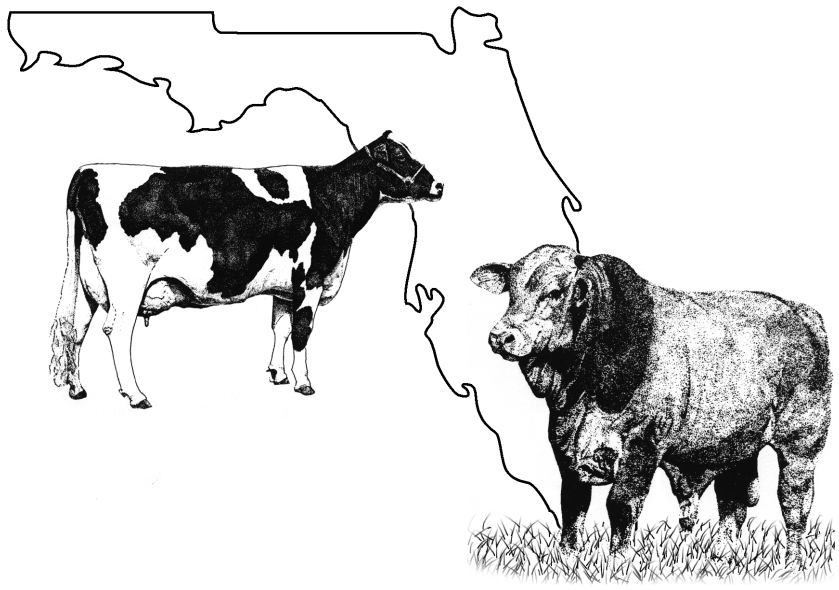
2016 Florida Ruminant Nutrition Symposium

27th Annual Meeting



February 15 - 17, 2016
Best Western Gateway Grand
Gainesville, Florida

PROCEEDINGS

UF UNIVERSITY of FLORIDA
IFAS
Department of Animal Sciences

PROCEEDINGS

2016

27th ANNUAL FLORIDA RUMINANT NUTRITION SYMPOSIUM

February 15 - 17, 2016
Best Western Gateway Grand Hotel
Gainesville, Florida

Department of Animal Sciences
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Daniele.luchini@addisseo.com

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Villa Rica, GA 30180
470-330-5196
Joseph.berry@adm.com

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Brett.bell@adm.com

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Cedar Rapids, IA 52404
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Dgreene@diamondv.com

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Valdosta, GA 31605
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D_waag@elanco.com

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513-844-6680
Atbyrd@fuse.net

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Roger.boland@mcness.com

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Jsparkman@hjbaker.com

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Harrisburg, PA 17111
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Faith.daniels@kemin.com

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6120 West Douglas Avenue
Milwaukee, WI 53218
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Rschmidt@lallemand.com

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Heath.graham@zoetis.com

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February 15, 2016
Balchem Mini Symposium – "Making Milk Protein"

2:00PM	**Dr. Clay Zimmerman**, Welcome and Introductions, Balchem Animal Health and Nutrition
2:30PM	**Dr. Charlie Will**, Select Sires Inc., "Breeding for Milk Protein"
3:15PM	**Dr. Mike Van Amburgh,** Cornell University "Using Precision Feeding to Improve Nitrogen Efficiency"
4:00PM	**Dr. John A Lucey**, University of Wisconsin, "The Future of Milk Protein as a Function Food"
5:00PM	Poolside Barbecue

February 16, 2016
Pre-Conference Symposium Sponsored by Quality Liquid Feeds – "New Nutritional Concepts for Maintaining Animal Performance During Heat Stress"

8:00AM	Continental Breakfast
8:45AM	**Dr. Randy Davis**, Quality Liquid Feeds, "Welcome and Objective for the Pre Conference Symposium"
8:45AM	**Dr. Randy Davis or Dr. Cory Berg**, "Quality Liquid Feeds Overview"
9:10AM	**Dr. Sha Tao,** University of Georgia, "Reducing the Impact of Heat Stress on Dry Cows and Fresh Cows"
10:00AM	**Dr. William Seymour**, Novus International, "Role of Methionine and Methionine Precursors in Transition Cow Nutrition with Emphasis on Liver Function"
10:50AM	**Dr. Stephen Emanuele**, Quality Liquid Feeds, "Why Feed a Molasses-Based Liquid Supplement When Corn is Cheap and Milk Price is Low"
11:45AM	Buffet Lunch

February 16, 2016
Ruminant Nutrition Symposium

9:00AM Registration (until 5:30PM)

11:45AM Buffet Lunch

1:00PM **Dr. Geoffrey Dahl,** University of Florida – Welcome

1:10PM **Dr. Monty Kerley**, University of Missouri, "Opportunities to Improve Feed Efficiency of Beef Production"

1:50PM **Dr. Amy Radunz**, University of Wisconsin at River Falls, "Impact of Maternal Nutrition on Calf Performance"

2:30PM **Dr. Charles Staples**, University of Florida, "Effect of Increased Supplementation of Vitamin E during Heat Stress"

3:10PM Refreshment Break

3:40PM **Dr. Kevin Harvatine**, Penn State University, "Managing Milk Fat Depression"

4:20PM **Dr. Adam Lock**, Michigan State University, "Fatty Acid Digestibility and Impacts on Responses of Dairy Cows"

5:10PM Welcome Reception

February 17th, 2016

6:30AM Continental Breakfast

8:00AM **Dr. Galen Erickson**, University of Nebraska, "Protein Nutrition Evaluation and Application to Growing and Finishing Cattle"

8:40AM **Dr. Tara Felix**, Penn State University, "Alternative Feeds for Beef Cattle"

9:20AM **Dr. Shawn Donkin**, Purdue University, "Control of Hepatic Gluconeogenesis during the Transition Period"

10:00AM Refreshment Break

10:30AM **Dr. Laura Hernandez**, University of Wisconsin-Madison, "Novel Concepts Regarding Calcium Homeostasis during the Transition Period"

11:10AM **Dr. José Santos**, University of Florida, "Dietary Manipulations and Interventions to Improve Ca Metabolism During Transition"

11:50AM Ruminant Nutrition Symposium Adjourns

Symposium Speakers

Guest

Mike Van Amburgh, Cornell University
Cory Berg, Quality Liquid Feeds
Randy Davis, Quality Liquid Feeds
Shawn Donkin, Purdue University
Stephen Emanuele, Quality Liquid Feeds
Galen Erickson, University of Nebraska
Tara Felix, Penn State University
Kevin Harvatine, Penn State University
Laura Hernandez, University of Wisconsin-Madison
Monty Kerley, University of Missouri
Adam Lock, Michigan State University
John Lucey, University of Wisconsin
Amy Radunz, University of Wisconsin at River Falls
William Seymour, Novus International
Sha Tao, University of Georgia
Charlie Will, Select Sires Inc.
Clay Zimmerman, Balchem Animal Health and Nutrition

University of Florida
Department of Animal Sciences

José Santos
Charles Staples

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Contact: Dr. José E.P. Santos
Department of Animal Sciences
P O Box 110910
Gainesville, FL 32611-0910
Tel: (352) 392-1958 Ext. 251
Fax: (352) 392-5595
Email: jepsantos@ufl.edu

27th Annual Florida Ruminant Nutrition Symposium

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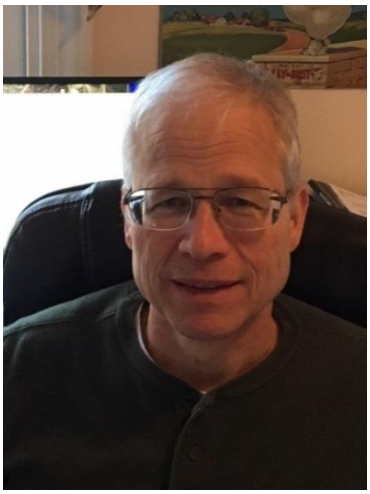
Title and Presenter ***Page***

BIOGRAPHIES

Dr. Sha Tao is an Assistant Professor in the Department of Animal and Dairy Sciences at the University of Georgia. He is originally from Kaifeng, China. He obtained his B.S. in Agriculture in 2004 and M.S. in Animal Sciences in 2007 at the Henan University of Technology, China where he studied feed technology of non-ruminants. Sha completed his Ph.D. in 2012 at the University of Florida in the Animal Molecular and Cellular Biology program under the supervision of Dr. Geoffrey Dahl. After graduation, he kept working in the same area as a post-doc associate at the University of Florida. Sha's Ph.D. and postdoc research focused on the effect of heat stress during the dry period on the mammary gland development, metabolic adaptations to lactation, and calf performance. In 2014, he moved to the University of Georgia at Tifton, GA as an assistant professor of heat stress physiology in dairy cattle. At Tifton, Sha keeps an active program to research the impact of heat stress on the cow's metabolic responses, mammary gland development and performance at various stages of her life cycle.

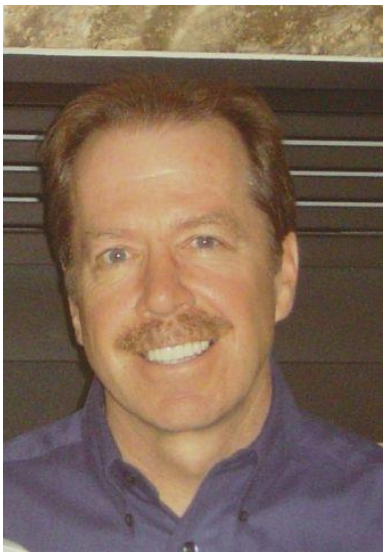


Dr. William Seymour is a Ruminant Technical Manager for Novus International. He is responsible for providing technical support for Novus products including Alimet, MFP, Mintrex chelated trace minerals and Agrado Plus antioxidant blend. He has prior experience as a dairy and ruminant nutritionist with Southern States and Tennessee Farmers Cooperatives, as Technical Services Manager for Roche Vitamins/DSM and as Manager of Dairy and Livestock Research for Agway and Cooperative Research Farms. He joined Novus in 2014. He received a B.S. in Animal Science from Cornell University and a Masters and Ph.D. in dairy and animal sciences from Virginia Tech. He resides in Ashland, VA with his wife Erin. They have two adult children Andrew and Grace Seymour.

Dr. Stephen Emanuele is a senior scientist and technical advisor for Quality Liquid Feeds. Dr. Emanuele received his BSc degree in Animal Sciences from the University of Rhode Island, the MSc degree in Dairy Science from the University of Maryland, and the PhD degree in Animal Sciences from the University of Florida. Dr. Emanuele has been working in the animal nutrition industry for over 26 years and currently he is responsible for leading the dairy research program at Quality Liquid Feeds. At QLF, he is responsible for training dairy field technical specialists and district sales managers, providing technical service to consultants, nutritionists, veterinarians and large dairy operations, conduct field experiments, and develop new products based on market needs. His most recent work was an analysis of data from 27 research publications to determine the optimal concentration of sugar, starch and soluble fiber in the dairy cow diet to maximize the production of milk components

Dr. Monty Kerley is a Professor of Animal Sciences in the Division of Animal Sciences at the University of Missouri. Dr. Kerley has a BS from Southern Illinois University-Carbondale, and MSc and PhD degrees from the University of Illinois-Urbana. His research is focused upon understanding nutritional and biological influences on beef cattle efficiency. His laboratory is studying relationships between mitochondrial respiration and metabolic efficiency of cattle. His laboratory is also conducting research to determine optimum amino acid to energy ratios that maximize gain efficiency and optimum degradable protein levels that maximize microbial efficiency in the rumen, which minimizes nitrogen waste, allows roughage removal from concentrate diets, and reduces waste and gas excretion. This research involves modeling bacterial flow from the rumen and developing empirical equations that optimize diet formulation for animal performance. Dr. Kerley is also conducting research evaluating by-products for beef feeding applications. Dr. Kerley is faculty coordinator of University of Missouri Beef Research and Teaching Farm and University of Missouri Feedmill. He teaches undergraduate ruminant nutrition, graduate ruminant nutrition, and graduate nutrition seminars. During his career at University of Missouri, Dr. Kerley has trained Doctorate and Masters students, has published referred articles, book chapters and abstracts, and has been awarded patents. He has directed and participated in externally-funded research. Dr. Kerley has received the AFIA Ruminant Nutrition Award, CAFNR Outstanding Researcher Award, and Gold Chalk Award in Graduate Teaching.

Dr. Amy Radunz is an Assistant Professor in the Department of Animal and Food Science at the University of Wisconsin River Falls. She earned her BS and MSc degrees at North Dakota State University. After earning her MSc, she worked in academic positions at Washington State University and The Ohio State University in the area of beef cattle production focused on extension, research and teaching. She then received her PhD degree from The Ohio State University in the area of ruminant nutrition and meat science. Dr. Radunz then took a faculty position at University of Wisconsin-Madison as the state's Beef Cattle Extension Specialist. In 2012, she moved to the University of Wisconsin-River Falls in a teaching position where she primarily teaches classes in beef cattle production and livestock evaluation. Her research has focused in the areas of late gestation nutrition impact on offspring performance and carcass composition and nutrition during early pregnancy on conception rates in beef cattle.



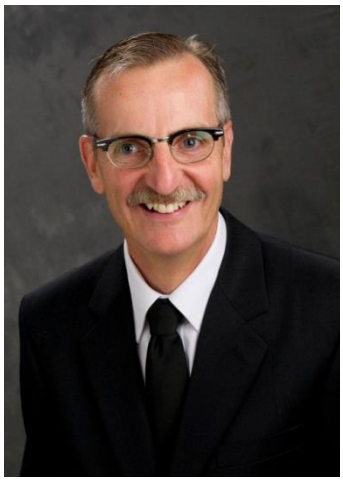
Dr. Charles Staples is a Research Foundation Professor in the Department of Animal Sciences at the University of Florida. Charlie earned his Animal Science degrees at New Mexico and Illinois. He was hired by the University of Florida as a dairy cattle nutritionist and has served at the rank of Professor since 1995. He teaches both undergraduate and graduate level nutrition courses. His research areas focus on the effects of dietary nutrients on production and reproductive performance of lactating dairy cows and on improving forage utilization by dairy animals. Based upon his research, Staples was the recipient of the American Feed Industry Association Award and the Nutrition Professionals Applied Dairy Nutrition Award from the American Dairy Science Association and a University of Florida Research Foundation Professorship.

Dr. Kevin Harvatine is an Associate Professor in nutritional physiology at Penn State University. He received his BS in Animal Sciences from Penn State, the MSc degree in dairy cattle nutrition from Michigan State University and the PhD degree in nutritional physiology from Cornell University. Kevin's research integrates traditional ruminant nutrition and modern molecular biology approaches to investigate the regulation of metabolism and develop dietary intervention strategies to improve dairy production. Specific research objectives include investigation of dietary factors that modify ruminal fatty acid biohydrogenation, regulation of synthesis of milk components, and basic regulation of lipid synthesis with the continual goal of developing feeding strategies to improve the efficiency and performance of dairy cows.

Dr. Adam Lock is an Associate Professor in the Department of Animal Science at Michigan State University. Originally from the United Kingdom, Dr. Lock received his PhD from the University of Nottingham and completed a post-doctorate at that institution as well as at Cornell University. He had a research and teaching appointment at the University of Vermont from 2006 to 2009 before moving to his current research and extension appointment at Michigan State University in the fall of 2009. Dr. Lock has developed his expertise in ruminant nutrition and physiology. His research and extension programs focus on both dairy production and human nutrition and health, and the interface between these two disciplines. The central theme is fatty acid digestion and metabolism in the dairy cow and the impact of bioactive fatty acids on animal production and human health. He is recognized for his ability to communicate to many sectors, from dairy farmers to dietitians and was awarded the 2011 American Dairy Science Association Young Scientist Award, which recognizes outstanding research by a young dairy scientist during the first 10 years of their professional career.

Dr. Galen Erickson is the Nebraska Cattle Industry Professor of Animal Science, Professor, and Beef Feedlot Extension Specialist at the Animal Science Department, University of Nebraska-Lincoln. His program focuses on byproduct feeds utilized by beef cattle, nutrition-environmental interactions with a focus on nitrogen and phosphorus, grain feeding and growth promotion for feedlot cattle. His awards include: Midwest American Society of Animal Science Young Outstanding Researcher Award (2009); the American Society of Animal Science Early Career Achievement Award (2009); Gamma Sigma Delta Research Award of Merit (2013); the University of Nebraska Darrell W. Nelson Graduate Student Advising Award (2015); and the American Feed Industry Association Ruminant Animal Nutrition Award (2015). He is serving on the beef NRC committee. He has supervised 49 graduate student degree programs, published 114 journal articles, 6 book chapters, 340 abstracts, and 342 producer publications. He received his BS degree from Iowa State University (Ames) in 1995 and his MSc and PhD degrees from the University of Nebraska-Lincoln in 1997 and 2001.

Dr. Tara Felix grew up on a small hobby farm in Northwest Pennsylvania. She attended Penn State University and graduated in 2006 with her bachelor's degree in Animal Biosciences. She went south for some warmer weather and a chance to study with world renowned mineral nutritionist, Dr. Lee McDowell. Upon successful defense of her thesis (entitled "Effects of dietary aluminum source and concentration on mineral status of feeder lambs"), Tara graduated from the University of Florida with her master's degree in 2008. Pursuing her passion for beef cattle, Dr. Felix traveled north again, really just following the great football, to The Ohio State University. There she spent 3 years writing her dissertation with Dr. Steve Loerch on "Eliminating barriers to increased distillers grains use in ruminant diets." Upon graduation Dr. Felix accepted a position at the University of Illinois at Urbana-Champaign. At IL, she spent 4 years and obtained over a million dollars to fund her research investigating the effects of alternative feeds on growth performance and ruminal metabolism of beef cattle. In January 2016, Dr. Felix rejoined the faculty at her first alma mater, Penn State University, in a 25% teaching and 75% extension appointment. As part of her extension appointment, she plans to continue her applied research on feed additives and other technologies used to promote beef cattle performance and improve nutrient digestion, and on novel feed ingredients.



Dr. Shawn S. Donkin received the BSc degree from McGill University (Montreal) in 1982 and worked in the feed industry and as a dairy herd manager before pursuing graduate education. He earned an MSc degree in Dairy and Animal Science from The Pennsylvania State University in (1987) and a PhD in Dairy Science from the University of Wisconsin-Madison (1992). He has been a member of the faculty of Purdue University since 1995 was promoted to Professor of Animal Sciences (2006). Dr. Donkin has developed an internationally-recognized research program to determine the control of liver function and importance to food animal production, animal well-being, and human health. This research, which is both applied and basic in scope, has resulted in feeding recommendations for optimal health and productivity in dairy cattle and has created knowledge of the underlying biology of nutrient metabolism. Dr. Donkin's laboratory was among the first to describe the molecular events that control glucose and nitrogen metabolism in dairy cattle and developing calves. Ongoing fundamental studies explore the role of nutrition, physiological changes, and environmental stressors on genes critical to health and productivity. Ongoing applied nutrition studies evaluate alternative energy and protein feeds for transition and lactating cows. Dr. Donkin has mentored 10 PhD students, 12 MSc students, and 2 postdoctoral fellows and authored over 55 referred publications, 90 abstracts, 8 extension publications, and 4 book chapters. He teaches courses in Ruminant Nutrition and Physiology, Dairy Cattle Management and Nutritional Biochemistry and Physiology.

Dr. Laura L. Hernandez is an Assistant Professor in the Dairy Science Department at the University of Wisconsin-Madison. She received her PhD in 2008 from the University of Arizona under the direction of Dr. Bob Collier. Her area of research has focused on how serotonin controls the mammary gland and various aspects of lactation. Dr. Hernandez combines basic research from the cell to whole-animal level in a variety of mammalian species to broaden the focus on the importance of the mammary gland and its contributions to and regulation of a successful lactation in dairy cattle. The outcomes of her novel research are aimed at demonstrating the presence of factors (specifically serotonin) produced within the mammary gland that can control the animal's physiology while lactating, particularly during the transition period when cows are the most metabolically and physiologically challenged. She specifically focuses on the interaction of serotonin and calcium metabolism during the transition period. Her research has determined that serotonin is an important regulator of mammary gland and maternal calcium homeostasis during lactation.

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

Reducing the Impact of Heat Stress on Dry Cows and Fresh Cows

Sha Tao[1]., A. P. A. Monteiro*, X-S. Weng*, J. K. Bernard*, J. Laporta†, G. E. Dahl†,*
**Department of Animal and Dairy Science, University of Georgia*
†Department of Animal Sciences, University of Florida

Introduction

Summer heat compromises a lactating cow's performance from different perspectives, such as decreased feed intake, altered metabolism, reduced milk production, impaired reproductive performance and increased disease incidence (Fuquay, 1981; Kadzere et al., 2002; West, 2003). Compared with lactating cows, dry cows produce less metabolic heat (West, 2003) and have a higher upper critical temperature (Hahn; 1997). Thus, heat stress management for dry cow is often overlooked, but substantially influences the cow's future performance. To avoid the negative impact of heat stress on dry cows, appropriate cooling should be applied during the entire dry period. Nutritional supplementation during dry and transition period may also be utilized to improve cow performance during the summer but more research need to be conducted in this area. This paper will focus on the impact of prepartum heat stress on performance during the dry period and the subsequent lactation performance. Treatments discussed in this manuscript were applied during the prepartum period only; after calving, all animals were managed in the same manner with cooling.

Prepartum Heat Stress Effects on Feed Intake and Metabolic Adaptation

Heat stress decreases dry matter intake (**DMI**) of dry cows, but to a lesser extent compared with lactating cows. Relative to cooled cows (**CL**), the non-cooled heat-stressed (**HS**) cows have ~15% decrease in DMI during the dry period (Table 1). As a result, they gain less body weight during the prepartum period partly due to the slower fetal growth (Tao et al., 2011). In addition to the reduced feed intake, heat stress alters the cows' metabolic responses. Recent studies suggest that, even a 30% decrease in DMI, heat-stressed cows have blunted adipose tissue mobilization in early (Lamp et al., 2015) and mid-lactation (Wheelock et al., 2010), and enhanced the whole body glucose utilization in mid-lactation (Wheelock et al., 2010) compared with pair-fed cows under thermo neutral condition. However, during the dry period, heat stress affects the cow's metabolism differently. Heat stress has no impact on the cow's adipose tissue mobilization (Lamp et al., 2015) and glucose clearance after a glucose tolerance test (Tao et al., 2012) during the dry period, presumably because of a lower energy demand for fetal growth compared with lactating. In contrast, relative to those under thermal neutrality with similar intake, the heat-stressed dry cows have more pronounced protein mobilization perhaps to support the fast fetal growth during late gestation (Lamp et al., 2015). Prepartum cooling improves feed intake before calving, and it has no carryover

[1] Contact at: Animal and Dairy Science Department University of Georgia - Tifton Campus, 2360 Rainwater Road, Tifton, GA 31793-5737. E-mail: stao@uga.edu.

effects on cow's DMI in early lactation (first 2-3 weeks postpartum) but increases the concentrations of nonesterified fatty acids (**NEFA**) and beta hydroxybutyric acid (**BHBA**) in blood (do Amaral et al., 2009; Tao et al., 2012) and peripheral tissue insulin resistance (Tao et al., 2012) to support the higher milk production. However, on the other hand, as the lactation advances, prepartum cooled cows will consume more feed relative to non-cooled prepartum cows in order to meet the nutrient demand for higher milk production.

Prepartum Heat Stress Effects on Mammary Development and Subsequent Milk Production

Late gestation heat stress has profound effects on milk production in the subsequent lactation. When active cooling (such as soakers and fans) is applied during the entire dry period, compared with HS cows, prepartum CL cows produce ~ 4 kg/d (12%) more milk in the next lactation (Table 2). Additionally, this increase in milk yield by cooling dry cows persists through the entire lactation, indicating an improved mammary function rather than the metabolically related galactopoietic effect (such as bovine somatotropin). On the other hand, when cows are cooled only during the close-up period, the increase in following milk yield is still apparent but to a lesser extent. From limited studies, relative to HS, the close-up CL cows have ~ 2.2 kg/d (5.8%) increase in milk yield during the next lactation (Table 2). This positive effect of prepartum cooling on subsequent lactational performance can be attributed to the improved mammary growth during the late gestation. The dry period is characterized by extensive mammary involution during the first couple weeks after dry off and the following mammary growth (Capuco et al., 1997), and heat stress influences both cellular processes. Indeed, HS cows have lower mammary cell proliferation ~2-3 weeks before calving compared with CL (Tao et al., 2011), which is partly due to a lower placental production of estrone-sulfate (Collier et al., 1982). Although often overlooked, mammary involution, including apoptosis and autophagy, during the early dry period may be important in modulating subsequent mammary growth and milk production. A recent study conducted at the University of Florida showed that the mammary gland of the HS cow has reduced autophagy during the early dry period compared with CL (Ramirez-Lee et al., 2015). The result from this study suggest that heat stress blunts mammary involution after dry-off, which perhaps negatively affects the subsequent period of mammary growth and milk production; however, more research is needed to evaluate this hypothesis. Relative to multiparous cows, prepartum cooling of nulliparous heifers has received less attention and only two publications have compared the lactational responses from applying heat abatement during summer to heifers (~1 month before calving). In general, prepartum cooling of nulliparous have shown less consistent benefits to subsequent lactation (Table 2), although, more controlled studies are warranted.

Prepartum Heat Stress Effects on Immune Function and Disease Incidence

Immune dysfunction is well characterized during the transition period and partly responsible for the increased disease incidence in early lactation. Various studies

indicate that prepartum heat stress exaggerate the dysfunctional immune system during late gestation and early lactation. Compared with prepartum CL cows, HS cows have higher blood count of leukocyte but smaller proportion of CD4+ T lymphocyte (Gomes et al., 2014). Also, immune cells of HS cows have weaker proliferative response and TNFα production when stimulated by a mitogen in vitro (do Amaral et al., 2010), suggesting a compromised cell-mediated immunity. The humoral immune response is also altered by heat stress during the transition period. After challenged with ovalbumin, the HS cows have less IgG production during the dry period (do Amaral et al., 2011) and early lactation (Gomes et al., 2014) compared with CL cows. In addition, the nonspecific innate immunity is impaired by prepartum heat stress as evidenced by the lower ability of neutrophils from HS cows to phagocytize and destroy pathogens relative to those from CL cows in early lactation (do Amaral et al., 2011).

With the compromised immunity during the transition period, it is expected that the prepartum HS cows would experience a higher disease incidence during early lactation. When comparing dry period seasonal effects on the occurrence of health disorders in the first 60 DIM in Florida, Thompson et al. (2012) found that cows dried off in the hot months (June, July and August) had increased incidences of mastitis, respiratory problems and retained fetal membranes in early lactation compared with those dried in cool months (December, January and February). Although confounded with the seasonal effects during early lactation and photoperiod during the dry period, these data may suggest the negative impact of prepartum heat stress on future disease susceptibility. In contrast, in the controlled experiments, studies (Santos et al., 2014; Thompson et al., 2014a) suggest that prepartum CL cows have similar incidence of diseases in early lactation but a slight increase in the incidence of metritis. The increase in metritis incidence is unexpected considering the improved measures of immunity of CL compared with HS cows during the transition period, but deserves further investigation.

Prepartum Cooling and Body Temperature

Evaporative cooling is by far the most efficient approach to minimize the negative impact of heat stress during the dry period on dairy cows. Active cooling including sprinklers and fans is widely used to abate heat from lactating cows, but not often applied in dry cow barns, especially during the for far-off period. However, successful decrease in the dry cow's body temperature by cooling results in a significant return on investment. In a free stall setting, implementation of active cooling effectively reduces dry cow's body temperature by ~ 0.4 °C (0.7 °F; 102.7 vs. 102.0 °F, Table 3) and respiration rate by 21 breath/min (72 vs. 51 breath/min), which are much smaller compared with the difference (~ 1.0 °C = 1.6 °F) of body temperature between non-cooled and cooled lactating cows, but enough to elicit the strong positive influence on subsequent lactation milk production.

Feeding Betaine-Containing Molasses to Transition Dairy Cows During Late Summer

Nutritional supplementation of heat-stressed lactating cows has been widely studied, but the related research for dry cows under heat stress is somewhat limited, especially during the far-off period. Betaine, also called tri-methyl glycine, is a natural compound either produced endogenously by choline oxidation (Zeisel, 2013) or found in feed ingredients, such as sugar beet solubles, which has the most abundant betaine content (Eklund et al., 2005). Betaine has two main functions in an animal's body. It is a powerful osmolyte to reduce dehydration and stabilize protein when a cell is under stress condition. Additionally, it serves as a methyl donor when fed to animals and is a key component in one carbon metabolism (Eklund et al., 2005; Bertolo and McBreairty, 2013). Supplementing 100 g/d rumen-unprotected betaine increased milk production in mid-lactation cows (Peterson et al., 2012) under a thermal-neutral condition. However, its role in transition dairy cows, especially during summer, remains unknown.

To investigate the potential impact of supplementation of betaine containing diet during the dry and transition period, a study (Monteiro et al., 2015) was conducted at the University of Georgia-Tifton Campus during late summer 2014. In early September, cows were randomly assigned to betaine or control groups either at dry off (n = 10/treatment) or 24 d before calving (n = 8/treatment) based on their previous mature equivalent milk yield. Cows were fed common diets supplemented with either a 28% CP molasses-based liquid supplement made from sugar cane or a 28% CP liquid supplement made of molasses from sugar cane (67%) and condensed beet solubles containing ~30% betaine (33%) for control and betaine cows, respectively, until 8 weeks postpartum. The liquid supplement was fed at a rate of 1.1 and 1.4 kg DM/d for pre and postpartum cows, respectively. Dry cows were housed in the same free-stall barn without supplemental cooling and lactating cows were cooled by misters and fans and milked thrice daily. Feed intake was recorded daily during the entire experimental period and plasma samples were harvested weekly from a subset of animals. For those enrolled at close-up, no treatment effects were observed for milk production and composition, feed intake, and blood metabolites during pre- and postpartum periods. In contrast, cows that received betaine diets starting at 56 d before the expected calving date had improved milk production (44.2 vs. 41.5 kg/d), milk fat concentration (4.78 vs. 4.34%), and 3.5% FCM (50.0 vs. 47.0 kg/d) during the first 8 weeks postpartum compared with control cows; however, no change in DMI was detected during both pre- and postpartum periods. As a consequence, betaine-fed cows had increased plasma concentrations of NEFA and BHBA during the early lactation. However, the experimental period for dry cows in this study spanned from Sep to Nov, 2014, and the environmental condition (average temperature and humidity index or THI < 68) during this period was not suitable to conclude the potential impact of betaine containing diet during the summer time. Thus, supplementation of betaine containing molasses during dry and lactating period improves performance during early lactation, but the potential impacts of feeding betaine to transition cows during hot summer need to be further studied.

Conclusions

It is important to recognize the negative impacts of dry period heat stress on cow performance, immunity and metabolism, and the significance of prepartum cooling in transition cow management. A special attention should also be given during the early dry period to ensure a smooth transition from lactating to non-lactating stage. In addition to cooling, other strategies, such as nutritional supplements, should be explored to future overcome the negative impacts of prepartum heat stress.

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Table 1. Summary of studies on effects of prepartum heat stress (HS) and cooling (CL) on dry matter intake (DMI)

	DMI, kg/d			
Period	HS	CL	Difference (kg or %)	Reference
Dry	11.3	12.2	0.9 or 8%	Adin et al., 2009
Dry	12.0	14.1	2.1 or 18%	do Amaral et al., 2009
Dry	8.4	9.8	1.4 or 17%	do Amaral et al., 2011
Dry	8.9	10.6	1.7 or 19%	Tao et al., 2011
Dry	10.2	11.1	0.9 or 9%	Tao et al., 2012
Dry	10.4	12.3	1.9 or18%	Thompson et al., 2014b
Average	10.2	11.7	1.5 or 15%	

Table 2. Summary of studies on effects of late gestation heat stress (HS) or cooling (CL) on milk production

	Milk production			
Period	HS	CL	Difference (kg or %)	Reference
Dry	37.2	40.7	3.5 or 9%	Wolfenson et al., 1988
Dry	25.4	28.1	2.7 or 11%	Avendaño-Reyes et al., 2006
Dry	39.3	41.4	2.1 or 5%	Adin et al., 2009
Dry	26.2	33.7	7.5 or 29%	do Amaral et al., 2009
Dry	32.2	34.5	2.3 or 7%	do Amaral et al., 2011
Dry	28.9	33.9	5.0 or 17%	Tao et al., 2011
Dry	43.2	45.6	2.4 or 6%	Thompson et al., 2011
Dry	27.7	34.0	6.3 or 23%	Tao et al., 2012
Dry	30.2	33.8	3.6 or 12%	Thompson et al., 2014b
Average	32.3	36.2	3.9 or 12%	
Close-up	38.7	40.1	1.4 or 4%	Urdaz et al., 2006
Close-up	32.1	33.5	1.4 or 4%	Adapted from Wang et al.,2010
Close-up	36.9	38.7	1.8 or 5%	Adapted from Gomes et al.,2013
Close-up	40.5	44.6	4.1 or 10%	Karimi et al., 2015
Average	37.0	39.2	2.2 or 6%	
Late gest.	23.3	25.6	2.3 or 10%	Adapted from Wang et al.,2010
Late gest.	25.1	25.5	0.4 or 2%	Adapted from Gomes et al.,2013
Average	24.2	25.6	1.4 or 6%	

Table 3. Summary of studies on effects of prepartum heat stress (HS) and cooling (CL) on rectal temperature (RT, °C) and respiration rate (RR, breaths/min)

	RT			RR			
Measurement	HS	CL	Diff.	HS	CL	Diff.	Reference
1400h	39.2	38.8	0.4	---	---	---	Wolfenson et al., 1988
1400h	39.3	39.0	0.3	74	67	7	Avendaño-Reyes et al., 2006
1500h	38.8	38.5	0.3	57	45	12	Adin et al., 2009
1430h	39.2	38.8	0.4	---	---	---	do Amaral et al., 2009
1430h	39.4	39.0	0.4	78	56	22	do Amaral et al., 2011
1430h	39.4	39.0	0.4	78	46	32	Tao et al., 2011
1430h	39.9	39.4	0.5	78	45	33	Thompson et al., 2014b
1430h	39.3	39.0	0.3	69	48	21	Tao et al., 2012
Average	39.3	38.9	0.4	72	51	21	

SESSION NOTES

Role of Methionine and Methionine Precursors in Transition Cow Nutrition with Emphasis on Liver Function

William M. Seymour[1]
Ruminant Technical Manager
Novus International, St. Charles, MO

Introduction

Methionine is an essential and multifunctional nutrient in vertebrate diets. In addition to its role in protein synthesis, methionine functions as a methyl donor and precursor to a number of other antioxidant and lipotropic compounds including cysteine, taurine, glutathione, metallothionine, choline, carnitine, creatine and S-adenosyl methionine (**SAM**), a universal methyl donor (Lehninger, 1977). Methionine precursors 2-hydroxy-4-(methylthio)-butanoic acid (**HMTBa**) and its isopropyl ester can also serve this function (Zanton et al., 2014). In addition these compounds exert beneficial effects on the rumen fermentation and microbial protein synthesis (Lee et al., 2015; Baldin et al., 2015).

Methionine is of particular interest in dairy cattle nutrition. Historically research has focused on the role of methionine as a co-limiting amino acid for milk protein synthesis, and in supporting milk fat synthesis and metabolic balance (Polan et al., 1991; McCarthy et al., 1968). More recently this view has expanded to include other metabolic functions of methionine such as its role in supporting liver function, oxidative balance and immunity (Osorio et al., 2013). In non-ruminants methionine deficiency produces fatty liver disease including depletion of cellular antioxidants (glutathinone), methyl donors, SAM, and general hepatic inflammation and fibrosis (Schugar and Crawford, 2012). This paper will focus on the nutritional role of methionine in the transition dairy cow.

Challenges to the Transition Cow

The dairy cow faces a major metabolic and immunologic challenge at calving in which essential nutrients can play a significant role. Methionine status can potentially be related to three important metabolic indices in transition cows: energy balance (lipid metabolism), protein balance, and antioxidant balance (Pedernera et al., 2010). Dysfunction in any of these spheres of metabolism may result in sub-clinical or clinical disorders or diseases (fatty liver, ketosis, impaired passive transfer of immunity to calf, reduced immune function and disease resistance in cows) (Bell, 1995; Overton and Waldron, 2004; Drackley, 2011; Lean et al., 2013). This up close view of metabolic function has to be put into the context of dairy farm management where dry matter

[1] Contact at: 20 Research Park Dr., St. Charles, MO 63304, Work Phone: 636-851-7134; E-mail: William.Seymour@novusint.com.

intake of cows near and just after calving is a most critical factor. Dry matter intake is in turn influenced by management and environmental factors such as stocking density, heat stress, rumen adaptation, calcium metabolism and overall level of disease challenge. These factors must be addressed in order to gain the most value from supplementing essential nutrients like methionine in the transition period (Overton and Waldron, 2004; Lean et al. 2013).

Liver function is central to metabolism and especially critical in transition dairy cows. During the first 3 to 6 weeks after calving, a typical Holstein cow will mobilize 40-60 kg of body fat and 20 to 25 kg of body protein (Bell, 1995; Komaragiri and Erdman, 1997). The liver both participates in and regulates the majority of this intense metabolic activity aimed at mobilizing and directing nutrient flow to the mammary gland and supporting organs like the G.I. tract. Therefore any diminution in liver function can impair lactation performance and reproduction. Methionine has been shown to be essential for normal liver function and health in several species (Katoh, 2002; Schugar and Crawford, 2012).

Prepartum Protein Status and Supplementation

The increasing demand for amino acids for fetal growth, colostrum synthesis, mammary gland, liver and G.I. tract coupled with the natural reduction in dry matter intake prior to calving can result in negative protein balance (Jaurana et al., 2002). Ideally this would be kept to a minimum to avoid depletion of body protein stores. As a result a number of studies have been conducted to test the effects of prepartum protein supplementation on subsequent performance of dairy cows (Lean et al., 2013).

Kokkonen (2014) conducted a meta-analysis of 15 published studies with 47 treatment comparisons. Prepartum diet crude protein levels varied from 9.7 to 20.6% of dry matter and diet RUP from 2.9 to 10.6% of DM. Confounding factors (length of supplementation, length of postpartum measurements, forage base, energy intake and parity) were included in the analysis with random study effects. A sensitivity analysis for study bias was performed in which each study was excluded, in turn, from the data analysis and the results compared to analysis of the full data set. Milk protein synthesis and postpartum dry matter intake increased in response to increasing prepartum protein supplementation in cows fed higher fiber, mixed forage diets including straw, but tended to decrease in cows fed corn silage-based diets supplemented primarily with soybean meal. The author speculated that corn silage-based diets supported greater rumen microbial protein yields and that use of soybean meal as the source of supplemental protein may have introduced a methionine limitation, based on the amino acid composition of corn and soy proteins (Kokkonen, 2014).

A recent study conducted by Osorio et al. (2013, 2014) tested the effect of supplementing each of two methionine sources (HMTB isopropyl ester and rumen-protected methionine) to dairy cows in late gestation and early lactation. The control diet was methionine limiting in relation to lysine. Supplementing an additional ~7 grams per day of metabolizable methionine from either source improved measures of antioxidant

capacity ($P < 0.04$) and tended ($P < 0.07$) to reduce acute phase inflammatory protein response (an indicator of liver function), increase plasma carnitine and improve white blood cell function. Postpartum dry matter intake and milk component yields were significantly improved in supplemented cows (Osorio et al., 2013). These results demonstrated a beneficial response to methionine supplementation during transition and early lactation and linked the response to indicators of methionine metabolism (Osorio et al., 2014).

Literature Summary of the Effects of Methionine Supplementation Pre- and Postpartum on Dairy Cow Performance

A simple summary of 10 published studies with 14 treatment vs. control comparisons was conducted for this review. Data were expressed based on the quantity of additional metabolizable methionine (**mMet**) supplied per cow per day prepartum (8-28 days) from various sources (both protected D-L methionine and methionine analogues). While methionine was also supplemented postpartum in the studies, the level of prepartum supplementation fit the performance data more closely. Prepartum supplementation rates varied from 3 to 12 grams per day of additional mMet; average milk yield ranged from 63 to 95 lbs per cow per day; and length of postpartum supplementation from 28 to 140 days. Milk protein yield and dry matter intake displayed the best fits of the data based on regression analysis. Both followed a polynomial response with a maximum response at ~ 7.5 g/cow/day of mMet prepartum ($R^2 = 0.44$ and 0.32, respectively). Milk fat yield and milk production were poorer fits. While this data set and the analysis are inadequate to draw conclusions the results suggest that optimum supplementation levels lie between 5 and 10 grams of additional mMet per cow per day during the prepartum period as previously suggested by Luchini and Loor (2015).

Summary

Performance of dairy cows is affected by methionine status during the transition period. Recent studies confirm effects of methionine on metabolic processes beyond basic requirements for milk protein synthesis. Various forms of methionine have proven effective in supporting improved cow performance. It appears that 5 to 10 grams of supplemental metabolizable methionine given prepartum will support optimal performance postpartum, although further research is undoubtedly required.

