying urea partition to the GIT, future nutrient requirement models will be more capable of predicting for maximum N efficiency.

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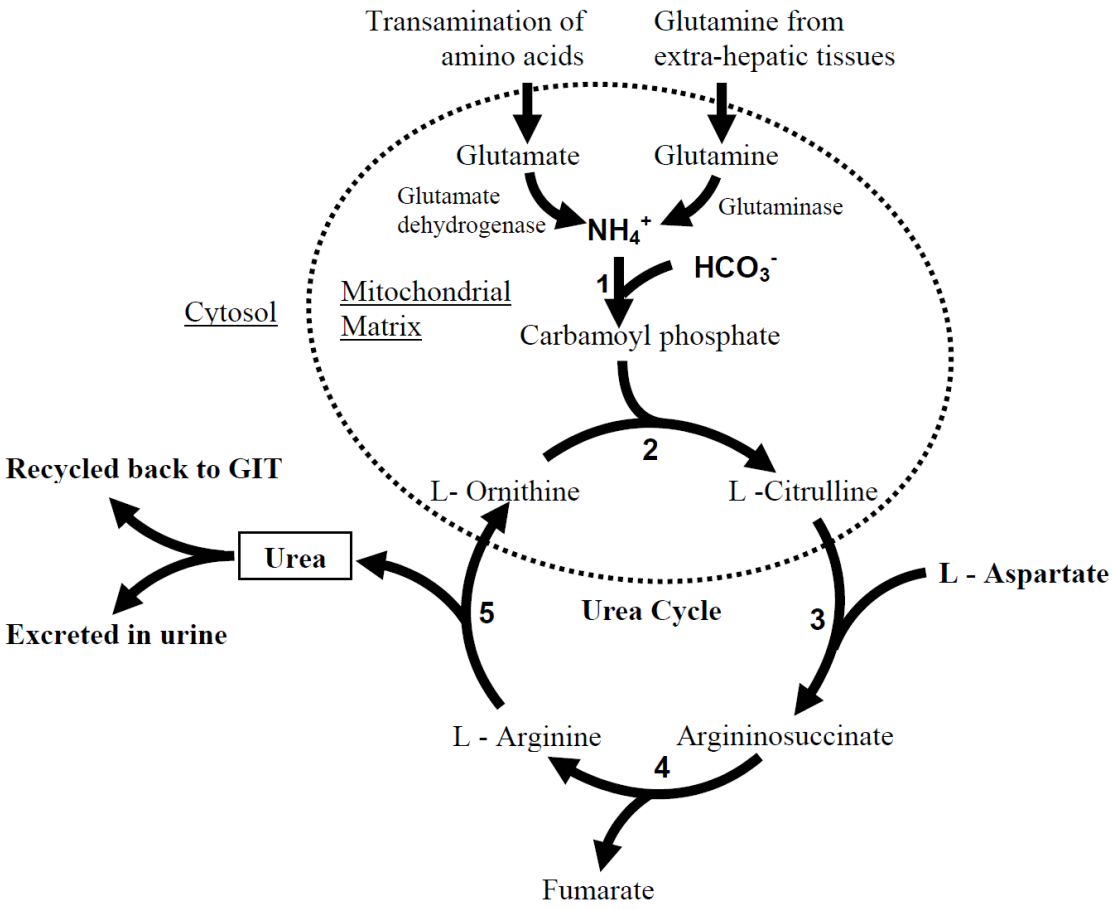

Figure 1. Reactions and Intermediates of urea biosynthesis. Mitochondrial NH_4^+ and cytosolic aspartate provide the two N atoms for urea synthesis. Five enzymes are involved in urea cycle. 1. Carbamoyl phosphate synthase I, 2. Ornithine transcarbamoylase, 3. Argininosuccinate synthase, 4. Argininosuccinate lyase, 5. Arginase. (Adapted from Sunny, 2004).

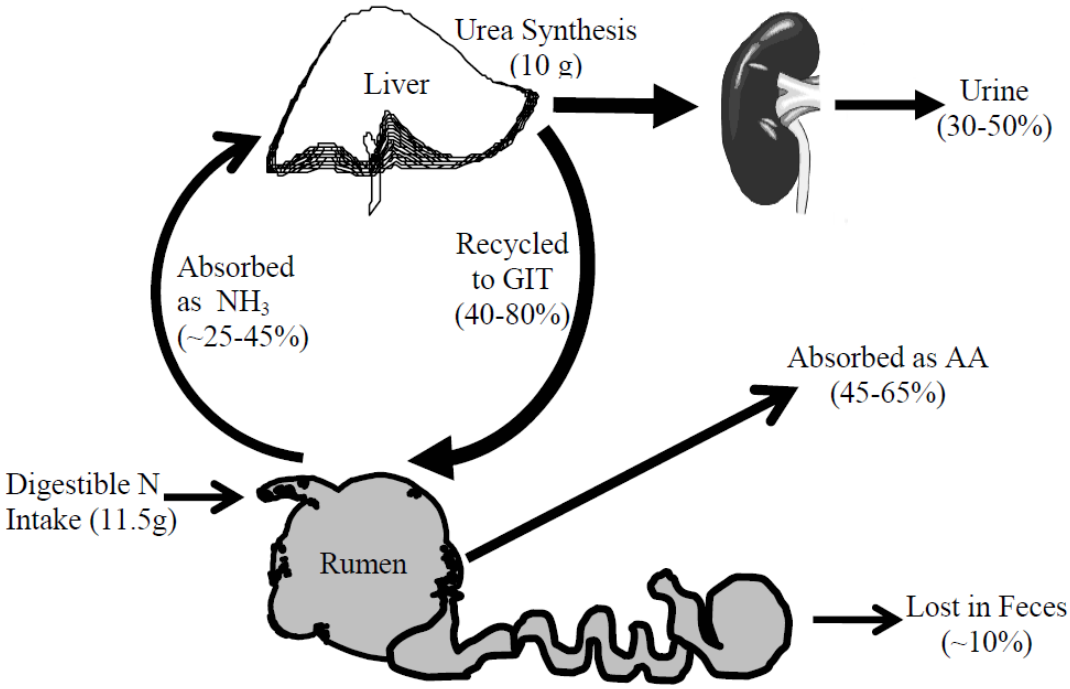

Figure 2. Fates of urea-N (g/d) synthesized and recycled in sheep fed pelleted barley and grass hay diet. Of the total urea synthesized in the liver (10g), 30-50 % is excreted in the urine and 40-80 % is recycled back to the GIT. Of this portion recycled back to the GIT, 25-45 % is reabsorbed to liver as ammonia where it is reutilized for the synthesis of urea, 45-65 % is absorbed as amino acids which is utilized for productive purposes and around 10 % is excreted in feces (Adapted from Lobley et al., 2000).