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SESSION NOTES

Why Feed a Molasses-Based Liquid Supplement When Corn is Cheap and Milk Price is Low

Stephen M. Emanuele[*1] *and Charles J. Sniffen*[§]
**Quality Liquid Feeds*
[§]Fencrest, LLC

Introduction

Sugar has been consumed by dairy cattle since the beginning of time. They have come from the pastures which are naturally high in sugars. When we feed fermented forages most of the sugars have been converted to fermentation acids. The rumen and the cow have evolved to use plant sugars. The most prevalent form of added sugar fed to cattle has been molasses. The main sugars in molasses are sucrose (65%), fructose and glucose. Historically, the reason for feeding added sugar has mostly been as a sweetener or to improve palatability of feeds that are being fed. This concept has now changed. There are now research papers showing advantages to feeding sugars. More importantly though is the fact that we now have laboratories analyzing total sugars in forages and byproducts. Sugar addition to rations has had a mixed history. It has only been in recent years that consideration has been given to the addition of sugar to the ration as a nutrient to benefit rumen function as well as the metabolism of the cow. We need to start thinking in terms of individual sugars.

The addition of sugar to dairy cattle diets can be very positive. Trials have reported increased milk yield and milk fat percent or increased NDF digestibility (Broderick and Radloff, 2003, Broderick and Smith 2001, Varga etal. 2001, Oldick etal. 1997). Yet, the addition of sugars to dairy cattle diets has not always improved milk yield, ruminal microbial protein yields or milk components (Hristov and Ropp 2003; McCormick etal. 2001, Morales etal. 1989). The reported variation in response to sugars in dairy cattle diets can be explained by four processes that occur in the rumen. These processes are:

A. A shift in the end products of ruminal sugar fermentation based on bacterial growth rate and rumen pH.
B. Not all sugars are used with the same efficiency by rumen bacteria for growth.
C. Establishment of a viable population of anaerobic fungi in the rumen.
D. Wasting of energy by rumen bacteria (energy spilling) when the supply of fermentable carbohydrates exceeds the needs for microbial growth.

Below is a partial list of sugars found in the feedstuffs that we feed.

Monosaccharaides

[1] Contact at: Quality Liquid Feeds, 3586 Hwy 23 North, P.O. Box 240, Dodgeville, WI 53533. Tel: (608) 935-2345; E-mail: semanuele@qlf.com.

5 carbon sugars – Arabinose, Ribose and Xylose
6 Carbon sugars – diverse across all plants; Glucose, Fructose, Galactose and Mannose

Disaccharides

Sucrose – found in all plants and consists of Glucose + Fructose
Maltose – found in all plants and consists of Glucose + Glucose
Lactose – found in milk and consists of Glucose + Galactose

Trisaccharides

Raffinose – found in cottonseeds and sugar beet pulp and consists of Galactose + Fructose + Glucose
Maltotriose – found in corn distillers grains and consists of Glucose + Glucose + Glucose

Tetrasaccharides

Stachyose - found in soybeans, consists of Galactose + Galactose + Glucose + Fructose

Polysaccharides

Fructans – mainly in grasses and consists of fructose
Galactans – mainly in alfalfa and soybeans and consists of galactose
Pectins – mainly in alfalfa and soybeans and contains arabinose + galactose
Cellulose – all plants and contains long chains of glucose
Hemicellulose – all plants, higher concentration in grasses and contains arabinose, xylose, mannose, galactose, and glucuronic acids
Starch – diverse among plants and contains long chains of glucose
Sugar alcohols – polyol – forage fermentation product - Mannitol – 6 carbon

It would be a mistake to assume that all sugars have the same fermentation rate in the rumen and that all sugars are used with the same efficiency by rumen bacteria. Yet, that is exactly what we do when we formulate dairy cattle diets.

The most common rapidly available sugars in forages and various grains are sucrose and glucose. However when forages are fermented these sugars disappear, leaving residual sugars from the fermentation. These residual sugars are the breakdown products from hemicellulose which are mainly the 5 carbon sugars shown above. There has been some preliminary work that would indicate that the rumen microbes use the different monosaccharide's with different efficiencies. We need to know more about the utilization of the water soluble disaccharides and oligosaccharides in the rumen and by the cow. The sugars found in cane molasses are mainly sucrose, glucose and fructose. Molasses contains 50% sugar on an as fed basis and 70% of the sugar is sucrose. Cellulose, starch and sugars all end up eventually as hexoses. These hexoses then are metabolized to pyruvate which can be metabolized to acetate, propionate, butyrate or lactate. Ruminal conditions must exist where the majority of hexose is fermented to acetate, propionate, and butyrate but not lactate. The energy generated from this

fermentation must be used for bacterial growth if sugars are to be used successfully in dairy feeding programs.

Rumen Diversity Influences the Efficiency of Sugar and Starch Utilization

Table 1 provides a global, but simplified view of the rumen ecology. This is a balance that can be disturbed by many factors:

1. Inadequate effective fiber to produce adequate buffering
2. High fermentable starch levels in the ration, which lead to periods of low ruminal pH
3. Ration sorting, which can lead to rumen acidosis and sore feet
4. Excessive rumen degraded protein containing peptides with Histidine
5. Over-crowding or high stocking density, which leads to slug feeding

The bacteria that digest fiber basically need a rumen pH that is over 6.0 for a significant part of the day for good fermentation. There needs to be adequate NH_3 at all times at a concentration high enough that there will be a gradient that will be adequate to bathe the colonies growing within the fiber matrix. There is an absolute requirement for isoacids for continued fermentation of fiber. Note that the bacteria that use protein as their substrate produce the primary source of the isoacids from the branched chain amino acids, leucine, isoleucine and valine. These bacteria have a long doubling time. This is influenced by nutrient supply and then to surface area. However, the down side in chopping or grinding the forages too fine is an increased wash out from the rumen and a depressed digestibility. There is a balance and the balance is to maintain a rumen mat.

The starch and sugar bacteria have very high growth rates. They can drive ruminal pH down very quickly. There is a balance that can be achieved where there first is a balanced nutrient flow to these bacteria that will stimulate a coupled fermentation – one that drives microbial mass and not microbial waste in the form of VFA. Next, there is the importance of enhancing the secondary fermenters which will use the lactic acid moderating the pH drop that occurs after an ingestive episode. This requires a malic acid source. A major organic acid found in molasses is malic acid.

A significant part of the normal rumen ecology is composed by protozoa which, under normal conditions, make up 40-45% of the microbial mass (Table 1). In contrast to the bacterial population, only 10% of this population washes out of the rumen to contribute to the microbial protein supply. This is in contrast to 75 to 85% of the bacterial population washing out of the rumen, with the rest lysing and being predated on by the protozoa. We say that the microbial mass that stays in the rumen contributes to the recycled N pool. It is now suggested that we are under estimating the contribution of this pool to the N needed by the bacteria in the rumen. It should be pointed out that many years ago classic studies conducted by Reis were done in sheep in Australia demonstrating the positive wool growth in sheep resulting from depopulating the rumen of protozoa. These studies pointed out the large pool of bacteria that the

protozoa consumed daily in the rumen. The Fungi make up only 3-8% of the microbial mass. It is proposed that they have a key role in opening up the fiber that will enhance colonization by the fiber bacteria. They are stimulated by sugar, which research suggest will result in improved fiber digestion.

It is the understanding of these interactions that will help us in developing better rations going into the future. The balance of the carbohydrates with different fermentation rates and the different protein sources with different fermentation rates must be balanced. Unfortunately we have yet to reach the point where we can effectively do this. It is a dynamic second order system. We have improved our ability to measure and define the amount of each protein and carbohydrate fraction, but in our nutrition models we assume that a cow eats 24 meals a day, the same size and evenly spaced. We know this not to be the case. Does this mean that we should abandon the use of nutrition models? No, but it does require that as nutritionists, we admit that these models do not account for associative feed effects or the impact of ration sorting and meal pattern on nutrient utilization in the rumen.

Impact of Sugar on Animal Performance

There have been several studies over the years that have demonstrated the value of adding sugar to rations. Most of these studies have been with the addition of molasses or sucrose directly. Several of the studies replaced the starch with the sugars, keeping the NFC constant. The results were always positive not only in milk yield but also in components. In studies with fermenters, the work showed increases in fiber digestion. It has been suggested that the fungi which play a role in opening up the fiber, are stimulated by the 6-carbon sugars. This is important because it fits well with the rapidly degraded protein (mostly the soluble) to give an early stimulation to fiber digestibility. Additionally, if we can reduce the starch in the ration, we will have better control of rumen pH, an additional enhancement for fiber digestibility. In the original nutrition models, it was assumed that all of the sugar was broken down in the rumen. This assumption is not correct. This is important because of the positive impact that the digestible sugars can play in the metabolism at the mammary gland. It is important to note that added sugars, in the form of sucrose or sucrose equivalents (glucose + fructose) are about 84% degraded in the rumen.

Figure 1 displays results from some of the work conducted in France by Dr. Rulquin and colleagues at INRA. This suggests that it is important not only to enhance rumen function but also having an optimum amount of digestible sugar that will enhance milk true protein yield. This means that we need to consider the feeding of additional sugars in the rations. Many rations that are fed in this country have silages as their forage base. This usually results in rations with a 3 to 4% total sugar as measured by the 80% ethanol procedure. Unfortunately, this procedure does not identify the individual sugars. A high percentage of these sugars are the 5-carbon sugars discussed earlier. These sugars are not very digestible in the rumen or in the small intestine. It is recommended that we should add about 3 to 5% additional sugar in the form of 6-carbon sugars. This will result in a total sugar in the ration of 6 to 8% of the DM. If we

assume that 35% of this sugar will escape then added to the base sugar level plus what we will derive from the escaped starch, we will approach the levels suggested by the work of Rulquin shown above. Research using molasses based liquids shows 84% utilization in the rumen.

Harris and Van Horn (1983) suggested that at 8% or less of the total ration dry matter molasses would contain the same productive energy as ground corn. This would be equal to 4 pounds of molasses on an as fed basis, when DMI was 50 pounds. Feeding 4 pounds of cane molasses would supply 2 pounds of sugar. At dietary concentrations above 8% of ration dry matter, the value of molasses declined relative to corn. Recent trials suggest that Harris and Van Horn were correct. In a recent meta-analysis of 25 published research trials, 3.5% FCM yield, milk protein yield, milk fat yield, were maximized when the diet contained 5 – 7% supplemental sugar (Emanuele et al., 2015). Non-linear analysis of the database indicated that for cows producing 90 to 100 pounds of milk, the ideal dietary sugar content was 7.14% (Emanuele, 2016, Leading Dairy Producer Conference, Wisconsin Dells, WI.). For all cows, the ideal dietary sugar content was 6.75% of diet DM (Emanuele et al., 2015).

Broderick and Smith (2001) replaced high moisture corn with dried molasses. Their diets contained 0, 4, 8, or 12% dried molasses. Their diets contained 60% forage with 67% of the forage from alfalfa silage and 33% from corn silage. When high moisture corn was replaced with dried molasses at 4 or 8% of diet DM, DMI increased ($P = 0.04$) (Table 2). The magnitude of the increase in DMI was 2.4 pounds. At least some of the nutrients from the increased DMI were used for fat synthesis because 3.5% FCM was increased when diets contained 4 or 8% dried molasses. The magnitude of the increase in 3.5% FCM was 4.4 pounds. Fat yield (lb/day) was increased when diets contained 4 or 8% dried molasses but not at 12% dried molasses. Rumen ammonia concentration was decreased when dried molasses replaced high moisture corn ($P = 0.05$). The magnitude of the decrease was 1.4 units (11.3 vs. 9.9 mM).

Based on this trial, dry molasses should not exceed 8% of diet DM. The amount of forage in the diet may influence the amount of sugar or molasses that can be used in the diet. Broderick and Radloff (2003) fed diets to high producing dairy cows that contained 50% forage on a dry matter basis (Table 2). The forage component of the diet was 60% alfalfa silage and 40% corn silage. They replaced high moisture corn with liquid molasses. Diets contained 0, 3, 6, or 9% liquid molasses. Dry matter intake and milk yield were maximized when the diet contained 3% liquid molasses on a dry matter basis ($P < 0.01$). Some of the additional energy derived from the additional DMI appears to be used for fat synthesis because 3.5% FCM was increased 4 pounds compared to the control diet. Yield of all milk components was maximized when the diet contained 3% liquid molasses. Based on the reported dry matter intake, the amount of liquid molasses in the diet was 1.75 – 1.84 pounds on a dry matter basis. This would be equivalent to 2.33 – 2.45 pounds of liquid molasses on an as fed basis. The amount of sugar added to the diet from the molasses would be 1.2 pounds on an as fed basis.

Molasses was compared to molasses and animal fat (Oldick et. al. 1997). The treatments in this trial were control without molasses, molasses only, molasses and animal fat at 2, 4 and 6 pounds of ration dry matter. The molasses and fat liquid supplements were included in the diets at 2.5, 4.9 and 7.4% of the diet dry matter. The molasses only diet contained molasses at 3.4% of diet dry matter. All treatments had similar energy density. Cows on the control diet had an average milk production of 71.6 pounds. Milk response to the molasses only treatment was 2.9 pounds greater than the control diet. Molasses did not increase dry matter or net energy intake but did increase milk yield. There are two possible explanations for the occurrence. One possibility is that the energy from molasses was used with greater efficiency for growth by rumen bacteria than the energy from other dietary carbohydrates. A second possibility is the presence of an associative effect. Adding molasses to the diet may improve the ruminal digestion of NDF. This hypothesis is supported by recent observations from Varga and coworkers (2001). They reported that when starch was replaced with sucrose, NDF digestibility was increased. At the greatest concentration of sucrose, 7.5% of diet DM; NDF digestibility was increased 8.5% compared to the control diet, which did not contain supplemental sucrose.

Growth Rate Dependent Shifts in Fermentation Products Can Explain the Variable Response to Sugar Addition in Dairy Diets

In the trials conducted by Broderick and Smith (2001) and Broderick and Radloff (2003), the response to sugar additions to the diet was not linear (Table 2). The response was quadratic because positive responses were reported at low inclusion levels of sugar addition and negative responses were reported at high inclusion levels. One explanation for the quadratic response to sugar addition is that some ruminal bacteria change their fermentation products based on their growth rate. When the rate of ruminal fermentation is rapid and starches and sugars are readily available in the rumen, *Strep. bovis* and *Selenomonas ruminantium* shift their fermentation from acetate, propionate and formate to lactate (Russell 1998, Russell 2002 pg.71-72). Both *Strep bovis* and *S. ruminantium* can grow very rapidly in the rumen. It is likely that at the higher levels of molasses, these bacteria shifted their fermentation to lactate with a reduction in acetate and propionate production. The shift to lactate fermentation is also influenced by the supply of amino acids in the rumen. When amino acid nitrogen availability is low, these organisms will use ammonia nitrogen as a nitrogen source. When they use ammonia nitrogen as a nitrogen source, the shift to lactate fermentation occurs at a slower growth rate (Russell, 1998). To prevent a shift to lactate production, sugars need to be added to dairy diets in moderate amounts and in combination with protein sources such as soybean meal and canola meal.

When feeding trials have been conducted, it has been assumed that all sugar sources would support the same amount of microbial growth and have similar fermentation rates. We now know that this is not a correct assumption. Bond and coworkers (Bond et. al. 1998) reported that *Streptococcus bovis* cannot utilize pentose (5-carbon sugars) and the growth rate of *Strep. bovis* is 40% slower on lactose than on glucose (Figure 2). *Ruminococcus albus* and *Ruminococcus flavefaciens* are the major

species of cellulolytic cocci in the rumen. These cellulose fermenting cocci do not grow on pentose, growth on glucose is slow but they will grow well on cellobiose (Russell, 2002, pg. 19). Cellobiose is a disaccharide made up of glucose units with a beta 1-4 linkage. *Ruminobacter amylophilus*, a starch digesting rumen bacteria will ferment maltose but not glucose (Russell 2002, pg. 21). It appears that certain sugars will stimulate the growth of specific rumen bacteria and that some sugars will not support the growth of major ruminal bacteria species.

All sugars are not equal when it comes to supporting microbial growth in the rumen (Van Kessell and Russell 1995). Strobell and Russell (1986) examined the effect of pH and carbohydrate source on yield of microbial protein from in vitro fermentation. They reported that the yield of microbial protein declined as pH was reduced from 6.7 to 6.0. There was an interaction between pH and carbohydrate source. When pH of the fermentation was 6.0, the yield of microbial protein was lowest on pectin and xylan compared to cellobiose, sucrose, starch or a mixture of carbohydrate sources. When the pH of the fermentation was maintained at 6.7, the yield of microbial protein was greatest on cellobiose, sucrose or a mixture of carbohydrate sources, and intermediate on starch or pectin and least on xylan. This trial suggests that 5-carbon sugars (xylan) will support less microbial growth in the rumen compared to 6-carbon sugars. McCormick and coworkers (2001) reported differences in fermentation between cornstarch, lactose and sucrose. Their diets contained 50% forage and 50% concentrate. They replaced ground corn with either lactose or sucrose at 2.5 and 5.0% of diet DM. Total organic acid production and fermentation pH was not different for any of the diets. Ammonia N concentration in mg/dl was lower on the sucrose supplemented diets compared to the other diets ($P = 0.06$). This would suggest that the rate of fermentation was faster on the sucrose supplemented diets compared to ground corn or lactose diets. The rate of protein fermentation would have been rapid on all diets because the major rumen degradable protein source in these diets was freeze-dried fresh ryegrass. In this study, treatment differences between ground corn and lactose were not significant for the parameters reported.

Impact of Sugar and Molasses on Ruminal pH and Fiber Digestion

If neutral detergent soluble carbohydrates (NDSC) differ in their rate and pattern of fermentation, we can indirectly measure these differences by measuring ruminal pH and volatile fatty acid production. The impact of NDSC on ruminal pH will depend on the amount of NDSC in the diet and the type of forage. When molasses or sucrose were fed at amounts greater than 12% of diet dry matter, rumen pH was depressed within one hour after feeding (Moloney et al. 1994, Khalili and Huhtanen 1991a). The reduction in ruminal pH lasted for up to four hours after feeding. If sodium bicarbonate was fed in the diet along with sucrose, the depression in ruminal pH was prevented (Khalili and Huhtanen 1991a). When molasses-based liquid supplements or dry sugar are used in dairy rations and fed at amounts less than 8% of diet dry matter rumen pH was not depressed compared to the control diet (Table 3; Piwonka and Firkins 1993, Maiga et al. 1995, McCormick et al. 2001, Varga et al. 2001).

The effect of molasses and sugar on fiber digestibility will depend on the composition of the ration and the level of molasses or sugar in the ration. When molasses is used at 12% or greater of diet dry matter, it will decrease dry matter and fiber digestibility (Khalili and Huhtanen 1991b, Moloney et al. 1994, Petit and Veira 1994). When used at less than 8% of diet dry matter, in dairy and beef diets, molasses-based liquid supplements or sugar did not depress fiber digestion compared to control diets (Piwonka and Firkins 1993, Oldick et al. 1997, Varga et al. 2001). These results support the earlier work of Foreman and Herman (1953). They observed that feeding molasses at rates of one or two pounds of dry matter did not decrease cellulose digestibility compared to diets without molasses. The effect of sugar or molasses on fiber digestion will depend on the effective fiber level in the ration, ration particle size and forage form (hay or silage). In dairy rations, which are formulated to meet or exceed the fiber requirements of dairy cows, molasses or sugar should not depress fiber digestion when used at less than 8% of the diet dry matter.

Since 1987, there have been several trials, which have examined the effect of sugar or molasses on microbial protein production in the rumen (Table 4). In all trials, feeding sugar or molasses increased the supply of microbial protein compared to the control treatment (Khalili and Huhtanen 1991a, Huhtanen 1988, Piwonka and Firkins 1993, Rooke and Armstrong 1989).

The increase in microbial protein was greatest when the molasses or sugar was fed in combination with casein, soybean meal or sodium bicarbonate. This is expected because casein and soybean meal would provide amino acids and peptides for the rumen bacteria and increase microbial growth rate. Sodium bicarbonate would increase liquid turnover rate in the rumen and would increase the microbial growth rate. Supplementation of grass silage-based diets with a source of readily available carbohydrate (sugar) has been found to increase the flow of microbial protein and non-ammonia nitrogen to the small intestine (Chamberlain et al. 1985, Huhtanen 1987, Rooke et al. 1987).

Non-ammonia nitrogen (NAN) includes microbial protein and natural protein. It is a measure of the total natural protein reaching the small intestine. In these three trials feed intake was restricted and sugar infused directly into the rumen. The increase in microbial protein production when sugar was infused is not surprising. The grass silage fed in these trials contained significant amounts of rumen degradable protein. The fermentation of this silage in the rumen would lead to elevated concentrations of rumen ammonia. In order for the rumen bacteria to capture this ammonia, they needed a supply of rapidly fermentable carbohydrate. The sugar infused into the rumen supplied the rapidly fermentable carbohydrate and stimulated microbial growth. This increased the microbial protein flow to the small intestine. Direct evidence for increased capture of ruminal ammonia by rumen bacteria was observed in all three trials because ruminal ammonia concentration was decreased when sugar supplements were included in the diet. The amount of non-ammonia nitrogen reaching the small intestine was increased when molasses or sugar replaced starch in the diet. Unfortunately dairy producers do not get paid based on the amount of microbial protein their cows produce each day.

Does an increase in the supply of microbial protein or non-ammonia nitrogen translate into an increase in animal performance?

Summary

Molasses-based liquid supplements and sugar are readily digestible sources of energy for dairy cattle. When added to dairy rations at 6 to 8% of the total ration dry matter, molasses-based liquid supplements and sugar may increase dry matter intake, fat-corrected milk yield, milk protein and milk fat yield and NDF digestibility. The mode of action appears to be through enhancing NDF digestibility, altering the ruminal microbial population and possibly providing an increased supply of nutrients for fat synthesis. Sugar or molasses, when fed at less than 8% of diet dry matter, can be used with the same efficiency as corn for milk production. Physical factors of the ration can influence responses to molasses or sugar. In rations with less than 19% ADF, and small particle size, use of sugar and molasses based liquid supplements may not increase feed intake and milk production. Response to liquid supplements and sugar has been greater when the ration contains adequate amounts of rumen degradable amino acids and peptides. Research trials published since 1983 suggest that molasses and sugar do more than just increase ration palatability, they can play a greater role in dairy rations by altering ruminal microbial populations and possibly increasing microbial growth in the rumen of dairy cattle.

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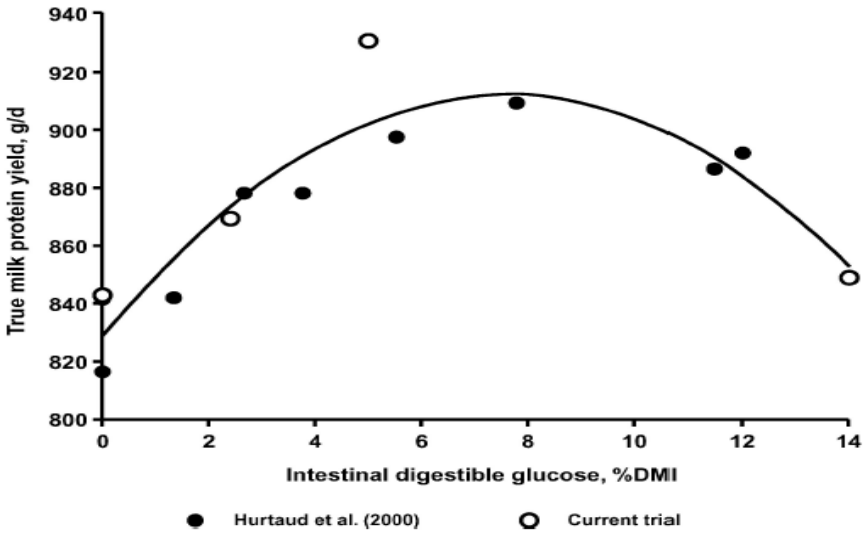

Figure. 1. Adapted from Rulquin et al. (2004).

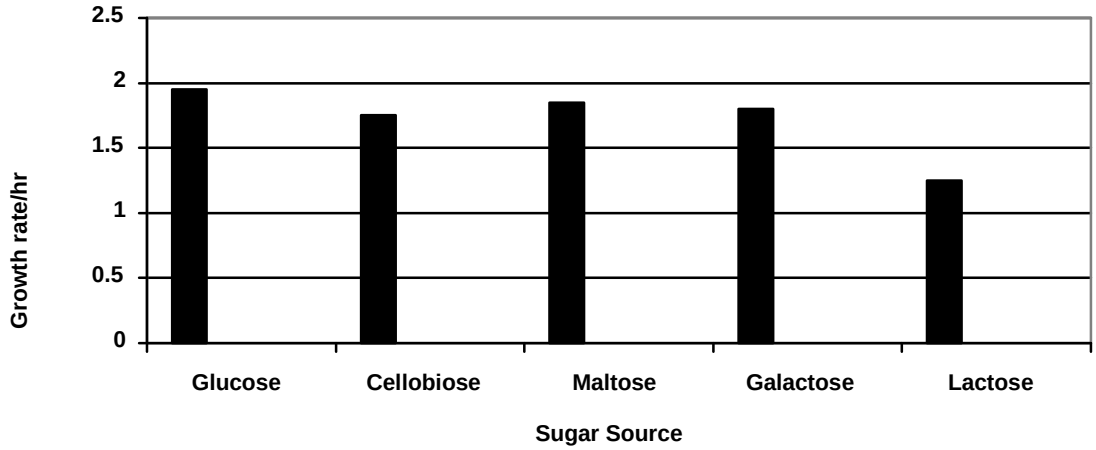

Figure 2. Growth rate of *Streptococcus bovis* on different sugars.

Table 1. Rumen ecology

Microbe	Primary substrate	Optimum rumen pH	Primary requirement	Main fermentation products	Doubling time
Bacteria	About 630 different bacteria (50% of microbial mass)				
Fiber and pectin	Fiber and pectin	6.3 to 6.8	NH_3 , isoacids	Acetate	8 – 10 h
Protein *C. aminophilum*	Protein	6 to 7	Protein, peptides, NH_3	NH_3, Isoacids	4 – 8 h
Allisonella histaminiformans	Histidine	4.5 to 6.5	Histidine, peptides from silage	Histamine	Rapid
Starch, *S. Bovis*	Starch and sugars	5.5 to 6.5	Peptides, AA, NH_3	Propionic, Lactic	15m –30m
Secondary - *M. elsdenii*, Methanogens	Lactic, H_2	6 to 6.8	Peptides, AA, malic	Propionic, CH_4	2 – 4 h
Protozoa	About 30 different protozoa (40 to 45% microbial mass)				
	Starch, sugars	6.3 to 7.0	Peptides, AA, Bacteria	Propionic, H_2	15 – 24 h
Fungi	About 14-15 types of fungi (3 – 8% microbial mass)				
	Fiber	6 to 7	NH_3, AA, sugars	lactic, acetic, H_2,	15 - 24h
Bacterial viruses (5 –7 types and .0000001% TMM); Yeasts (0.1 to 0.2% TMM)					

TMM = total microbial mass.

Table 2. Effect of sugar or molasses on lactating cow performance

Experiment	Forage source	Treatments	Dry matter Intake, lb/d	3.5% FCM yield, lb/d	Treatment effects
Broderick and Radloff (2003)	Alfalfa silage and corn silage, 50% forage diet	HM corn	56.4	97.6	Significant quadratic effects
		Liquid molasses			
		3% diet DM	61.5	100.2	
		6% diet DM	58.2	98.2	
		9% diet DM	57.7	93.0	
Broderick and Smith (2001)	Alfalfa silage and corn silage, 60% forage diet	HM corn	55.3	91.2	Significant quadratic effects
		Dry molasses			
		4% diet DM	56.8	92.5	
		8% diet DM	57.7	95.6	
		12% diet DM	57.3	87.0	
McCormick et al. (2001)	Chopped ryegrass, 50% forage diet	Ground corn	50.2	84.4	No effect on milk yield or DMI
		Sucrose			
		5% diet DM	50.3	83.5	
Oldick et al. (1997)	Corn silage, alfalfa haylage	Ground Corn	47.4	72.0	No effect on DMI
		Molasses	46.5	74.5	Milk yield increased ($P < 0.05$)
		Molasses + Fat	49.2	78.5	
Maiga et al. (1995)	Corn silage and alfalfa hay	Corn	51.0	70.3	Sugar supplements with fat equal to corn + fat
		Molasses + Fat	54.0	74.2	
		Dry Whey + Fat	54.0	74.9	
		Corn + Fat	53.5	74.2	

HM = high moisture.

Table 3. Effect of Sugar and Molasses on Rumen pH When Fed at Less than 8% of Diet Dry Matter

Experiment	Forage Source	Treatments	Rumen pH	Treatment Effects
McCormick 2001 In Vitro Trial	Freeze-dried ryegrass	Ground Corn Lactose Sucrose	6.77 – 6.78	No effect of carbohydrate source
Varga 2001 In Vitro Trial	Alfalfa Silage Corn Silage 2:1 ratio	Starch Starch + Sucrose Sucrose	5.97	No effect of carbohydrate source
McCormick 2001 In Vivo Trial	Chopped Ryegrass	Ground Corn Sucrose	6.19 – 6.21	No effect of carbohydrate source
Maiga 1995 In Vivo trial	Corn Silage Alfalfa Hay	Corn Corn + Molasses Corn + Whey	6.68 – 6.85	No effect of carbohydrate source
Piwonka 1993 In Vivo Trial	Corn Silage Orchardgrass Hay	Barley Barley + Dextrose	6.47	No effect of carbohydrate source
Chamberlain 1985 In Vivo Trial	Grass Silage	Barley Barley + Molasses Beet Pulp Beet Pulp + Molasses	6.33 6.21 6.40 6.45	Within carbohydrate source, Barley or Beet Pulp, pH was not different

Table 4. Effect of Molasses or Sugar on Microbial Protein Production

Experiment	Treatments	Animal	Microbial N g/d	Treatment effects
Rooke and Armstrong 1989	Sucrose Sucrose + Urea Sucrose + Casein Sucrose + Soybean meal	Non-lactating cows	105 108 126 112	Sugar effect significant when fed with casein or soybean meal
Piwonka 1993	Barley Barley , 4.4% of diet DM + Dextrose, 5.6% of diet DM	Holstein heifers	64 74	Sugar effect is significant, microbial N increased 15.6%
Khalili 1991a	Barley Barley + Sucrose Barley + Sucrose + Buffer	Dairy steers	72 90 94	Sugar effect is significant, microbial N increased 25% - 30%
Huhtanen 1988	Barley Barley + Molasses Beet Pulp Beet Pulp + Molasses	Dairy steers	71 74 60 75	No effect with barley diets Effect is significant with beet pulp diets
Hall and Herejk 2001 In Vitro Trial	Bermuda grass (BG) NDF BG NDF + Pectin BG NDF + Sucrose BG NDF + Starch	Rumen microbes	0.014 0.030 0.026 0.034	Sucrose = Pectin Starch effect significant compared to Sucrose

SESSION NOTES

Opportunities to Improve Feed Efficiency of Beef Production

Monty Kerley[1]
Division of Animal Sciences
University of Missouri

Introduction

Beef cow-calf efficiency has been a topic of several review and symposium papers. A primary focus of many discussions on beef cowherd efficiency has centered on reproductive success and calf yield per cow, or more simplistically calf weight divided by cow weight. Reproduction and weaning weight are important but research has also demonstrated potential benefit from selecting for metabolic efficiency and strategic supplementation to enhance production efficiency. Efficiency is improved when reduction of inputs is achieved without detriment to product value and/or when product value is enhanced without increased input costs. In cow-calf production maintenance cost of the cow is the primary expense and most probable means of reducing input costs. Since reproduction and calf weight determines value methods, to enhance either can benefit cowherd productivity. Potential of selection for residual feed intake to reduce cow maintenance costs and nutritional strategies to enhance reproductive development and conception to improve production efficiency as well as calf growth efficiency will be discussed.

Nutritional Strategies to Improve Efficiency

Effective Energy

Energy requirement of beef cattle is typically based upon net energy (**NE**) requirements for maintenance and growth functions. Even when total digestible nutrients (**TDN**) or metabolizable energy values are used instead of NE, the same base assumptions are made regarding energy requirement for maintenance and growth. Emmans (1994) developed an alternative method of assessing energy requirement (Effective Energy, **EE**) in cattle that has been found to more accurately predict energy requirement. Primary difference between NE and EE was the argument that NE assigned a single energy requirement value to protein and lipid accretion when energetic expenditure for accretion of these tissues was not the same. Emmans (1994) separated energy requirements into separate functions of protein and lipid retention. Effective energy requirement is a function of maintenance energy requirement (**MH**), energy required for protein retention (**PR**) and lipid retention (**LR**) and energy lost as methane (EE = MH + PR + LR + MTHE). Research conducted at University of Missouri has shown that EE estimates of energy requirement were more accurate in predicting intake than NE estimates, with NE typically over predicting energy requirement by 20% or greater. Accurate prediction of energy requirement is important if diets are to be

[1] Contact at: Division of Animal Sciences, University of Missouri, 111 Animal Sciences Ctr., Columbia, MO 65211-0001. E-mail: kerleym@missouri.edu.

balanced that allow an animal to maximize feed efficiency. Research with other species has shown that intake of diets inadequate in supply of amino acids will result in satiety signals being controlled by amino acid intake rather than energy intake. In other words, the animal will consume a diet above its energy requirement to meet its most limiting amino acid requirement. I propose this routinely occurs in most ruminant diets, whether based on forage or grain, and that feed efficiency cannot be maximized until all required nutrients are balanced relative to energy density of the diet.

Cattle Do Not Have a Protein Requirement

Beef cattle may well be the only significant food production species left that balances diets for crude protein requirement, or more recently metabolizable protein requirement. The error with this approach is that cattle do not have a protein or metabolizable protein requirement but rather have a requirement for amino acids. Rumen bacteria have a requirement for ammonia (**RDN**) and amylolytic rumen bacteria have a requirement for peptide/amino acid nitrogen (**RDP**). Non-protein nitrogen, such as urea, can supply RDN and protein fermented in the rumen can supply RDP and RDN. Because research has yet to show microbial requirement of essential amino acids to meet RDP requirements, an argument could be made for a general protein requirement by the rumen, but cattle are similar to swine and poultry in having a requirement for essential amino acids to meet maintenance and growth functions. Balancing diets to meet amino acid requirements is more difficult for cattle than other species due to microbial fermentation of protein in the rumen. Meng et al. (1999) developed equations to predict microbial amino acid flow postruminally based upon mass of substrate fermented and rate of passage from the rumen. These same equations can be used to estimate RDN and RDP requirements of rumen microbes. From these estimates diets can be balanced to meet ammonia and peptide/amino acid requirements of rumen microbes and predict amino acid flow postruminally.

Amino Acid to Effective Energy Ratio

Maximum feed efficiency in cattle will occur when amino acids are supplied to meet amino acid requirement for maintenance and growth. Amino acid requirement will be dependent upon energy intake. As energy intake increases potential for growth or milk production increases and demand for amino acids subsequently increases as well. Our research has shown that balancing absorbable amino acid supply to energy intake can enhance reproductive development of heifers and two-year olds. Outcomes of balancing amino acid to EE (AA:EE) is improved reproduction, increased feed efficiency of replacement heifers and potential for reduced age at first calving.

Forage protein is extensively fermented in the rumen with little rumen undegradable protein. Therefore, the majority of protein flowing post ruminal is microbial origin. Methionine is the first-limiting amino acid in microbial protein for growing cattle. Our research has led us to conclude that most forages are limiting in methionine rather than energy. Because methionine is limiting, protein growth (lean) potential is limited.

This led us to hypothesize that rumen escape methionine supplementation could enhance growth and development of heifers and two-year old cows.

Post ruminal supply of methionine to developing heifers was shown to improve reproductive development. Hersom et al. (2009) compared corn gluten meal and Alimet [methionine hydroxy analogue; DL-2-hydroxy-(4-methylthio) butanoic acid] or Alimet alone on developing heifer growth, efficiency and reproductive development. Table 1 presents results from feeding Alimet to heifers. Heifers were given 0, 7 or 15 grams of methionine per day via supplement (2.5 Kg soy hull based) to an *ad libitum* hay diet. Experiment was conducted over 85 days. Supplementing methionine increased daily gain linearly over the first 30 days and increased reproductive tract score on day 85. Supplementation increased feed efficiency the first 30 days of study and increased feed efficiency numerically at day 85. The most important aspect of developing heifers is to have a reproductively mature tract at breeding season. This research was designed to test if heifers would respond to amino acid supplementation when receiving a diet promoting acceptable weight gain. Data were interpreted to demonstrate that methionine supplementation did improve reproductive development. Data were also used to support hypothesis that early stage development is important in achieving reproductive maturity. Primary difference due to methionine supplementation was measured first 30 days of study, yet significant difference was measured in reproductive tract score at day 85 with heifers fed 15 grams of Alimet having a more mature reproductive tract score. Supplying 15 grams of Alimet would yield approximately 7 grams of methionine post ruminal. When estimating amino acid to energy requirement for these heifers the control diet was deficient approximately 7 grams of methionine, the first limiting amino acid.

A similar strategy was evaluated using two-year old lactating cows. It was hypothesized that requirements of these cows due to milk production and growth could create a deficiency for amino acids. To test this hypothesis, two-year old lactating cows were given pasture only or fed corn-based supplement (approximately 1 Kg per head per day) with 100 or 200 grams per head per day of blood meal. Similar to the heifer study, methionine was predicted to be first limiting amino acid. When cows were fed a supplement with 100 grams of blood meal, hay intake increased numerically and daily gain of cows decreased, whereas milk intake and daily gain of calves increase (Table 2). When 200 grams of blood meal was provided, then daily gain of cows increased, milk intake and daily gain by calves were similar to those of calves nursing cows with no supplement. Cows used in this study averaged 74 days postpartum. Data were interpreted that supplementing young cows with energy alone (deficient in limiting amino acid) resulted in increased milk production at the expense of cow weight gain. Since this animal is still growing to reach mature size, weight loss as measured in this study could hypothetically influence reproduction. The difference between inadequate and adequate amino acid supply at similar energy supplementation was 0.1 Kg weight loss per day and 0.3 Kg weight gain per day. Over a 90 day period this would equate to a body condition score change of 1 to 1.5. Similar to results measured in developing heifers, diets that balance amino acid to energy ratio can potentially have benefits in two-year old cows. This is important since it typically is not the worst heifers that fail to rebreed.

Nutrition management is important for this stage of production not only to enhance probability of conception for the next year but also because successful rebreeding of two-year olds generally result in cows being reproductively sound beyond seven years.

Maximizing Calf Feed Efficiency

Formulating diets and supplements to balance amino acid to energy ratio for calves has been shown to improve growth performance and positively impact feedlot performance and carcass value. Studies were conducted with weaned steers where moderate quality hay was fed *ad libitum* and supplement fed at 0.75 or 1.5% of body weight. Supplement consisted of either dried distillers grains (**DDG**) or DDG, soyhulls, rumen-protected soybean meal, blood meal and corn oil (**BAL**). The BAL supplement was formulated to balance amino acid to energy ratio. Hay offered to steers was tall fescue and moderate in quality (10% crude protein, 62.8% neutral detergent fiber). When steers were fed supplement at 0.75% of body weight it was predicted that post ruminal amino acid supply was adequate with DDG supplementation to meet requirement, and data supported this prediction with daily gain and feed efficiency being 2.6 and 7.1 for both treatments (Table 3). However, when supplementation was increased to 1.5 % of body weight it was predicted that amino acid supply would not meet requirement for growth potential which data supported. When steers were fed a supplement formulated to balance amino acid to energy ratio, daily gain was increased 0.2 Kg per day compared to DDG supplement and feed efficiency was improved by 0.4 Kg less feed per Lb of gain. Using a live weight gain value of $1.60 per Lb, feeding a balanced supplement at 1.5% of body weight allowed supplement cost to be increased $275 per ton compared to supplement fed at 0.75% and $151 per ton more than DDG. In these calves supplemental feed to gain was 5.6:1.

A similar study approach was conducted with calves (Table 4). Calves were weaned in early April and continuously grazed, rotationally grazed or fed 1.2 Kg of supplement balanced for predicted amino acid requirement, and compared to growth performance of calves maintained with cows through July. Supplement consisted of corn, DDG, blood meal, feather meal and **MFP** (flavored methionine hydroxy analogue; DL-2-hydroxy-(4-methylthio) butanoic acid). Calves provided with supplement had similar weights and gains as calves nursing their dams, with calves not supplemented having lower body weights and daily gains as would be expected. The primary goal of this research was to demonstrate potential of supplement to mimic performance of nursing calves. A second goal and more to the point of this paper is performance potential for balanced supplements. Compared to gains by rotationally grazed calves and continuously grazed calves, supplement feed efficiency was 3.4 and 2.7 Lbs of feed per Lb of gain. Formulating supplements to balance amino acid supply to energy potential for growth can improve calf feed efficiency.