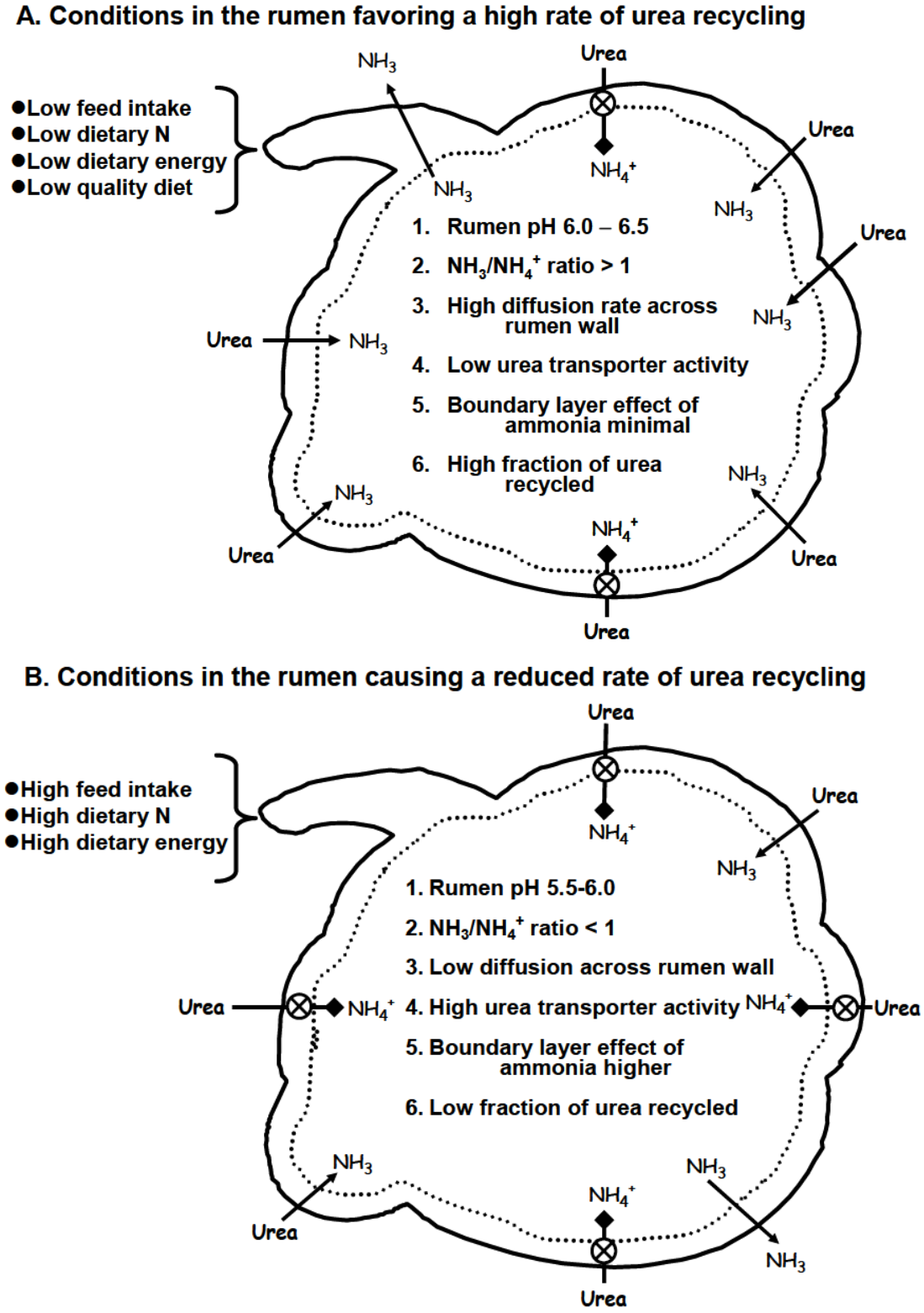

Figure 3. Ruminal conditions to promote or reduce urea recycling in response to diet and composition (Adapted from Bequette and Sunny, 2005).

SESSION NOTES

Digestion and Nutrient Flow in Continuous Culture System and Animal Responses. Do They Match?

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Introduction

This paper is a summary of one of our recent studies, for more details and complete data, please see Brandão et al. (2020). Studies aiming to determine feed digestion, the effects of feed additives, or to study ruminal fermentation, often require determination of ruminal fermentation end-products and nutrient flow using a cannula fitted in the abomasum or duodenum (Ahvenjärvi et al., 2000). The omasal sampling technique (**OST**), described by Huhtanen et al. (1997) and modified by Ahvenjärvi et al. (2000) is a well-accepted technique to assess ruminal fermentation and nutrient flow. It has been successfully used for estimating nutrient flows and ruminal metabolism of nitrogen (Reynal et al., 2003), carbohydrates (Owens et al., 2008), fatty acids (Sterk et al., 2012), and minerals (Tuori et al., 2006). Although this technique provides valuable results and is considered adequate to estimate ruminal fermentation and nutrient flow, it is laborious and expensive. Therefore, alternative techniques capable of accurately simulating ruminal fermentation are warranted.

The dual-flow continuous culture system (**DFCCS**) was described by Hoover et al. (1976) aiming to simulate the continuous differential flows of liquid and solids from the rumen. It provides a closer response to in vivo fermentation than closed vessel incubations (Hoover et al., 1976). The system consists of a long-term fermentation, with periods varying from 8 (Calsamiglia et al., 2002) to 11 days (Dai et al., 2019). It has been used mainly to evaluate the effect of feedstuffs and additives on fermentation, digestion, nutrient flow and N metabolism in dairy (Brandão et al., 2018) and beef (Amaral et al., 2016) diets. One of the most important advantages of the DFCCS compared with other in vitro systems is the continuous removal of fermentation end-products, which reduces issues with accumulating fermentation products, such as VFA and ammonia (**NH_3**) that can potentially inhibit fermentation. Additionally, the system allows for intense sampling, determination of degradation rates, and testing feed additives in early developmental stages that are not yet produced in large scale, under a constant dry matter intake and passage rate.

However, studies quantitatively comparing ruminal fermentation data originated from DFCCS to OST are still scarce. Hristov et al. (2012) compared the variability of data from continuous culture systems with in vivo data; however, that study included a wide variety of different in vitro systems and compared them with in vivo total tract digestibility, which can be different when compared to ruminal digestibility. Therefore,

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we hypothesized that ruminal carbohydrate and N metabolism have similar responses to the independent variables used in this study when estimated using DFCCS and OST. The objective of the study was to summarize the literature and evaluate carbohydrate and N metabolism using a meta-analytical approach to compare two methods: DFCCS and OST.

Carbohydrates

True organic matter (**OM**) digestibility was positively associated with the two carbohydrate independent variables used in the present study: Neutral detergent fiber (**NDF**) degradability (quadratically), non-fiber carbohydrates (**NFC**) concentration (linearly; Brandão et al. 2020). Changes in ruminal NDF degradability directly reflect on true ruminal OM digestibility considering that the rumen is the main site of fiber digestion (Broderick et al., 2010). Dietary NFC concentration was positively associated with true OM digestibility possibly due to its high ruminal fermentability. Offner and Sauvant (2004) reviewed 87 studies from beef and dairy cattle fed a wide variety of starch sources and reported ruminal starch degradability of 71.0%. Therefore, increases in dietary NFC concentration can positively impact true OM digestibility by providing a greater amount of highly fermentable carbohydrates.

The NDF degradability was used as an independent variable in Brandão et al. (2020) and it was also used as dependent variable and regressed against dietary NFC concentration in Brandão et al. (2020). Typically, dietary NFC concentration increases at the expense of NDF, which results in a diet with lower fiber. In in vivo studies, the association of highly fermentable carbohydrates with lower dietary NDF results in a drop in ruminal pH (Oba and Allen, 2003), which can affect fiber degrabability. In DFCCS studies, pH is commonly controlled, suggesting that this negative association between NDF degradability and dietary NFC concentration is more sensitive to changes in substrate, rather than only pH.

Concentration of volatile fatty acids (**VFA**) in the rumen is affected by several factors, including (but not limited to) rate and extent of OM digestion. Highly digestible feeds are degraded in the rumen producing VFA as end-product (Russell et al., 1992). Therefore, in agreement with the digestibility responses, total VFA concentration increased in response to increase in NFC concentration and NDF degradability (Brandão et al. 2020). Total VFA concentration was not affected by method when regressed with all independent variables used, suggesting that estimates of VFA concentration might be close when made using DFCCS and OST. The rate of VFA absorption by ruminal wall can affect ruminal total VFA concentration (Hall et al., 2015); however, in DFCCS there is no absorption and the VFA are removed through digesta outflow. We speculate that the digesta outflow rate in DFCCS is similar to the rumen hall absorption rate and the rate of VFA that is washed out from rumen via passage rate, which may explain the lack of method effect on the estimates of VFA concentration.

Chemical structure of the protein and its interaction with carbohydrates are important factors that determine ruminal crude protein (**CP**) degradability (NRC, 2001). Part of the total CP in feeds is bound to the plant cell wall and it can be slowly degraded or be of low biological availability (Sniffen et al., 1992). As plants mature, the contribution of this slowly degraded CP portion increases. Therefore, if a large portion of CP is bound to plant cell wall (primarily lignin), CP degradability tends to decrease due to reduced microbial access to nitrogenous compound. This may explain the observed positive association between true CP degradability and NDF degradability (Brandão et al. 2020). Therefore, this positive association between degradability of true CP and NDF is likely a reflection of a more digestible feed.

Molar proportion of butyrate usually does not change often and ranges from 10 to 20% (Bergman, 1990). In the present study, molar proportion of butyrate only responded to increments in dietary NFC concentration and had an overall study corrected mean of 11.7%, which is within the literature values. Considering that NFC encompasses starch and sugars, it has been shown in continuous culture (Vallimont et al., 2004) and in vivo (DeFrain et al., 2004) that increasing dietary NFC concentration might result in greater butyrate concentration. Additionally, the positive and linear response of butyrate to dietary NFC concentration is possibly also associated with accumulation of lactate in the rumen due to presence of large amounts of rapidly fermentable carbohydrates (Nagaraja and Titgemeyer, 2007). Increased concentration of butyrate has been observed in animals fed high grain diets (Coe et al., 1999).

Ruminal lactate metabolism can generate acetate, propionate, butyrate, and to a lesser extent caproate and valerate (Marounek et al., 1989); however, the primary end-product varies depending on ruminal pH (Satter and Esdale, 1968), in which when the pH is acidic butyrate is preferably produced from acetate (Satter and Esdale, 1968). It has been proposed that butyrate can be produced from acetate utilizing the two hydrogens atoms released by the oxidation of lactate to pyruvate; therefore, butyrate formation might work as a hydrogen sink (Esdale et al., 1968).