Effect of Starch on Forage Digestion

Fiber fermentation is impeded by starch fermentation, an effect regarded as negative associative effects. It is believed that starch fermentation reduces pH below a

critical point conducive to fiber-fermenting bacteria, resulting in reduced fiber (forage) digestion. Grigsby et al. (1993) demonstrated that negative associative effects occurred when corn exceeded 0.6% of body weight in growing steers fed hay however positive associative effects occurred when corn was fed at 0.2 to 0.4% of body weight (Table 5). Calves fed corn at 0.2% of body weight had increased fiber digestion and calves fed corn at 0.2 and 0.4% of body weight had increased microbial protein flow post ruminal compared to calves receiving no corn and soyhulls only. When calves were fed corn at 0.6% of body weight fiber digestion was reduced compared to no corn supplement and microbial protein flow post-ruminally was lower than for calves fed 0.4% of body weight corn. This research has been used to base corn inclusion level in supplements for forage-fed cattle.

Supplementing Lipid to Increase First-Service Conception Rates in Mature Cows

Lipid supplementation (linoleic and linolenic acids) was shown to improve reproductive measurements in cattle (sperm quality, egg quality and embryo transfer success). Response was hypothesized to occur from provision of essential fatty acids influencing prostaglandin metabolism. Mature cows were fed whole soybeans (1.6 Kg per head per day) 30 days prior to calving until approximately 15 days post-calving or at calving for 45 days post-calving (Table 6). These time points were used to coincide with estimated timing of egg development for breeding season initiation 60 days post-calving. A control treatment of soyhulls and soybean meal similar in energy and amino acid content was also fed. Cows supplemented soybeans 30 days prior to calving had a 76% first-service conception rate compared to control treatment (62%) or to cows supplemented soybeans at calving (60%). Overall conception rate was not different among treatments, with 93% of cows supplemented 30 days prior to calving conceiving and 86 to 87% of cows fed other treatments conceiving in a 60 day breeding season. Interpreted from these data was that essential fatty acid supplementation could increase first-service conception rate, with an increase of 22% in this study. Subsequent on-farm studies showed similar results with other vegetable oil sources. While overall conception rate was not statistically increased, a 22% increase in first-service conception rate could increase weaning weight approximately 10 Lbs averaged across a herd. Lipid supplementation provides an additional means of potentially improving reproductive performance.

Summary of Nutritional Strategies to Improve Reproductive Efficiency

Nutritional management of developing and first-calf heifers should be to support their growth and development and, for first-calf heifers, to support demands for milk production. The argument made in this presentation is to ensure that supplements are formulated to adequately supply amino acid requirements based on energy intake by the animal. Likewise, the argument for diets/supplements to be formulated for nursing and weaned (preconditioning or stocker programs) calves to balance amino acid supply with requirement based on energy intake is made. If amino acids are not supplied at levels required then efficiency will be compromised. The best check for producers to use would be an assessment of feed to gain ratio. If creep feed or supplements do not

yield feed to gains less than 5:1 it would be potentially beneficial to challenge diet/supplement formulation. Research to date has demonstrated that energy requirement is best described by EE and that maximum feed efficiency cannot be attained if amino acid supply is not balanced with EE intake.

Potential to Select for Metabolic Efficiency

Residual feed intake (**RFI**) is a measure of efficiency referenced by Koch et al. (1963) who attributed this measure as a means of selecting for metabolic efficiency. An attribute assigned to RFI is that it identifies efficiency independent of growth. Consequently selection for this trait should allow greater feed efficiency to be achieved in cattle without concomitant selection for increased frame size. Research conducted to date has proven this to be true. A majority of research conducted in the US has focused on impact RFI selection has on feedlot performance, which has proven to be potentially substantial by improving feed efficiency up to 20%. Because RFI is a measure of cellular energy metabolism efficiency its impact on the cow herd should be equally substantial.

Research was conducted comparing correlation between RFI phenotype of cows measured as a heifer and as a mature cow (Minton, 2010). In this study correlation of heifer RFI to cow RFI was only 0.17, however correlation of RFI category (low, average or high) between heifer and cow was significant at 0.31. Dry matter intake measured as a heifer and as a cow was significantly correlated (0.42). Since RFI is a function of dry matter intake correlation of intake between heifer and cow time periods would be expected and is interpreted that heifer RFI has relevance to cow efficiency. When cow RFI and performance was averaged within heifer RFI category (Table 7) cow RFI and dry matter intake followed heifer phenotype trends with no difference in average daily gain. Heifers that were phenotyped as low, average and high RFI had an average RFI value as a cow of -1.1, -0.2 and 1.5, respectively. Dry matter intake by cows increased as heifer RFI category increased, with cows consuming 2.4, 2.5 and 2.7% of body weight in dry matter, respectively. Meyer et al. (2007) phenotyped cows for RFI and then measured forage intake on pasture by low and high RFI phenotypes (Table 8). Low RFI cows consumed 20% less forage than high RFI cows. In this study, reduced intake by low RFI cows did not affect weight change or calf daily gain. Using 20% reduced forage intake could lead one to interpret that selection for RFI would allow every sixth animal to graze for free. While a conclusion that carrying capacity could be increased would be logical, practical outcome from selection for feed efficiency is more likely to be better body condition score and reproductive performance when forage/feed resources are limiting.

Preliminary research has shown milk production differs among RFI phenotypes. Two experiments were conducted measuring milk production of cows categorized by heifer RFI phenotype. When lactating cows were grouped by heifer RFI category there was no difference in dry matter intake among RFI category but body weight change was less for low RFI than other groups, milk production decreased numerically as RFI increased and milk production efficiency improved as heifer RFI improved (Table 9).

The second experiment was conducted with grazing dairy cattle and showed a similar trend (Table 10).

Summary of RFI Selection Potential to Increase Cow Herd Efficiency

Cow maintenance energy requirement is the greatest expense in a beef herd. Intake will vary by 40% between the most and least efficient animal in a herd, suggesting that potential for improvement is substantial. Selection for RFI can improve cow efficiency resulting in reduced forage needs by as much as 20%. Selection for efficiency in the cow herd also results in progeny that are more efficient. Using RFI as a selection trait is still relatively new in the beef industry; however, impact has promise of being substantial. A commercial seedstock producer has reduced average intake 15% compared to population average intake and low RFI animals consume 32% less feed than population average. Research to date leads to the conclusion that potential to improve cow herd efficiency via RFI selection is great.

Conclusion

This paper was written with the intent to provide a brief overview of concepts that will be discussed in the presentation. Goal of presentation will be to identify specific steps that can be taken through nutritional management and selection to improve production efficiency and animal efficiency. Nutritional management discussion will include approaches to balance amino acid supply with energy supply, potential to enhance reproductive development and mature cow conception and diet/supplement formulation that maximizes calf growth efficiency. Selection discussion will include potential of RFI phenotyping to improve cow efficiency.

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Table 1. Effect of Alimet[1] supplementation on growth and reproductive tract development of beef heifers

	Supplement (g Alimet/head/d)		
	0	7.5	15
ADG d 0 to 30, Kg/d	0.8[c]	1.0[a,b]	1.2[a]
Total ADG d 0 to 85, Kg/d	0.8	0.8	0.9
Total gain in 85 d, Kg/d	47	49	53
Begin weight, Kg	374	369	374
End weight, Kg	421	418	426
Pelvic area	184	186	183
Reproductive tract score	4.2	4.2	4.5
Hay disappearance d 0 to 30, Kg/d	12.1	11.6	11.3
Hay disappearance d 0 to 85, Kg/d	12.5	12.7	12.8
Feed to gain, d 0 to 30	16.6	12.3	9.5
Feed to gain, d 0 to 85	16.7	16.5	15.1

[a,b,c] Means with unlike superscripts differ (P < 0.08).

[1] Methionine hydroxy analogue [DL-2-hydroxy-(4-methylthio) butanoic acid].

Table 2. Effects of supplemental energy source and level of undegradable intake protein on cow-calf weight changes, calf milk consumption and cow forage and total organic matter (OM) intake while grazing endophyte-infected (E+) tall fescue pasture

	Pasture only	Pasture and Supplement[a]				Effect[b]			
		Soybean hulls		Cracked corn					
		100	200	100	200	E	P	I	S
						———Pr > F[c]———			
Cows									
n	6	6	6	6	5	—	—	—	—
Initial weight, Kg	461	472	456	487	420	—	—	—	—
Gain, Kg	0.6	4.0	-5.7	-5.2	18.9	NS	NS	0.02	NS
ADG, Kg/d	0.01	0.07	-0.1	-0.1	0.33	NS	NS	0.02	NS
Calves									
N	6	6	6	6	5	—	—	—	—
Initial weight, Kg	89	99	102	105	94	—	—	—	—
Gain, Kg	32	28	35	38	29	NS	0.08	NS	NS
ADG, Kg/d	0.55	0.67	0.51	0.67	0.51	NS	0.08	NS	NS
Milk intake, Kg/d[d]	2.1	4.5	3.5	4.1	2.5	NS	0.07	NS	0.06
Cow intake, g of OM/Kg body weight									
N	6	6	6	6	5	—	—	—	—
Forage	16.0	17.7	15.2	14.8	15.9	NS	NS	NS	NS
Total	16.0	20.0	17.9	16.9	18.3	NS	NS	NS	NS

[a] Supplements consisted of cracked corn or soybean hulls as an energy source with either 100 g/d or 200 g/d of ruminally undegraded intake protein.
[b] Contrasts: E = cracked corn vs soybean hulls; P = 100 vs 200 g/d of UIP; I = interaction between source of energy by level of UIP; S =E+ vs average of supplement treatments.
[c] Only those contrasts for which P < 0.20 are shown. NS = not statistically significant
[d] Milk intake determined on July 16.

Table 3. Growth performance of stocker calves fed different levels of supplement balanced or unbalanced for amino acid to energy ratio

	0.75% body weight		1.5% body weight	
	DDG[1]	BAL[1]	DDG	BAL
Initial weight, Kg	272	273	273	271
End weight, Kg	382[c]	386[b,c]	395[b]	411[a]
ADG, Kg	1.18[c]	1.22[b,c]	1.32[b]	1.50[a]
Feed:Gain	7.1[a]	7.1[a]	6.7[a,b]	6.3[b]

[a,b,c] Means with unlike superscripts differ (P<0.05).
[1] DDG = dried distillers grains; BAL = balanced supplement.

Table 4. Potential for supplementation to improve growth performance of nursing calves

	Weaned			
	No supplement		Supplement	Cow-calf pairs
	Continuous	Rotation		
April BW, Kg	206[c]	205[b]	206[a]	205[a]
June BW, Kg	244[b]	255[b]	279[a]	279[a]
July BW, Kg	245	269	303	303
April to June ADG, Kg	1.6[c]	2.2[b]	3.0[a]	3.2[a]
June to July ADG, Kg	0.1[b]	0.7[a]	1.1[a]	1.1[a]

[a,b,c] Means with unlike superscripts differ (P<0.05).

Table 5. Effect of supplemental corn intake on digestion of Bromegrass hay by steers

	Corn (% of body weight)				P value (Q)
	0	0.2	0.4	0.6	
Rumen digestibility					
Dry matter, %	65.7	66.9	58.2	52.2	<0.01
NDF, %	55.8	59.9	53.6	47.3	<0.01
Microbial N flow, g/d	136	156	181	153	<0.01

Table 6. Effect of whole soybeans on reproduction in mature cows

	30 days prior		Calving	
	Soybean	Control	Soybean	Control
Cycling, %	75	56	50	46
First Service, %	76	62	60	56
Pregnant, %	93	86	87	86

Table 7. Performance traits of cows averaged across heifer residual feed intake (RFI) category

	Heifer RFI Category		
	Low RFI	Average RFI	High RFI
RFI	-1.10[b]	-0.22[ab]	1.46[a]
Dry matter intake, Kg	16.4	17.4	17.6
Daily gain, Kg	1.0	1.0	0.9
Initial body weight, Kg	615	632	594

[a,b] Means with unlike superscripts differ (P<0.05).

Table 8. Performance traits of cows grazing pasture phenotyped as low or high residual feed intake (RFI)

	Low RFI	High RFI
RFI	-0.42[b]	5.1[a]
Initial body weight, Kg	569	557
Dry matter intake, Kg	12.4	15.6
Calf daily gain, Kg	0.85	0.95

[a,b] Means with unlike superscripts differ (P<0.05).

Table 9. Milk production of cows grouped by heifer residual feed intake (RFI) category (beef)

	Low RFI	Average RFI	High RFI
RFI	0.1	-0.6	0.9
Intake, Kg	20.6	20.1	20.8
Milk, Kg	11.2	10.4	9.7
Milk efficiency	1.8[c]	1.9[b]	2.1[a]

[a,b,c] Means with unlike superscripts differ (P<0.05).

Table 10. Milk production of cows grouped by heifer residual feed intake (RFI) category (dairy)

	Low RFI	Average RFI	High RFI
RFI	-0.86	0.04	0.84
Intake, Kg	27.2	28.2	27.8
Milk, Kg	16.4[a]	15.7[ab]	14.9[b]

[a,b] Means with unlike superscripts differ (P < 0.05).

SESSION NOTES

Impact of Maternal Nutrition on Calf Performance

Amy Radunz[1]
University of Wisconsin-River Falls

Introduction

Beef cattle production in the United States is currently facing many challenges. The nation's cow herd has continued to decline since its peak in 1975 (NASS USDA, 2010). A major reason for this decline in cow numbers is increased input costs, of which feed represents the greatest portion. Therefore, investigations on how to more efficiently produce beef from conception and how the environment may influence the final phenotype of the animal are critical. The phenotype of the animal is a combination of genetics and environment. While the genes the animal inherits from his/her parents cannot be changed, how the genes are expressed can be influenced by environmental factors, such as nutrition, from the time of conception. Most of the research in beef cattle feedlot nutrition has focused on post-weaning environmental influences on the final phenotype of the calf in regards to growth and carcass traits; however, recent research in the area of developmental programming has started to provide more insight into the impacts of early postnatal period and even gestational environment on lifetime productivity of humans and livestock species. Research has begun to demonstrate that maternal nutrition during gestation may impact progeny postnatal growth, health, feed efficiency, and carcass composition as outlined here.

Developmental Programming

The fetal origins hypothesis was first proposed by Dr. Barker based on epidemiological studies investigating low nutrient intake by pregnant mothers experience during the 1944 Dutch Famine of World War II and the resulting long-term health implication of their children. Dr. Barker reported low caloric intake during gestation induced drastic changes in developmental programming, thereby impacting the future health of offspring observed by an increased incidence of clinical conditions as obesity, insulin resistance, and type 2 diabetes (Barker et al., 2002).

Similarly, in domestic animal studies, maternal nutrition during gestation has been reported to impact postnatal body composition, insulin sensitivity, and growth rate, which all have implications for production efficiency and meat quality (Ford et al., 2007; Radunz et al., 2010 and 2012). Factors such as maternal nutrition, environment, or stressors during gestation can change nutrient supply to the fetus, which can then affect growth and development of organs, skeletal muscle tissue, and adipose tissue. These changes to the development of the fetus can alter postnatal skeletal muscle growth, fat

[1] Contact at: Animal and Food Science Dept., Ag Science Building; Phone: 715-425-3704; E-mail: amy.radunz@uwrf.edu.

deposition, insulin resistance, or hypertension of offspring, which can impact economically important traits such as growth rate, health, and carcass composition. To date, most of the research using animals have been focused on human health implications, but recent studies have investigated beef cow gestation nutrition and management to provide evidence of its implications to offspring productivity.

One of the mechanisms by which developmental programing may be explained is epigenetics. Epigenetics encompasses changes to marks on the genome early in development that are copied from one cell generation to the next, which may alter gene expression, but do not involve changes in primary DNA sequence. Epigenetic mechanisms such as DNA methylation and histone modifications (e.g., acetylation, methylation, and phosphorylation) can change genome activity under some environmental (nutrition or toxicants) conditions (Bollati and Baccarelli, 2010). A greater understanding of how maternal nutrition induces epigenetic modifications to adipose tissue would provide critical information in understanding pathways influencing postnatal adipose deposition.

Developmental windows of muscle and adipose tissues, economically important tissues in meat animal production, occur during gestation, which could influence production efficiency and carcass composition of the individual. Muscle hyperplasia (increasing cell number) starts in early gestation and terminates during mid-gestation. Any impact on muscle growth after this point time is achieved by hypertrophy (increasing cell size). Adipose tissue growth primarily begins during late gestation and adipose tissue hyperplasia can continue until maturity, however at a diminishing rate as the animal becomes older. Previous research in early weaning and high starch diets in beef cattle has already provided evidence of postnatal environmental influences on adiposity. In addition to these tissues, other developmental windows occur throughout gestation for the placenta, specific tissues, and organs. Therefore, not only does the type of environmental stress influence the final phenotype but also at what time in development the stress occurs. For example, nutrient restriction followed by adequate nutrition in early gestation results in larger birth weights in sheep compared to adequate nutrition, whereas nutrient restriction in late gestation results in lower birth weights (Munoz et al., 2008). Collectively, these studies and other research has provided evidence that the developmental windows occur from periconception to early postnatal life of the animal (Fowden et al., 2006).

Post-Weaning Growth Traits

Milk production postpartum can be influenced by prepartum nutrition of the dam, which could have development programming implications. Cows receiving an energy-deficient gestation diet verses a high-energy diet, had lower milk production (Corah et al., 1975). Additionally, cows allowed limited vs. ad libitum grazing access prepartum had a 9% decrease in early lactation milk production (Kearnan and Beal, 1992). Over and under nutrition of ewes resulted in decreased IgG concentration, nutrient content, and volume of colostrum in milk (Swanson et al., 2007). Studies have reported prepartum restriction of nutrients resulted in a decrease in colostral IgG (Shell et al.,

1995) and absorption of IgG by calves. Effective passive transfer of IgG in colostrum is vital to calf health and immunity (Perino et al., 1995). These studies indicate that late gestation nutrition impacts mammary gland development, which could impact postpartum milk production and passive immune transfer thus impacting postnatal growth and health.

Most studies investigating gestational nutrition on progeny performance have used gestating sheep as a biomedical model and have focused on the effects of global under-nutrition (McMillen et al., 2001; Ford et al., 2007) and global over-nutrition (Wallace et al., 2002; Long et al., 2010). These studies have reported an association between maternal nutrient intake and progeny's postnatal body composition and glucose metabolism as well as indicated that timing and duration of nutrient modification during gestation differentially impacts outcomes. In cattle, providing a high vs. low energy diet during late gestation in beef cattle was reported to increase calf birth weight and subsequent weaning weight (Corah et al., 1975). The impact of global nutrient restriction during early gestation (d 32 to 115) has varied. Long et al. (2008) observed that nutrient restriction of cows did not influence birth weights or postnatal growth calves. In contrast, Underwood et al. (2008) reported greater postnatal growth and feed efficiency in steers born from cows that were nutrient restricted during a similar period of gestation (d 31 to 120). Nutrient restriction (55% global restriction) during early to mid- gestation in sheep (d 28-78) resulted in male offspring having similar birth weights, but lighter weights at slaughter, greater amounts of internal fat, and less muscle mass (Ford et al., 2007). In these studies, nutrient restriction appears to have provided adequate energy for fetal growth, possibly at the expense of the dam's tissue because nutrient partitioning during pregnancy favors the fetus at the expense of the dam and the placenta efficiency may be different (Barcroft, 1946).

While studies investigating maternal over- or under-nutrition are valuable, the investigation of specific diet components could provide greater insight into mechanisms of developmental programing. A few studies in beef cattle have investigated specific diet components during gestation on postnatal progeny production traits. A series of reports from the University of Nebraska has demonstrated that cows supplemented protein on dormant winter range in late gestation had steer progeny with greater postnatal growth rate and intramuscular fat deposition than progeny from cows not supplemented protein (Stalker et al., 2006; Martin et al., 2007; Larson et al., 2009). Previous research has focused on developmental programming affects elicited by comparing energy sources fed at isoenergetic which differ in energy substrate supply (corn, hay, and corn dried distillers grains [DDGS]) during late gestation in sheep and cattle (Radunz et al., 2012). When corn (high starch) and DDGS (high fiber and fat and excess protein) energy sources verses hay (high fiber) were fed to dams during late gestation this resulted in progeny with greater birth weights. In addition research at Purdue University demonstrated that feeding diets containing excess protein during the third trimester resulted also in greater birth weights (Gunn et al., 2012). Collectively, these studies indicate that maternal nutrition in late gestation influence fetal growth.

Carcass Traits

Comparable to human health research, the desired impact is to deposit less external and internal fat during growth, not only because of the consequences to the efficiency of growth but also impacts on such economically important traits as carcass value and reproduction. One distinct difference in humans compared to beef cattle is concerning the intramuscular (*i.e*. marbling) adipose depot. In beef cattle, marbling has greater economic value than in other livestock species (*i.e.* pigs, sheep), because this fat depot is a major determinant in USDA Quality Grade and is used to determine carcass value. Approximately 80% of fetal adipose tissue is deposited in the final few weeks of gestation, but the development of these adipocytes starts earlier in gestation (Symonds et al., 2007). Adipose tissue growth occurs through two mechanisms: 1) preadipocyte proliferation; impacting capacity to form new mature adipocytes (hyperplasia) and 2) increased size and lipid storage capacity of mature adipocytes (hypertrophy). Adipocyte hyperplasia occurs primarily during late fetal development and early postnatal life in humans (Martin et al., 1998) and bovines (Zhu et al., 2008). This process is highly sensitive to the nutritional environment and to the prevailing concentrations of insulin-like growth factors, glucose, insulin, and glucocorticoids (Martin et al., 1998). Although preadipocytes can proliferate and differentiate in adults, their capacity appears to be limited with most of the developmental work completed early in life (Martin et al., 1998). Therefore, evidence supports that the fetal and early postnatal periods are critical stages of adipose tissue programming which impact later fat deposition.

Research from the University of Nebraska (Stalker et al., 2006; Stalker et al., 2007; Larson et al., 2009) provides supporting evidence that late gestation is a critical period for marbling development (Table 1). Over a 3-year period, steer calves from cows grazing native range with or without protein supplementation were followed from birth to slaughter. Native range was determined not to meet the protein requirements of the cow during late gestation and protein supplementation was provided to one group to meet those requirements. The calves born to cows supplemented with protein had carcasses with higher marbling scores, with a greater percentage of carcasses graded USDA Choice, and 60 lbs heavier hot carcass weights.

Additionally, Radunz et al. (2012) reported similar results indicating an important role of late gestation nutrition on marbling development and fetal growth. Calves born to cows fed corn had the least marbling and lower percentage of carcasses grading in the upper 2/3 of USDA Choice compared to calves born to cows fed distiller's dried grains with solubles or hay at the same fat endpoint. These results suggest that the amount of marbling in the carcass may not only be determined by genetics, postnatal nutrition, and postnatal management but also could be determined by what the cow is fed during gestation. In the following research using the same model in sheep by Radunz, fetal adipose and *longissimus* muscle tissues collected at birth provide the first evidence that maternal diet can influence the expression of imprinted genes associated with adipose tissue development and growth, which could explain difference in postnatal fat deposition.

Conclusions

One of the major challenges in gaining more knowledge in the area of developmental programming in beef cattle production is the time and resources needed to collect the data. Therefore, at this time, more questions may be raised than answered. The research presented here indicates maternal nutrition can impact postnatal growth and fat deposition in ruminants. More specifically, late gestation maternal nutrition may have a significant impact on intramuscular fat deposition in beef cattle. The question remains to be answered whether this is the result of changes in maternal body condition score, substrate supply to the fetus, quantity and/or quality of protein supply or other dietary factors. In order to improve efficiency of beef cattle production, more research is warranted to investigate the impacts of the environment, such as nutrition, during gestation and early postnatal life of cattle on lifetime productivity.

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Table 1. Effects of cow protein supplementation during late-gestation on progeny feedlot performance and carcass traits

	Stalker et al. 2006		Stalker et al. 2007		Larson et al. 2009	
	NS[1]	S[1]	NS	S	NS	S
Weaning weight, lbs	463[a]	476[b]	463[a]	476[b]	514[a]	529[b]
Dry matter intake, lb/d	18.7	18.8	24.6[a]	26.6[b]	19.8[x]	20.3[y]
Feed:Gain	5.41	5.46	6.97	7.19	5.37	5.38
HCW, lbs	800	814	765[a]	805[b]	805[a]	822
USDA Choice, %	85	96	---	---	71[a]	85[b]
Marbling Score[2]	467	479	449	461	445 [a]	492

[ab] Means differ, $P \leq 0.05$.
[xy] Means differ, $P \leq 0.10$.
[1] NS = non supplemented and S = supplemented (contained = 62% dried distillers grain plus solubles, 11% wheat middlings, 9% cottonseed meal, 5% dry corn gluten feed, 5% molasses, 2% urea, 6% vitamin and trace mineral premix, and monensin).
[2] 400 - 499 = Low Choice; 500-599 = Average Choice.

Table 2. Effects of cow late gestation dietary energy source on progeny feedlot performance and carcass traits

	Grass Hay[1]	Corn	DDGS	*P*-value
Birth weight, lbs	85.5[a]	95.0[b]	91.0[b]	0.01
Weaning weight, lbs	548	569	564	0.09
ADG, lbs/d	3.37	3.46	3.41	0.48
Dry matter intake, lbs/d	19.3	19.6	19.6	0.78
Gain:Feed	0.174	0.178	0.176	0.43
Days on Feed, d	178	168	170	0.10
Hot carcass weight, lbs	688	688	675	0.59
12th rib fat, inhes	0.48	0.50	0.51	0.71
Ribeye area, inches^2	12.0	12.0	11.9	0.79
Yield grade	2.82	2.82	2.85	0.93
Marbling score[2]	549[a]	506[b]	536[ab]	0.03

[1] Diets fed to cows during late gestation. HAY = ad libitum grass hay; CORN = limit-fed corn diet; and DDGS = limit-fed dried distiller's dried grains with solubles.
[2] Slight = 300 to 399, Small = 400 to 499, Modest = 500 to 599.

SESSION NOTES

Effect of Increased Supplementation of Vitamin E During Heat Stress

C.R. Staples[1], G.C. Gomes, J.E. Zuniga, L.F. Greco, L.D.P. Sinedino, E. Karadaya, E.S. Ribeiro, N. Martinez, R.S. Bisinotto, F.S. Lima, M.A. Engstrom, and J.E.P. Santos*
*University of Florida, Gainesville and *DSM, Parsippany, NJ*

Introduction

Vitamin E is an antioxidant that plays important roles in the maintenance of cellular membranes, immunity, and reproduction (National Research Council [**NRC**], 2001). The form that is most common in feeds and is most biologically active is α-tocopherol. Unlike vitamin A, it is not thought to be degraded by ruminal microorganisms. A specific requirement for vitamin E has not been defined yet because titration studies are lacking. The recommended rate of supplemental vitamin E is 1.6 and 0.8 IU/kg of body weight for pregnant dry cows and lactating cows, respectively. A 650 kg cow supplemented at the recommended guideline of the 2001 Dairy NRC would consume daily ~1,000 IU prepartum and ~ 500 IU postpartum. Cows fed fresh forages will require less supplemental vitamin E than this. Unlike plasma retinol concentrations, plasma α-tocopherol concentrations do reflect vitamin E intake. Based upon optimizing neutrophil function and minimizing clinical mastitis, the minimal acceptable concentration of plasma α-tocopherol for the dairy cow within a day or two after calving was proposed to be 3 to 3.5 ug/ml of plasma (Weiss, 1998). Cows at later stages of lactation may have a different minimal acceptable concentration as they may be under less immunological stress. Life changes that increase metabolic demands, such as parturition and copious amounts of milk production, increase oxygen requirements substantially. As a result, the production of reactive oxygen species (**ROS**) such as O_2^-, OH^-, H_2O_2, and lipid peroxy radical (**LOO$^-$**) increases. Oxidative stress results when ROS are produced faster than they can be neutralized by antioxidants (Sies, 1991). Oxidative stress usually occurs during the periparturient period (Ronchi et al., 2000) and may contribute to periparturient disorders (Brezezinska-Slebodzinska et al., 1994) and be associated with metabolic diseases (Ronchi et al., 2000). The predominant antioxidant in biological cell membranes is α-tocopherol. Heat stress may aggravate oxidative stress (Bernanucci et al., 2002). Feeding additional vitamin E as an antioxidant in the summer during the periparturient period may be needed due to the greater oxidative stress caused by elevated temperature and humidity.

Experimental Design

Objective was to evaluate vitamin E supplementation above NRC recommendations during the close-up dry period and during lactation to Holstein cows (36 primiparous and 34 multiparous) managed either in free-stall barns using fans and sprinklers to evaporatively cool the cows or in shaded outdoor lots without fans and sprinklers during the prepartum period. After calving, all cows were housed together in

[1] Contact at: Department of Animal Sciences, University of Florida, 2250 Shealy Drive, Gainesville, FL 32911. E-mail: chasstap@ufl.edu

a free-stall facility equipped with fans and sprinklers. All-rac-alpha-tocopherol (DSM, Parsippany, NJ) was mixed with ground corn and dried molasses and top-dressed daily [2](100 g of mix/cow) on the first feeding of the day of the total mixed ration. Amounts of vitamin E supplementation were either 1,000 IU prepartum and 500 IU postpartum as recommended by the NRC (2001) for feeding of dairy animals or 3,000 IU prepartum and 2,000 IU postpartum (greater than current published recommendations for vitamin E). In summary, the 4 treatments were 1) prepartum shade only and NRC amounts of vitamin E, 2) prepartum shade only and a 3 to 4 fold increase in vitamin E supplement, 3) prepartum evaporative cooling and NRC amounts of vitamin E, and 4) prepartum evaporative cooling and a 3 to 4 fold increase in vitamin E supplement. The treatments started at 4 weeks prepartum and continued through 15 weeks of lactation. Measurements included heat stress responses prepartum, intake of feed, body weight, yields of milk and milk fat and protein. Blood was collected on days -30, -14, 3, 7, 14, 21, 28, 35, and 42 relative to calving for analyses. Cows were bred using timed artificial insemination at 46 and 64 ± 3 days in milk. Conceptuses were collected by uterine flushing 15 days after each AI and their length measured.

Results and Discussion

Prepartum Responses

During the prepartum period, the environmental temperature-humidity index (**THI**) averaged 74.8 ± 4.9 and cows were exposed to a THI greater than 70 during 85% of the day. Therefore the study was conducted during conditions of heat stress. Providing fans and sprinklers to one group of cows kept them cooler than the group only given shade. Prepartum evaporative cooling in free-stalls reduced vaginal temperature from 102.9 to 102.2^{o}F (mean of measurements taken regularly between noon and 7 p.m.), reduced respiration rates from 69 to 43 breaths per minute (measured at 3 p.m.), reduced plasma concentrations of nonesterified fatty acids (**NEFA**) from 0.28 to 0.14 mM, and increased intake of feed dry matter (**DM**) by 15%, from 8.3 to 9.5 kg/day across parities ($P < 0.05$). As expected, cows offered only shade were hotter, breathed faster, and ate less feed compared with cows provided shade, fans, and sprinklers. Vaginal temperatures, plasma NEFA concentrations, and DM intake were not affected by the amount of vitamin E top-dressed. Multiparous cows experienced greater oxidative stress compared with primiparous cows based upon greater plasma concentration of lipid hydroperoxides (3.08 vs. 2.48 uM). The larger body size and greater feed intake (9.7 vs. 8.1 kg/day) of the older cows likely caused them to experience greater oxidative stress.

Plasma concentrations of vitamin E were lowest at 3 days in milk (Figure 1). All treatment means at this time were at or below the minimum acceptable concentration for the periparturient dairy cow (3.0 to 3.5 ug/mL) as suggested by Weiss (1998) who based these minimum blood values upon animals having good neutrophil function and reduced clinical mastitis. Greater prepartum heat stress did not affect vitamin E

[2] Contact: Department of Animal Sciences, University of Florida, 2250 Shealy Drive, Gainesville, FL 32608. Phone: 352-392-1958 ext. 253. E-mail: chasstap@ufl.edu

concentrations at this early time postpartum but greater vitamin E supplementation increased plasma concentrations from 2.8 to 3.4 ug/mL ($P < 0.001$) at 3 days in milk. This difference was even greater at 7 days in milk being 3.1 vs. 4.0 ug/mL for normal compared with high vitamin E treatments, respectively. By 14 days in milk, all treatment group means surpassed 4.0 ug/mL with the exception of the multiparous cows in the cooled normal vitamin E group which only averaged 3.2 ug/mL. If using minimum plasma vitamin E concentrations stated by Bill Weiss at Ohio State University as a guideline, multiparous cows may benefit from supplementation of vitamin E above NRC recommendations during the first 2 weeks postpartum.

In this study, incidence of retained fetal membranes (RFM; membranes retained for more than 24 hours after parturition) was 8.6%, incidence of metritis (fetid, watery, uterine discharge during the first 12 days postpartum) was 18.6%, and incidence of clinical mastitis (first 6 weeks postpartum) was 14.3%. The incidence of these maladies were not affected by the amount of vitamin E top-dressed in the study. In other studies, giving more vitamin E had benefited reproductive health. Injecting 3,000 IU of vitamin E within 1 to 2 weeks of calving reduced the incidence of RFM of heifers (LeBlanc et al., 2002) and of all parities (Erskine et al., 1997). Although the incidence of RFM, metritis, or mastitis was not affected by the amount of vitamin E top-dressed in our study, the mean plasma concentrations of vitamin E were lower in cows afflicted with these maladies. Plasma concentration of vitamin E averaged across -14, 3, and 7 days relative to calving was 3.6 and 2.5 ug/mL ($P < 0.001$) for healthy and RFM cows, 3.7 vs. 3.2 ug/mL ($P = 0.03$) for healthy and metritic cows, and 3.7 vs. 3.2 ug/mL ($P = 0.03$) for healthy and mastitic cows, respectively. Dry matter intake during the last 14 days before calving did not differ between healthy and sick cows, being 9.9 and 9.8 kg/day for healthy and RFM cows for example.

Postpartum Responses

Milk Production and Composition and Feed Intake. The benefit of providing fans and sprinklers to multiparous cows during the close-up dry period was clear. Multiparous cows fed the recommended amount of vitamin E produced an average of 36.0 kg/day of 3.5% fat-corrected milk if provided only shade during the close-up dry period. However, if the multiparous cows were provided shade, fans, and sprinklers during the close-up dry period, they produced 39.9 kg/day of 3.5% fat-corrected milk, an increase of 3.9 kg/day due to prepartum cooling. This milk advantage due to cooling of multiparous cows during the close-up period was similar to the increase in milk production reported when multiparous cows were cooled for the whole dry period (Tao and Dahl, 2013). Feeding more vitamin E than currently recommended appeared to have the same positive effect on milk yield as prepartum cooling in our study. Multiparous cows offered shade only during the close-up period and fed the normal amounts of vitamin E produced an average of 36.0 kg/day of 3.5% fat-corrected milk. If the supplementation of vitamin E increased to 3,000 IU prepartum and 2,000 IU postpartum, average milk production increased to 39.5 kg/day, an increase of 3.5 kg/day. So milk production by multiparous cows was greatest under two very different management conditions. Milk yield by multiparous cows was the same using 1)

evaporative cooling conditions plus supplementing recommended amounts of vitamin E and 2) shade only plus supplementing extra vitamin E. These increased amounts of milk yield were supported by increased amounts of feed DM intake; that is, multiparous cows ate more feed DM after calving if cooled in the close-up period or if fed more vitamin E and housed with only shade compared with shaded cows fed recommended amounts of vitamin E. Multiparous cows benefited from consuming more vitamin E only when provided shade alone during their late dry period in the summer season.

The story for prepartum cooling and vitamin E supplementation was much different for primiparous cows. Evaporative cooling during the close-up period of primiparous cows did not result in greater milk production or greater feed intake postpartum as it did for multiparous cows. The milk secreting cells of the mammary gland of primiparous cows may be more resistant to decreased cell proliferation due to heat stress compared with multiparous cows. The effect of supplementing more vitamin E to primiparous cows provided only shade was negative and the opposite of the positive effect on multiparous cows. For primiparous cows not cooled with fans and sprinklers prepartum, supplementing with more vitamin E reduced postpartum DM intake from 19.8 to 17.9 kg/day (a 1.9 kg/day decrease) and reduced yield of 3.5% fat-corrected milk from 27.7 to 22.5 kg/day, a decrease of 5.2 kg/day. As a result, the conversion of feed into 3.5% fat-corrected milk was decreased from 1.49 to 1.33 kg of milk per kg of feed. Feeding more vitamin E to evaporatively cooled primiparous cows in the dry period did not affect their postpartum intake of DM or their production of milk.

The opposite responses by primiparous and multiparous cows to additional vitamin E supplementation was unexpected. The reasons for the different responses may result from differences in stress between parities and therefore a difference in need for additional vitamin E. Multiparous cows appeared to be under greater stress than primiparous cows. After calving, the loss of body weight was greater (35 vs. 12 kg) and the time it took to recover the lost body weight was greater (15 vs. 5 weeks) for multiparous compared with primiparous cows (Figure 2). The average energy balance during the first 15 weeks postpartum also was less for multiparous compared with primiparous cows (-0.8 vs. 3.6 Mcal/day). This greater metabolic stress of multiparous cows is supported by the greater blood concentrations of NEFA (fat mobilized from adipose tissue; 0.36 vs. 0.21 mM) and the ketone body, beta-hydroxybutyric acid (BHBA; produced by the incomplete oxidation of mobilized fat; 0.65 vs. 0.53 mM). During the close-up dry period, the multiparous cows also seemed to be under greater stress. They had increased concentration of lipid hydroperoxides in their blood during the prepartum period. These lipid hydroperoxides form as a result of oxidative stress in the cells of the cow. Oxidative stress results from increased demand for oxygen under conditions described in the Background section of the paper. Excess free radicals form as a result of uncontrolled oxidative stress. These free radicals can react with lipids in cell walls. Vitamin E is stored in the lipid portion of cell walls, ready to react with free radicals so as to prevent damage and death to the cells. As vitamin E donates its hydrogens to free radicals to form harmless water and to stop lipid peroxidation, a vitamin E radical forms called tocopheroxyl radical. This vitamin E radical itself can be dangerous to the cell if the vitamin E is not returned to its normal state with the help of

vitamin C. Supplementing additional vitamin E can be a good thing if the cow is under additional oxidative stress or it may be a bad thing if oxidative stress is not under control. In our study, the multiparous cows appeared to be under more stress compared to the primiparous cows and therefore benefited from consuming more of the important antioxidant vitamin E. The primiparous cows likely consumed more vitamin E than they required and may have experienced tissue damage at the cellular level resulting in lowered performance.

Mean concentration of milk fat was 3.60% across all treatments and all weeks of lactation. Neither prepartum cooling nor increased vitamin E supplementation affected milk fat concentration of primiparous cows (mean of 3.56%). For multiparous cows, the story was different. Cooling multiparous cows prepartum resulted in greater milk fat concentration, increasing from 3.52% to 3.73%. This agrees with others who reported improvements in milk fat % if cows were evaporatively cooled prepartum (Avendano-Reyes et al., 2006; do Amaral et al., 2009). Interestingly, the feeding of additional vitamin E to noncooled multiparous cows increased milk fat % to the same extent (from 3.52% to 3.73%) as if they had been evaporatively cooled. In other words, feeding additional vitamin E had the same positive effect on milk fat % as did prepartum cooling. Providing additional vitamin E during the late dry period when cows were only offered shade from the heat may have helped maintain good development of the mammary gland cells. Concentration of milk true protein (mean of 2.94%) was not affected by evaporatively cooling or by the amount of vitamin E supplemented.

Immune responses. The white blood cells are largely responsible for keeping the cow heathy. In this study, we examined the activity of one kind of white blood cells called neutrophils. Neutrophils travel to sites of infection through the blood stream in order to engulf and then kill the invading pathogens. We collected blood from the cows and added the bacteria, *E. coli*, to the blood to assess how effectively the neutrophils would destroy this pathogen. First we will look at the prepartum period. Providing extra vitamin E to shade-only primiparous cows reduced the proportion of neutrophils killing bacteria from 49 to 41%. However the opposite occurred for shade-only multiparous cows. Feeding extra vitamin E tended to increase the proportion of neutrophils killing bacteria from 33% to 43% (vitamin E by cooling by parity interaction, $P = 0.07$). This response of benefit to extra vitamin E by multiparous cows and of harm to primiparous cows is similar to what occurred with milk production. What about the postpartum period? When NRC amounts of vitamin E were fed, a greater proportion of the blood neutrophils tended to engulf and kill the bacteria in the postpartum period if the cows were evaporatively cooled prepartum than if the cows were not evaporatively cooled prepartum (39.5% vs. 33.7%). These positive results due to prepartum cooling agree with those of do Amaral et al. (2011). However, if vitamin E was fed above NRC recommendations, the proportion of neutrophils that killed bacteria tended to be reduced from 39.5% to 33.2% when cows were evaporatively cooled but the proportion was not affected under greater heat stress conditions (vitamin E by cooling interaction, $P = 0.09$). Feeding extra vitamin E may have acted as a prooxidant and reduced the killing activity of neutrophils when cows were under less stress.

Conceptus Length. 15 days after insemination, conceptuses were collected from 32 animals and measured for length. The current thinking is that conceptus length may be a good indicator of development and that longer conceptuses are more likely to survive and go on to become calves. Providing more vitamin E to cows that had been exposed to greater heat stress prepartum resulted in longer conceptus length (28 vs. 63 mm) whereas conceptus length was shorter if cows were evaporatively cooled prepartum and fed more vitamin E (38 vs. 8 mm; VitE by cooling interaction, $P < 0.01$). The impact of feeding more vitamin E on development of the conceptus was dependent upon the prepartum environmental conditions in this small data set. Feeding excess vitamins E and C to mice also had negative effects on mice, reducing the percentage of viable fetuses (Tarin et al, 2002). Feeding more vitamin E to multiparous cows tended to double the length of the conceptus at 15 days of life whereas it tended to reduce the length of the conceptus of primiparous cows.