Nitrogen

Carbohydrates, primarily rapidly fermentable carbohydrates such as NFC, determine the energy available for microbial growth and microbial N yield (Schwab et al., 2006). Therefore, the negative and linear response of NH_3-N to increases in dietary NFC concentration (Brandão et al. 2020) is likely associated with greater microbial protein synthesis, which results in lower NH_3-N accumulation (Oba and Allen, 2003; Schwab et al., 2006). Indeed, bacterial N/total N increased as dietary NFC concentration increased (Brandão et al. 2020), while nonammonia nonmicrobial N relative to the total N was not associated with dietary NFC concentration. Interestingly, even though bacterial N/total N increased in response to NFC, efficiency of microbial protein synthesis was not associated with dietary NFC concentration. This suggests that yield of microbial protein increased due to abundance of substrate; however, the efficiency of converting OM truly digested into microbial N did not change. Bach et al. (2005) observed that bacterial N flow decreased as ruminal pH increased; however,

efficiency of microbial protein synthesis was not associated with rumen pH which commonly respond to changes on carbohydrates fermentability. Additionally, ruminal NH_3-N was insensitive to efficiency of microbial protein synthesis (Brandão et al. 2020), and similar response has been reported by Bach et al. (2005) and Oba and Allen (2003). When true CP degradability was used as dependent variable (Brandão et al. 2020), it was not affected by method, demonstrating that estimate of true CP degradability using DFCCS is similar to OST.

Passage rate and digestion are two competitive process (Mertens, 1977) that affect ruminal fermentation, and it is interesting to note that in DFCCS studies passage rate is constant, while in OST it is variable and largely affected by intake and diet characteristics. Furthermore, it is possible that the continuous removal of fermentation end-products reduces the issue with accumulation of products that can inhibit fermentation, resulting in responses similar to in vivo.

Dependent Variables Affected by Method

When efficiency of N utilization was regressed with efficiency of microbial protein synthesis (**Figure 3F**) we observed a linear and positive association. However, when Bach et al. (2005) regressed these two variables they reported a quadratic response, with a maximum point at 29 g microbial N/kg OM fermented and 69 g of microbial N/100 g rumen available N. This different response may be attribute to: 1) in their study they included a smaller number of observations (n = 136) than the present study; 2) They had no studies with efficiency of N utilization < 40% (However, even without points below 40% efficiency of N utilization, we would likely still have a linear association); 3) It is possible that some studies used in Bach et al. (2005) were also used in the present study; however, we have additional data from 2005 to 2019; and 4) we included DFCCS and OST data in our analysis, while Bach et al. (2005) used only DFCCS. Additionally, our equation has smaller root mean square of the error (4.63 versus 6.54), wider range of efficiency of N utilization, and greater R^2, suggesting a better fit of the model. Therefore, it is possible that our result is more robust than reported by Bach et al. (2005).

In the NRC (2001), efficiency of microbial protein synthesis was regressed against apparent ruminal N balance and had a negative linear relationship, with efficiency of microbial protein synthesis ranging approximately from 12 to 54 g of microbial N/kg of fermented OM, which is similar to our efficiency of microbial protein synthesis range (**Figure 3F**). That demonstrates that if available ruminal N is relativity high and fermented OM low, then microbial use of N and energy utilization becomes uncoupled. This result supports the linear association observed in the present study when regressing efficiency of N utilization against efficiency of microbial protein synthesis. At low efficiency of N utilization, efficiency of microbial protein synthesis is also low, likely due to the low energy available. Also, if there is abundance of energy available and N is not limiting, the greater the energy available, the greater the efficiency of converting N into microbial N. The NRC (2001) assumes a fixed efficiency of N utilization of 85% and efficiency of microbial protein synthesis of approximately 21

g of microbial N/kg of fermented OM. In our dataset when efficiency of N utilization is 85%, the projected efficiency of microbial protein synthesis is approximately 46 g of microbial N/kg of fermented OM for DFCCS and 38 g of microbial N/kg of fermented OM for OST. Even though the NRC (2001) recommends a fixed efficiency of microbial protein synthesis, they acknowledged that efficiency of microbial protein synthesis is responsive to dietary manipulations. Therefore, efficiency of microbial protein synthesis provides valuable insights on microbial energy use, and efficiency of N utilization is an indicator of N use. These two variables are complementary and their use in association is generally recommended.

Molar proportion of acetate reached the maximum point at approximately 70% NDF degradability, and molar proportion of propionate presented minimum point at approximately 69% NDF degradability (Brandão et al. 2020). Suggesting that the NDF degradability point that maximizes acetate is similar to the point that minimizes propionate (**Figure 1A and B**). Due to the method having an effect only on the estimate of $ß_0$, the NDF degradability point that maximizes acetate and minimize propionate is the same for DFCCS and OST. Similarly, when molar proportions of propionate and acetate were regressed with dietary NFC concentration and efficiency of microbial protein synthesis (Brandão et al. 2020), OST had greater estimate of $ß_0$ for acetate and lower propionate than DFCCS.

Acetate was consistently lower and propionate greater in DFCCS. Method affected the $ß_0$ estimate of acetate and propionate in six, out of the eight regressions reported in Brandão et al. (2020). This shift in the intercept demonstrates that even though the magnitude of the response was different, the functional relationship was maintained between methods. There are some evidences in the literature that molar proportion of acetate is lower in DFCCS due to a decrease in fibrolytic population (Mansfield et al., 1995). Our results are in agreement with Hristov et al. (2012), that also observed lower molar proportion of acetate in continuous culture studies when compared to in vivo.

Concentration of NH_3-N was related linearly and positively with true CP and NDF degradability (**Figure 2**). The study corrected means of NH_3-N concentration were 12.2 mg/dL and 8.4 mg/dL in DFCCS and OST, respectively, resulting in a 30% difference. Increments in NDF degradability resulted in greater NH_3-N accumulation in DFFCS, and this was the only variable with non-significant $ß_0$ but significant $ß_1$ between methods (Brandão et al. 2020). This result can be partially explained by the fact that in a DFCCS, there is no NH_3-N absorption through the wall, and the only way out of the system is through overflow. While in vivo, NH_3-N is absorbed through the portal-drained viscera, extracted by the liver and converted to urea (Lapierre and Lobley, 2001). Urea is then excreted in the urine or recycled via saliva or other sections of the gastrointestinal tract (Gozho et al., 2008).