Summary and Conclusions

Daily intake of supplemental vitamin E was increased from 1,000 to 3,000 IU during the close-up prepartum period and from 500 to 2,000 IU postpartum for primiparous and multiparous cows housed with shade only or with shade, fans, and sprinklers during the last 4 weeks of pregnancy. Milk production and milk fat concentration of multiparous cows housed without fans and sprinklers in the prepartum period was negatively affected. However, both milk production and milk fat concentration were restored if cows were provided with more vitamin E. No benefit of extra vitamin E was detected if multiparous cows were evaporatively cooled prepartum. Production of fat-corrected milk of primiparous cows was reduced if they were fed vitamin E above NRC recommendations regardless of prepartum cooling method. Based upon lower plasma concentrations of NEFA, less loss of body weight, and less negative energy balance, primiparous cows were under less metabolic stress postpartum than were multiparous cows. Feeding 3 to 4 times the recommended amount of the antioxidant vitamin E to these lower-stressed cows may have caused vitamin E to form many tocopherol radicals that damaged cell membranes and hurt performance rather than act as an antioxidant and help performance as it did with the multiparous cows. The combination of increased thermal stress prepartum and metabolic stress due to greater milk production postpartum may have created a scenario in which the requirement for an antioxidant, vitamin E, was increased for multiparous cows.

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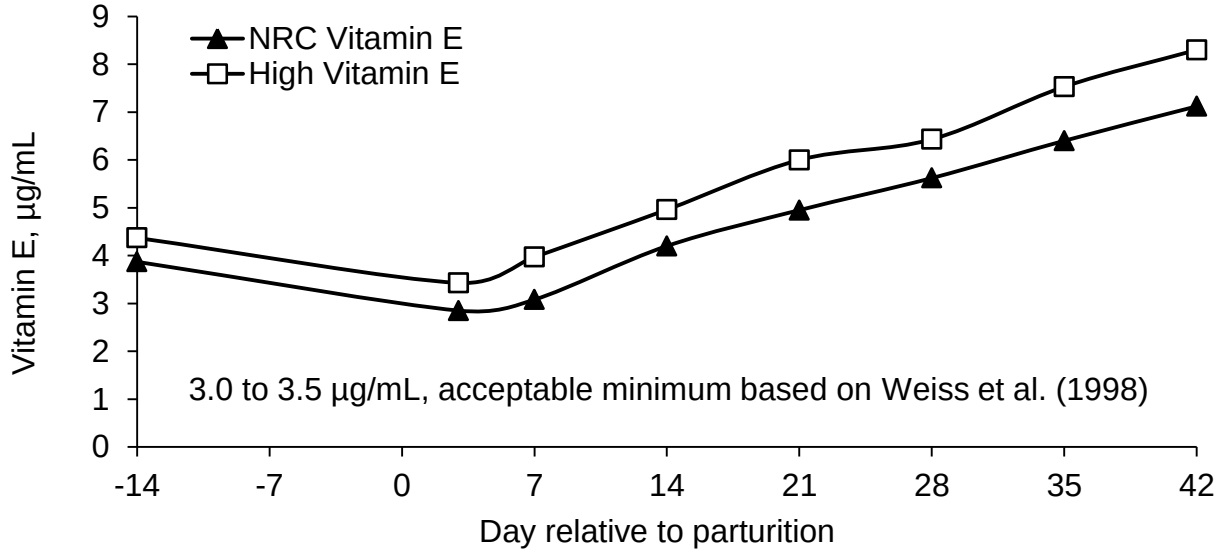

Figure 1. Concentrations of vitamin E in plasma of Holstein cows fed recommended amounts of vitamin E (1,000 IU/day close-up prepartum and 500 IU/day postpartum; "NRC Vitamin E") or 3,000 IU/day in the close-up and 2,000 IU/day in the postpartum periods ("High Vitamin E").

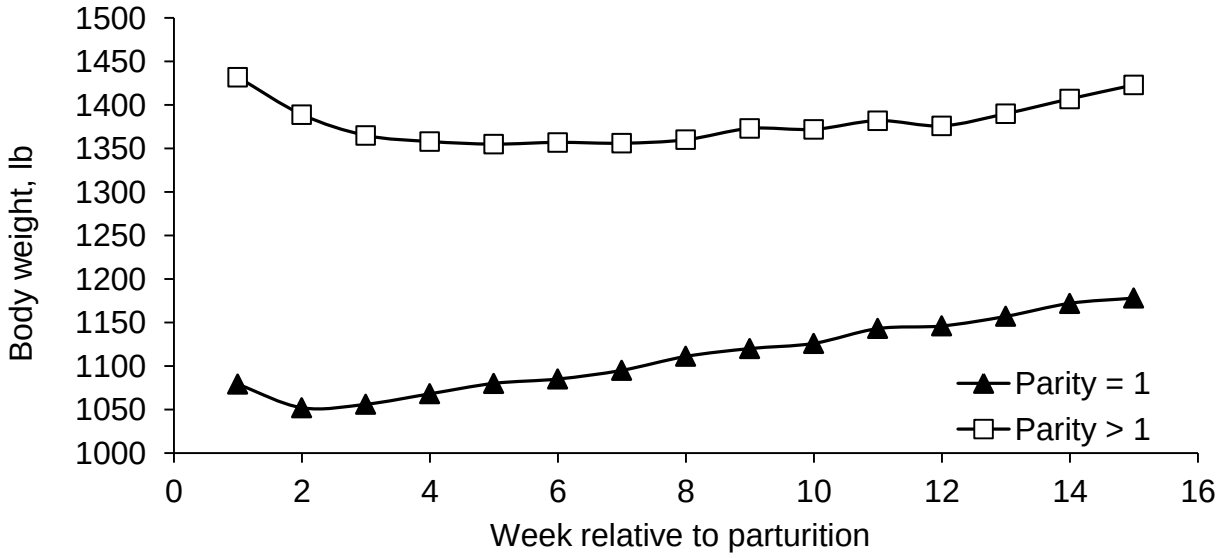

Figure 2. Body weight of primiparous and multiparous lactating Holstein cows from calving through 15 weeks of lactation.

SESSION NOTES

Managing Milk Fat Depression

Kevin J. Harvatine[1]
Department of Animal Science
The Pennsylvania State University

Introduction

Milk fat concentration is variable within and between farms and is modified by genetics, season of the year, and physiological state, but is especially responsive to diet. Synthesis of milk fat is an energy demanding process, but also represents a significant portion of the economic and nutritional value of dairy products. First described over one and a half centuries ago, diet-induced milk fat depression (**MFD**) is characterized by a decrease in milk fat yield of up to 50% with no change in milk yield or yield of other milk components. Milk fat depression is classically observed in ruminants fed highly fermentable diets or diets high in plant oils. Varying levels of MFD are commonly experienced today in both intensively and extensively managed dairy herds, and this represents a level of milk fat production below the genetic potential of the cow. Milk fat depression is also a useful variable for evaluating herd management. In many cases onset of diet-induced MFD is an indication of modified ruminal fermentation and in more pronounced cases this can be associated with ruminal acidosis and reduced efficiency. Therefore, maintaining optimal milk fat synthesis has value beyond the milk fat sold. Although the past two decades have provided extensive knowledge of the causes and mechanisms of MFD we continue to experience the condition because of the requirement to feed high-energy diets and the desire to maintain optimal milk production. Additionally, numerous dietary factors commonly interact to cause MFD, making prediction difficult. We have investigated the time course of induction and recovery of MFD that provides insight into identifying causative factors and setting expectations for correction of MFD. We have also demonstrated that a rumen available methionine analog reduces the risk of MFD and that feeding management has an important role.

Historical Theories of Milk Fat Depression

The investigation of diet-induced MFD has a rich history that has included many theories to explain reduced milk fat synthesis. Most of these theories postulated that limitations in substrate supply for milk fat synthesis caused MFD, generally based on changes in absorbed metabolites as a consequence of alterations in ruminal fermentation including a decrease in the acetate to propionate ratio (Bauman and Griinari, 2001). This formed the basis for one of the most widely known substrate

[1] Contact at: Department of Animal Sciences, 324 Henning Building, University Park, PA 16802; Tel: (814) 865.6334; E-mail: kjh182@psu.edu.

supply limitation theories that proposed that acetate supply was limiting milk fat synthesis during diet-induced MFD. However, the reduced ratio of acetate to propionate with highly fermentable diets is predominantly due to increased ruminal production of propionate (Davis and Brown, 1970). Overall, several decades of research has tested numerous theories based on substrate limitations and found little to no evidence for their support in classical diet-induced MFD which is associated with highly fermentable and high unsaturated fat diets (extensively reviewed by Bauman and Griinari, 2003; Shingfield and Griinari, 2007; Bauman et al., 2011). Acetate supply does not appear to be limiting during classical diet-induced MFD; however, acetate supply may have a small impact on milk fat synthesis. Sheperd and Combs (1998) increased milk fat yield 230 g/d with ruminal infusion of 2.2 kg/d of neutralized acetate. We have recently observed a 178 g/d increase in milk fat with infusion of 420 g/d of neutralized acetate (Urrutia et al., 2015). Importantly, these were increases in milk fat above normal levels (>3.55%). Acetate appears to provide an opportunity for small changes in milk fat outside of conditions of classical diet-induced MFD.

Davis and Brown (1970) recognized that *trans*-C18:1 fatty acids (**FA**) were increased in milk fat of cows with low-milk fat syndrome. They suggested that *trans*-FA originated from incomplete ruminal biohydrogenation of unsaturated FA and might contribute to the development of MFD. Subsequent studies have demonstrated a clear relationship between specific *trans*-FA and MFD (see reviews by Bauman and Griinari, 2003; Shingfield and Griinari, 2007; Bauman et al., 2011). Investigations over the past twenty years have clearly established that diet-induced MFD is associated with rumen production of unique FA from ruminal metabolism of dietary polyunsaturated fatty acids (**PUFA**). Referred to as the *biohydrogenation theory*, the basis for diet-induced MFD relates to an inhibition of mammary lipid synthesis by specific FA that are intermediates in the biohydrogenation of dietary PUFA, and these are only produced under certain conditions of altered ruminal fermentation (Figure 1, Bauman and Griinari, 2003). *Trans*-10, *cis*-12 conjugated linoleic acid (**CLA**) was the first of these to be recognized and it has been extensively investigated at the whole animal and molecular level (reviewed by Bauman et al., 2011). In experiments abomasally infusing *trans*-10, *cis*-12 CLA, a curvilinear relationship ($R^2 = 0.96$) exists between the dose of *trans*-10, *cis*-12 CLA and the reduction in milk fat secretion; as little as 2.5 g/d delivered post-ruminally caused a 20-25% reduction (de Veth et al., 2004). A curvilinear relationship also exists between milk fat content of *trans*-10, *cis*-12 CLA and the reduction in milk fat [$R^2 = 0.93$; (de Veth et al., 2004). Milk FA profile provides a sensitive indicator of rumen outflow of FA as 85% of the preformed FA originate directly from chylomicrons absorbed in the gut (Palmquist and Conrad, 1971).

Ruminal Biohydrogenation

Ruminant diets are low in total fat, although forages, oilseeds, fat supplements, and some byproducts can result in a significant intake of PUFA. Dietary FA are metabolized in the rumen resulting in a large difference between the FA profile of the diet and the FA absorbed. Most FA in the diet are esterified and these are hydrolyzed in the rumen and the resulting unsaturated FA are isomerized (double bond position

changed) and biohydrogenated (double bond removed; Figure 1). The extent of biohydrogenation and the intermediates formed are determined by the properties of the fat source, retention time in the rumen, and characteristics of the microbial population (Allen, 2000, Palmquist et al., 2005). Dietary factors that modify ruminal fermentation (ex. high starch, high oil, monensin) also modify ruminal FA metabolism through associative effects that presumably result in a microbial population that utilizes the alternative pathway of PUFA biohydrogenation.

Ruminal biohydrogenation may be simply described as a function of the available FA pool size, ruminal retention time, and bacterial biohydrogenation capacity (Harvatine and Bauman, 2007). Microbial biohydrogenation is a multi-step process for which the kinetics are not well documented. Harvatine and Allen (2006b) used the pool and flux method (Firkins et al., 1998) to observe *in vivo* ruminal FA kinetics of a cottonseed-based diet that included a fat supplement. Dietary FA had a slow ruminal passage rate (6.4 to 7.4%/h) indicating a long average rumen retention time. In contrast, the fractional biohydrogenation rate of linoleic acid was high (14.6 to 16.7%/h). Interestingly, the biohydrogenation of *trans* C18:1 FA was also very high (33.4 to 48.4 %/h), although a decrease in the biohydrogenation rate of *trans*-C18:1 FA was associated with an increased duodenal flow of biohydrogenation intermediates and diet-induced MFD. *In vivo* ruminal FA kinetics clearly demonstrates that ruminal FA metabolism is responsive to associative dietary factors and that the long retention time provides ample time for metabolism of fat sources that are not rapidly available in the rumen.

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. Bailey et al. (2005) conducted an economic analysis of the variation in milk production and composition using monthly milk records of all herds in the Mid-East milk market over two years and reported a wide distribution in milk fat concentration and that one-third of the herds experienced a reduction in milk fat for 1 to 3 months. Significant variation in milk fat composition exists within herds because of stage of lactation, genetics, physiological state, management, and their interactions. This variation is well demonstrated by a 905-cow example herd with low milk fat (herd average = 3.2%). The 25^{th} and 75^{th} percentiles of milk fat concentration were 2.6% and 3.6%, respectively. We also observed a relationship between milk fat concentration and milk yield with higher producing cows having lower milk fat concentration. Although significant variation is not explained by milk yield, there was a large slope to the regression line and milk fat concentration of cows less than 75 lbs averaged 3.81%, cows 75 to 95 lbs averaged 3.19%, and cows above 95 lbs averaged 2.90%. 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 MFD.

Dietary Risk Factors For Milk Fat Depression

Prediction of the occurrence of MFD is complex because it is not directly caused by a single dietary factor; rather it is the result of the interaction of numerous factors that reduce the rate of biohydrogenation and shift biohydrogenation to the alternate pathway. It is preferable to think of dietary risk factors that move a diet along a continuum from low to high risk. Below is a summary of major risk factors. This is not a complete list, but highlights the most important issues.

Diet Fermentability

The microbial population is driven by the substrate available and by the rumen environment and is directly dependent on the concentration of starch and NDF and the rates and extent of ruminal digestion. Maximizing fermentablity is important for energy intake, but care should be given to minimizing sub-acute ruminal acidosis. Milk fat depression more commonly occurs with corn silage compared to haylage-based rations and with more rapidly digested starch sources such as high moisture corn compared to dry ground corn. Low milk fat is commonly associated with sub-clinical and clinical ruminal acidosis, but MFD is frequently observed without a reduction in rumen pH (Harvatine and Allen, 2006a). Rumen pH is dependent on the VFA profile, rate of production, and rate of absorption, buffer secretion, and presence of dietary buffers and varies by approximately 1 to 1.2 pH units over the day (Allen, 1997). Providing multiple sources of starch and fiber with overlapping rates of digestion is the safest approach. Additionally, sugar substituted for dietary starch may reduce risk without loss of digestibility (Mullins and Bradford, 2010).

Diet Polyunsaturated Fatty Acids

Unsaturated fatty acids have a dual impact on ruminal biohydrogetion in that they modify the microbial population and increase the amount of substrate that must be biohydrogenated. It is important to know the total amount of unsaturated fat and also the source, which dictates the FA profile and rate of FA availability. Fish oil has the greatest impact, but is not commonly found in excessive amounts in diets. Cotton, soy, corn, and many other plant oils are high in linoleic acid (C18:2) and incorporation of these grains, oils, and their byproducts increases the risk of MFD. The concept of Rumen Unsaturated Fatty Acid Load (RUFAL, Jenkins, 2011) is a simple and insightful calculation that is complemented by consideration of the fat source. There are significant differences in the rate of ruminal FA availability, for instance cottonseed and whole roasted soybeans are expected to have a much slower release of FA in rumen than distiller's grains, ground sources, or oil supplements.

Fat is commonly supplemented to increase diet energy density and many protected fat supplements are available. Supplements that are high in saturated fat (palmitic and stearic) do not increase the risk of MFD; however calcium salts of FA are available in the rumen and can reduce milk fat (Lundy et al., 2004; Harvatine and Allen, 2006b). The calcium salt slows the release of unsaturated fat in the rumen and does reduce the impact of these oils compared to free oil, but does not provide a high level of

rumen protection. The impact of calcium salts depends on the FA profile and interaction with other factors (Harvatine and Allen, 2006a; Rico and Harvatine, 2011).

Rumen Modifiers

Many supplements have a large impact on the rumen microbial population. Monensin is the most common rumen modifier associated with MFD (Jenkins, 2011). However, it is only a risk factor and can be safely used in many diets. Other rumen modifiers may reduce risk, although varying levels of evidence supports their effectiveness. We observed that 2-hydroxy-4 (methylthio) butanoic acid (HMTBA) decreased the risk of diet-induced MFD in two separate experiments (Baldin et al., 2014; Baldin et al., 2015), although the mechanism is not clear. Additionally, a direct fed microbial product was shown to stabilize rumen biohydrogenation during a high diet fermentability challenge (Longuski et al., 2009) and others may have similar impacts.

Feeding Strategies

Slug feeding grain is commonly associated with sub-clinical rumen acidosis and MFD. Many assume that TMR feeding eliminates this issue since every bite has the same nutrient composition. However, the rate of intake of fermentable organic matter is very variable over the day due to sorting and variable rates of intake. Generally, cows sort for more fermentable feed particles early in the day, but also consume feed at approximately a four times higher rate after delivery of fresh feed and during the late afternoon (Niu et al., 2014). We have observed increased milk fat with feeding cows in four equal meals every six hours compared to twice per day (Rottman et al., 2014). Fresh feed delivery is a strong stimulus for feeding and feeding times may be selected to move intake into lower intake periods of the day.

Interaction of Milk Production Level and Response to Diet

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 (2006a) compared saturated (highly saturated prilled free 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 (control 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 while 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 salt of unsaturated FA, while the high producing cows decreased milk fat over 20%. A similar response was observed by (Rico et al., 2014) when comparing a high palmitic acid supplement (87% C16:0; Berga-Fat F100) to calcium salts of palm FA (Megalac) where low producing cows numerically increased milk fat with both treatments, but high producing cows decreased milk fat and increased *trans*-10 C18:1 in milk fat when fed the unsaturated palm FA. Collectively these 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.

How to Predict the Occurrence of Milk Fat Depression

The complexity of dietary fermentability and associative effects makes prediction of MFD difficult. It is arguably impossible to balance a diet that maximizes milk yield and energy intake without incorporation of numerous risk factors. Ruminant nutrition is best practiced as a continuous experiment that monitors cow response to diet modification (Allen, 2011). It is important to compare nutrient concentrations and model predictions to benchmarks and experience with similar diets. However, even with the best feed analysis, software, and experience the interaction of diet ingredients and risk of the diet is best determined by the cow and observed by titration and observation. Book values are expected to reasonably represent the FA profile of most feedstuffs. The FA concentration of byproducts should be closely monitored and we have also observed significant variation in the FA concentration of corn silages. If MFD is experienced on the farm it may be advisable to conduct a FA analysis of major forage grain sources.

The Time Course of Induction and Recovery

Traditionally, dietary factors that cause low milk fat have almost exclusively been studied through induction of MFD. This is useful because it tells us what dietary factors cause MFD, but it does not directly tell how to recover or accelerate recovery once you have MFD. The mammary gland is acutely sensitive to absorption of CLA with reduced milk fat synthesis observed within 12 h of abomasal infusion (Harvatine and Bauman, 2011). We have conducted time course experiment to characterize the timing of induction and recovery of diet induced MFD and repeatedly observe that milk fat yield decreases progressively and reaches a nadir in 7 to 10 d when feeding high risk diets [Figure 2; (Rico and Harvatine, 2013)]. When cows are returned to a recovery diet, milk fat progressively increases with a major portion of recovery occurring in approximately 10 d. Knowing the time course is very important in identifying what may have caused MFD and setting expectations and monitoring recovery from the condition.

Rapidly Recovering Milk Fat

When milk fat moves below the herd's goal, the logical approach is to systematically remove risk factors. The challenge is which risk factors to remove without loss of milk or energy intake. A multi-step approach may be best. First, determine the diet unsaturated FA level and availability. In the short term, minimizing unsaturated FA intake may be the best first step. Secondly, determine if diet fermentability is higher than optimal. In some cases reducing fermentability may reduce

sub-clinical acidosis and improve rumen function without loss of milk yield. If diet fermentablity appears within safe limits a reduction may result in decreased milk yield, so monitor production closely after making modifications. Lastly, determine if a rumen modifier can be added to stabilize fermentation. It is important to have reasonable expectations on the time-course of recovery. Dietary changes are expected to result in observable improvements in 10 to 14 d, but complete recovery will require nearly 3 weeks and maybe longer with more modest dietary changes.

Other Important Impacts on Milk Fat

Seasonal Variation in Milk Fat

Most dairy producers and nutritionists recognize a seasonal change in milk fat that is sometimes attributed to changes in forage sources, weather, or herd days in milk. A very repeatable seasonal pattern is observed in milk fat and protein concentration in all milk markets including Florida (Figure 3). In most regions of the country, fat concentration peaks in December or January and reaches a nadir in July. The range for annual cycle is approximately 0.25 percentage units. This highly repeatable pattern is reasonably independent of year-to-year differences in forage quality and weather. This seasonal variation should be incorporated into the expected milk fat concentration when setting production goals and troubleshooting milk fat production.

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 higher milk yield at the morning milking, but 0.32 and 0.09 percentage unit higher 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 2X and 3X/d. We have also observed milk yield and milk composition at each milking while milking every 6 h and feeding cows 1X/d at 0800 h or in four equal feedings every 6 h (Rottman et al., 2014). We observed a daily rhythm of milk fat synthesis and feeding 4x/d decreased the amplitude of the rhythm. This demonstrates that the daily rhythm is partially dependent on the timing of intake. We are exploring nutritional opportunities based on these rhythms including the timing of feed deliver and feeding multiple diets over the day.

Conclusions

Milk fat depression results from an interaction between ruminal fermentation processes and mammary tissue metabolism. Investigation of milk fat synthesis over the past 100 years has resulted in numerous theories. To date, the biohydrogenation theory is the only proposed mechanism that has provided causative evidence and

withstood rigorous examination. The mechanism by which biohydrogenation intermediates reduce milk fat synthesis has and will continue to provide insight into the regulation of milk fat synthesis. Milk fat depression continues to be a real-world condition that reduces the efficiency and productivity of dairy cows, but understanding its fundamental basis will allow for effective management and intervention strategies. Management of the risk factors associated with MFD is required to reach both milk and milk fat yield goals. The time course of induction and recovery can be utilized to both identify contributing factors and set expectations for recovery. Lastly, the seasonal and circadian pattern of milk fat synthesis explains variation observed between summer and winter and between milkings and should be considered in monitoring and setting production goal.

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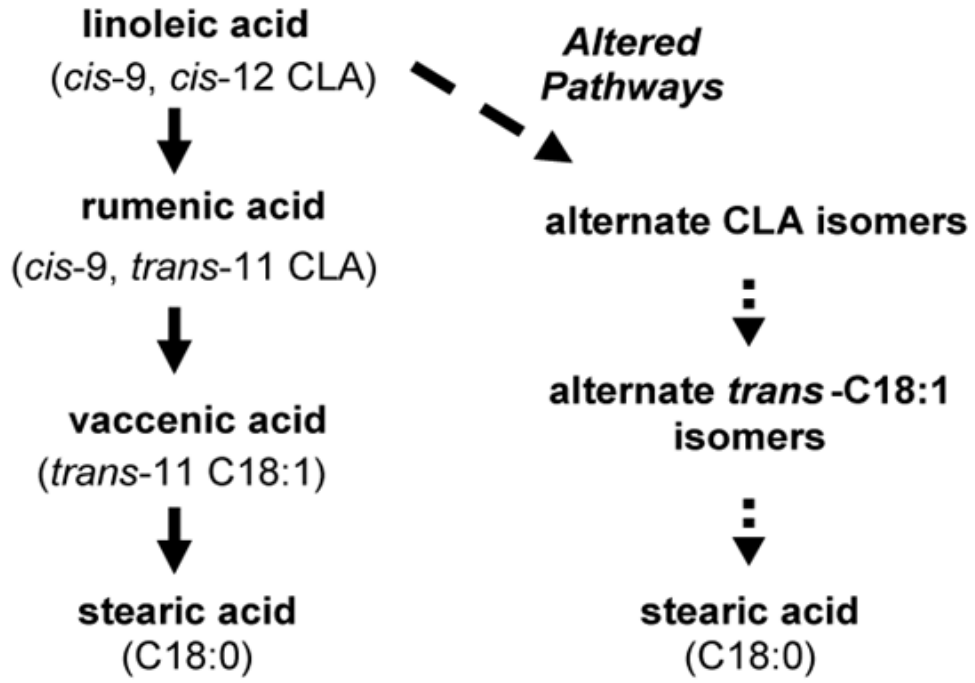

Figure 1. Biohydrogenation pathways during normal and altered ruminal fermentation. Adapted from Griinari and Bauman (1999).

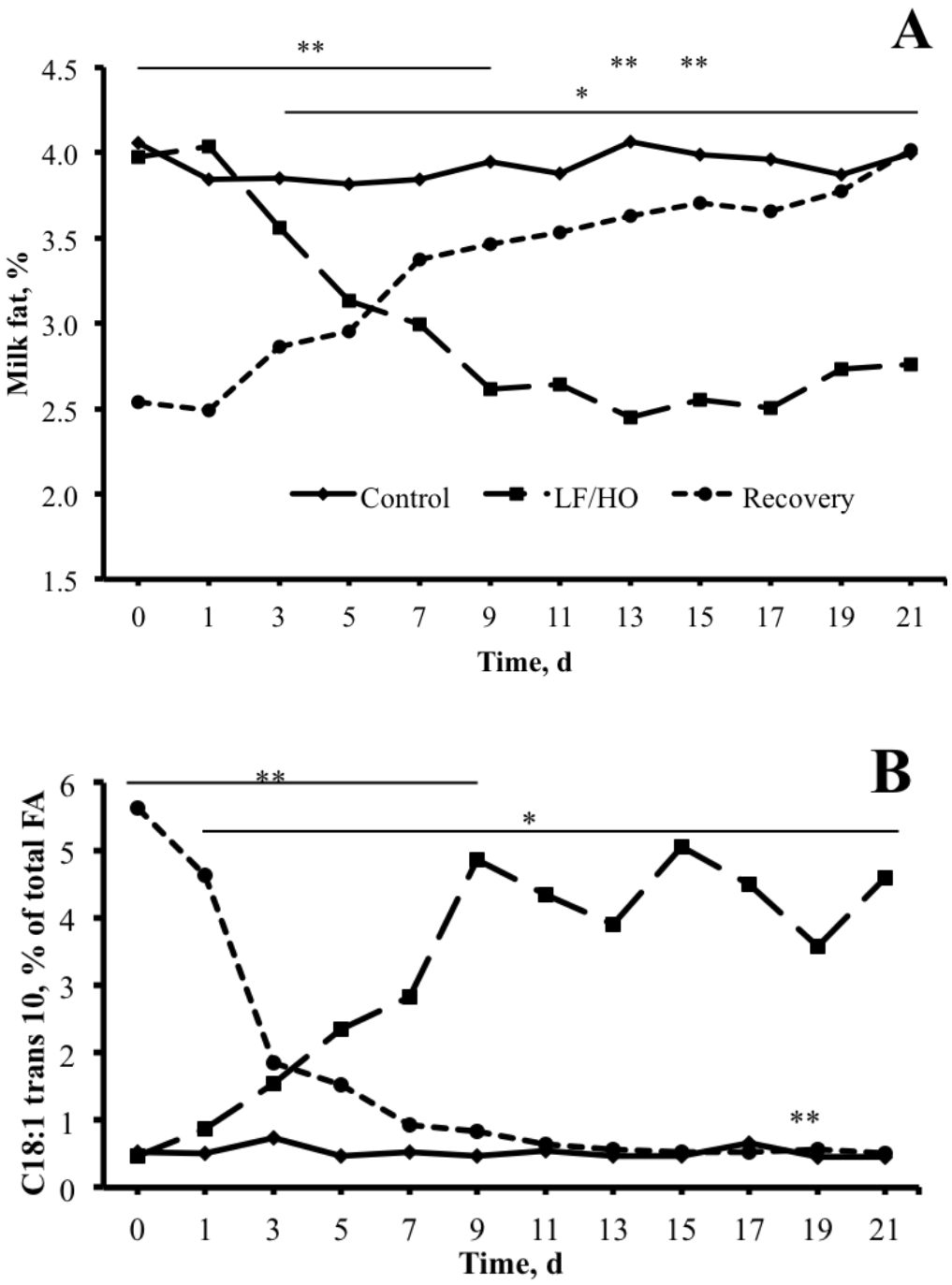

Figure 2. Temporal changes during induction of and recovery from milk fat depression. Panel A: Milk fat percent. Panel B: *trans*-10 C18:1 in milk fat.

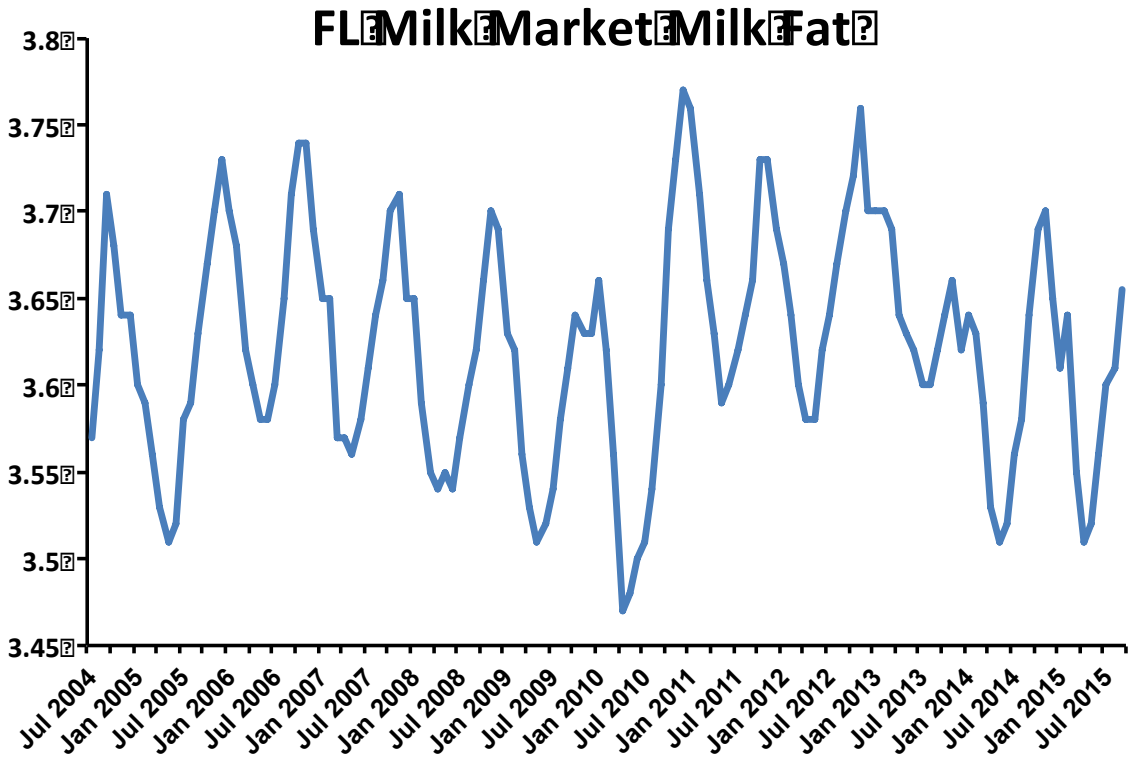

Figure 3. Seasonal pattern of milk fat in the Florida Milk Market from July 2004 to July 2015.

SESSION NOTES

Fatty Acid Digestibility and Impacts on Responses of Dairy Cows

Adam L. Lock[1] and Jonas de Souza
Department of Animal Science
Michigan State University

Introduction

The addition of supplemental fatty acid (**FA**) sources to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production. The ability to understand and model FA, the effects of individual FA, and different FA supplements on production parameters has direct impact on dairy industry recommendations and the usefulness of FA supplementation strategies. The emphasis of the current paper is on biological processes and quantitative changes during the metabolism of FA in the rumen and the effect this has on FA availability to the dairy cow, the digestibility of these FA, and their overall impact on performance. We will focus on recent research supplementing palmitic acid (C16:0) and stearic acid (C18:0)-enriched supplements on feed intake, digestibility, milk production, and milk composition.

Fatty Acid Metabolism in the Rumen

As well as being derived from specific supplements, FA in the dairy cow's diet are also present in forages and concentrates. Each feed/fat source is composed of a different mix of individual FA. The majority of FA in dairy cow diets contain 16 and 18-carbons. Generally, most cereal grains and seeds contain a high concentration of linoleic acid (C18:2 n-6), whereas linolenic acid (C18:3 n-3) is typically the predominant FA in forage sources. For example, corn, cottonseed, safflower, sunflower, and soybean oils are high in C18:2 n-6, whereas linseed is high in C18:3 n-3. Unsaturated FA are toxic to many rumen bacteria, thus an extensive metabolism of dietary lipids occurs in the rumen that has a major impact on the profile of FA available for absorption and tissue utilization (Palmquist et al., 2005). The two major processes that occur are hydrolysis of ester linkages in lipids found in feedstuffs and the biohydrogenation of unsaturated FA. A series of recent in vitro studies concluded that biohydrogenation occurs to enable rumen bacteria to survive the bacteriostatic effects of unsaturated FA, and that the toxicity of unsaturated FA is probably mediated via metabolic effects rather than disruption of membrane integrity. Furthermore, it appears that the degree of toxicity of different unsaturated FA varies for individual ruminal bacteria species; all the main species that comprise the ruminal cellulolytic bacteria appear vulnerable to inhibition by unsaturated FA (Maia et al., 2007, 2010). Biohydrogenation of unsaturated FA results in the conversion of unsaturated FA to saturated FA, mainly C18:0, through a series of biohydrogenation intermediates (conjugated C18:2 and *trans* C18:1 FA). The major substrates are 18:2 n-6 and 18:3 n-3 and the rate of rumen biohydrogenation is in the

[1] Contact at: 2265 Anthony Hall, Dept. of Animal Science, 474 S Shaw Ln, East Lansing, MI 48824-4463; Tel: (517) 802-8124, E-mail: allock@msu.edu.

range of 70-95% and 85-100%, respectively (Jenkins et al., 2008); thus C18:0 is the predominant FA available for absorption by the dairy cow under typical feeding situations (Bauman and Lock, 2006).

Fatty acid supplements are often used as a means to increase the energy density of the diet and many of these are referred to as inert. In this case inertness simply means that the FA supplement has minimal effects on rumen fermentation. Although deemed inert at the level used, they can still be hydrolyzed, if a triglyceride, or biohydrogenated, if unsaturated. Often, calcium-salts of palm FA or canola are referred to as 'protected'. However, these are not protected from rumen biohydrogenation, but rather are considered to be ruminally inert with regard to their effects on the microbial population (Palmquist, 2006).

Fatty Acid Metabolism in the Intestine

The lipid material that reaches the intestine consists of approximately 80-90% free FA attached to feed particles. The remaining lipid components are microbial phospholipids plus small amounts of triglycerides and glycolipids from residual feed material. These esterified FA are hydrolyzed by intestinal and pancreatic lipases (Doreau and Ferlay, 1994). FA absorption occurs predominantly in the jejunum region of the small intestine. Prior to reaching the jejunum, two secretions, bile and pancreatic juice, are added to the digesta in the duodenum. Before FA absorption can occur, it is necessary for the lipid material absorbed onto the feed particles to be solubilized into the aqueous environment. In ruminants, micelle formation is the key to this process and, therefore, key to efficient FA absorption (Lock et al., 2005).

During FA digestion in the small intestine, bile secretions supply bile salts and lecithin, and pancreatic secretions provide enzymes to convert lecithin to lysolecithin and bicarbonate to raise the pH. Lysolecithin acts as an amphiphile (substance with both water and lipid-loving capacity) and further increases the solubility of saturated FA (Freeman, 1969). Lysolecithin together with bile salts desorb FA from feed particles and bacteria, allowing the formation of the micelles (Lock et al., 2005). Once micelles are formed they facilitate transfer of water-insoluble FA across the unstirred water layer of intestinal epithelial cells, where the FA and lysolecithin are absorbed.

Impact of Supplemental 16- and 18-Carbon Fatty Acid on Fatty Acid Digestibility

Under typical feeding situations, C18:0 is the predominant FA available for absorption by the dairy cow, regardless of the diet fed. As result, this FA has a critical impact on total FA digestibility as we observed in a recent meta-analysis and meta-regression examining the intestinal digestibility of long-chain FA in lactating dairy cows (Boerman et al., 2015). We observed similar digestibility values among 16- and 18-carbon FA in the control diets (non-fat supplemented diet), which suggests that at low levels of FA intake, the potential differences in FA digestibility frequently presented in the literature between saturated and unsaturated FA is minimal (Figure 1A). However, when we compared the digestibility of 16- and 18-carbon FA to the digestibility of C18:0

in diets supplemented with fat across the entire data set, we observed modest differences between C18:0 and unsaturated FA (Figure 1B). Implications for differences among FA was highlighted when we generated best-fit equations for the relationship between flow and digestibility of FA (Boerman et al., 2015). We observed a negative relationship between the total flow and digestibility of FA (Figure 2A). Furthermore, the decrease in total FA digestibility appears to be driven by the digestibility of C18:0 because a negative relationship between the duodenal flow and digestibility of C18:0 was observed (Figure 2B). The exact mechanisms for the reduction in digestibility are not understood; however, potential causes include limits in lysolecithin or competition for absorption sites (Drackey, 2000). Additional research to understand the observed reduction in C18:0 digestibility and how this may be overcome or improved is required.

Our recent FA digestibility research has utilized and focused on C16:0 and C18:0-enriched supplements. Of particular importance, Boerman and Lock (2014b) fed increasing levels of a C18:0-enriched supplement (85% C18:0) to dairy cows and observed no positive effect on production responses, which was likely associated with the pronounced decrease in total FA digestibility as FA intake increased (Figure 3A). Similarly, de Souza et al. (2015) fed increasing levels of a C16:0-enriched supplement (87% C16:0) to dairy cows and even though a positive effect was observed on production response up to 1.5% diet dry matter, we observed a decrease in total FA digestibility as FA intake increased (Figure 3B). Considering the results presented in Figure 3, given that the range on FA intake is similar across both studies, the decrease in total FA digestibility is more pronounced when there is increased intake/rumen outflow of C18:0 rather than C16:0, similar to our observations in Figure 2. The exact mechanisms for these differences in digestibility are not understood; however, potential causes include the lower solubility C18:0 than C16:0, which would be more dependent of lysolecithin for absorption.

To further understand what factors influence FA digestibility, we recently utilized a random regression model to analyze available individual cow data from 5 studies that fed a C16:0-enriched supplement to dairy cows (unpublished results). We observed that total FA digestibility was negatively impacted by total FA intake, but positively influenced by the intake of *cis*-9 C18:1. This suggests that a combination between 16-carbon and unsaturated 18-carbon FA may improve FA digestibility, but reasons for this effect needs to be further determined.

Fatty Acid Metabolism in the Mammary Gland

Lipids in milk are primarily in the form of triglycerides (98%) with phospholipids and sterols accounting for 1.0 and 0.5 % of total lipids, respectively. Bovine milk is extremely complex and contains about 400 FA, a large proportion of which are derived from lipid metabolism in the rumen (Jensen, 2002). Milk FA are derived from 2 sources; <16 carbon FA from de novo synthesis in the mammary gland and >16 carbon FA originating from extraction from plasma. 16-carbon FA originate from either de novo or preformed sources. Substrates for de novo synthesis are derived from ruminal fiber digestion and dietary FA supply preformed FA for direct incorporation into milk fat

(Palmquist, 2006). Microbial synthesis of branched and odd-chained number FA in the rumen and absorption of biohydrogenation intermediates also contribute to the diversity of FA secreted in milk fat. Under typical conditions, about half of the FA in milk are synthesized de novo, 40 to 45 % originate from FA in the diet, and less than 10% are derived from mobilization of adipose tissue (Palmquist and Jenkins, 1980). However, nutrition can substantially alter the balance between mammary de novo FA synthesis and uptake of preformed FA. C16:0, C18:0 and *cis*-9 C18:1 are the major FA in milk fat. The relatively high melting point of C16:0 and C18:0 requires the production of de novo synthesized FA or the conversion of C16:0 and C18:0 to *cis*-9 C16:1 and *cis*-9 C18:1, respectively, in the mammary gland in order to maintain fluidity.

Effect of Fatty Acid Supplementation on NDF Digestibility

The amount of FA that are included in the diet is relatively small for lactating dairy cattle, and changes in FA digestibility therefore may have minimal effects on overall DM digestibility and digestible energy intake. Even significant reductions in individual FA digestibility estimates may have little effect on reducing total DM digestibility compared with reductions in digestibility of more abundant feed ingredients. Changes in intake and digestibility of other nutrients, such as NDF, due to fat supplementation may affect positively or negatively the digestible energy value of the fat supplement.

Weld and Armentano (2015) performed a meta-analysis to evaluate the effects of fat supplementation on DMI and NDF digestibility of dairy cows. Supplementation of fat supplements high in medium chain FA (C12, C14) decreased both DMI and NDF digestibility. Addition of vegetable oil decreased NDF digestibility by 2.1 percentage units, but did not affect DMI. Although feeding calcium-salts of palm FA distillate decreased DMI by 1.45 kg/day, it increased NDF digestibility by 2.2 percentage units. Overall, the authors concluded that the addition of a fat supplement, in which the fatty acids are C16 or greater in length, has minimal effects on NDF digestibility.

We recently utilized a random regression model to analyze available individual cow data from 6 studies that fed a C16:0-enriched supplement to dairy cows (unpublished results). We observed that NDF digestibility was positively impacted by total C16:0 intake and DMI was not affected. This suggests that the increase in NDF digestibility when C16:0-enriched supplements are fed to dairy cows is not explained through a decrease in DMI. Reasons for this effect needs to be further determined.

Overall Impact of Fatty Acid Supplementation on Production Responses

There is a wide range of FA supplements available for lactating dairy cattle. For example, calcium-salts of free FA and prilled saturated free FA are two common types of supplements used in the dairy industry and they differ in FA content and FA profile. Calcium-salt supplements typically contain 80-85% FA and these provide approximately 50% saturated and 50% unsaturated FA. By comparison prilled saturated free FA contain approximately 99% FA which are approximately 90% saturated, 10%

unsaturated. A summary of the FA profile of some commonly used supplements is provided in Table 1. Although in general FA supplementation has been shown to increase milk yield, milk fat yield, and the efficiency of milk production, great variation has been reported in production performance for different FA types, and indeed the same supplement across different diets and studies. This is evident in a meta-analysis examining the effect of FA supplementation to diets of dairy cows (Rabiee et al., 2012). In general milk production and milk fat content and yield increased, DMI and milk protein concentration decreased, and milk protein yield was not affected by FA supplementation. There was a wide range of responses (~5 standard deviations) for all variables, indicating varied and marked biological effects of the different FA supplements (Rabiee et al., 2012).

Utilizing a larger data set than Rabiee et al. (2012), we recently performed a meta-analysis of production responses to commercially available FA supplements (Boerman and Lock, 2014a). Available data were collected from 133 peer-reviewed publications of which 88 met our selection criteria, comprising 159 treatment comparisons. Calcium-salts of palm FA distillate (PFAD; n=73), saturated prilled FA (PRILLS; n=37), and tallow (n=49) supplemented at ≤ 3% diet DM were compared to non FA supplemented diets used as controls. Treatment comparisons were obtained from either randomized design (n=99) or crossover/Latin square design experiments (n=60). Preliminary results from the meta-analysis are shown in Figure 4.

Overall, FA supplementation increased yield of milk and milk components and reduced DMI. However type of supplement influenced response with PRILLS not reducing DMI, tallow having no effect on milk fat yield, and PFAD having no effect on milk protein yield. It is important to note that the majority of the studies reported in Figure 4 simply compared a single commercial FA supplement with a non FA supplemented control diet. This makes direct comparisons between different FA supplements difficult to interpret and importantly provide accurate answers to commonly asked questions (by farmers and nutritionists) as to which are the best FA supplements to use. There are limited reports in the published literature that have undertaken direct comparisons between different commercially available FA supplements. Results from the meta-analysis also suggest that responses to FA supplements interact with other dietary components, and this should be examined further.

Impact of Supplemental C16:0 and C18:0 on Production Responses

In the 1960's Steele and co-workers performed a series of studies using relatively pure sources of C16:0 and C18:0 and and their findings suggested that C16:0 supplementation induces a higher milk fat response (concentration and yield) when compared to C18:0 supplementation. More recent work from Enjalbert et al (1998) suggests that the uptake efficiency of the mammary gland is higher for C16:0 than for C18:0 and *cis*-9 C18:1. We have recently carried out a series of studies examining the effect of individual saturated FA on production and metabolic responses of lactating cows (Lock et al., 2013; Piantoni et al., 2013; Rico et al., 2014; Piantoni et al., 2015). These results indicate that C16:0 supplementation has the potential to increase yields of

milk and milk fat as well as the conversion of feed to milk, independent of production level when it was included in the diet for soyhulls or C18:0 (Table 2).

Rico et al. (2013) fed increasing levels of a C16:0-enriched supplement (87% C16:0) to dairy cows and observed a quadratic response with a positive effect on milk fat yield, 3.5% fat-corrected milk and feed efficiency up to 1.5% diet DM (Table 3). Furthermore, we recently utilized a random regression model to analyze available individual cow data from 10 studies that fed a C16:0-enriched supplement to dairy cows (unpublished results). We observed that energy partitioning toward milk was increased linearly with C16:0 intake, as a result of a linear increase in milk fat yield and 3.5% fat-corrected milk with increasing intake of C16:0.

Piantoni et al. (2015) reported that C18:0 increased DMI and yields of milk and milk components, with increases more evident in cows with higher milk yields, indicating that there was significant variation in response. Reasons why only higher yielding cows responded more positively to C18:0 supplementation than lower yielding cows remains to be determined. However, when we directly compared C16:0 and C18:0 supplementation the yield of milk fat and 3.5% FCM increased with C16:0 regardless of level of milk production (Table 2, Rico et al., 2014). In a recent dose response study with mid lactation cows, feeding a C18:0-enriched supplement (85% C18:0) increased DMI but had no effect on the yields of milk or milk components when compared to cows fed a non-FA supplemented control diet (Table 4), which is probably associated with the decrease in FA digestibility (Figure 3A, Boerman and Lock, 2014b).

There is mechanistic data to support the concept that individual FA can impact milk fat synthesis differently. Hansen and Knudsen (1987) utilized an in vitro system and reported that C16:0 stimulated de novo FA synthesis and incorporation into triglycerides whereas other FA were either neutral or inhibitory. In addition, there were only minor differences in the esterification efficiency into triglycerides of various FA, except for C16:0, which was a better substrate than the other FA tested. These results, in association with the digestibility results, suggest that C16:0-enriched supplements improve performance of dairy cows, while understanding factors that affect the digestibility of C18:0 with increasing intake/duodenal flow may allow the development of strategies to overcome this possible limitation.

Conclusions

The addition of supplemental FA to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production. Although in general FA supplementation has been shown to increase milk yield, milk fat yield, and the efficiency of milk production, great variation has been reported in production performance for different FA supplements, and indeed the same supplement across different diets and studies. Just as we recognize that not all protein sources are the same it is important to remember that not all FA supplements are the same. The key is to know what FA are present in the supplement, particularly FA chain length and their degree of unsaturation. The digestibility of the FA supplement, as well as its potential

interaction with other dietary factors is important to determine the energetic value of the supplement. Once this information is known it is important to consider the possible effects of these FA on DMI, rumen metabolism, small intestine digestibility, milk component synthesis in the mammary gland, energy partitioning between the mammary gland and other tissues, and body condition. The extent of these simultaneous changes along with the goal of the nutritional strategy employed will ultimately determine the overall effect of the supplemental FA, and the associated decision regarding their inclusion in diets for lactating dairy cows.

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Table 1. Fatty acid composition of common fat supplements (Data from our laboratory)

Fatty Acid, g/100 g	Tallow	Ca-salt PFAD	Saturated free FA	C16:0-enriched
C14:0	3.0	2.0	2.7	1.6
C16:0	24.4	51.0	36.9	89.7
C18:0	17.9	4.0	45.8	1.0
C18:1	41.6	36.0	4.2	5.9
C18:2	1.1	7.0	0.4	1.3

Table 2. Summary of DMI, milk production and composition, body weight, and BCS for cows supplemented with C16:0 and C18:0 supplements. The C16:0 supplement contained ~ 99% C16:0 and the C18:0 supplement contained ~ 98% C18:0

	Piantoni et al. (2013)[1]			Piantoni et al. (2015)[2]			Rico et al. (2014)[3]		
Variable	Control	C16:0	SEM	Control	C18:0	SEM	C16:0	C18:0	SEM
DMI, kg/d	27.8	27.8	0.54	25.2^{n}	26.1^{m}	0.42	32.1	32.3	0.44
Milk yield, kg/d	44.9^{b}	46.0^{a}	1.7	38.5^{n}	40.2^{m}	0.71	46.6	45.8	2.02
Fat yield, kg/d	1.45^{b}	1.53^{a}	0.05	1.35^{n}	1.42^{m}	0.03	1.68^{y}	1.59^{z}	0.05
Milk fat, %	3.29^{b}	3.40^{a}	0.11	3.60	3.59	0.12	3.66^{y}	3.55^{z}	0.09
Protein yield, kg/d	1.38	1.41	0.04	1.14^{n}	1.19^{m}	0.02	1.50	1.49	0.05
Milk Protein %	3.11	3.09	0.05	3.00	2.99	0.05	3.24	3.29	0.05
3.5% FCM	42.9^{b}	44.6^{a}	1.35	38.6^{n}	40.5^{m}	0.76	47.5^{y}	45.6^{z}	1.64
3.5% FCM/DMI	1.54^{b}	1.60^{a}	0.03	1.53	1.55	0.04	1.48^{y}	1.40^{z}	0.05
Body weight, kg	722	723	14.7	727	730	12.8	720	723	13.6
BCS	2.99	2.93	0.15	2.67	2.67	0.11	2.93^{z}	2.99^{y}	0.11

[1]Treatments were either a control diet (with 2% of diet DM as added soyhulls) or a C16:0-supplemented diet (with 2% of diet DM as C16:0). Means within a row with different superscripts ($^{a, b}$) differ ($P < 0.05$).

[2]Treatments were either a control diet (with 2% of diet DM as added soyhulls) or a C18:0-supplemented diet (with 2% of diet DM as C18:0). Means within a row with different superscripts ($^{m, n}$) differ ($P < 0.05$).

[3]Treatments were either a C16:0-supplemented diet (with 2% of diet DM as C16:0) or a C18:0-supplemented diet (with 2% of diet DM as C18:0). Means within a row with different superscripts ($^{y, z}$) differ ($P < 0.05$).

Table 3. Dry matter intake, milk production and composition, body weight, and BCS for cows supplemented with increasing levels of a C16:0-enriched supplement (Rico et al., 2013). The C16:0 supplement contained 87% C16:0

	C16:0 supplementation, % diet DM					
Variable	0%	0.75%	1.50%	2.25%	SEM	*P*-value
DMI, kg/d	28.8	28.8	28.6	27.4	0.83	0.05
Milk yield, kg/d	43.7	43.5	44.5	42.5	1.73	0.06
Fat yield, kg/d	1.63	1.69	1.78	1.70	0.09	0.01
Milk Fat, %	3.78	3.88	4.01	4.03	0.17	0.01
Protein yield, kg/d	1.36	1.36	1.40	1.32	0.06	0.08
Milk Protein, %	3.17	3.15	3.18	3.16	0.07	0.32
3.5% FCM, kg/d	45.3	46.1	48.0	45.9	1.91	0.02
3.5% FCM/DMI	1.57	1.60	1.68	1.68	0.07	0.21
Body weight, kg	703	705	701	701	25.7	0.76
BCS	2.66	2.48	2.71	2.84	0.05	0.94

Table 4. Dry matter intake, milk production and composition, body weight, and BCS for cows supplemented with increasing levels of a C18:0-enriched supplement (Boerman and Lock, 2014b). The C18:0 supplement contained 85% C18:0.

	C18:0 supplementation, % diet DM				SEM	*P*-value
Variable	0%	0.80%	1.50%	2.30%		
DMI, kg/d	28.5	29.1	29.6	30.0	0.61	0.13
Milk Yield, kg/d	38.3	38.6	38.2	37.8	1.65	0.51
Fat Yield, kg/d	1.43	1.40	1.40	1.42	0.04	0.61
Fat, %	3.79	3.72	3.74	3.82	0.08	0.29
Protein Yield, kg/d	1.33	1.33	1.32	1.30	0.05	0.49
Protein, %	3.49	3.50	3.48	3.49	0.05	0.91
3.5% FCM/DMI	39.8	39.4	39.3	39.3	1.40	0.77
FCM/DMI	1.43	1.39	1.35	1.33	0.04	0.03
Body weight, kg	738	739	735	737	12.0	0.58
BCS	3.44	3.40	3.39	3.42	0.08	0.37

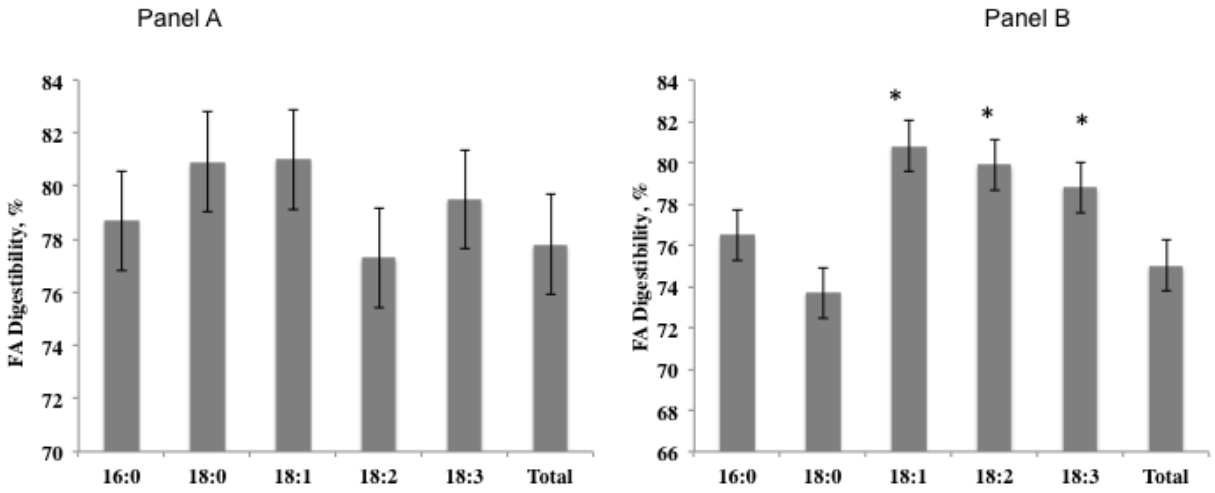

Figure 1. Meta-analysis of intestinal digestibility of FA in lactating dairy cows (Boerman et al., 2015). Apparent intestinal digestibility estimates from nonfat supplemented (control) treatments (Panel A; n = 16) and from control and fat supplemented treatments (Panel B; n = 43). Results are from 15 published studies that measured duodenal flow and intestinal digestibility of FA in dairy cows. * Refers to comparing individual FA digestibility against C18:0 ($P < 0.05$).

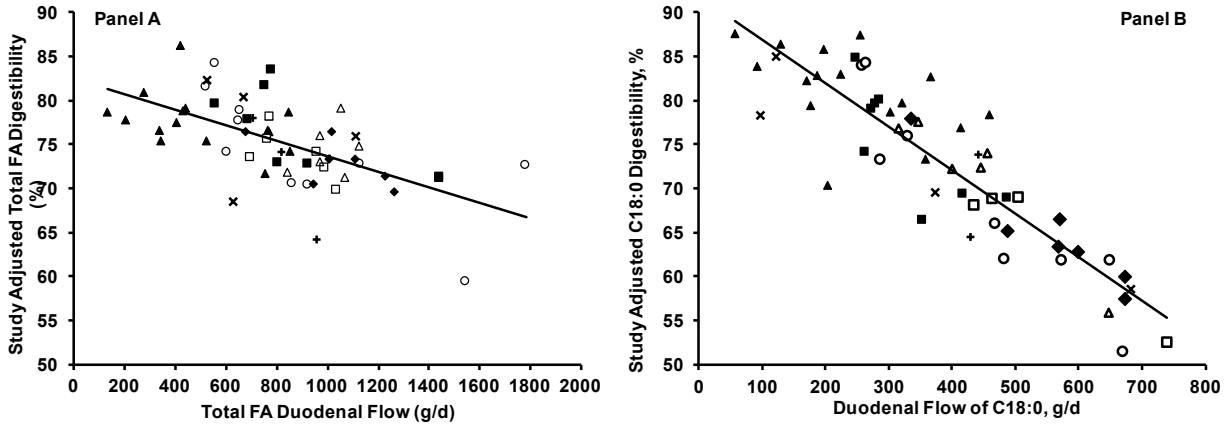

Figure 2. Relationship between study adjusted total FA intestinal digestibility and total FA duodenal flow (Panel A) and study adjusted C18:0 intestinal digestibility and duodenal flow of C18:0 (Panel B). Results from a meta-analysis using 15 published studies that measured duodenal flow and intestinal digestibility of FA in dairy cows (Boerman et al., 2015). Control treatments represented by black triangles; animal-vegetable fat treatments represented by black diamonds; calcium salt treatments represented by black squares; tallow treatments represented by open circles; vegetable oil treatments represented by open triangles; seed meal treatments represented by open squares; whole seed treatments represented by black addition sign; and other treatments represented by black multiplication sign.

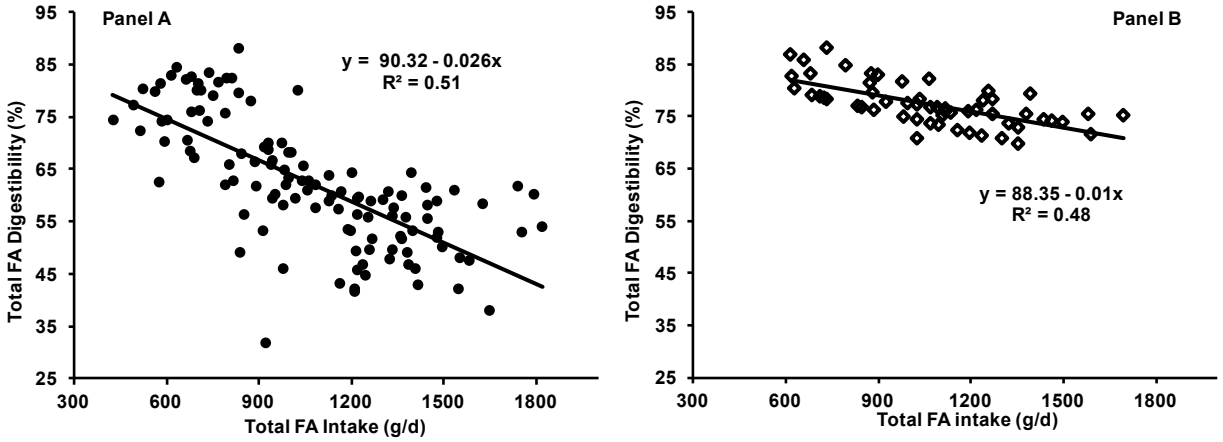

Figure 3. Relationship between total FA intake and total FA digestibility of dairy cows supplemented with either a C18:0-enriched supplement (Panel A) or a C16:0-enriched supplement (Panel B). Results in Panel A utilized 32 mid-lactation cows receiving diets with increasing levels (0 to 2.3% dry matter) of a C18:0-enriched supplement (85% C18:0) in a 4 X 4 Latin square design with 21-d periods (Boerman and Lock, 2014b). Results in Panel B utilized 16 mid-lactation cows receiving diets with increasing levels (0 to 2.25% dry matter) of a C16:0-enriched supplement (87% C16:0) in a 4 X 4 Latin square design with 14-d periods (de Souza et al., 2015).

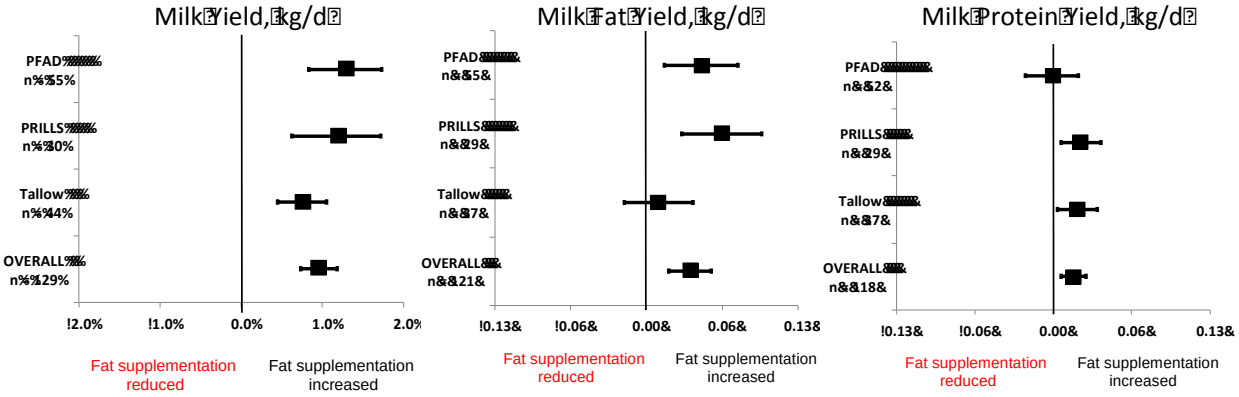

Figure 4. Effect of commercially available FA supplements on yield of milk, milk fat, and milk protein (Boerman and Lock, 2014a). All data reported in peer-reviewed journals in which FA supplements were included at ≤ 3% diet DM compared to control with no added FA supplement. All studies had to have measurements of variance reported. **PFAD** – calcium salts of palm FA distillate (~ 50% 16:0, ~ 50% unsaturated 18-carbon FA); **PRILLS** – saturated FA prills (> 80% saturated FA [16:0 and/or 18:0]); **Tallow** – animal fat labeled as tallow (~ 50% 16:0 and 18:0, ~ 45% 18:1). Data analyzed using Comprehensive Meta-Analysis (CMA) version 2.0 (Biostat, Englewood, NJ), calculating difference between FA supplemented and control diets using a random effects model.

SESSION NOTES

Protein Nutrition Evaluation and Application to Growing and Finishing Cattle

Galen E. Erickson[1], A. K. Watson, J. C. MacDonald, and T. J. Klopfenstein
Department of Animal Science
University of Nebraska-Lincoln

Protein Systems and Metabolizable Protein

Ruminants require amino acids to be supplied to organs and tissues for maintenance and productive functions (e.g., growth, lactation, pregnancy, etc). This paper will focus on growing and finishing cattle, thus maintenance and growth will be the primary target. Amino acid requirements are generally based on evaluation of amino acid uptake and retention of amino acids by organ/tissues and arterial-venous difference. Amino acids are classified into two categories, either essential (need to be absorbed or provided) or non-essential amino acids that can be synthesized. The requirements for essential amino acids are based on amino acids provided at the small intestine for absorption.

Burroughs et al. (1974) proposed a metabolizable protein (**MP**) system for ruminants because amino acids supplied at the small intestine are not just a function of dietary supply, which is different than non-ruminants. The MP system is in contrast to the crude protein (**CP**) system which refers to measuring protein based on the nitrogen content and a simple conversion by multiplying %N by 6.25. The logic for CP system is that the average of all amino acids contains 16% N by weight. Using a CP system simply refers to formulating diets based on a total %CP in the diet, which is still the most common approach used in the beef industry (Vasconcelos and Galyean, 2007). Clearly, the CP system is not logical in ruminants (although easy and simple) because not all protein are created equal in that certain amounts are required for meeting the microbial protein requirements and certain amounts are required in different scenarios in the small intestine that originate from the diet. While many nutritionists use terms like “natural” protein and non-protein nitrogen when supplementing protein, the MP system needs to be adopted.

In ruminants, predicting the amino acids supplied at the small intestine is complex. Dietary protein (including non-protein nitrogen such as urea) can be degraded in the rumen and resynthesized by microbes into new amino acids and proteins. This fraction of protein is referred to as rumen degradable protein (**RDP**) which is synonymous with degradable intake protein (**DIP**) used in the 1996 beef NRC. In essence, RDP supplies protein to meet the protein requirement for microbial growth or production of more microbial protein. However, microbes also require energy for growth (i.e., more microbial protein). Either protein (RDP) or energy limits microbial growth, just

[1] Contact at: C220 Animal Science; P. O. Box 830908, Lincoln, NE 68583-0908; Tel: (402) 472-6402; E-mail: gerickson4@unl.edu.

like energy or protein can limit growth of cattle themselves. The goal with formulation of diets is to ensure that energy is limiting microbial growth (not RDP). Meeting the RDP requirement is essential to maximize energy fermentation and microbial protein supply. Once the supply of microbial protein is predicted, then the balance of protein needed yet by the animal can be formulated.

For most feeds, a portion of protein "escapes" degradation by microbes, or "bypasses" the rumen intact either as proteins or amino acids. These fractions are called rumen undegradable protein (**RUP**), which is synonymous with undegradable intake protein (**UIP**) used in the 1996 beef NRC. The amount of RUP that is needed should be calculated as the difference between MP required at the small intestine minus predicted microbial supply.

Using the MP system illustrates that once RDP requirements are met, adding additional protein that is degradable will have no benefit on microbial growth, or the animal. Excess RDP (as ammonium in the rumen) is absorbed, converted to urea and excreted as urea in the urine. Adding more is useless, and in fact detrimental from a N excretion and ammonia loss perspective. Predicting RDP requirements is also challenging as dietary energy supply to microbes needs to be predicted, in addition to microbial efficiency. Microbial efficiency is simply the proportion of microbial protein relative to ruminally digested organic matter. In beef cattle, we have typically used total digestible nutrients (**TDN**) as a proxy for ruminally digested organic matter. As TDN increases, energy supply for the microbes increases, which increases the RDP requirement, but also may influence microbial efficiency of converting energy into protein from microbial growth. There are instances where TDN can increase without increasing ruminally digestible organic matter, such as the case with fat. Using fat-free TDN may be logical. Similarly, fermented feeds have already been partally fermented anaerobically, which logically leaves less energy available to rumen microbes. In many cases, lowering microbial efficiency has been recommended for fermented, ensiled feeds such as corn silage. Figure 1 provides the recommended microbial efficiencies we have used at the University of Nebraska-Lincoln. The1996 beef NRC predicts a decrease in microbial efficiency as dietary TDN is decreased below 65 due to decreased passage and predation. At dietary TDN above 77, microbial efficiency decreases due to low rumen pH and energy demands for microbes to maintain pH versus growth. These recommendations are based on numerous studies as outlined by Patterson et al. (2006) and the 1996 NRC. A microbial efficiency of13% agrees well with the 2001 Dairy NRC, Burroughs et al. (1974) and European data. In contrast, Galyean and Tedeschi (2014) recently proposed a simpler equation that is approximately 9% microbial efficiency which is not impacted by dietary TDN (Figure 2). Their review included data from 66 published papers with cannulated cattle fed diets that vary from 46 to 90% TDN.

Predicting microbial supply (and thus requirement for RDP) is essential in ration formulation. Microbial supply is dictated by rumen available energy and microbial efficiency, which dictates RDP required. Microbial supply also indirectly determines amount of supplemental RUP required. If the MP requirements are met by supply of

microbial protein and RUP in the feed, then adding supplemental RUP is not necessary. In general, RDP supplementation is essential for high-grain finishing diets. In general, rapidly growing cattle fed forage-based diets that require a relatively large amount of MP cannot sufficiently maximize gain or efficiency without adding supplemental RUP. These will be presented in greater detail in the growing or finishing sections.

While overfeeding RDP (best example is urea) does not contribute more MP once microbial requirements are met, overfeeding RUP does contribute more and more MP as dietary supply increases. Overfeeding RUP from supplemental protein sources that contain a large proportion of RUP (as % of CP) has been very uncommon in the beef industry. Most supplemental protein sources that are good sources of RUP are very expensive, which has lead nutritionists and researchers to focus on providing the minimum amount to meet (and not exceed) requirements. There is one exception to this historical limitation. Distillers grains plus solubles provides the most cost-effective source of RUP simply because it contains approximately 30% CP which is approximately 65% RUP (% of CP) and yet has been priced relative to corn grain the past 10 years, as supply increased dramatically. As a result, overfeeding RUP has been possible even when RDP “appears” to be limiting. Historically, RDP was cheaper to overfeed than protein sources high in RUP, yet excess RDP has no “value” to cattle when overfed. When RUP is overfed relative to MP requirements, then excess protein is still absorbed. If the amino acids are not needed (i.e., required), then excess MP is deaminated and the urea recycled to the rumen (to supply RDP) or the large intestine or saliva. Once those pools have been recycled and if RDP is still not needed based on concentration gradients, then any excess is excreted as urea in the urine. Cattle are very efficient at recycling N in the form of urea to ensure adequate RDP before excreting excess. When RUP is overfed, most nutritionists readily understand the concept of N recycling. However, two key things are misunderstood related to overfeeding RUP. The first misconception is that the energy cost of recycling N and synthesizing urea is a large cost and will influence performance. The data do not support an appreciable or measurable energy cost related to deamination of excess RUP and synthesis of urea for recycling or excretion. The second missed issue related to excess RUP is the energetics and use of amino acids as an energy source. By definition, excess RUP used for energy bypassed ruminal fermentation and thus bypassed the associated energy losses of carbohydrate fermentation in the rumen. Some inefficiencies are noted when even starch is converted to VFA and subsequently used for energy. This inefficiency is greater for fiber fermented in the rumen due to the increased molar proportion (and presumably production) of acetate relative to propionate (keep in mind that fiber would be of little value unless fermented in the hindgut, so energy inefficiency is necessary with fiber fermentation). Regardless, RUP used as an energy source has approximately 140 to 160% the energy of corn starch, which can be calculated, has been measured in finishing cattle (Carlson et al., 2016), and has been known for a very long time (Kleiber cited data from 1918 in dairy cattle).

Applied Protein Supplementation for Growing Cattle

Cows and backgrounding cattle are fed diets consisting primarily of forages. Table 1 provides CP, RDP, and RUP contents of selected feeds. Most forage protein is very degradable, whereas corn protein is generally high in proportion of RUP. The exception for corn protein is high-moisture ensiled corn, which increases degradability. Degradability of protein (and energy) in the rumen is also increased by moisture content or harvesting early (Benton et al., 2005). In addition, the RUP digestibility is certainly not 80% as assumed by the 1996 NRC. Data using mobile bag technique and removal of bacterial protein (using a NDF procedure) suggest intestinal digestibility of RUP originating from forages is low, and decreases as forage maturity increases or in dormant, low-quality forage (Haugen et al., 2006). In addition to forages being low in proportion of RUP (% of CP), most is indigestible. If forage-based diets are fed to young, growing cattle, then RUP supplementation is likely to improve gain and efficiency because growing cattle have greater MP requirements, which cannot be met by naturally occurring RUP originating from the forages.

A few sources of RUP are available, but most of these are very expensive sources of protein. Over the past decade, a large increase in ethanol production has led to a large increase in supply of competitively priced distillers grains plus solubles. Distiller's grains normally average approximately 30% CP (Buckner et al., 2011; Spiehs et al., 2002) that is 63% RUP (Castillo-Lopez et al., 2013). The benefit of distiller's grains plus solubles is that pricing is normally competitive to corn which makes the price competitive to other protein sources, plus is a good RUP source. The amino acid balance is less ideal as a protein source in grain fed cattle (but is overfed to compensate for amino acid balance). However, in forage diets, the amino acid profile is beneficial, and again, protein is normally overfed to add energy in addition to protein. Lastly, in forage-based diets, no difference has been observed between wet (**WDGS**), modified (**MDGS**; partially dried), or dried distillers grains plus solubles (**DDGS**; Ahern et al., 2015) in terms of feeding value compared to corn (about 130% of corn energy when supplemented in a forage diet. In feedlot diets, WDGS is better than MDGS, which is better than DDGS with feeding values of 135-140, 120-125, and 110-112% of corn for WDGS, MDGS, and DDGS, respectively (Bremer et al., 2011; Nuttelman et al., 2011; Nuttelman et al., 2013; Watson et al., 2014).

Three examples of recent research from the University of Nebraska will be used to document the impact of RUP supplementation in growing cattle fed or grazing forage based diets. Growing calves were fed diets of ground cornstalks (64.5%) with 30% corn distillers solubles (liquid feed from dry mill ethanol plants) which should be a diet deficient in MP, and sufficient in RDP. Calves were then supplemented with either a combination of soyhulls and urea or 2.0% treated soybean meal (Soypass) and 1.3% corn gluten meal. Soypass is 50% CP and 65% RUP (% of CP) and the corn gluten meal used in this study was a branded product (Empyreal, Cargill) that is 75% CP and 65% RUP (% of CP). Both feeds are excellent and concentrated sources of RUP. Steer calves started at 617 lb and gained either 1.27 or 1.45 lb/d ($P = 0.14$) for cattle not given RUP (Control) or given RUP (Table 2). Calves tended ($P = 0.08$) to eat more if fed the Control compared to those given RUP. As a result, calves given RUP were more efficient ($P = 0.02$) than Control. For calves grazing corn residue, supplementation is

common. A question is what supplementation is ideal for growing calves grazing residue, particularly whether RUP is limiting growth. Tibbitts et al. (2016) grazed calves weighing 516 lb initially with no supplement, corn, corn plus urea, DDGS, or RUP supplementation with soybean meal and soypass (SBM treated to increase RUP). Supplementation was formulated to provide the same amount of energy across supplements (Table 3). Calves given no supplement lost weight over the 60 day grazing. Supplementing corn to provide 3 lb of TDN allowed calves to gain 0.31 lb/d versus 0.53 lb/d with corn plus urea to meet the RDP requirement. However, feeding the same amount of energy from DDGS increased gain to 1.32 lb/d due to the RUP being provided. Lastly, feeding soypass and SBM yielded the best ADG at 1.48 lb/d which may be due to meeting both the predicted RDP and MP requirements. It is unclear whether the calves fed DDGS gained less than the SBM and Soypass treatments due to incorrect estimation of TDN of SBM/Soypass or whether calves fed DDGS were deficient in RDP that limited microbial growth or energy utilization of the diet. Either way, RUP has tremendous value with grazing calves. The best evidence of RUP supplementation benefiting growing calves is some recent data with calves fed corn silage growing diets. Corn silage growing diets is a common method used for backgrounding calves. Corn silage contains approximately 75% TDN which means if protein requirements are met, then gains and feed conversion will be better than many backgrounding programs. Another unique attribute of corn silage is the protein is mostly RDP which allows for greater opportunity for RUP supplementation to increase ADG and improve conversion. Hilscher et al. (2016) recently evaluated 0, 2.5, 5.0, 7.5 or 10.0% supplemental RUP which was a blend of Soypass and branded corn gluten meal (Empyreal, Cargill Inc.). Gain and feed conversion both improved linearly as supplemental RUP increased (Table 4). The trial was designed to determine a breakpoint for RUP supplementation or a point where ADG and F:G are improved and plateau. The greatest response was for the first 5% RUP, but ADG and F:G continued to improve but at a diminishing rate, which lead to a linear response to supplemental RUP.

These data suggest that with growing cattle, performance is dependent on energy content of the diet, but increasing gain may be realized with supplemental RUP even with adequate RDP in the diet. More research should optimize use of supplemental RUP sources and amounts in different backgrounding situations. Knowing the RDP/RUP makeup of feed ingredients is critical, as well as the energy content.

Applied Protein Supplementation for Finishing Cattle

Finishing cattle are different than backgrounding cattle as diets are mostly corn or corn and corn milling byproduct based. Because diets are high in grain, ruminally digestible organic matter or energy available in the rumen is quite high. In fact, ruminal acidosis is a result of too much and too rapid of energy digestion in the rumen. Much of the research focused on finishing cattle was targeting RDP supplementation to maximize gain and G:F (feed efficiency). Few studies have evaluated RUP supplementation as corn fed as either dry-rolled or steam-flaked corn is relatively high in RUP which may negate the need for supplemental RUP, except early in the feeding

period. Few operations will phase feed (add different supplement ingredients with stage of growth) as most operations want to feed one base finishing diet.

Three studies have evaluated RDP supplementation with finishing diets based on dry-rolled corn (Shain et al., 1998; Milton et al., 1997) or dry-rolled corn, high-moisture corn, and steam-flaked corn (Cooper et al., 2002). The requirement for RDP in dry-rolled corn diets is approximately 6.8% of diet DM, 10.1% of diet DM for high-moisture corn, and 8.3% of diet DM for steam-flaked corn based on these studies. The differences reflect changes in energy available in the rumen. A few examples will be presented where protein supplementation has been evaluated in finishing cattle in addition to some phase-feeding of RUP for finishing cattle.

Take Home Points or Considerations

1. Overfeeding RDP above requirements has no value.
2. Overfeeding RUP has value, but has historically been cost prohibitive to use excess protein as an energy source.
3. TDN is only a proxy for ruminally digested organic matter.
4. Predicting microbial protein supply and efficiency of microbial protein production under diverse dietary regimens with large numbers and production settings would be beneficial.
5. Measuring microbial flow is a major challenge, and requires the use of microbial markers and flow markers that can be a challenge.
6. The metabolizable protein system is only partially adopted or considered. There is a need to make modelling the MP system easier and accurate for adoption by commercial nutritionists.
7. Growing cattle respond to RUP supplementation in many cases, but each situation varies and needs evaluation. Forages are generally high in RDP (as % of CP) and RUP that is present in forages is low in digestibility relative to concentrates.
8. Finishing cattle certainly require RDP due to energy available in the rumen. While models suggest that cattle should respond to RUP supplementation early in the feeding period, data are variable. Because corn (and corn byproducts) are relatively high in RUP and digestibility of RUP is high, RUP supplementation later in the feeding period has limited value.
9. A long-term need exists to adopt a metabolizable amino acid system in the beef industry, similar to the trend in the dairy industry.
10. Development of models is very useful, but should be informed and developed from research data and experimentation.

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Table 1. Selected feeds with CP, RUP (% of CP), and RUP digestibility

Feedstuff[1]	CP	RUP (% of CP)	RUP dig. UNL	RUP dig. NRC[2]
Corn	8.8	60	95	90
SBM	52.9	30	98	93
SoyPass	48.9	72	97	93
Blood Meal	100	90	90	80
CGM	68.2	70	95	92
DDGS	30.8	65	89	80
Sorghum silage	9.0	20	36	55
Alfalfa hay	19.8	13	38	70
Bromegrass hay	8.3	26	44	65
Sweet Bran	23.8	25	81	-

[1] Corn = dry-rolled corn; SBM = soybean meal; SoyPass = nonenzymatically browned SBM; CGM = corn gluten meal; DDGS = dry distillers grains plus solubles; Sweet Bran = Branded type of wet corn gluten feed.
[2] NRC predicted digestibility of RUP based on the 2001 Dairy NRC.

Table 2. Performance of growing calves fed harvested cornstalks with distillers solubles (30%) with (+RUP) or without (Control) supplemental RUP as 2.0% Soypass and 1.3% corn gluten meal (Empyreal, Cargill Inc.). Adapted from King et al. (2016)

	Control	+RUP	SE	P-value
Initial BW, lb	617	618	4.9	0.91
Ending BW, lb	724	740	7.5	0.14
DMI, lb/d	13.8	12.7	0.5	0.08
ADG, lb	1.27	1.45	0.07	0.14
Feed:Gain	10.5	8.65	-	0.02

Table 3. Performance of growing calves grazing corn residue and individually supplemented with corn, corn plus urea, DDGS, or a soypass/SBM blend. Supplements were formulated to provide equal energy (TDN) and vary in protein (none, RDP, or RDP/RUP). Adapted from Tibbitts et al. (2016)

	No Suppl	Corn	Corn+urea	DDGS	Soypass/SBM	SEM
Suppl. DM, lb	-	3.75	4.0	3.0	3.5	
TDN, %	-	83%	78%	104%	90%	
TDN, lb	-	3.11	3.12	3.12	3.15	
Initial BW, lb	516	516	516	516	516	3.5
Ending BW, lb	504	539	559	629	640	4.9
ADG, lb	-0.18[e]	0.31[d]	0.53[c]	1.32[b]	1.48[a]	0.06
RDP bal, g/d	-150	-259	0	-225	12	-
MP bal, g/d	61	78	75	229	364	-

Table 4. Performance of backgrounding calves on corn silage based diets and individually supplemented with 0 to 10% supplemental RUP. Supplement was included at 12% and soyhulls and some urea was replaced with a 60:40 blend of SoyPass and Empyreal (Cargill Inc.). Adapted from Hilscher et al. (2016)

	Supplemental RUP					Contrast	
	0%	2.5%	5%	7.5%	10%	Linear	Quad
Initial BW, lb	595	597	597	596	600	0.98	0.60
Ending BW, lb	791	824	855	842	868	<0.01	0.88
DMI, lb/d	16.9	18.3	18.9	17.4	18.4	0.05	0.84
ADG, lb	2.51	2.91	3.31	3.15	3.43	<0.01	0.82
Feed:Gain	6.74	6.26	5.71	5.52	5.35	<0.01	0.57

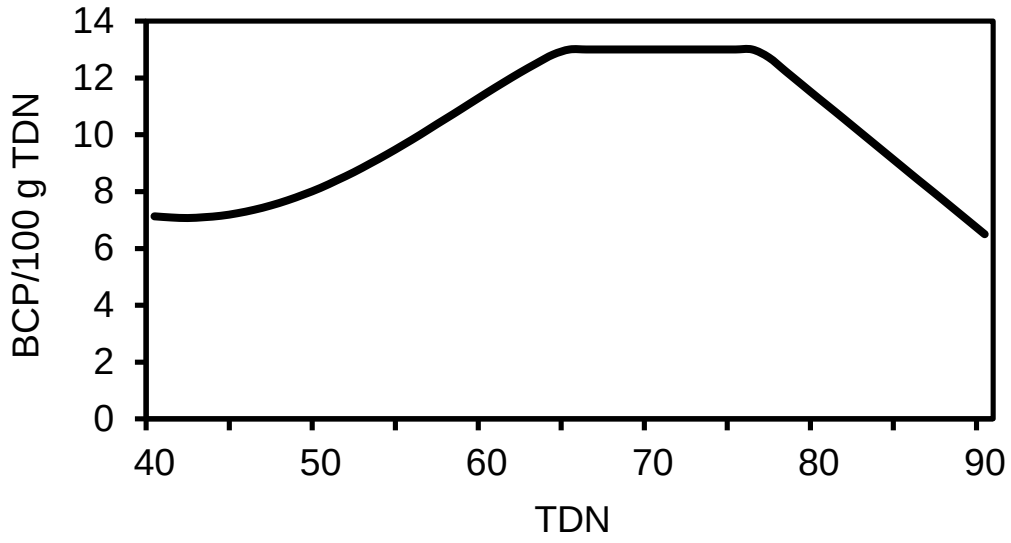

Figure 1. Microbial efficiency expressed as microbial protein (BCP) per 100 g of TDN. Efficiency varies as dietary TDN varies. Adapted from Patterson et al. (2006) and 1996 NRC. If TDN <65% then BCP=2.619948 + 1.78321X-.095981X^2 + .001777X^3 - .000010524X^4.