In an experiment aiming to study the effect of increasing N intake on urea kinetics and recycling, Marini and Van Amburgh (2003) using Holstein heifers, reported N recycling ranging from 83% in a low N diet and 29% in high N diets. Additionally,

Lapierre and Lobley (2001) reported that in cattle, on average 30 to 40% of the N intake is recycled and returned to the gut as urea. In DFCCS studies, urea is commonly added in the artificial saliva aiming to simulate N recycling (Hannah et al., 1986). This practice is important when low CP diets are used, and similar to in vivo, the contribution of urea recycling is important to ensure microbial growth. Satter and Slyter (1974) using continuous culture fermenters, suggested a minimum of 5 mg/dL of ruminal NH_3-N to ensure microbial growth. Therefore, in experiments in which the diet provides enough N, the addition of urea might result in a greater NH_3-N input in the system than the microbial population is capable of converting into microbial N.

In our dataset, dietary CP averaged 16.8% for DFCCS (min = 4.0, max = 28.7, SD = 3.2) and 16.3% for OST (min = 9.9, max = 23.8, SD = 2.1). Considering a hypothetical scenario of 1) 0.4 g/L of urea added via saliva the fermenters; 2) passage rate of 10%/h liquid and 5%/h for solids; and 3) fermenter volume of 1,830 mL. A total of 1.728 g urea (0.795 g of N) is added to each fermenter daily. Therefore, if the experimental diet was formulated containing 16.8% CP, the urea input by saliva represents approximately 28% of additional N input, which represents 28% of N recycling. This N recycling value is in agreement with in vivo data (Lapierre and Lobley, 2001; Marini and Van Amburgh, 2003).

The ruminal nitrogen balance in vivo is represented by the N input via dietary, rumen wall, saliva, and endogenous and output from rumen wall and flow to omasum. In a DFCCS the only way out of N is by overflow. Therefore, we speculate that the N added in the artificial saliva used in DFCCS should represent the balance (N recycling minus N output), instead of only the N recycling. The NRC (2001) assumes that at an apparent N balance of zero, approximately 15.2% of RDP is lost in the rumen. Considering N recycling of approximately 28%, the balance would be approximately 13%. We speculate that by reducing urea in the artificial saliva to approximately 0.19 g/L, the ruminal NH_3-N values obtained using DFCCS might be more closely related to OST. We also hypothesize that considering that N recycling is regulated by N intake (Marini and Van Amburgh, 2003), the amount of urea added in the artificial saliva needs to be adjusted according to factors such as CP level and extend of ruminal protein degradation. It is possible that depending on the diet fed to the fermenter, this addition of 0.4 g/L urea exceeds the ruminal microbial ability to metabolize NH_3, resulting in accumulation, which might explain the difference on NH_3-N observed between the two methods. Therefore, studies adjusting the amount of urea added in the artificial saliva in DFCCS are warranted.

In summary, out of 41 regressions developed in the present study, method only affected 14 estimates of $ß_0$ and 2 estimates of $ß_1$. Because the majority of method effects were only observed in the estimate of the intercept, it is likely that treatment effects observed in DFCCS are likely maintained when tested in vivo; however, the magnitude of the response may be different. In those cases, results need to be interpreted cautiously when extrapolating DFCCS data to in vivo, especially regarding NH_3-N concentration. Our results indicate that the DFCCS provides valuable estimates

of ruminal fermentation, and that overall, the functional responses observed in DFCCS studies are similar to OST.

Conclusions

This meta-analysis was performed aiming to compare ruminal fermentation responses in vitro using the continuous cultures system with responses obtained from in vivo studies using omasal sampling technique. Overall, method affected OM digestibility, molar proportion of acetate and propionate; however, the difference was observed only in the estimates of intercept. Even though we observed a method effect for molar proportion of acetate and propionate, total VFA concentration was not affected by method. Method only affected nonammonia nonmicrobial N relative to the total N when regressed with NDF degradability, while bacterial N/total N /total N was affected by method when regressed with NDF degradability and efficiency of microbial protein synthesis. Furthermore, true CP degradability and efficiency of microbial protein synthesis responses were not affected by method.

Concentration of NH_3-N was the only variable that had method effect on estimate of intercept and slope, demonstrating that estimation of NH_3-N using DFCCS needs further adjustments and studies investigating this response are warranted. Therefore, even though we observed differences in the estimate of $ß_0$ for some variables, in most cases the magnitude of the response was small, and the biological value of this difference is likely minimum. Most importantly, the functional responses to different dietary NFC concentration, efficiency of microbial protein synthesis, and NDF and true CP degradability are overall maintained in the DFCCS compared to OST.