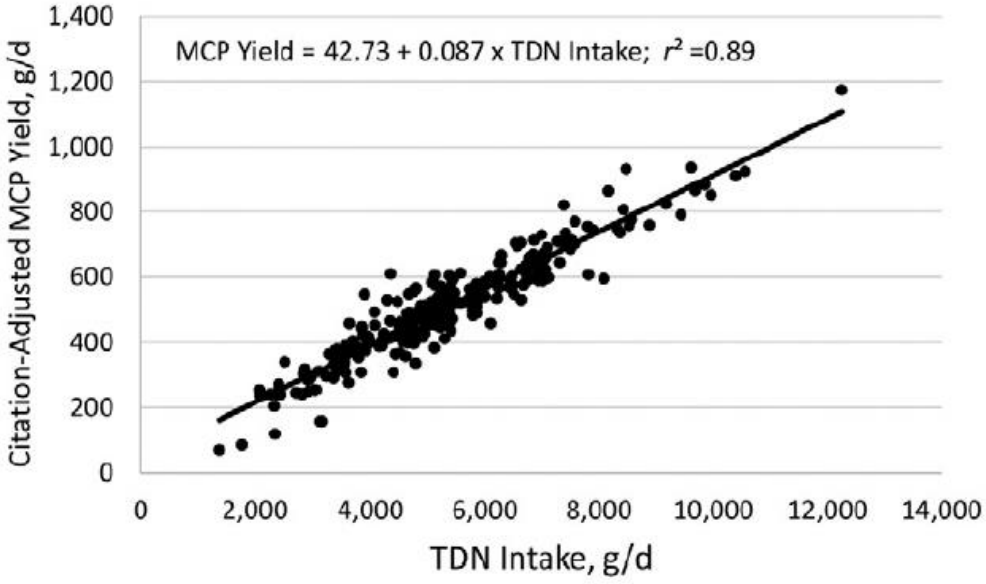

Figure 2. Proposed microbial efficiency of BCP = 42.73 + 0.087 (TDN intake, g) by Galyean and Tedeschi (2014) based on literature review including all studies.

SESSION NOTES

Alternative Feeds for Beef Cattle

Tara L. Felix[1]
Department of Animal Science
The Pennsylvania State University

Introduction

As a beef cattle nutritionist, two things have been drilled into to me from early on: 1) the cheapest way to feed the cow is to let her "harvest" her own feed by grazing, and 2) corn is the cheapest source of energy for fed cattle. However, fluctuations in corn and land prices in more recent years have raised questions regarding the truth in these old adages. The objectives of these proceeding are to explore the historic feeding strategies for cattle, discussing their benefits and limitations, and provide information on alternative feeds for beef cattle producers to take advantage of.

Forages

Although beef cattle producers and nutritionists have always relied on cow grazing to reduce input costs in their systems, many challenges are associated with this scenario. While the cows cheapest source of energy may be the one she harvests herself (i.e. grazed forages), the quantity and quality of those forages do not always match the cows requirements (Sollenberger and Chambliss, 1991). More often than not, calving seasons correspond more closely with poor forage quality and limited quantity. This is a major challenge to overcome because calving is also the time of the greatest energy and protein requirements of the dam (NRC, 2000). Therefore, while the cow is undergoing uterine involution, nursing a calf, and attempting to return to estrus and rebreed, she is often doing so with a limited supply of nutrients from pasture (Whittier et al., 1993). In addition, harvested forages may be fed to growing calves in confinement feeding systems (Murphy and Loerch, 1994). Feeding forages to growing cattle allows the deposition of lean gain, without the worries of over-fattening calves. However, there are challenges to feeding forages in confinement as they can be bulky, difficult to handle, and may have poorer quality. When harvested forages are used to supplement cattle requirements, they can be quite costly to an operation, depending on the source, year, and so on.

Corn

Cattle can be fed unprocessed, whole kernel corn to supplement their energy need; and, when corn was $2/bushel, this was a cheaper alternative to some harvested forages (Wright, 2005). Traditionally, corn grain is processed prior to inclusion in beef cattle diets, particularly in feeder cattle diets, to improve starch digestion, feed efficiency, and physical characteristics of the diet (Owens et al., 1997). However, the

[1] Contact at: Department of Animal Science, 324 Henning Building, University Park, PA 16802; Tel: (814) 865-0065; E-mail: tfelix@psu.edu.

slower rate of fermentation of the whole kernel, may improve rumen synchrony when feeding corn in combination with a forage (i.e. as a supplement to grazing). Much of the rationale for corn processing then centers on traditions and perceptions. Ørskov (1986) reported that processed corn may be favored over feeding whole corn because of the visual appearance of whole kernels in the feces. i.e. more kernels are perceived to be in the feces of cattle fed whole kernel corn than those fed processed corn. However, researchers have quantified the excretion of whole kernels and found that less than 2% of the kernels consumed were present in the feces (Gorocica-Buenfil and Loerch, 2005). In feedlot settings, up to 80 or 90% of the ration DM would have been corn as recently as the late 90's. However, in the current U.S. bioenergy environment, there is a "newfound" reliance on corn grain to provide energy for fuel. This shift in emphasis on corn for fuel instead of feed has driven up the competition for corn grain and, thus, the cost.

Alternative Feeds

Due to the increasing costs associated with corn grain and harvested forages (and land!), beef cattle producers are turning to alternative feeds to meet cattle protein and energy requirements. The best alternative feeds for beef producers are those that are cheap and readily available. To remain economically viable, secondary products of other industries can, and should, be taken advantage of in beef cattle diets. These proceedings will focus on alternative feeds for beef cattle systems with added emphasis on products available throughout the Southeastern United States.

There are a large variety of alternative feeds in the United States. These feeds are sometime referred to as "millfeeds", as they are the products of processing materials, be they plant or animal, for human use. These feeds have also been termed by-products or co-products, and, as in this proceeding, alternative feeds. These terms may be used interchangeably. It is important to note that as grain processing is an ever-changing field, so are the alternative feeds from those processes. Thus, one critical component to using these alternative feeds effectively is obtaining a chemical analysis and knowing the nutrient composition of the material, in order to best meet the animal's requirements. Each scenario may have a different "optimum inclusion" of the products and this optimum is dependent on cost, availability, roughage inclusion, water source, and the production system. Because of the challenges associated with consistency of alternative feeds and the lack of peer-reviewed publications on the topic, more of the published information regarding alternative feeds are found in extension-type articles. Therefore, these articles have been included and cited in an attempt to make the proceedings complete.

Distiller's grains with solubles. Among the most popular alternative feeds in the Midwestern United States are the co-products of corn processing. For example, distiller's grains with solubles (**DGS**) have been an important, low cost protein source for beef cattle producers for over 3 decades. Demand for DGS increased as the cost of corn reached up to $8/bu and they became an economically attractive source of energy. However, there are 3 major challenges when feeding large amounts of DGS. #1 Protein:

The “traditional” DGS diet may have contained approximately 25% DGS on a dry matter basis (**DMB**) and supplied approximately 14.3% crude protein (**CP**) on a DMB to the diet. When corn prices sky-rocketed though, it was not uncommon to see feedlot diets that included 50% DGS, increasing dietary protein to roughly 19% (DMB). That shift had some researchers questioning the long term ramifications of feeding so much excess protein, not only on the environment, but also on the animal. #2 Fat: Another challenge with using DGS as an energy source has been the fat content. Feeding fat in excess reduces fiber digestibility and cattle performance. Some DGS may contained as much as 10 to 12% fat (DMB). While fiber digestibility was not a major concern for feedlot owners, it had some cow producers turning to a lower fat alternative, like corn gluten feed (**CGF**). Fat content represents another avenue of income for ethanol companies, however, and many Midwestern plants now de-oil their DGS and sell a product containing as little as 3 to as much as 8% fat. #3 Sulfur: The 3rd major issue with feeding DGS has been sulfur content (Felix et al., 2011). Unfortunately, due to the use of sulfuric acid in the production of ethanol, this one may not be an easy fix. Some new investigations have looked at using phosphoric acid in place of sulfuric, but the efficiency of ethanol production using this technique has not been good enough for it to become an industry standard. That said, most plants will have a sulfur value on their DGS, but that value may vary within plants and between plants. The typical range of sulfur in DGS can be anywhere from 0.35 to 1.00% (DMB). The moral of this story is to test DGS and/or ask for the plants analysis of their DGS. Two important considerations with DGS are cost and availability. The cost of DGS follows the cost of corn. As corn price increases, price of DGS increases. In August of 2012, the drought in the Midwest had driven the cost of corn so high, that several ethanol plants were no longer running. Availability of DGS became a serious issue. Similarly, in the fall of 2015, demand for DGS was so great that it became, temporarily, more expensive than corn. Remember, the goal of alternative feeds should be to reduce input costs. In the Southeastern States, with less access to corn processing plants, reliance on DGS will likely not be the norm.

Other fibrous feeds can also be used with great success in cattle diets. These fibrous feeds include brewer’s grains, soybean hulls, cottonseed products, and citrus pulp. As these feeds are byproducts of their respective industries, as with any byproduct feed, the most important thing for a nutritionist to do is get a nutrient analysis of each new load.

Brewer’s grains. Brewers grains are the byproducts of brewing different grains, but predominately barley, for the beer industry. They are typically a regionally available feed that can be sourced relatively inexpensively. One of the reasons that brewers grains are growing in popularity with the beef industry is the increasing availability. From 2013 to 2014 alone the total number of breweries in the U.S. has grown by 18.6% (Brewers Association, 2014). Much of this growth has been realized in small operations, or “microbreweries and in regional craft breweries. In fact, in 2014, the growth of craft beer production rose 9.6% even while overall beer production fell 1.4% (Morris, 2014). For example, Florida’s craft brewing industry produces over a million barrels of beer annually (Brewers Association, 2014). Because of the unique nature of the beers these

breweries sell, nutritionists and producers wishing to capitalize on these byproducts should recognize the inherent variability from source to source *and* even within a source. The NRC (2000) states that brewer's grains contain 26 to 29% CP and 6 to 10% fat (DMB). However, reports of up to 12% fat, or greater, have been cited (Long et al., 2015). In many instances, the biggest challenges associated with sourcing brewers grains for cattle producers are the storage and handling of the product. Brewers grains will be cheapest when sourced wet and contain as much as 79% moisture in these circumstances (NRC, 2000). Handling and storing a product this wet, particularly in a warmer climate, presents challenges with runoff, spoilage, and equipment.

Soybean hulls. Another alternative feed to consider is soybean hulls. While whole soybeans have a number of antinutritional factors that can be discussed (such as phytoestrogens, goitrogens, etcetera), soybean hulls are heated and processed, thus, most of the antinutritional factors routinely attributed to soybeans are eliminated. However, caution should still be taken and soybean hulls should not be included at more than 30% of the diet as they have been known to cause bloat at these upper inclusions (Rankins, 2011). Because of the uniformity of the soybean industry, soybean hulls tend to be the most consistent of the alternative feeds and contain 12.2% CP (NRC, 2000).

Cottonseed byproducts. One of the more unique alternative feeds for the Southern States to capitalize on in particular are cottonseed byproducts. There are a number of cottonseed products to choose from as cattle feeds, including whole cottonseed, cottonseed hulls, cottonseed meal, and gin trash (Stewart, 2010). Whole cottonseed can come delinted or "fuzzy". Fuzzy cottonseed has been touted by some as a near perfect supplement for cattle (Blezinger, 1999) because it contains the meat and oil from the seed, as well as some additional fiber from the fuzzy lint that covers the seed. It typically contains 15 to 21% CP and 15 to 17% fat (DMB); however, its nutrient composition too can be affected by growing and harvest conditions, thus analysis is recommended. Because of the high fat concentration in whole cottonseed, their inclusion in the diet is often limited to 15% of the DM (Blezinger, 1999). Cottonseed hulls are simply the outer seed coat that is removed before the grain is processed for oil. Cottonseed hulls contain very little protein (approximately 4%; NRC, 2000) and are mostly fiber (90% NDF on a DMB; NRC, 2000). Therefore, they are more applicable in a situation where additional "filler", or fiber, is needed, such as a growing cattle diet or cows fed a mixed ration. Cottonseed meal is a popular source of protein for cattle feeders. At 36 to 41% protein, is a concentrated option for producers that do not have access to the Midwestern corn milling products. In addition, because its poor quality protein is not favored by swine and poultry nutritionists, cost tends to be favorable (Jurgens and Bregendahl, 2007). Finally, gin trash can be used as a cattle feed and is best recommended for cows in the last trimester, due to its poor digestibility and limited nutrient supply (Stewart, 2010). Gin trash is a feed that will provide an economic feed for cows without putting too much fat on them; however, it may not be palatable when first fed. Adaptation to gin trash is advisable then. Caution should be exercised when feeding cottonseed products, and largely centers around gossypol in the diet. Gossypol is a problem that nonruminant nutritionists are extremely familiar with. However, the

rumen microbes do degrade the majority of gossypol entering cattle. Still, gossypol poisoning may reduce fertility in breeding bulls, females seem more resistant to these effects, and it may reduce intake and gains in growing animals, if overfed (Poore and Rogers, 1998; Stewart, 2010).

Peanut byproducts. Similar to cottonseed, there are a variety of products available from peanut processing, including: hulls, skins, and meal. However, these products are less widely available and should be fed with some caution. For example, although peanut meal may contain as much as 45 to 55% CP (DMB), it is often lysine deficient. In addition, the protein that is present is often less digestible than other high protein supplemental feeds, likely due to the tannin concentration in peanuts. From a health standpoint, peanut products are prone to aflatoxin contamination (Kellems and Church, 2010). Aflatoxins can cause fertility issues, including abortions, and suppress growth. On top of the troubles with aflatoxin, associated with all peanut products, peanut hulls also have very little energy. Because of this, peanut hulls are generally only used when other alternative feeds with comparable fiber characteristics, like cottonseed hulls, are too expensive or unavailable (Blezinger, 2003).

Citrus byproducts. Citrus byproducts are unique to the Southeastern U.S. and CA. These byproducts can include citrus meal or citrus pulp, although citrus pulp is the more widely used product for cattle. Citrus pulp can be an excellent feed for cattle. The fiber fractions in citrus pulp are very digestible, making it a popular choice for growing animals. In fact, citrus pulp has been successfully fed to growing cattle at up to 50 to 60% of the diet (Kellems and Church, 2010). However, additional protein sources will need to be considered when feeding such great amounts to growing cattle and citrus pulp contains only 5 to 8% CP (DMB). In addition, citrus pulp is heavily used in the dairy industry due to its fiber concentration and palatability; thus, access for beef producers may be more limited (Jurgens and Bregendahl, 2007).

Conclusions

When feeding alternative feeds to cattle, they should always pencil in the operation. Myer and Hersom (2003) provide an excellent overview on determining the value of several alternative feeds relative to corn and cottonseed meal. The key for beef cattle producers and nutritionists alike will be to stay on top of new information. Most importantly, however, is to remember that byproducts are secondary to the plants processing the whole grains. Therefore, the composition of these byproducts should always be determined before decisions regarding which to use in the ration, and at what dietary inclusion they will be incorporated, are made. It is important to determine what works best in your production system. Cost should drive much of the decisions regarding “optimum” inclusion of alternatives feeds in beef rations. As is typical, these decisions will have to be made quickly to take advantage of opportunities as they arise and the need for rapid dissemination of new information on alternative feeds will be paramount.

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SESSION NOTES

Control of Hepatic Gluconeogenesis During the Transition Period

Shawn S. Donkin[1]
Department of Animal Sciences
Purdue University

Introduction

The importance of gluconeogenesis, a metabolic pathway that results in formation of glucose from non-carbohydrate carbon substrates, is underscored in dairy cattle by to the lack of intestinal glucose absorption that occurs as a consequence of the extensive fermentation of free dietary carbohydrate in the rumen. In early lactation greater than 90 % of whole animal glucose requirements are met through endogenous glucose production and liver is the primary site of synthesis for glucose that is available for metabolism and mammary lactose synthesis. Lactation and gestation impose the greatest demands on glucose economy of ruminants and ketosis and pregnancy toxemia are commonly linked to gluconeogenic insufficiency. Transition to lactation in dairy cattle represents one of the most dramatic changes in glucose metabolism. Understanding of the control of gluconeogenesis in transition cows has the potential to yield management strategies to increase glucose supply for optimal milk production and to alleviate clinical diseases linked to glucose insufficiency. This review will provide background information on control of gluconeogenesis with a primary focus on emerging information on control of gluconeogenesis in transition dairy cows.

What Is Gluconeogenesis and Why Is It Important?

Gluconeogenesis is the process of formation of new glucose in the body. This process occurs primarily in liver and to a lesser extent in kidney and serves to assemble small carbon-containing compounds into a six carbon glucose molecule. The resulting glucose is then available for distribution to other tissues in the body for immediate metabolism, lactose synthesis in the case of mammary tissue, or for storage. Because milk lactose is derived from blood glucose the capacity for milk production is directly determined by the capacity for gluconeogenesis in liver and the ability of other tissues to spare glucose use during lactation and make it available to the mammary gland. However the supply of glucose to several tissues is essential for their normal function. Red blood cells require glucose as an energy source, the brain and central nervous tissue oxidize glucose (Mayes, 1996), and in ruminants the source of the glycerol part of the triglyceride molecule in adipose tissue and milk is derived from glucose.

How Much Glucose is Needed by Dairy Cows?

Blood glucose concentrations are controlled within fairly narrow limits under normal physiological conditions. Whole body glucose metabolism is characterized by

[1] Contact at: Department of Animal Sciences, 915 West State Street, West Lafayette, IN 47907-2054. E-mail: sdonkin@purdue.edu.

the appearance of glucose in blood from either intestinal absorption or gluconeogenesis and the removal of glucose by peripheral tissues. Because of the appreciable fermentation of starch and free sugars in the rumen there is little glucose absorbed form the diet of dairy cattle. The concentration of glucose plasma is function of the entry of glucose from gluconeogenesis (and other sources) and the removal of glucose by extrahepatic tissues (Figure 1). Consequently changes in blood glucose concertation can reflect any of the possible combinations of contribution to, or pull form, the circulating glucose pool. Although feed processing may affect starch escaping form the rumen and availability for absorption as glucose numerous studies indicate that the net flux of glucose from the portal drained viscera (**PDV**; gut, pancreas, spleen and associated adipose tissue) is negligible (Reynolds, 2006).

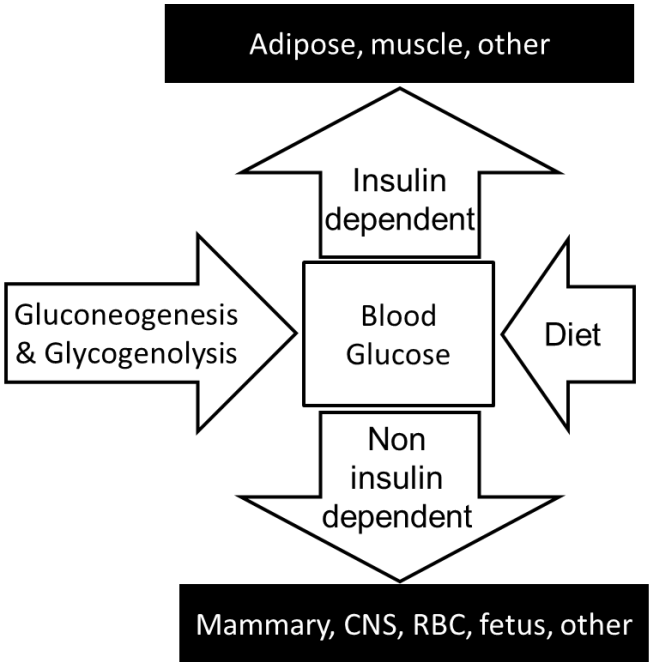

Figure 1. Factors controlling blood glucose concentrations. The appearance of glucose in blood is a function of glucose adsorption from glucose in the diet and gluconeogenesis. The removal of glucose from blood to tissues can be either insulin mediated (as per adipose tissue, muscle and other tissues) or non-insulin dependent (as per mammary tissue, central nervous system (**CNS**), red blood cells (**RBC**) and the developing fetus). The concentration of glucose in blood is the combined result of these physiological events.

Estimates of glucose needs for maintenance functions for dairy cows range between 200 g/d based on the data of Bickerstaff et al. (1974) to more than 400 g/d based on data from steers to estimate maintenance needs of cattle (Reynolds et al, 1991). During the transition to lactation the needs for gluconeogenesis increase abruptly from 1200 g/d at 21 days prior to calving to approximately 3 kg/d at 3 weeks postpartum as milk production increases to 80 lbs/d (36 kg/d) (Reynolds et al., 2003; Aschenbach et al., 2010). Estimates of glucose needs are derived from estimates of glucose needed for maintenance and the need for lactose synthesis as well as needs for oxidation by

mammary tissue. The latter has been estimate using the quantity of lactose produced per day multiplied by 1.5 to account for glucose use by mammary tissue other metabolism that does not directly yield milk lactose (Hanigan et al., 1992).

Adequate feed intake is needed to provide the precursors for gluconeogenesis from rumen fermentation. Consequently the capacity for glucose production is closely matched by the capacity for energy intake and in particular for energy sources that provide glucogenic precursors, particularly propionate. Because transition cows often voluntarily self-impose feed intake limitations there is a need to adapt liver metabolism to use alternatives sources of glucose carbon such as lactate and amino acids. This capacity is achieved through changes at the cellular and molecular levels in liver cells (hepatocytes) in response to signals received from extrahepatic tissues including increased circulating fatty acids, changes in other metabolites or hormones such as insulin, glucagon and glucocorticoids.

Substrates for Gluconeogenesis and Primary Control Points and Processes

The main substrates for glucose synthesis in fed ruminants are lactate, propionate and amino acids. Glycerol, from adipose tissue, can also contribute carbon for glucose synthesis during feed restriction and energy deficiency. Propionate contributes approximately 50% of the carbon for gluconeogenesis while lactate or amino acids contribute 10-15% each (Huntington, 1990). In addition the use of amino acids for glucose synthesis may be of considerable importance when intake is depressed in late gestation (Bell et al., 2000).

Pyruvate is a common entry point in the gluconeogenic pathway for lactate, alanine or other gluconeogenic amino acids. Alanine accounts for approximately 24% of the net portal appearance of amino acid nitrogen in fed ruminants (Reynolds et al., 1991). A lack of increase in hepatic glucose output in response to mesenteric vein infusions of alanine in cattle is accompanied by decreased liver extraction of lactate (Reynolds et al., 1991) and suggests a common point of regulation for lactate and alanine metabolism to glucose.

During gluconeogenesis pyruvate formed from lactate, alanine and other amino acids is transported into the mitochondria and carboxylated to oxaloacetate by pyruvate carboxylase (**PC**; Figure 2). In contrast, propionate is metabolized through part of the TCA cycle to oxaloacetate. Oxaloacetate can be metabolized to phosphoenolpyruvate (**PEP**) by phosphoenolpyruvate carboxykinase (**PEPCK**) or metabolized in the tricarboxylic acid (**TCA**) cycle. In turn, PEP carbon can be metabolized to glucose or recycled to pyruvate via pyruvate kinase (**PK**). In order for lactate carbon to be metabolized to glucose, the flux through PEPCK and PC must exceed the PK flux, whereas net flux of propionate carbon requires only a greater flux through PEPCK relative to PK and PC.

Hormonal control of metabolism is determined by changes in hormone concentrations and the ability of tissues to respond to those changes. The rates of

glucose production and utilization are regulated by insulin, glucagon and glucocorticoids in ruminants (reviewed by Brockman, 1986). Blood insulin concentrations are responsive to diet (Jenny and Polan, 1975), stage of lactation (Herbein et al., 1985), and dietary differences within developmental state (Breier et al., 1988). Developmental differences have been identified for the acute responsiveness of hepatocytes to insulin and glucagon (Donkin and Armentano, 1993; Donkin and Armentano, 1995). Data indicate that direct insulin and glucagon directly regulate in gluconeogenesis in ruminants, and insulin opposes the effects of glucagon and suggests PEPCK as a major regulatory site in this regard.

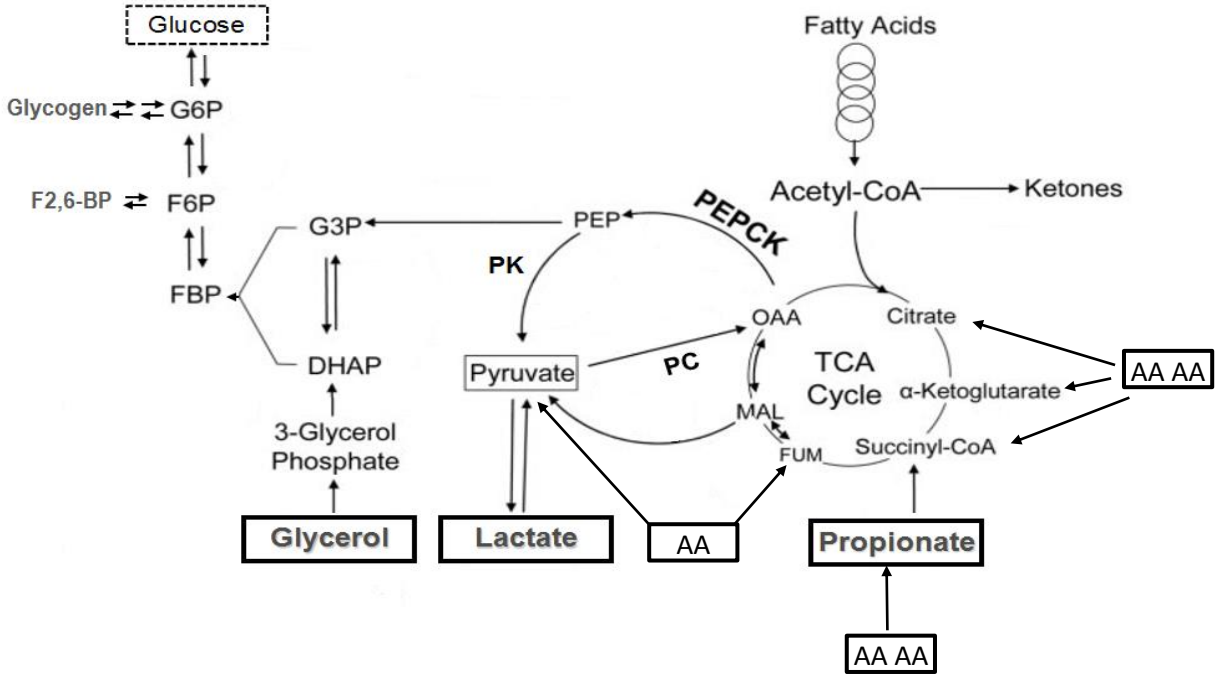

Figure 2. Metabolism of propionate, lactate, pyruvate, amino acids and glycerol to glucose in bovine liver and relationship to fatty acid oxidation. Abbreviations: Glucose-6-phosphate (**G6P**), Fructose-6-phosphate (**F6P**), Fructose-2, 6-bisphosphate (**F 2,6-BP**), Fructose-1,6, bisphosphate (**FBP**), dihydroxyacetone phosphate (**DHAP**), glycerol 3 phosphate (**G3P**), phosphoenolpyruvate (**PEP**) oxaloacetate (**OAA**) fumarate (**FUM**), malate (**MAL**), pyruvate kinase (**PK**), pyruvate carboxylase (**PC**), phosphoenolpyruvate carboxykinase (**PEPCK**), Amino acids (**AA**). Solid boxes indicate precursors for glucose synthesis.

Control of gluconeogenesis in non-ruminants occurs mainly in three distinct segments of the gluconeogenic pathway. First through the combined actions of PEPCK and PC, described above, which to oppose the actions of PK. These reactions generate PEP which is subject to further metabolism to dihydroxyacetone phosphate (**DHAP**) or glycerol-3 phosphate (**G3P**) and are substrates for aldolase in the formation of fructose 1,6, bisphosphate. The opposing reactions of fructose 1,6, bisphosphatase to form fructose-6-phosphate and phophofructokinase-1 to form fructose 1,6, bisphosphate

determine the relative flux of carbon to gluconeogenesis or glycolysis respectively. After subsequent isomerization of fructose-6-phosphate to glucose-6-phosphate the relative activities of glucokinase and glucose-6-phosphatase (**G6Pase**) determine the availability of free glucose for release from liver. The resulting free glucose in the endoplasmic reticulum is released from the hepatocyte through the action of facilitated glucose transporter 2. The combined flux through the three reaction loci that result in formation of PEP, fructose 1,6, bisphosphate and free glucose and their opposing reactions determines the net flux of non-carbohydrate precursors to glucose. Control of gluconeogenesis, like most metabolic processes, occurs through a combination of substrate availability, short term hormonal regulation, allosteric regulation, and regulation involving changes in gene expression. Overall homeostatic and homeorhetic control of gluconeogenesis occurs through combinations of these primary modes of metabolic control and action on the three reaction that distinguish gluconeogenesis and glycolysis. Although these reactions and their control have been broadly explored across species there a need for additional specific information for ruminants on all levels of control of gluconeogenesis.

Glucose precursors are supplied to liver through absorption across the rumen wall and other portions of the gastrointestinal tract into the hepatic portal vein. The provision of glucogenic precursors is critical for hepatic glucose production. In vitro experiments using bovine hepatocytes indicate maximal gluconeogenesis from propionate, lactate, and glycerol that occurs between 2 and 4 mM (Donkin and Armentano, 1994) which correspond to concentrations that are well above physiological levels for the hepatic portal vein (Reynolds et al., 1988a). Despite additional capacity for gluconeogenic precursor metabolism with increased substrate supply it appears that this is not a primary regulator of glucose output in vivo as short term infusion of alanine into the mesenteric vein in beef heifers (Reynolds and Tyrrell 1991) or propionate in mid-lactation dairy cows (Casse et al. 1994) had little impact on hepatic glucose output. In contrast, prolonged changes in supply of glucose precursors appear to alter the capacity for gluconeogenesis through changes in expression of key genes in gluconeogenesis (Karcher et al., 2007).

Gluconeogenesis is also controlled allosterically. Acetyl-CoA, is a known allosteric activator of PC and ruminant PC has served as a model to characterize these effects of acetyl-CoA on PC activity (Easterbrook-Smith et al., 1979). This allosteric activation and the increased non esterified fatty acid release from adipose tissue with feed restriction and transition to lactation (Greenfield et al. 2000; Velez and Donkin, 2005) and subsequent metabolism through β –oxidation in liver to acetyl CoA may be instrumental in amplifying changes in PC expression for increased gluconeogenesis from lactate and amino acids. In addition acetyl CoA acts as a allosteric inhibitor of pyruvate kinase. Additional potential for allosteric control of gluconeogenesis occurs through the repression of phosphofructokinase -1 (**PFK-1**) exerted by ATP and citrate and repression of hexokinase by glucose-6-phosphate. The allosteric repression of these reactions diminishes glycolysis and favors gluconeogenesis. One of the most potent allosteric regulators of gluconeogenesis is fructose 2,6 bisphosphate, a metabolite that simultaneously allosterically activates PFK-1 to stimulate glycolysis and inhibits fructose 1,6-bisphosphatase (**FBPase-1**) to reduce gluconeogenesis. The

accumulation of fructose 2,6-bisphosphate in liver is favored by high glucose and insulin concentrations whereas elevated glucagon leads to the reduction of fructose 2,6-bisphosphate to favor gluconeogenesis. The abundance of fructose 2,6-bisphosphate in liver is controlled by the phosphorylation state of the bifunctional enzyme phosphofructokinase 2/fructose-2,6-bisphosphatase (**PFK-2/FBPase-2**). Although the role of PFK-2/FBPase-2 regulation has been well described for nonruminants (Rider et al., 2004) the role of this enzyme in regulating gluconeogenesis in ruminants remains largely unexplored.

Gene Expression and Control of Gluconeogenesis

Long-term regulation of gluconeogenesis in nonruminants has been characterized by changes in the expression of genes encoding glucoregulatory enzymes, mainly PEPCK and pyruvate kinase (Pilkis and Claus, 1991). It is well established that insulin represses PEPCK whereas glucagon (or cAMP) and glucocorticoids induce the activity of the PEPCK enzyme by directly regulating expression of the gene through transcriptional modulation and mRNA stability (reviewed in O'Brien and Granner, 1990). Control of lactate use for glucose synthesis is distributed between pyruvate kinase and the reactions involving PC and PEPCK (Sistare and Haynes, 1985). Glucocorticoids have little effect on flux through pyruvate kinase; therefore, an increase in gluconeogenesis from lactate in response to glucocorticoid is mainly due to the combined increases in flux through reactions catalyzed by PC and PEPCK (Jones et al., 1993).

In cattle and sheep the activity of PC is responsive to nutritional and physiological states that impose the greatest demands for endogenous glucose production such as lactation and feed deprivation (Smith and Walsh, 1982). In contrast, the activity of PEPCK is relatively invariant between different nutritional and physiological states in ruminants. The expression and activity of PC is highest in liver, kidney, adipose, brain, adrenal gland, and lactating mammary tissue (Barritt, 1985). Short-term allosteric regulation of PC activity by acetyl-CoA has been well characterized and is likely under these conditions; however, sustained changes in the activity of the PC enzyme require parallel increases in PC mRNA (Zhang et al., 1995). Changes in PC abundance, through alteration in rates of synthesis, represent long-term regulation of lactate metabolism (Barritt, 1985). Data from our laboratory (Greenfield et al., 2000) indicate that expression of PC mRNA is dramatically increased across the transition to lactation whereas PEPCK is relatively unchanged during this period. In bovine liver, the capacity for gluconeogenesis from lactate appears to be directly related to the expression of PC mRNA (Velez and Donkin, 2005). The activity and mRNA abundance of PC are closely linked in transition dairy cows (Greenfield et al., 2000). Taken together these data indicate that the capacity for gluconeogenesis in transition cows is regulated at the level of PC mRNA abundance.

Pyruvate Carboxylase During Transition to Calving and Feed Restriction

The transition to lactation underscores the importance of gluconeogenesis in ruminants as hypoglycemia, ketosis, and related metabolic disorders are often observed when gluconeogenic capacity fails to adapt to the increased demands for glucose to support lactose synthesis and mammary metabolism. The importance of appropriate adaptation to the increased demands for glucose in the periparturient cow is highlighted by reports that incidence of ketosis in commercial dairy herds is 17 to 26 % (Dohoo and Martin, 1984; Melendez et al., 2006). The impact of proper nutritional management of the lactating cow in minimizing these disorders has been recognized previously (Zamet et al., 1979); the implications of appropriate management of the dairy cow during late gestation (Bell, 1995, Grummer 1995; Jouany, 2006) have become well recognized over the past two decades. Additional efforts are still needed to define and meet the nutritional requirements of the transition dairy cow in order to optimize animal health, production in the ensuing lactation, overall longevity, and animal well-being (NRC, 2001).

Because obligatory requirements exist for glucose as a substrate for brain, erythrocytes, kidney medulla and mammary tissue (Mayes, 1996) ruminants have evolved adaptations to reduced supply propionate from rumen fermentation such as during feed restriction. Under these conditions there is increased gluconeogenesis from lactate, amino acids and glycerol to meet glucose needs (Baird et al., 1980). An increase in PC mRNA coupled with increased metabolism of lactate to glucose in response to partial feed restriction in dairy cows (Velez et al., 2005) and a lack of effect on PEPCK expression suggests a critical role of PC in regulation of hepatic metabolism in ruminants especially during nutrient insufficiency.

We examined the mRNA abundance for PC and PEPCK in transition cows (Greenfield et al., 2000; Hartwell et al., 2001) and determined that PC responds to the onset of calving and that PEPCK expression is elevated after lactation is established. The activities of both enzymes are reflected by changes in mRNA abundance at calving (Greenfield et al., 2000; Agca et al., 2002). We have cloned and sequenced the promoter region of bovine PEPCK-C (Zhang et al., 2014). Comparisons between the promoter regions of rat and bovine PEPCK revealed no similarities in the overall nucleotide sequence. We have identified the transcription start site, linked the promoter to a luciferase reporter sequence, and generated a family of 5' promoter truncations. When expressed in liver cell cultures the bovine PEPCK promoter is responsive to propionate (Zhang et al., 2014), which suggests feed forward control of gluconeogenesis in bovine that is linked to rumen propionate production.

We have further explored control of PC and PEPCK with feed restriction and bST administration and determined that PC, but not PEPCK, is elevated during feed restriction (Velez and Donkin, 2005) and PEPCK, but not PC, is elevated with bST (Velez and Donkin, 2004). These data highlight the unique aspects of bovine metabolism with regard to control of glucose synthesis. When these data are considered collectively our current understanding of the major control points for gluconeogenesis in ruminants shows that PC plays a pivotal role in determining the rate of gluconeogenesis when intake is compromised such as during the transition to

calving, whereas PEPCK-C is linked to control of gluconeogenesis when feed intake is not constrained.

Additional Roles of Pyruvate Carboxylase in Liver Metabolism

The two possible fates of pyruvate in liver are conversion to acetyl CoA by pyruvate dehydrogenase followed by further metabolism through the TCA cycle, or conversion to oxaloacetate via PC as an intermediate in the synthesis of glucose. Likewise, the abundance of non-esterified fatty acids (**NEFA**), released from adipose tissue at calving, may be oxidized to acetyl CoA and further metabolized in the TCA cycle or alternatively, can be partially oxidized to ketones. Increased PC mRNA abundance and activity on the day of calving may provide an adaptive mechanism which allows pyruvate carbon to be channeled through oxaloacetate to maintain hepatic glucose output and simultaneously minimize ketogenesis from non-lipid precursors. The direct allosteric activation of PC by acetyl CoA (Mayes, 1996) would serve to further augment the impact of increased PC mRNA expression in the periparturient dairy cow.

An additional role for increased PC at calving and during feed restriction may be to generate mitochondrial oxaloacetate as a substrate in TCA cycle oxidation of NEFA. Although the pathogenesis of ketosis is not clearly understood, it is generally thought to be initiated by inadequate availability of endogenous glucose. Secondary signs of the disorder result in fatty liver and further impairments in gluconeogenesis and ammonia detoxification (Grummer, 1995). Increases in PC mRNA which coincide with increased clearance of lipid from liver in dairy cattle given glucagon infusions (Hippen et al., 1999; She et al., 1999) support a role for PC in this process.

Propionate and PEPCK Expression in Bovine

The effects of short chain (volatile) fatty acids to stimulate growth and differentiation of rumen epithelium have been recognized for some time (Van Soest, 1994). Recently the effects of short chain fatty acids, produced by colonic bacteria, have been recognized as beneficial in suppressing proliferation of colon cancers and regulating immune function (Sanderson et al., 2001). More recently propionate, acetate and butyrate have been identified as repressors (Tran et al., 1998) and as activators of gene expression (Drozdowski et al., 2002). The potency of volatile fatty acids to regulate gene expression is highlighted by striking example that the developmental switching of the globin gene can be halted and even reversed by infusion of butyrate in the sheep fetus (Perrine et al., 1990).