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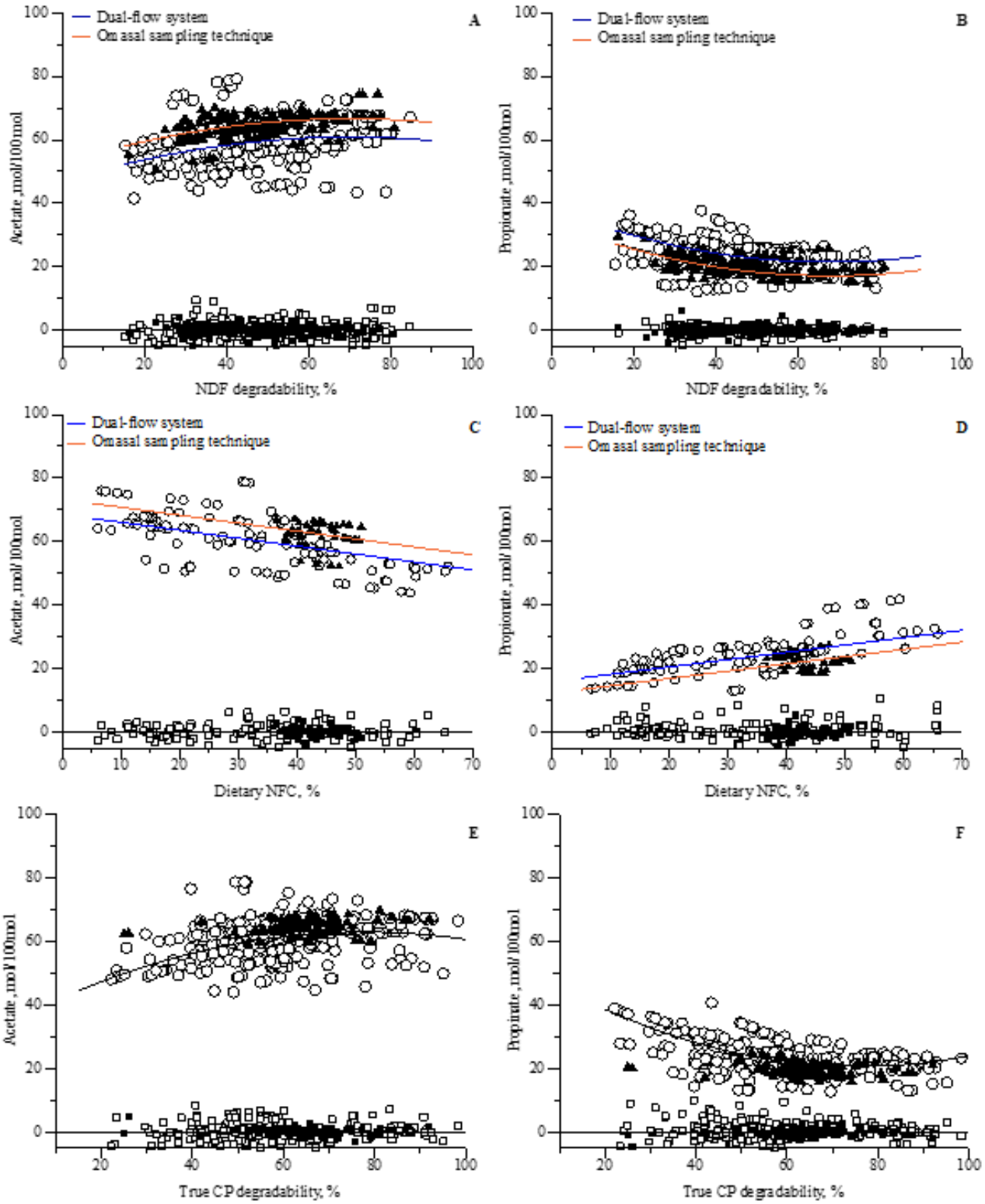

Figure 1. Adjusted ruminal molar proportion of acetate (A) and propionate (B) regressed with neutral detergent fiber degradability, regressed with dietary non-fiber carbohydrates

concentration (C and D), and regressed with true crude protein degradability (E and F). Data obtained from studies using dual-flow continuous culture (○) and their residuals (□); and from omasal sampling technique (▲) and its residuals (■). Residuals (observed – predicted) are represented in the 0-line X axis.

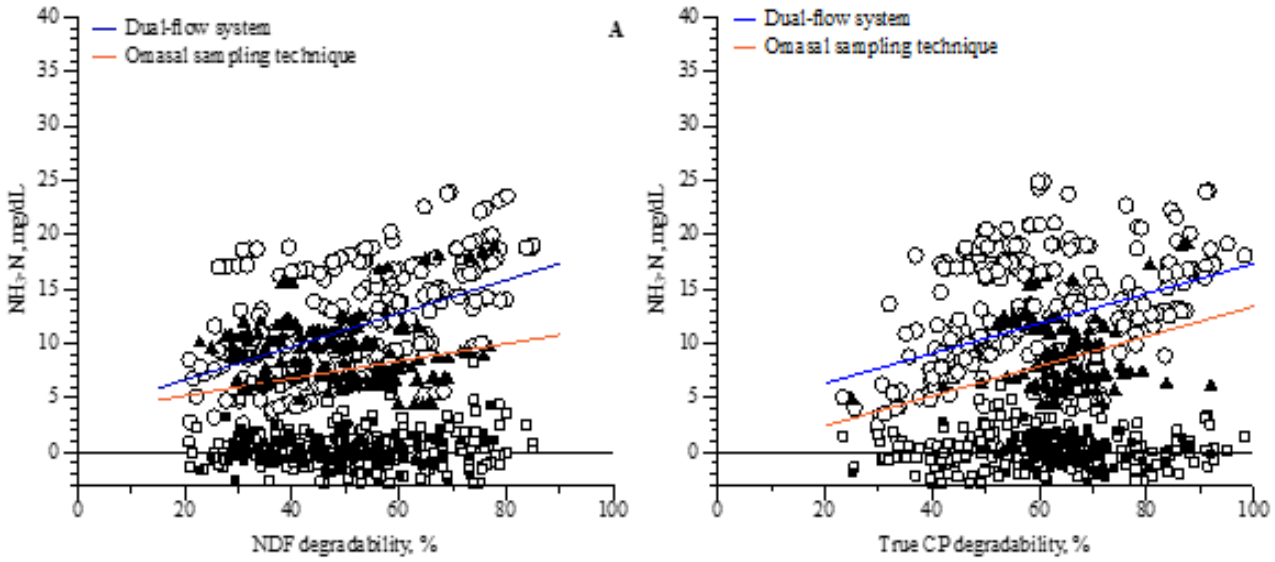

Figure 2. Adjusted concentration of ammonia regressed with neutral detergent fiber degradability (A) and true crude protein degradability (B). Data obtained from studies using dual-flow continuous culture (○) and their residuals (□); and from omasal sampling technique (▲) and its residuals (■). Residuals (observed – predicted) are represented in the 0-line X axis.