Expression of PEPCK was elevated up to 5 fold when rat hepatoma cells (H4IIE cells) were incubated for 4 h in the presence of 2.5 mM of short chain volatile fatty aicds (Massillon et al., 2003). Incubation of rat hepatoma (**H4IIE**) cells with acetate, propionate, caproate and valerate also increased glucose-6 phosphatase (**Gluc-6-Pase**) mRNA (Massillon et al., 2003). Promoter analysis indicates that these effects are mediated through specific transcription factors expressed in liver, intestine and kidney. These data indicate that short chain volatile fatty acids can affect gluconeogenesis

directly by genes that control the fate of glucose precursors (Van Schaftingen and Gerin, 2002).

Recent data from our laboratory supports an induction of PEPCK in liver in response to increased propionate supply to liver. Feeding monensin, a feed additive that causes a shift in rumen fermentation to favor increased propionate production. The data indicate an increase in abundance of PEPCK-C mRNA with prepartum monensin feeding. Prepartum feed intake did not differ for the monensin and control cows and PEPCK transcript abundance observed for monensin fed cows at -14 and +1 days relative to calving was similar to the expression observed during lactation when feed intake is much greater than the prepartum period. These data suggest that the effect of propionate on PEPCK expression is independent of level of feed intake but linked to the end products of rumen fermentation. Experiments outlined in this proposal will determine the effects of propionate in this regard.

Expression of PEPCK mRNA that is sensitive to propionate supply would serve to increase the gluconeogenesis from propionate and also gluconeogenesis from other substrates including lactate and alanine. Induction of PEPCK in bovine and increased capacity for gluconeogenesis from propionate represents a novel control mechanism for gluconeogenesis. The action of propionate to promote PEPCK expression would serve to couple the availability of the primary substrate for gluconeogenesis in ruminants to a novel feed-forward induction of gluconeogenic capacity.

Recent experiments in our laboratory have explored the potential for propionate to directly control PEPCK expression and gluconeogenesis. Postruminal propionate infusions over an 8-h period to augment the daily supply of propionate by 25% resulted in an increase in liver expression of PEPCK and flux of propionate to glucose despite elevated blood insulin concentrations. Separate experiments using the bovine PEPCK gene promoter revealed that propionate is a potent and direct activator of PEPCK gene expression. The effects of propionate suggest primary control that is dominant to glucagon, dexamethasone, and insulin in this regard. Follow up experiment have identified a specific region within the PPECK gene promoter responsible for this control. The mechanisms by which propionate acts to control expression of PEPCK, and consequently the capacity for gluconeogenesis, are the subject on ongoing investigations.

Strategies to Improve Glucose Economy of Transition Dairy Cows

Many of the feeding strategies that are already currently employed for transition cows are successful because they impact the ability of the cow to produce glucose in liver and supply it to other tissues. These include maintaining feed intake and increasing intake as soon after parturition as possible. Because energy intake and glucose production are closely correlated the inclusion of rumen fermentable feeds that increase in supply of propionate serve to promote glucose supply as long as overall intake is not compromised in response to the higher energy density ration. Feeds, feed additives, and feeding management strategies that promote maximal daily rumen propionate

production and absorption will directly impact the production of glucose by the transition cow. Our current work indicates that glucose production becomes more efficient with greater propionate supply. Feed additives, including ionophores, that shift fermentation pattern to promote greater propionate production per unit of feed intake have a direct positive effect on glucose status through promoting increased capacity for gluconeogenesis. Feeding management strategies that encourage more consistent and frequent feeding are likely to promote this process as well. Provision of alternative precursors for glucose including lactate, certain amino acids, and glycerol may be beneficial during the immediate postpartum interval as ability of the cow to metabolize these nutrients is elevated. Care should be exercised not to supply these at the expense of normal rumen fermentation or feed intake.

Summary, Conclusions, and Future Directions

Robust and appropriate responses and controls for gluconeogenesis are critical for optimal growth, lactation and well-being of ruminants used in production agriculture. Propionate, lactate, amino acid sand glycerol contribute to whole animal glucose metabolism although their contributions vary with physiological state and their supply. Features of ruminant metabolism results in aspects of the control points for gluconeogenesis in ruminants that appear to be unique compared with nonruminants particularly with respect to the response to reduced feed intake and to supply of gluconeogenic precursors, namely propionate. The adaptations to nutrient insufficiency associated with the transition to lactation results in increased capacity for lactate metabolism though changes in the PC gene and the adaptation to increased feed intake and greater ruminal propionate production appear to involve specific changes in PEPCK. These coordinated responses appear to be essential in maintaining tissue needs for glucose and involve responses at the level of their respective gene promoters. Although recent research has expanded knowledge of gluconeogenesis in ruminants to include the molecular control additional work needed to integrate the impact of genetics, breed type, pre- and postnatal nutrition and environment to control of this critical process and to permit application of this knowledge to precision management and feeding programs that optimize productivity, health, and profitability for ruminant production systems.

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SESSION NOTES

Novel Concepts Regarding Calcium Homeostasis during the Transition Period

Laura L. Hernandez[1] and Samantha R. Weaver
Department of Dairy Science
University of Wisconsin

Introduction

Adequate circulating calcium (**Ca**) concentrations throughout the transition period are necessary for a productive lactation, but large quantities of Ca are lost from maternal Ca pools into milk and colostrum. A rapid, substantial drop in maternal blood Ca causes 5-10% of cows to be afflicted with clinical hypocalcemia (**CH**) and an additional 50% to suffer from subclinical hypocalcemia (**SCH**). Subclinical hypocalcemia and CH are significant risk factors of early lactation culling/premature removal from the herd (DeGaris and Lean, 2008; Reinhardt et al., 2011; Roberts et al., 2012). Furthermore, SCH increases risks of developing ketosis; displaced abomasum; and metritis; SCH depresses immune function; prolongs the interval until pregnancy is achieved; decreases pregnancy rate; and reduces overall productivity (**Figure 1;** Kimura et al., 2006; Goff, 2008; DeGaris and Lean, 2008; Chapinal et al., 2011; Reinhardt et al., 2011; Chapinal et al., 2012; Martinez et al., 2012). During lactation, dietary Ca is not sufficient to maintain maternal Ca concentrations while supporting milk formation. Therefore, activation of maternal bone Ca mobilization during the dry period is critical for the prevention of post-partum SCH and CH. Using the estimates of Dr. Garrett Oetzel ($300 loss for each treatment of CH, and $125 loss for each treatment of SCH; Guard, 1996) the annual cost to the U.S. dairy industry, which has approximately 9,200,000 cows, is approximately $575,000,000 for SCH and approximately $325,000,000 for CH (NAHMS, 2007; Oetzel, 2013). SCH and CH are detrimental to animal health and welfare, and a formidable economic burden to U.S. farmers (Oetzel, 2013). Currently accepted practices for treatment and prevention of SCH and CH include oral Ca supplementation post-calving and anionic salt supplementation (DCAD) pre-calving (Oetzel, 2004; Oetzel, 2013). However, post-calving Ca supplementations, while critical for treatment, are not sufficient to prevent maternal hypocalcemia and its associated peripartum disorders. Use of anionic salts in the pre-partum period has reduced the incidence of SCH and CH. However, approximately 25% of cows will still be afflicted with SCH (Oetzel, 2004). Additional limitations in the use of anionic salts as a prevention strategy include cost, reduced feed palatability, and the difficulty of finding low potassium forages to include in the diet (Oetzel, 2004; Goff, 2004; Goff, 2008). The lack of adequate therapies targeted towards preventing hypocalcemia leaves a large percentage of the U.S. dairy cow population unprotected and new therapeutic strategies are lacking because the physiological mechanisms of SCH are not fully understood.

[1] Contact at: Department of Dairy Science, 1675 Observatory Drive, 864 Animal Sciences Building, Madison, WI 53706-1205; Tel: (608) 263-9867; E-mail: llhernandez@wisc.edu.

The Onset of Milk Production Drains Ca Pools in Dairy Cows

Colostrum and milk synthesis rapidly depletes Ca from the maternal circulation and therefore Ca must be mobilized from maternal bone to maintain adequate circulating concentrations. Circulating Ca concentrations are tightly regulated and controlled by several hormones including: Vitamin D, calcitonin, parathyroid hormone (**PTH**) and parathyroid hormone related-protein (**PTHrP**). Liberation of Ca from bone stores can only be triggered when circulating Ca concentrations dip below the animal's minimal threshold for Ca, via a classic negative feedback loop. Dietary Ca is insufficient to maintain maternal Ca homeostasis during milk synthesis. This is demonstrated by the fact that a dairy cow will lose 9-13% of her bone mass during the first 30 days of lactation. Bone loss during lactation is an evolutionary strategy of mammals used to support the cow as well as the mammary glands' demand for Ca for milk synthesis (**Figure 2**; Wysolmerski et al., 1995; Wysolmerksi, 2010; Goff, 2014).

The Mammary Gland Functions as an "Accessory Parathyroid Gland" during Lactation

The mammary gland produces the hormone PTHrP, which binds to receptors on bone to drive bone resorption and liberate Ca into the systemic circulation (Wysolmerski et al., 1995; Wysolmerski, 2010). PTHrP is only produced by the mammary gland during lactation. The Ca sensing receptor (**CaSR**) present in the mammary epithelium plays a crucial role in controlling maternal Ca concentrations during lactation. CaSR is highly expressed in the mammary gland during lactation, compared to virgin and pregnant time periods (VanHouten et al., 2003). Mammary PTHrP production is responsible for the mobilization of Ca from the bone during lactation, rather than the typical endocrine regulator of bone, PTH (Wysolmerski et al., 1995; VanHouten, 2005; Wysolmerski, 2010; Wysolmerski, 2012). Our lab made a novel discovery that serotonin is essential for the liberation of Ca from bone during lactation to sustain maternal Ca homeostasis in rodent models. This occurs through induction of PTHrP by the mammary gland (Hernandez et al., 2012; Laporta et al., 2014a, 2014b). Furthermore, we demonstrated that serotonin is critical for the expression of CaSR. This finding indicates that serotonin is crucial for mammary gland sensing of systemic Ca concentrations.

Mammary Gland Coordination with the Skeletal System Liberates Ca During Lactation

The skeletal system maintains its structural and functional roles via communication between two cell types, osteoblasts (**OB**), which are responsible for bone formation, and osteoclasts (**OC**), which are responsible for bone resorption, and thus Ca mobilization. PTH regulates this mechanism under non-lactating conditions. Research in humans and rodents has suggested the PTH action on bone is uncoupled during lactation (Wysolmerski, 2010; VanHouten and Wysolmerski, 2013). PTHrP signals through the same G-protein coupled receptor (PTH1R) that PTH does on the OB to decrease OB cell proliferation and up-regulate genes responsible for OC

differentiation during lactation. In rodents and humans, the mammary gland is the main source of PTHrP found in the circulation (Thiede, 1994; Wysolmerski et al., 1995; Wysolmerski, 2010; VanHouten and Wysolmerski, 2013). Mammary-derived PTHrP, not PTH, is the critical hormone responsible for induction of bone Ca mobilization during lactation (Wysolmerski et al., 1995).

Serotonin Regulates Mammary Gland Physiology During Lactation

Serotonin is synthesized in numerous tissues throughout the body and brain and is incapable of crossing the blood-brain barrier. Serotonin is synthesized from the amino acid L-tryptophan in a two-step process. The first step is production of 5-hydroxytryptophan (**5-HTP**) via the rate-limiting enzyme, tryptophan hydroxylase (**TPH**). The second step is the conversion of 5-HTP to serotonin by aromatic amino acid decarboxylase (Wang et al., 2002). TPH1 is the rate-limiting enzyme for serotonin production in non-neuronal tissues, while TPH2 is used to produce serotonin in neuronal tissues. Our laboratory and others have shown that serotonin regulates milk protein gene expression, as well as the disassembly of tight junctions that occurs during the involution process (Matsuda et al., 2004; Stull et al., 2007; Hernandez et al., 2008; Pai and Horseman, 2008). Furthermore, we have shown that the mammary gland expresses a unique pattern of serotonin receptors in rodent, bovine, and human mammary epithelium (Hernandez et al., 2009; Pai et al., 2009). The epithelial component of the bovine mammary gland expresses at least five serotonin receptor isoforms (5-HT1B, 2A, 2B, 4 and 7; Hernandez et al., 2009). Our lab determined that the 5-HT2B receptor subtype modulates serotonin's regulation of PTHrP production within the mammary gland in a rodent model (Hernandez et al., 2012; Laporta et al., 2013a; Laporta et al., 2014a,b). We also confirmed that circulating serotonin concentrations post-partum are positively correlated with circulating Ca concentrations on the first day of lactation in dairy cows (Laporta et al., 2013b). Furthermore, we showed that serotonin activates expression of various Ca pumps and transporters in the mammary gland to stimulate transport of Ca from blood to milk during mouse lactation (Laporta et al., 2014a). Ca transport into the mammary gland is thought to occur through the Ca^{2+} influx channel (**ORAI1**) and subsequent pumping into the milk by the apical plasma membrane Ca^{2+} ATPase (**PMCA2**; Cross et al., 2014).

Current research in humans and rodents implicates PTHrP in the regulation of maternal Ca homeostasis during lactation. Our laboratory has demonstrated the necessity of serotonin for regulation of Ca transport in the mammary gland during lactation. Furthermore, we have demonstrated that serotonin is necessary for the production of mammary PTHrP during lactation. Mammary PTHrP production is critical to the mobilization of Ca from bone tissue to support lactation. Therefore, delineation of the mechanisms regulating the mammary gland serotonin-PTHrP axis in the dairy cow could lead to development of novel therapeutic interventions to reduce the incidence of SCH and CH in the U.S. dairy cow population.

The following model for the regulation of Ca mobilization from bone by the mammary gland during the transition period has been proposed by our laboratory

(**Figure 3**).

New Ideas About Calcium and Serotonin

Our laboratory recently demonstrated that serotonin is necessary for mammary PTHrP synthesis in lactating rodents and mammary epithelial cells grown in lactogenic culture (Hernandez et al., 2012; Laporta et al., 2013a; Horseman and Hernandez, 2014). We also demonstrated that supplementation of a serotonin precursor, 5-HTP, to rats during the transition from pregnancy to lactation increased the post-parturition circulating serotonin, PTHrP, and Ca concentrations, and also increased total Ca content in milk (Laporta et al., 2013a). Furthermore, we observed increased OC numbers in the femurs collected from rats supplemented with 5-HTP, indicating this response was due to bone Ca mobilization.

In order to better understand the relationship between serotonin and maternal Ca homeostasis, we recently deleted TPH1 in mice.

TPH1 catalyzes the rate-limiting step in non-neuronal serotonin synthesis. TPH1 deficient mice have little to no circulating serotonin. Our goal was to make mice deficient in non-neuronal serotonin and delineate the potential molecular mechanisms underlying serotonin's regulation of Ca homeostasis during lactation. We demonstrated that i.p. injections of 5-HTP to these mice restored and even elevated circulating serotonin concentrations compared to wild-type dams. Our results also demonstrated that total Ca concentrations are decreased in TPH1 null mice and that Ca concentrations can be restored with i.p. injection of 5-HTP. RNA-sequencing analysis of mammary glands collected on d 10 of lactation from wild-type, TPH1 deficient mice and TPH1 deficient mice injected with 5-HTP revealed that serotonin is critical for the cellular response to Ca, along with a variety of further, yet unexplored, regulatory pathways (Laporta et al., 2015). Upon further analysis of the specific Ca pumps and transporters present in the mammary gland we observed that mRNA abundance of several Ca pumps and transporters was reduced in the TPH1 deficient mammary gland and restored by exogenous 5-HTP (Laporta et al., 2014a). These results indicate that peripheral serotonin is critical for maintaining circulating Ca concentrations and mammary gland Ca transport during lactation.

In order to evaluate the utility of the mammary serotonin-PTHrP axis in Holstein dairy cows, we performed several observational studies

In a small study of 42 multiparous Holstein dairy cows, we observed that serotonin and PTHrP concentrations on d 1 of lactation were positively correlated with total Ca concentrations (Laporta et al., 2013b; **Figure 4**). Additionally, we have observed that serotonin concentrations are dynamic over the course of a given lactation, and decrease around the time of calving (d 0-2 lactation), rebounding by approximately ten days into lactation (Moore et al., 2015; **Figure 5**). The overall average serotonin concentration in dairy cows is approximately 1700 ng/ml. However, the concentrations fluctuate dependent on stage of lactation. These results combined

with our rodent data support our hypothesis that serotonin and PTHrP are critical players in the regulation of Ca homeostasis in Holstein dairy cows.

Intravenous (IV) infusion of 5-HTP in late lactation, non-pregnant, multiparous Holstein dairy cows increases circulating serotonin concentrations and alters Ca dynamics

In order to demonstrate the role of serotonin in Ca homeostasis in dairy cows, we performed a preliminary experiment in which we infused 5-HTP IV for one hour daily for four days in late-lactation dairy cows at varying doses (0, 0.5, 1.0, or 1.5 mg/kg) to determine an optimum dose of 5-HTP necessary to produce significant changes in Ca. All three doses of 5-HTP significantly increased circulating serotonin concentrations (Laporta et al., 2015) to a similar extent in the two hours after dosing, with concentrations returning to baseline concentrations observed in the saline controls by two hours after infusion. In addition to serotonin concentrations, we measured circulating total Ca concentrations following the same time course post infusion. While initially counter-intuitive, our data demonstrated that total Ca concentrations decreased in immediate response to 5-HTP treatments (**Figure 6a;** Laporta et al., 2015). In order to determine where the circulating Ca was going after 5-HTP infusion, we measured urine Ca concentrations prior to the start of infusion and two hours after the end of the infusion. Our results indicate that there was a decrease in urine Ca output with higher doses of 5-HTP treatment (**Figure 6b**). This suggests that Ca is not being lost into the urine. Therefore, we measured total Ca concentrations in the milk during the infusion periods and observed that the highest dose of 5-HTP increased total milk Ca concentrations (**Figure 6c**). This supports the hypothesis that serotonin causes transient hypocalcemia by increased Ca transport into the mammary gland and subsequently into milk. Increased Ca transport into the mammary gland during lactation is critical for the stimulation of bone Ca mobilization by PTHrP because transient systemic hypocalcemia.

Use of 5-HTP before calving to prevent hypocalcemia

In order to determine if elevating serotonin concentrations in pre-fresh dairy cows would result in increased post-calving Ca concentrations, we treated multiparous Holstein cows with daily IV infusions of 1.0 mg/kg of 5-HTP beginning 7 d before the estimated calving date until calving. Our data demonstrates that IV infusions of 5-HTP pre-calving increased ($P = 0.04$) post-calving total Ca concentrations compared to saline treated controls (**Figure 7**). Furthermore, we measured deoxypyridinoline (**DPD**), a marker of OC activity and therefore bone resorption, in the urine. These data demonstrate that cows receiving 5-HTP before calving have increased bone resorption on at calving (**Figure 8;** $P = 0.01$). These results support demonstrate that 5-HTP treatment pre-calving can potentially improve post-calving Ca concentrations by increasing bone Ca resorption.

Interrelationship of a negative DCAD and serotonin

Given that 5-HTP treatment pre- calving was capable of increasing post-calving Ca concentrations, we wanted to determine if a common preventative treatment for SCH and CH, negative DCAD, controls Ca homeostasis via a serotonergic mechanism. To this end, we fed Holstein dairy cows a positive DCAD (+130 mEq/kg) diet for 21 days prior to calving or a negative DCAD (-130 mEq/kg) diet for 21 days prior to calving. Upon analysis of circulating serotonin concentrations from 9 days before calving through 6 days post-calving, we determined that a negative DCAD diet increased circulating serotonin concentrations pre-calving ($P = 0.05$; **Figure 9**). This suggests the resulting improvement in post-calving Ca concentrations (data not shown) in the cows receiving a negative DCAD diet pre-calving could be due to serotonin's control of Ca homeostasis. At this time we are unaware if negative DCAD works exclusively through a serotonergic mechanism. Furthermore, we do not know if a negative DCAD diet combined with 5-HTP treatment will have a synergistic effect on post-calving Ca concentrations.

Conclusion

In conclusion, we have demonstrated that serotonin plays a critical role in regulation of maternal Ca transport, maternal Ca homeostasis and mammary PTHrP production in the rodent. Additionally, our data demonstrate that mammary gland Ca transporter expression and induction of PTHrP production by the mammary gland during lactation are key regulators of maternal Ca homeostasis in rodent models. Furthermore, our rodent models indicate that the mammary gland is a significant source of serotonin during lactation. Our observational data in Holstein cows suggests that serotonin, PTHrP, and Ca are interrelated during the early days postpartum. Furthermore, our initial experiment exploring the effects of 5-HTP on maternal Ca homeostasis in late-lactation dairy cows supports the hypothesis that serotonin induces transient hypocalcemia by shuttling Ca into the mammary gland in order to stimulate mammary production of PTHrP, and the elevated PTHrP is critical to stimulate bone Ca resorption. Treating pre-partum Holstein dairy cows with 5-HTP resulted in improvement of post-partum Ca concentrations. Finally, using a current therapeutic intervention for prevention of SCH and CH in the dairy industry, feeding of a negative DCAD diet pre-partum, resulted in the increase of circulating serotonin concentrations. Taken together, our **preliminary** data in rodents and dairy cows support the hypothesis that mammary serotonin is critical to the induction of mammary PTHrP, and PTHrP entering the maternal circulation in turn restores maternal Ca homeostasis during lactation. Further delineation of these molecular pathways could lead to powerful, innovative preventative strategies against SCH and CH in dairy cows.

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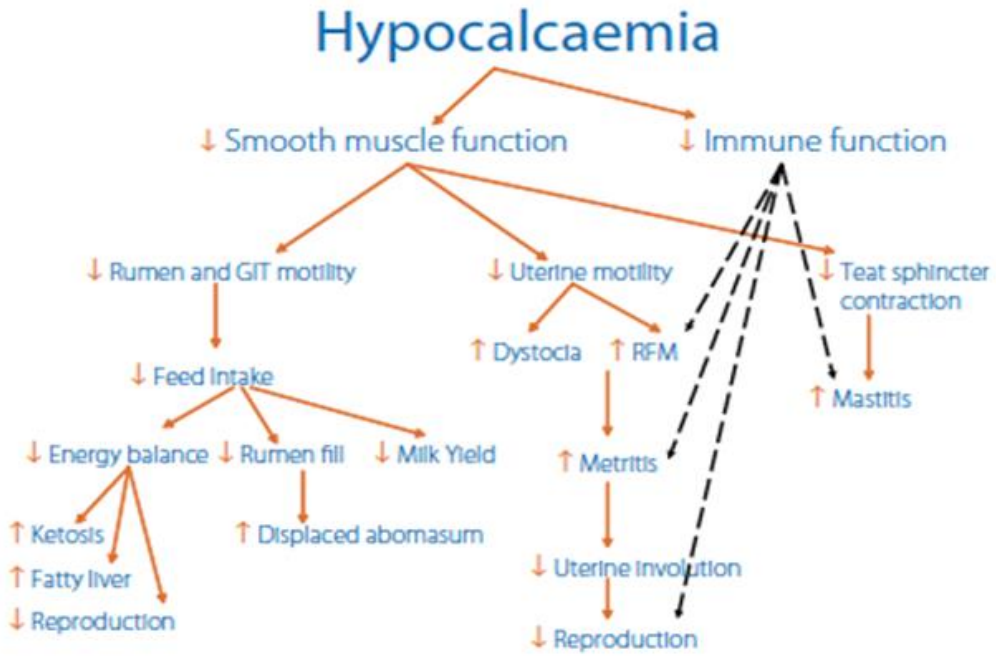

Figure 1. Hypocalcemia is a 'gateway' disease that leads to increased risks of other periparturient diseases. (DeGaris and Lean, 2008).

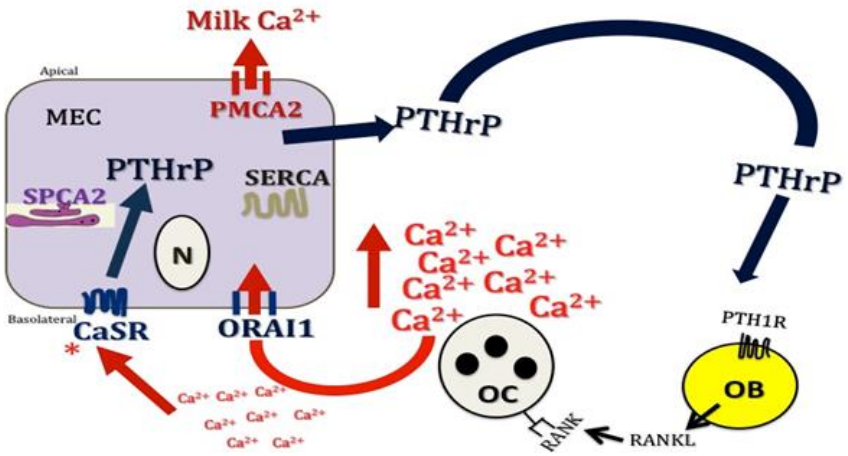

Figure 2. Maternal Ca homeostasis is regulated by the mammary gland-bone axis. During lactation, the Ca sensing receptor (CaSR) on the basolateral side of the mammary epithelial cell (MEC) during lactation detects low blood Ca concentrations due to the increased transport of Ca into the MEC by Ca release-activated Ca channel protein 1 (ORAI1). Ca is either secreted into the milk through the apical plasma membrane Ca ATPase 2 (PMCA2) or sequestered in the Golgi apparatus by secretory pathways Ca ATPase 2 (SPCA2) or endoplasmic reticulum by the sarco(endo)plasmic reticulum Ca ATPase (SERCA). Detection of systemic decreased Ca by CaSR results in parathyroid hormone related-protein (PTHrP) production. PTHrP is secreted into the circulation and will bind its receptor PTH1R on the osteoblast (OB) cell in the bone increasing production of receptor activated nuclear factor kappa B (RANKL), which binds its receptor (RANK) on the osteoclast (OC) cell in the bone tissue, activating Ca liberation from bone.

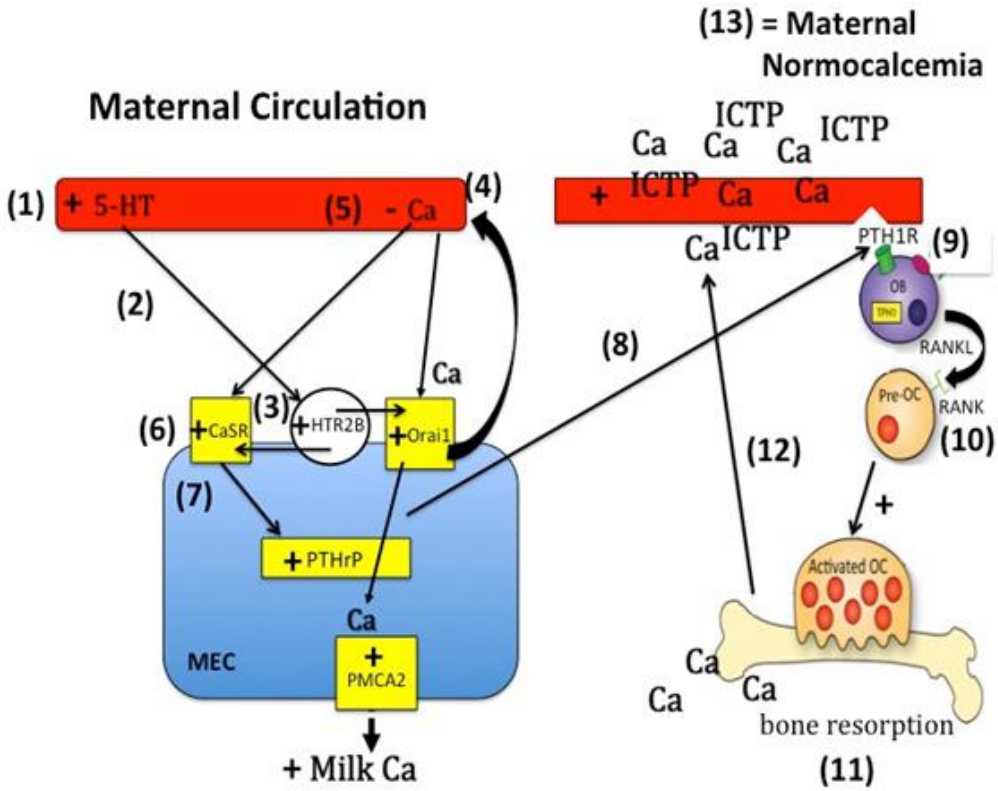

Figure 3. Proposed model of the regulation of maternal Ca homeostasis by serotonin (5-HT). **1)** Increased maternal circulating 5-HT, **2)** stimulates signaling via the 5HT2B receptor on the basolateral side of the mammary epithelium (MEC), **3)** increasing expression of Orai1 and CaSR, **4)** resulting in increased transport of Ca into the mammary gland, which leads to **5)** decreased systemic Ca concentrations, and **6)** this is detected by the CaSR on the basolateral surface of the mammary epithelium, resulting in **7)** stimulation of PTHrP production by the MEC. **8)** PTHrP is then secreted by the MEC and acts through **9)** PTH1R, its receptor present on the OB, stimulating production of RANKL, which binds to the RANK receptor on the OC, **10)** stimulating OC maturation and activation, allowing for bone resorption, **11)** resulting in the liberation of Ca from the bone, and **12)** resulting in increased systemic Ca and type I collagen fragment (ICTP) concentrations (a bone resorption marker),**13)** resulting in normocalcemia.

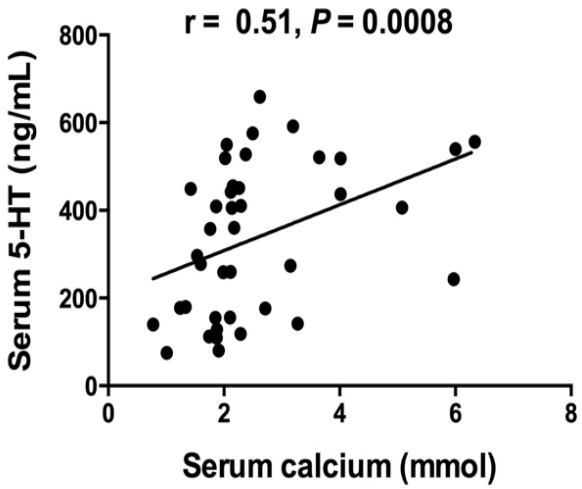

Figure 4. Circulating serotonin (5-HT) and total Ca concentrations are positively correlated on d1 lactation in multiparous Holstein cows (Laporta et al., 2013b).

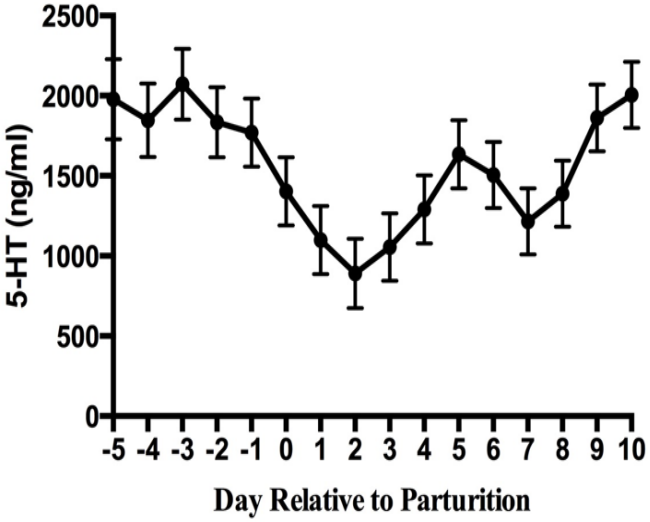

Figure 5. Circulating serotonin (5-HT) concentrations fluctuate around calving in multiparous dairy cows and serotonin decreases around the time of calving. (Moore et al., 2015).

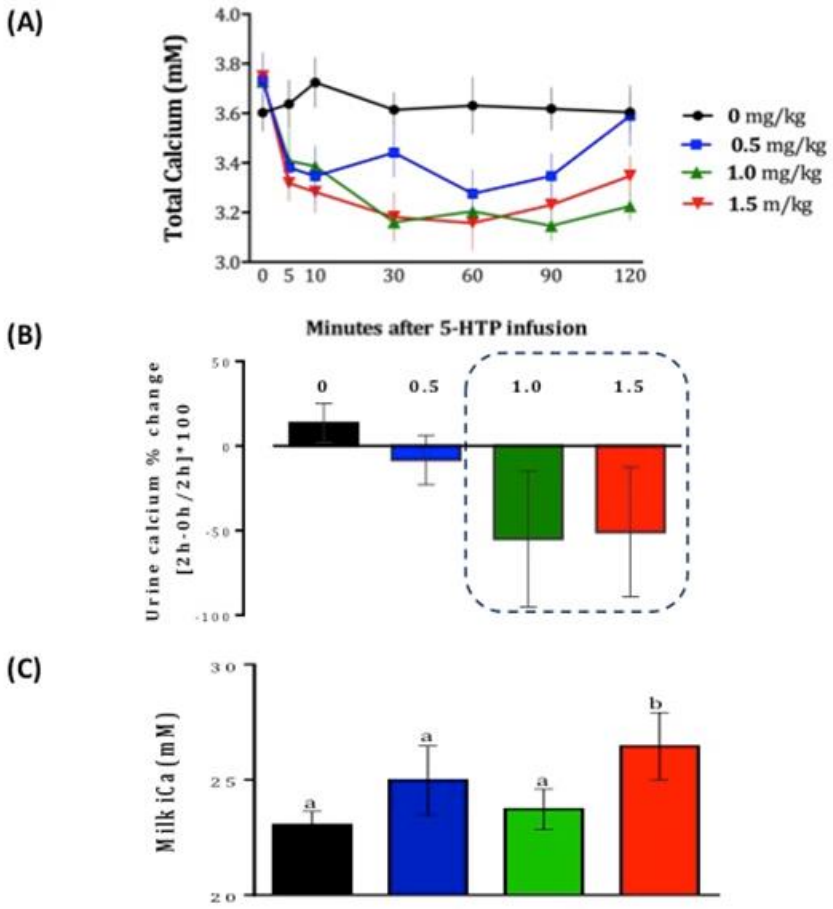

Figure 6. Increasing serotonin concentrations increases flux of Ca to the mammary gland from the circulation **(A)** I.V. infusion of 5-HTP to late lactation dairy cows at varying doses decreases circulating Ca concentrations in the first 2 h after dosing. **(B)** Urine calcium concentrations are decreased after infusion as dose of 5-HTP increases. **(C)** Milk Ca concentrations increase with increased infusions of 5-HTP. (Laporta et al., 2015).

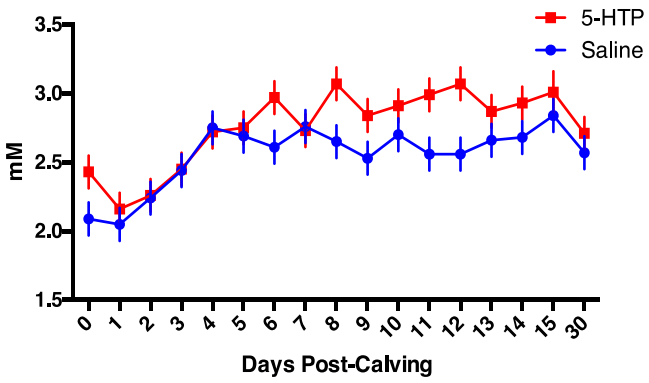

Figure 7. 5-HTP treatment pre-calving increases post-calving circulating Ca concentrations (Weaver et al., unpublished results).

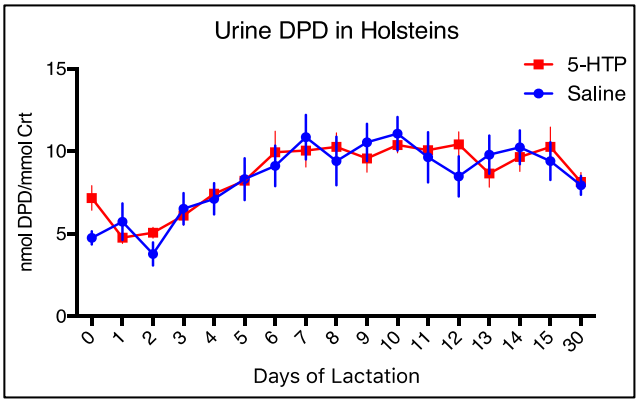

Figure 8. Urine deoxypyridinoline (DPD) concentrations post-calving in cows treated with saline or 5-HTP pre-calving. (Weaver et al., unpublished results).

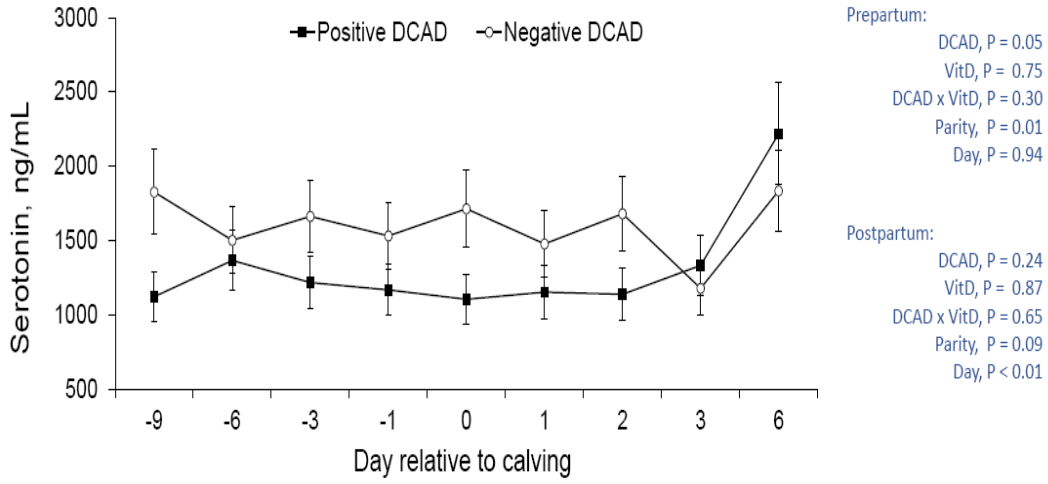

Figure 9. Feeding a negative DCAD for 21 days pre-calving increases circulating serotonin concentrations compared to feeding a positive DCAD for 21 days pre-calving (Martinez, unpublished results).

SESSION NOTES

Dietary Manipulations and Interventions to Improve Calcium Metabolism

José E.P. Santos[1], N. Martinez, A. Vieira-Neto, C. Lopera, and C. Nelson
Department of Animal Sciences
University of Florida

Introduction

Hypocalcemia is a common and important problem in dairy cows. It is usually defined as subclinical and clinical according to blood concentrations of total (**tCa**) or ionized (**iCa**) calcium (**Ca**) and the diagnosis of clinical signs. Cows with clinical hypocalcemia present muscle weakness and fasciculation, inability to control body temperature, anorexia, and or inability to stand which eventually leads to recumbency, coma, and death if untreated. Although the clinical form of hypocalcemia (**CH**) can result in death, it usually is easily controlled affecting only 3 to 5% of the postpartum dairy cows, primarily older cows. On the other hand, the subclinical form is much more prevalent and can have detrimental impacts to subsequent health. Depending upon how it is defined and the frequency of blood sampling for diagnosis, subclinical hypocalcemia (**SCH**) can affect 25 to 40% of primiparous and 45 to 80% of the multiparous cows. Cows with SCH have reduced dry matter (**DM**) intake, suppressed measures of innate and acquired immune function, compromised energy metabolism and increased incidence of other periparturient diseases. The reason why cows develop hypocalcemia is the combination of colostrum synthesis associated with the inability to quickly restore the blood pool of Ca either from bone remodeling, gut absorption, or renal reabsorption. Removing the mammary gland greatly diminishes the decline in blood Ca around parturition. Improper diets are a major risk factor for hypocalcemia. Dietary manipulations are opportunities to improve early lactation Ca homeostasis. Altering the mineral composition of the prepartum diet to result in a negative dietary cation-anion difference (**DCAD**) to induce a compensated metabolic acidosis has been shown to reduce the risk of CH and SCH. Furthermore, adjusting the concentrations of Ca, P, and Mg also are important for success of a negative DCAD diet. Other alternatives to control hypocalcemia include feeding very low concentrations of dietary Ca prepartum (<0.30%), intestinal sequestration of Ca by feeding synthetic zeolite, or administration of Ca salts immediately after calving. This manuscript reviews recent research on methods to control and reduce the impact of hypocalcemia in dairy cows.

Hypocalcemia

Dairy cows develop hypocalcemia because of the inability to restore blood concentrations of Ca with the onset of colostrogenesis and lactation. In general, blood

[1] Contact at: Department of Animal Sciences, University of Florida, 2250 Shealy Drive, Gainesville, FL 32911. E-mail: jepsantos@ufl.edu.

concentrations of Ca start to decline on the last day or two of gestation (Goff et al., 2002; Kimura et al., 2006) because of sequestration in the mammary gland for colostrum synthesis. Mastectomized Jersey cows did not experience any drop in concentrations of tCa in plasma with parturition, thereby reinforcing the concept that changes in Ca around calving are the result of mammary secretion into colostrum and milk, and not related to the endocrine changes with calving (Goff et al., 2002).