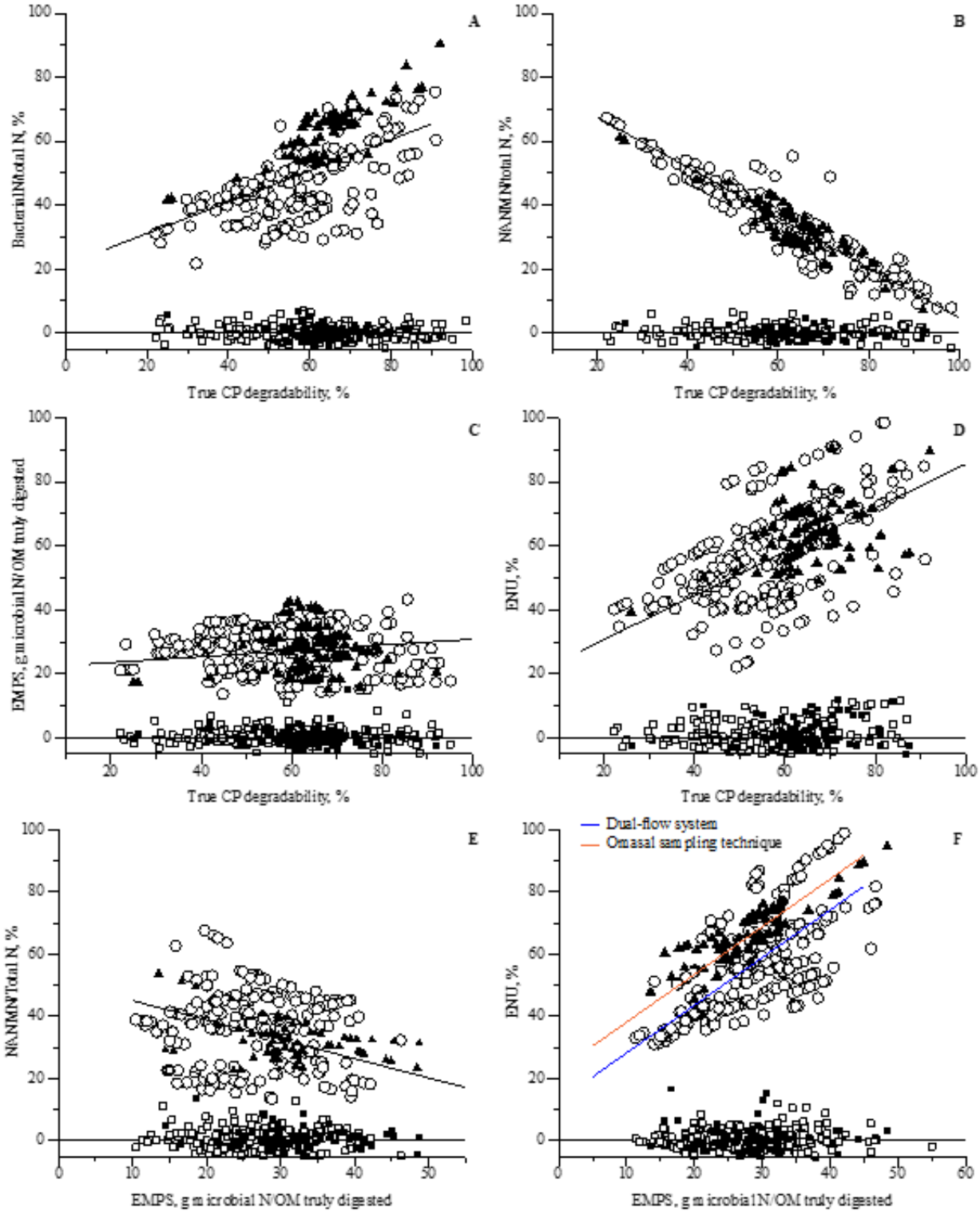

Figure 3. Adjusted proportion of bacterial nitrogen (A) and nonammonia nonmicrobial nitrogen (B) from total nitrogen flow, efficiency of microbial protein synthesis (C) and efficiency of

nitrogen use (D) regressed with true crude protein degradability. Adjusted proportion of nonammonia nonmicrobial nitrogen (E) and efficiency of nitrogen use (F) regressed with efficiency of microbial protein synthesis. Data obtained from studies using dual-flow continuous culture (○) and its residuals (□); and from omasal sampling technique (▲) and their residuals (■). Residuals (observed – predicted) are represented in the 0-line X axis.

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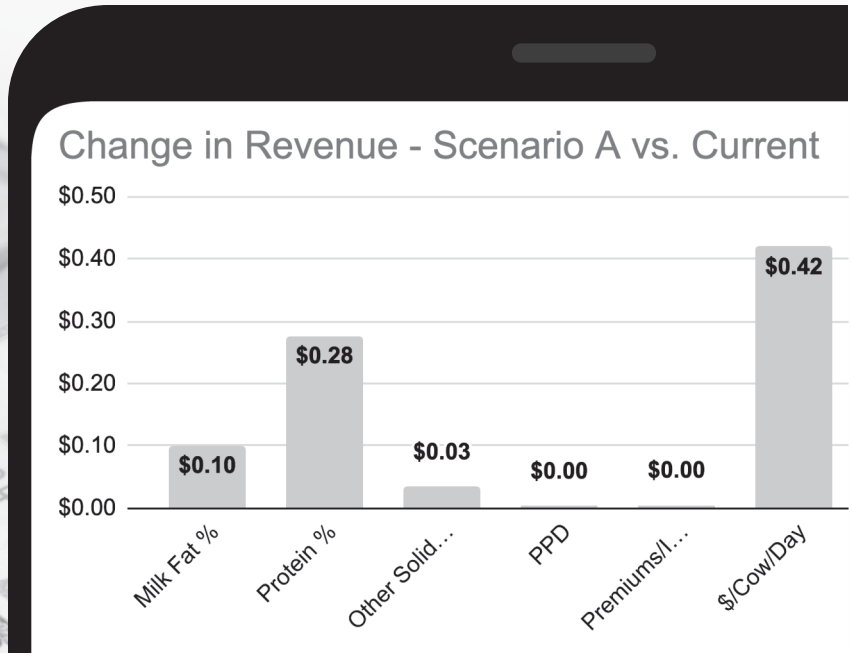

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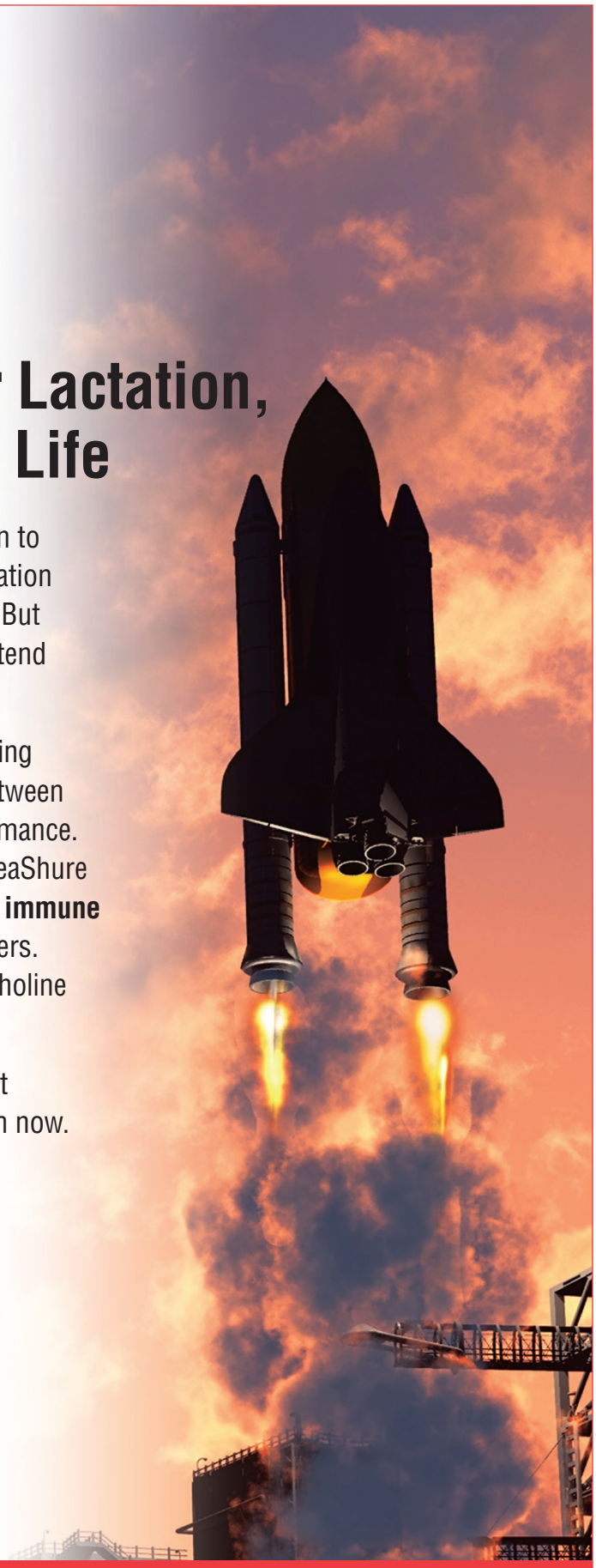

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A smart cow like me only goes for the best, most researched products. That's why the dairy portfolio from ARM & HAMMER™ keeps me at the top of my class. BIO-CHLOR™, CELMANAX™ and CERTILLUS™ build my resilience to challenges while MEGALAC®, FERMENTEN™ and DCAD Plus™ keep me performing my best. To top it all off, ESSENTIOM™ helps boost my immunity. Getting all your dairy solutions in one place? That's smart.

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- Increase milk production
- Improve nitrogen efficiency
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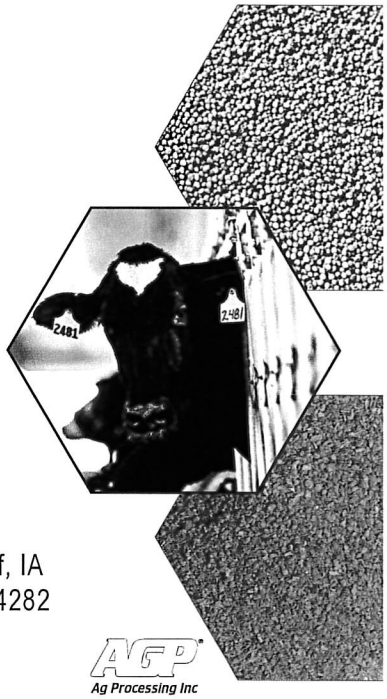

Production Locations

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– BRIAN BROWN, Sunburst Dairy, Belleville, Wisconsin
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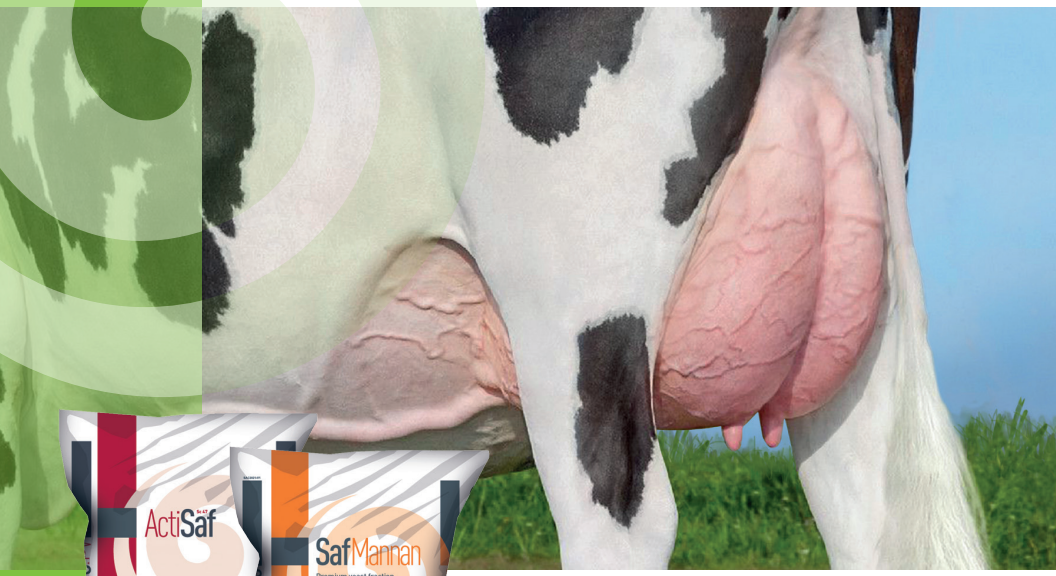

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*E. A. Horst et al., 2018, J. Dairy Sci. (Suppl. 2):383, 2018 and M. Al-Qaisi et al., 2017, AABP

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