In general, the dairy cow in late gestation and early lactation has very dynamic and responsive Ca homeostatic mechanisms to assure that the degree of hypocalcemia is controlled and does not lead to death. Nevertheless, whenever these homeostatic and homeorhetic mechanisms fail at the onset of lactation, then CH might occur. Therefore, milk fever or CH is caused by the inability of the cow to rapidly adapt to the increased needs of Ca and not because of a dietary deficit. It is known that CH is a problem that affects primarily older cows and prepartum nulliparous cows are almost never diagnosed with milk fever. It is thought that the greater colostrum synthesis associated with a less dynamic and active Ca resorption mechanism favors the occurrence of CH in older than younger cows. It has been estimated that the risk of CH increases 9% for every lactation in the life of a cow (DeGaris and Lean, 2008). Figure 1 illustrates the changes in colostrum yield and concentration of Ca in colostrum according to parity and prepartum DCAD fed (Martinez et al., 2014). It is clear that multiparous cows secrete larger quantities of Ca in the first milking (52% more or 9 g), and this difference is almost 3 times the entire plasma pool of a dairy cow. If one assumes that the plasma volume in adult Holstein cows is similar to those estimated for beef cattle and Guernsey cows, approximately 40 mL/kg of live weight (Reynolds, 1953; Springel, 1968), then typical postpartum cows would have approximately 27 L of plasma containing approximately 90 mg of Ca/L or a total of 2.43 g. It is not surprising that an additional 9 g of Ca secreted during the first milking would impose a greater challenge for multiparous cow to adjust their blood Ca concentration compared with the challenge faced by primiparous cows.

Although CH can be tragic, the most common presentation of this disorder is the subclinical form, in which blood Ca concentrations are below those considered normal, but the cow presents no visible clinical signs. In general, SCH is defined as tCa ≤ 2.0 m*M* or < 8 mg/dL by most authors (Goff et al, 2014; Reinhardt et al., 2011), although the rationale for such a cut-off has not been clearly defined. Recently, we reported that cows with serum tCa of < 2.15 m*M* (< 8.59 mg/dL) had a marked increase in the risks of metritis, puerperal metritis and other diseases in the first month postpartum (Martinez et al., 2012). More striking was the fact that the risk of uterine diseases greatly increased with a decrease in serum tCa concentrations in the first 3 DIM (**Figure 2**). Inducing SCH by intravenous infusion of a Ca-specific chelating agent in dry cows depressed DM intake almost 5 kg/d compared with saline, depressed rumen contractility, impaired insulin release and increased lipid mobilization (Martinez et al., 2014). Induction of SCH reduced iCa in immune cells, which impaired their ability to phagocytize and kill bacteria in vitro. It seems that SCH can have a range of detrimental effects on dairy cows. Therefore, managing blood Ca in dairy cows involves not only reducing the risk of CH but also preventing SCH.

Dietary Methods to Reduce Hypocalcemia

Three dietary strategies have been evaluated to reduce the risk of CH and SCH in dairy cows immediately after calving. Those strategies involve altering the mineral composition of the prepartum diet or adding sequestering agents that bind Ca in the gut and prevent its absorption.

Limiting Dietary Ca Intake

One of the oldest approaches to controling hypocalcemia in dairy cows is to limit the amount of Ca consumed and absorbed in the gut to induce a state of negative Ca balance in the last weeks of gestation. Work at Iowa State University in the early 1970's demonstrated that feeding prepartum diets with low Ca content effectively reduced the incidence of CH in cows considered to be highly susceptible to this metabolic disorder (Goings et al., 1974). When cows are fed diets that induce a negative Ca balance, they have increased concentrations of parathyroid hormone (**PTH**) which is critical to stimulate bone resorption, intestinal absorption, and renal reabsorption.

Yarrington et al. (1974) compared dairy cows fed a diet that supplied < 10 g of Ca and approximately 25 g of P per day (low Ca diet) with cows fed 25 g of Ca and 25 g of P per day prepartum (control diet). The authors challenged the cows at approximately 10 d prepartum with intravenous EDTA for 4 h, a salt that chelates iCa in blood and increases renal excretion inducing a period of SCH. They observed that cows fed the low Ca diet had a faster return of blood Ca to the normal concentrations after EDTA challenge than cows fed the control diet. Cows fed the low Ca diet had increased markers of bone remodeling suggesting greater Ca resorption activity. In fact, hydroxyproline concentrations in urine increased to a greater extent in cows fed the low Ca than the control diet. Hydroxyproline is an amino acid that is a major component of the protein collagen and increased concentrations in urine are an indication of bone resorption. One of the interesting findings was that the Chief cells in the parathyroid glands of cows receiving the low Ca diet were characterized as being in an active synthesizing phase with alterations of the organelles indicating increased secretory activity of PTH. It seems that feeding diets that induce a negative Ca balance promotes increased mobilization of Ca from bone in order to maintain normal concentrations in blood when intestinal absorption is inadequate. Upregulating these mechanisms before calving likely explain the reduced susceptibility of dairy cows to CH when fed low Ca diets.

The challenge with low Ca diets is that they have to induce a negative Ca balance. In late gestation, dairy cows require approximately 20 g of absorbable Ca to meet the needs for endogenous losses in feces (~ 10 g/d), uterine and fetal accretion (~ 9.5 g/d) and urinary loss (~ 0.5 g/d). Most feeds and Ca sources have bioavailability estimated at 50 to 70%; therefore, to induce a negative Ca balance in prepartum cows with typical intake of 10 to 12 kg of DM per day in the last two weeks of gestation, a diet would have to contain no more than 0.25% Ca to assure that intestinal absorption would

be less than the 20 g needed. Obviously, this is not simple to achieve because most forages, grains, and by products fed to dairy cows have at least 0.25% Ca.

Feeding Zeolite to Sequester Ca in the Gut

Feeding low Ca diets prepartum reduces the risk of hypocalcemia in dairy cows, but achieving diets that induce a negative Ca balance is challenging. One approach to reduce Ca availability is to feed supplements that sequester Ca in the gut, therefore, preventing its absorption. One such strategy that has been investigated is the addition of Zeolite to prepartum diets. Zeolites are complex structural compounds consisting of interlocking molecules of SiO_4 and AlO_4 in the form of a honey-comb. The aluminum silicate formed carries negative charges that attract positively charged ions such as Ca^{2+} and Mg^{2+} in the gut.

Thilsing et al. (2006) investigated the binding capacity of zeolite to Ca, P, and Mg in vitro using a system to mimic the conditions of the rumen of cattle. The addition of zeolite to the incubation containing rumen fluid reduced supernatant Ca and Mg, but had no effect on P. When the pH of the rumen fluid was reduced by addition of HCl then Zeolite was capable of binding P. The authors suggested that the ability of Zeolite to bind Ca when the pH of the suspension is similar to that of the rumen, or P under more acidic pH would be desirable to prevent hypocalcemia in cattle. A reduction in the intestinal absorption of Ca and P prepartum would improve the ability of the cow to mobilize Ca at the onset of lactation. However, Zeolite also bound Mg, which would be unfavorable to prevent CH and SCH, and could potentially induce hypomagnesemia in cows fed diets low in Mg.

The concept of feeding Zeolite has been evaluated in dairy cattle in a few experiments and the results are promising. In general, cows are fed 0.7 to 1.0 kg of Zeolite which represents 6 to 10% of the total DM intake of a prepartum cow in the last 2 weeks of gestation. Feeding Zeolite during the last 2 weeks of gestation increased plasma tCa concentration on the day of calving and the first few days postpartum (Thilsing et al., 2002). Cows fed Zeolite had increased concentration of $1,25(OH)_2$ Vitamin D_3 in serum prepartum, the active form of Vitamin D_3. However, feeding Zeolite depressed DM intake, a common finding with feeding large quantities of minerals to cattle, and reduced concentrations of Mg and P in serum. Although sequestering Ca prepartum seems effective at improving Ca metabolism at the onset of lactation, it poses issues with DM intake and blood Mg concentrations.

Altering the Dietary Cation-Anion Difference

In the US, the most widely implemented method to reduce the risk of hypocalcemia in dairy cows is to alter the mineral composition of the prepartum diet to induce a compensated metabolic acidosis in the last weeks of gestation. This concept is based on the fact that cows under metabolic acidosis have increased sensitivity to PTH (Goff et al., 2014) and induction of metabolic acidosis with acidogenic salts increases the expression of PTH receptor in the kidney of cattle (Aris et al., 2016). In general, the

maximal PTH response to Ca chelation occurs during metabolic acidosis compared with normal blood pH (Lopez et al., 2002).

When salts containing strong anions are fed, the premise is that the rate and extent of absorption of the anion is greater than that of the cation in the salt. In some cases, the cation in the salt can be utilized by the rumen microbes such as in the case of NH_4Cl. The most common strong anions fed are Cl^- and S^{2-} in the form of SO_4^{2-}. It is thought that Cl^- is more bioavailable than S^{2-} so it has greater acidifying power. When salts of Cl and SO4 are fed, for instance $CaCl_2$, more equivalents of the anion (Cl^-) from the molecule is absorbed than the cation (Ca^{2+}). This causes an imbalance in charges in the epithelial cell of the intestine (increase in negative charges), which forces secretion of HCO_3^- into the intestinal lumen or retention of H^+ ions. The end result is a loss of HCO_3^- and an increase in H^+ concentration which ultimately results in a state of metabolic acidosis. If compensated, then minor changes in blood pH occur, with changes in blood HCO_3^- and the partial pressure of CO_2 caused by changes in respiration rate. A common finding is aciduria because of the increased H^+ excretion in urine as part of the compensatory mechanism.

One of the problems with metabolic acidosis is that, if uncompensated, it can impair insulin signaling and exacerbate the status of insulin resistance that occurs in late gestation and early lactation. Excessive feeding of strong anions or errors during diet formulation because of inaccurate mineral composition or mixing of ingredients can potentially result in excessive intake of Cl and SO_4, which can depress blood pH and influence energy metabolism in dairy cows. Cows fed large quantities of acidogenic salts had impaired glucose utilization after a glucose tolerance test suggesting interference with insulin action on peripheral tissues (Bigner et al., 1996). Therefore, it is critical that prepartum diet formulation using negative DCAD be based on continuous monitoring of the chemical composition of the ingredients, and not on tabular values for mineral content.

Formulating diets to achieve a negative DCAD follows some logical concepts. The first is to limit the intake of strong cations. Ingredients should be selected such that low K and Na intakes are prioritized. It makes little sense to feed diets that are high in K and Na and then try to counter act their effects by feeding large quantities of strong anions. Acidogenic salts are known to depress DM intake either because of palatability issues or because of the metabolic acidosis they induce (Charbonneau et al., 2006).

When proper feed selection is made, then most diets should require fewer than 2 equivalents (**Eq**) of strong anions fed to achieve a desirable negative DCAD. An Eq is a unit of electrical charge and refers to the atomic mass of a given mineral divided by its valence. In the case of Cl-, the atomic mass is 35.5 and the valence is 1, so 1 Eq of Cl^- is the same as 35.5 g. One Eq of anion supplies enough charges capable of neutralizing 1 Eq of cation. For instance, let's suppose that the target DCAD of the diet fed to prepartum cows is -100 mEq/kg. The negative value indicates that the diet provides more Eq of anions (negatively charged ions) than cations (positively charged ions). In other words, for this diet, each kg of diet DM contains an excess of 100 mEq (or 0.1 Eq)

of anions relative to cations. Now, let's assume that the expected DM intake for the prepartum cows will be 12 kg. Therefore, each cow will consume an excess of 1.2 Eq of anions relative to cations (12 kg x 0.1 Eq = 1.2 Eq anions). In order for 2 Eq of anions to reduce the dietary DCAD to -100 mEq/kg, then the basal diet cannot have a DCAD > +100 mEq/kg. This is why it is critical to select ingredients with low concentrations of K and Na or with relatively low DCAD. This will assure that inclusion of acidogenic salts will be minimal which, in turn, should reduce the risk of suppression of DM intake and increase efficacy of the program to prevent hypocalcemia. Remember, it is not the relative DCAD of the diet that is critical, but the total Eq of strong anions relative to strong cations consumed by the cow. If a diet contains a DCAD of -100 mEq/kg, but the cow only eats 6 kg/day, the acidifying ability will be limited because the relative excess intake of anions will be only 0.6 Eq. On the other hand, a diet with DCAD of -80 mEq/kg fed to cows consuming 13 kg of DM will induce a more exacerbated metabolic acidosis because the relative excess intake of anions will be 1.04 Eq.

It is important to mention that the ideal DCAD to prevent hypocalcemia is unknown. Most suggestions range from -50 to -150 mEq/kg, a range that would result in a differential of 1.2 Eq of anions consumed per day. Reducing the DCAD improves blood Ca concentrations (Charbonneau et al., 2006) and this effect can be seen in Holstein cows fed diets containing +130, -80, -130, and -180 mEq/kg. However, the association between DCAD and blood Ca is nonexistent if we look only at the data from cows fed the negative DCAD (-80 to -180 mEq/kg) suggesting equal ability to prevent hypocalcemia within that range (**Figure 3**).

Manipulating DCAD only is not enough. Prepartum diets should have limited concentration of P. Increasing dietary intake of P to amounts above 50 g/day during the prepartum period can increase the risk of CH (Lean et al., 2006). Increase in blood P concentrations, induced by release of Ca and P from bone resorption, is controlled initially through PTH by increased filtration and urinary P excretion. In addition, fibroblast growth factor (**FGF**) 23 produced by osteocytes and osteoblasts regulates blood P concentration (Bergwitz and Jüppner, 2010). Under high blood P concentrations, FGF23 expression is up-regulated, which helps increase urinary P loss to maintain blood P under a tight range. However, FGF23 suppresses the activity of 1-α hydroxylase in the kidneys, the key enzyme responsible for synthesis of active vitamin D3. Therefore, if blood phosphate increases because of overfeeding P, then circulating concentrations of $1,25(OH)_2$ vitamin D_3 decreases, which may result in hypocalcemia (Bergwitz and Jüppner, 2010). Therefore, it is recommended that diets for prepartum cows should contain no supplemental P and concentration of 0.30% P in the dietary DM is more than enough to meet the P needs of the pregnant cow.

Another aspect is the concentration of Mg in the diet. It is clear that NRC (2001) recommendations for Mg in diets of prepartum cows are inadequate. Magnesium is important not only to prevent hypomagnesemia, but also to enhance the ability of the cow to mobilize Ca from bone when stimulated by PTH. Magnesium is involved in the second messenger system of PTH and low blood Mg impairs Ca resorption (Robson et al., 2004). It is unclear what the ideal Mg content in the diet should be, but most

recommendations are around 0.40 to 0.45% of the DM. This is based on the idea that intakes of 40 to 50 g of Mg/d will provide sufficient soluble Mg to increase ruminal concentrations that will favor passive diffusion. This high concentration of Mg might be more important when the main source fed is in the oxide form. It is known that MgO is poorly soluble and bioavailability usually is less than 50%.

Finally, debate exists about the ideal concentration of Ca in the diet when cows are fed rations containing a negative DCAD. Lean et al (2006) conducted a meta-analysis to critically and systematically review the literature on hypocalcemia in dairy cattle. They demonstrated that the risk of CH is low when dietary Ca < 0.6%, then the risk increases with increasing dietary Ca up to 1.5%, after which it decreases again. The lowest risks of CH were detected when dietary Ca was either < 0.6% of > 2.0%. Because diets that induce metabolic acidosis rely on increased Ca resorption from bones, it is poorly justifiable to overfeed Ca. The reduced risk of CH when diets contain > 2.0% Ca are likely the result of transcellular transport because of diffusion from the lumen of the rumen and intestine to the vascular space through cellular junctions because of the large differential concentration between the lumen and the interstitial space. However, it is important to remember that Ca is a cation and large increases in Ca feeding with intestinal absorption will attenuate the acidifying effect of the acidogenic salts fed.

Duration of Feeding Acidogenic Salts Prepartum and Urinary pH

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

Because cows fed acidogenic salts excrete acidic urine, measuring urine pH has become a common method to monitor the effectiveness of feeding such diets. Cows should be on the diet for at least 48 h for urine pH to drop and become somewhat stable. Recommendations for urininary pH vary, and most are based on personal observation to prevent CH (6.2 to 6.8; Jardon, 1995). Charbonneau et al. (2006) clearly showed that as the dietary DCAD decreases, so does urinary pH. Very likely, when urinary pH drops below 5.00, the cow responds by reducing DM intake which prevents further increments in the acidotic state. We have found little or no association between

average urinary pH measured twice weekly in the last 2 weeks of gestation and blood iCa concentration in the first 2 DIM (**Figure 3**). It seems that urinary pH is a good monitor to evaluate the degree of metabolic acidosis, but once it is below 7.0 it is not necessarily associated with improvements in blood Ca in early lactation. It important to mention that within a given diet, substantial variability exists in mean urinary pH in the last 2 weeks of gestation, particularly for cows fed diets of very low DCAD (**Figure 4**). Therefore, one should be cautious of making dietary changes based on small changes in urinary pH or over-interpret the variations in urinary pH among cows fed a given diet.

Supplementing Ca after Calving

One method commonly used to prevent CH is the administration of injectable or oral Ca salts immediately after calving. In many cases, those injectable solutions (Ca borogluconate) or oral gels or bolus (primarily Ca chloride or combinations with other salts) are administered repeatedly in the first day or two after calving. The premise is that the oral Ca will increase blood concentrations of Ca that will prevent cows from developing CM. However, in recent years, the use of oral Ca products have become an alternative to minimize the risk of SCH in postpartum dairy cows.

Oetzel and Miller (2012) administered 43 g of Ca as an oral bolus containing $CaCl_2$ and $CaSO_4$ to dairy cows in the first hours after calving and repeated the application 8 to 35 h after calving. The authors observed no change in blood concentration of iCa, likely because sampling time was too late relative to treatment. In general, 43 g of oral Ca as salts of Cl and SO4 increase blood iCa for only 2 h. Therefore, if samples are not collected within that time frame, no differences will be observed. Administration of oral Ca benefited subpopulations of cows, those that were lame at calving and cows of greater potential for milk yield based on the previous lactation mature equivalent (**ME**) for milk yield. Lame cows that received oral Ca had reduced incidence of health events in the first month of lactation. Cows with a previous lactation 305-d ME of at least 5% above the mean value for the herd produced more milk in the first month postpartum if treated with Ca at calving.

We have recently completed 2 experiments to characterize blood concentrations of minerals and acid-base status after oral dosing of Ca salts and to determine the effects of oral Ca on mineral and metabolic status, incidence of diseases, and reproductive and productive performance (Martinez et al, 2016). In experiment 1, 18 Holstein cows on the day of calving were assigned to receive a single dose of 0, 43, or 86 g of Ca as an oral bolus. Blood was sampled before, and at 0.5, 1, 2, 4, 8, 12, and 24 h after treatments to characterize acid-base status and mineral concentrations. Concentrations of iCa increased for 2 h in cows supplemented with 43 g of Ca and fewer than 8 h in cows supplemented with 86 g of Ca (**Figure 5**). The changes in iCa concentrations, from pre-treatment to 0.5 h after 86 g of Ca supplementated on d 0 were of 0.11 ± 0.03 m*M* in multiparous and 0.25 ± 0.03 mM in primiparous cows.

In experiment 2, 450 Holstein cows considered of low (LRM; normal calving) or high risk (HRM; dystocia, twins, stillbirth, retained placenta and/or vulvo-vaginal

laceration) of metritis on the day of calving were blocked by parity as primiparous or multiparous and then randomly assigned to control, no Ca supplementation; 86 g of Ca on d 0 and 1 postpartum (CaS1); or 86 g of Ca on d 0 and 1 postpartum followed by 43 g/d on d 2 to 4 postpartum (CaS4). Blood was sampled before and 30 min after treatment on d 0, and 30 min after treatments on d 1 to 4, and on d 7 and 10 for determination of concentrations of minerals and metabolites and blood acid-base responses. Disease was evaluated for the first 30 DIM. Milk yield was measured daily for the first 5 months of lactation. Reproduction and cow survival were evaluated for the first 210 DIM. On the day of calving and before any treatment, 48, 49 and 36% of the control, CaS1 and CaS4 cows had SCH (tCa < 2.125 m*M*). Oral Ca reduced the incidence of SCH (control = 69.3%; CaS1 = 57.5%; CaS4 = 34.2%). Calcium supplementation decreased the prevalence of SCH on d 0 and 1 posptarutm in all cows. Stopping oral Ca in CaS1 on d 1 postpartum, however, caused a rebound in SCH on d 2 to 4 postpartum in primiparous cows. To our surprise, oral Ca tended to increase the incidence of metritis (control = 22.7%; CaS1 = 35.9%; CaS4 = 31.2%), primarily because of an increment in LRM primiparous cows (control = 17.9%; CaS1 = 35.7%; CaS4 = 42.9%). Oral Ca increased morbidity in primiparous cows (control = 38.1%; CaS1 = 61.8%; CaS4 = 60.3%) but had no impact on multiparous cows (control = 38.2%; CaS1 = 35.1%; CaS4 = 30.1%). On the other hand, oral Ca reduced the incidence of certain diseases (mastitis + lameness + digestive problems) in multiparous cows (control = 16.3%; CaS1 = 6.2%; CaS4 = 5.3%). The body condition did not differ among treatments, and cows lost on average 0.44 units of body condition in the first month of lactation. Calcium supplementation did not affect milk yield in the first 5 months postpartum. However, as reported by Oetzel and Miller (2012) for multiparous cows, Ca supplementation was beneficial to milk yield in the first 30 DIM for cows of greater production potential (above the mean 305-d ME milk yield of the previous lactation; 14,003 kg), but detrimental to multiparous cows below average production potential. Calcium supplementation to primiparous cows reduced pregnancy per artificial insemination (**P/AI**) at the first AI (control = 55.8, CaS1 = 31.5, CaS4 = 37.0%) and at all AI (control = 48.5, CaS1 = 34.6, CaS4 = 38.5%); however, Ca supplementation to multiparous cows improved P/AI at the first AI (control = 32.1, CaS1 = 38.6, CaS4 = 41.3%) and at all AI (control = 28.1, CaS1 = 35.3, CaS4 = 40.5%). These responses of P/AI to Ca supplementation resulted in extended median days to pregnancy (control = 75, CaS1 = 100, CaS4 = 94 d) and a smaller proportion of pregnant cows (control = 89.3, CaS1 = 83.9, CaS4 = 83.9%) in primiparous cows, but fewer days to pregnancy (control = 115, CaS1 = 94, CaS4 = 94 d) and increased proportion of pregnant cows in multiparous cows (control = 67.0, CaS1 = 77.2, CaS4 = 74.3%).

Collectively, these results indicate that responses to oral Ca supplementation are conditional on parity and production potential of cows. Oral Ca supplementation in the first days of lactation might be beneficial to health, production and reproduction in multiparous cows, particularly those of greater production potential that might be at a greater risk for SCH. On the other hand, the work by Martinez et al. (2016a,b) suggests that dosing primiparous cows with oral Ca for 2 to 5 consecutive days should be discouraged. It increased the risk of diseases, depressed reproduction, and had no benefit on production.

Conclusions

Hypocalcemia is a prevalent metabolic disorder in dairy cows in early lactation. It is not related to inadequate intake of Ca but caused by the inability of the cow to mobilize Ca from bones quickly at the onset of colostrogenesis and lactation. Hypocalcemia increases the risks of numerous other health problems resulting in economic losses to dairy producers. Dietary manipulation remains the method of choice to minimize the incidence of hypocalcemia. It involves limiting the intake of Na, K and P, and then manipulating the remainder of the macrominerals to achieve a negative dietary DCAD. Alternative methods such as the use of Ca sequestering agents are available, but they also present challenges as does feeding of acidogenic salts prepartum. In some cases, supplementing Ca in an oral bolus can benefit multiparous cows but the practice is discouraged in primiparous cows. Alternative dietary and pharmacological methods are currently under investigation. Manipulating the source of vitamin D3 fed prepartum or administering to dairy cows immediately after calving show some promise.

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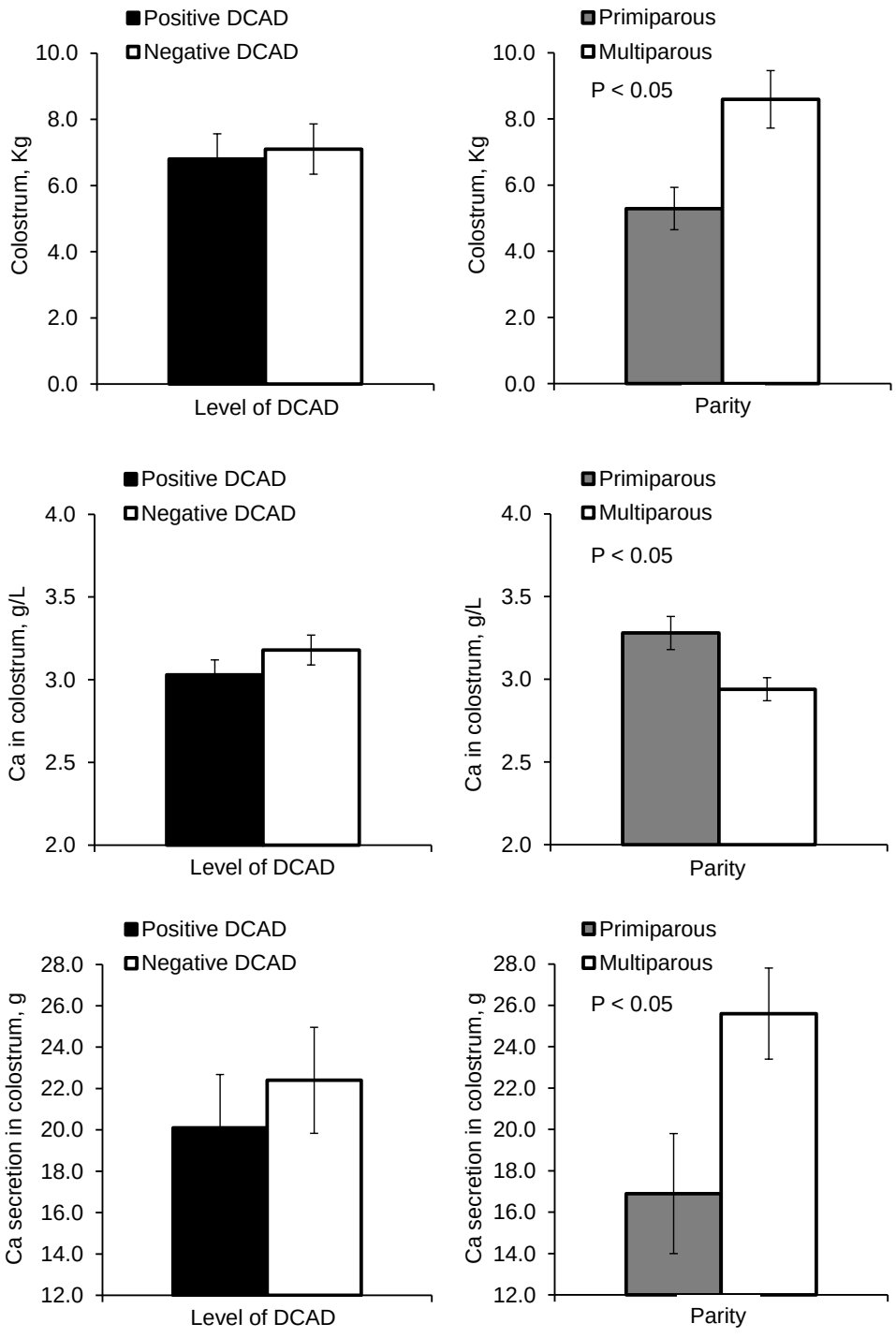

Figure 1. Colostrum yield (kg), concentration of Ca in colostrum and Ca secretion in colostrum of primiparous and multiparous cows fed a diet prepartum containing either +130 mEq/kg (positive DCAD) or -130 mEq/kg (negative DCAD). Adapted from Martinez et al. (2014).

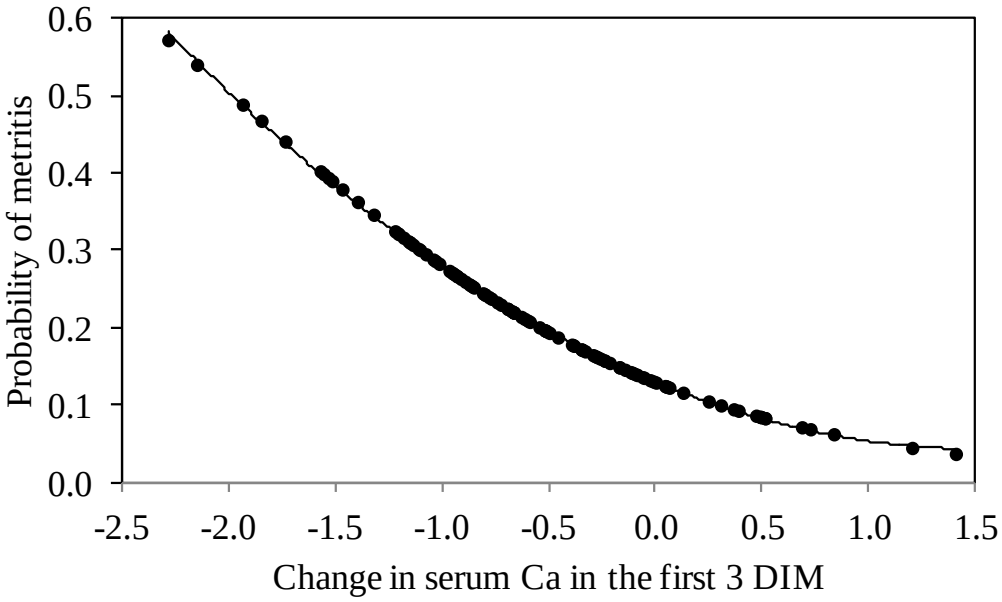

Figure 2. Probability of metritis relative to the change in serum tCa concentrations between the d of calving and the lowest value within the first 3 DIM. As serum tCa decreased, the risk of metritis increased ($P < 0.05$). Adapted from Martinez et al. (2012).

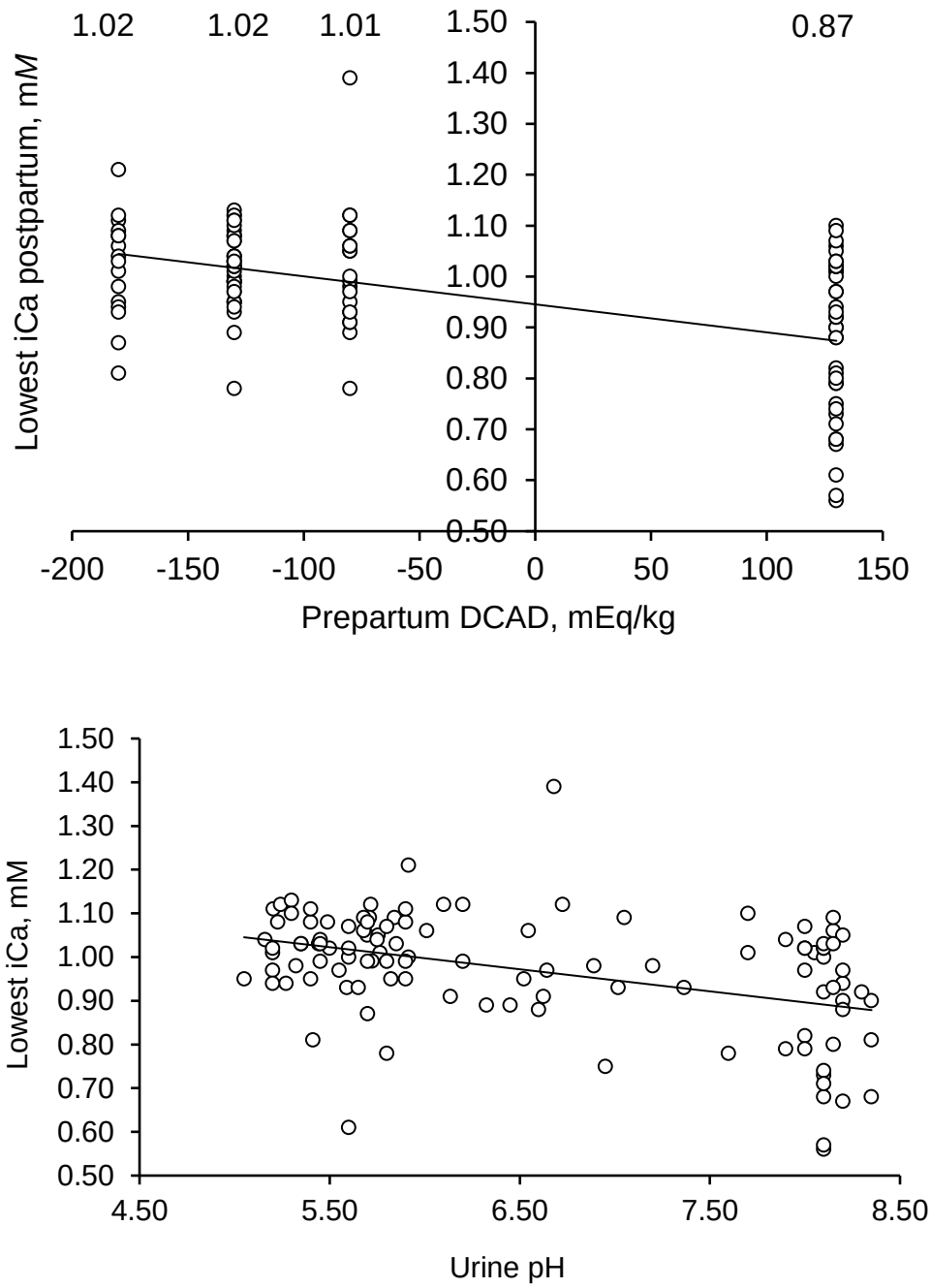

Figure 3. Lowest blood iCa concentration in the first 2 DIM (0, 1, and 2 DIM) of Holstein cows fed diets that differed in DCAD (+130, -80, -130, -180 mEq/kg) and according to the average urine pH in the last 2 weeks of gestation. Average values for iCa are depicted for each dietary DCAD. Santos et al. unpublished results.

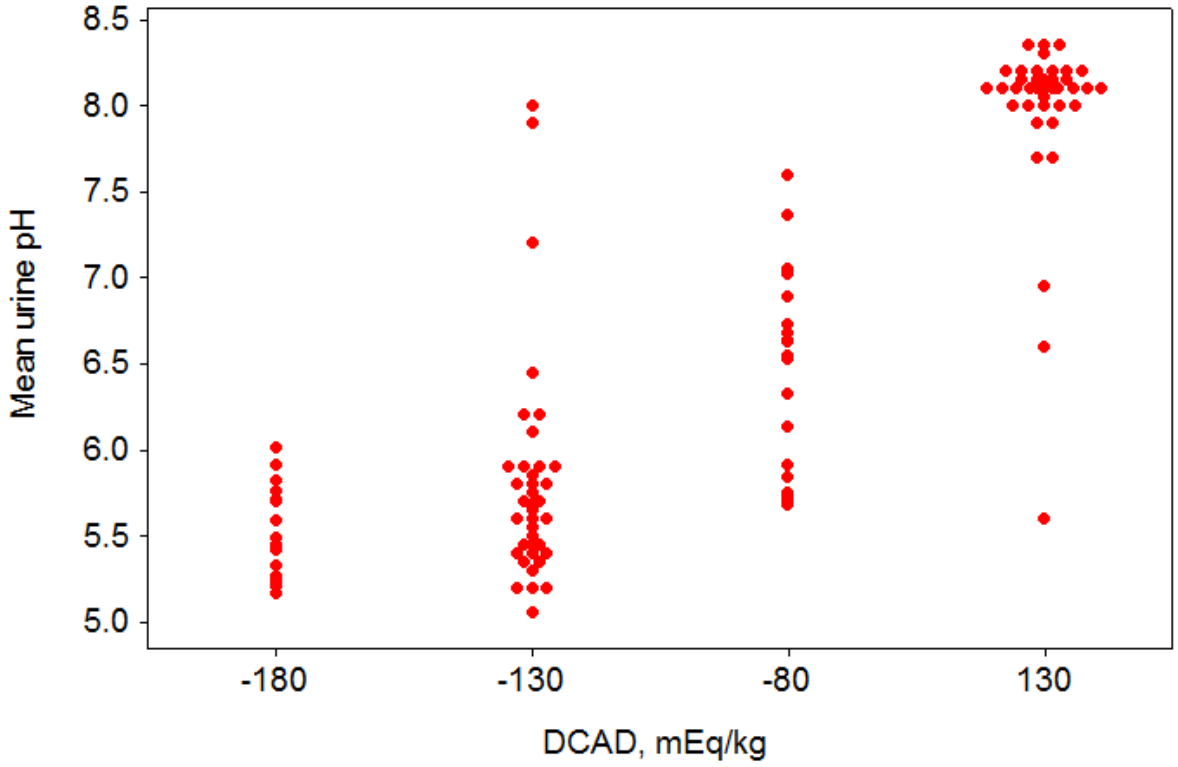

Figure 4. Distribution of mean urine pH in Holstein cows in the last 2 weeks of gestation (mean of 4 measurements per cow) according to the DCAD of the diet offered.

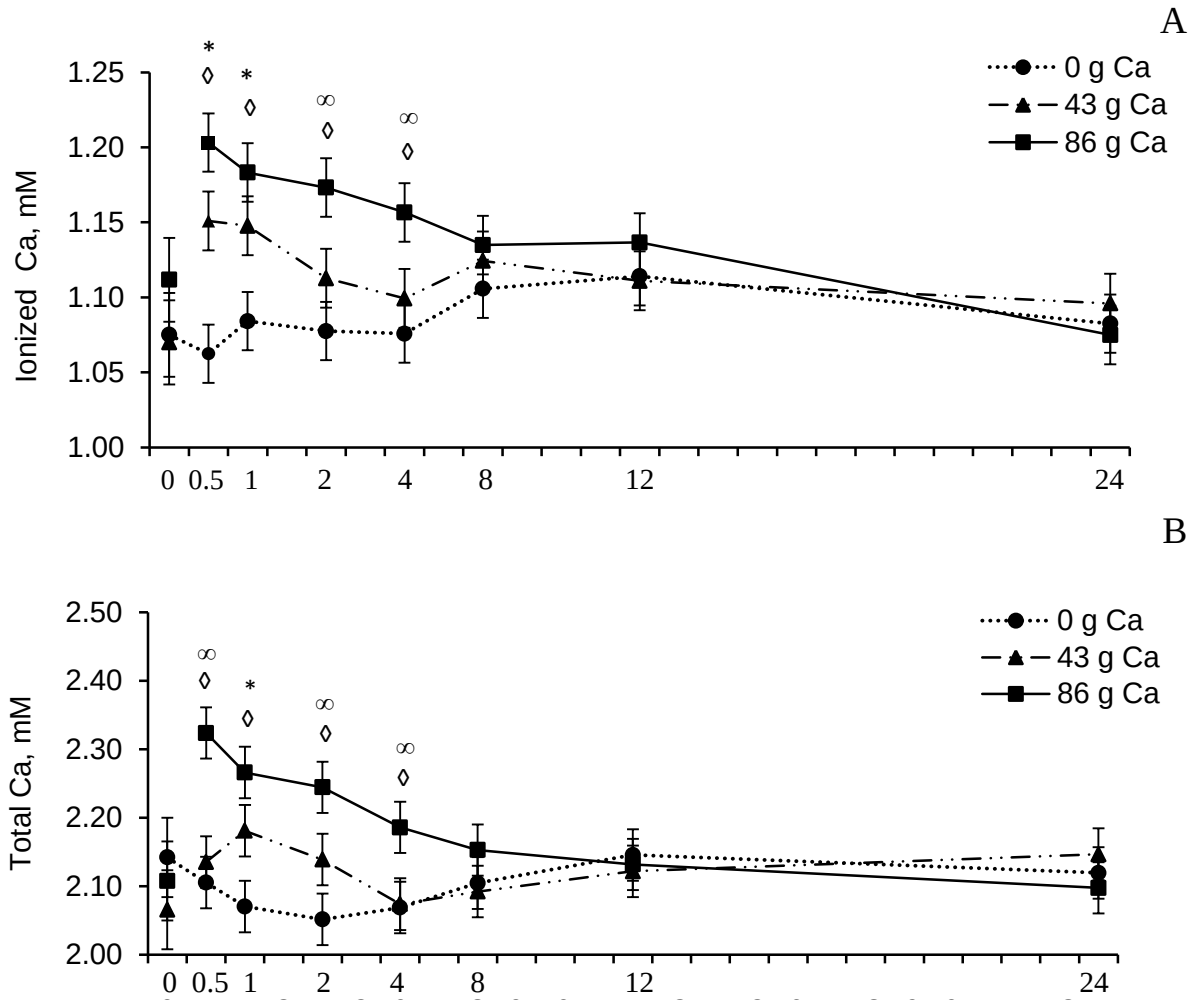

Figure 5. Characterization of blood ionized Ca (iCa; A) and serum total Ca (tCa; B) concentrations after oral supplementation with 0, 43, or 86 g of oral Ca in Holstein cows. Administration of oral Ca increased ($P < 0.01$) blood iCa and tCa, and the increments were dose-dependent. A dose of 43 g of oral Ca increase blood iCa and serum tCa for 2 h, whereas 86 g of oral Ca increased iCa and tCa for 4 to 8 h (Adapted from Martinez et al., 2016).

SESSION NOTES