PROCEEDINGS

2014

25th ANNUAL FLORIDA RUMINANT NUTRITION SYMPOSIUM

February 4 - 5, 2014
Best Western Gateway Grand Hotel
Gainesville, Florida

Department of Animal Sciences
University of Florida
Institute of Food and Agricultural Sciences
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25th ANNUAL FLORIDA RUMINANT NUTRITION SYMPOSIUM
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University of Florida, IFAS

Tuesday, February 4, 2014—*Pre Conference Symposium (Prince Agri Products Inc.*)

9:00AM Program Introduction — **Dr. Jim Chapman** — Prince Agri Products Inc.

9:10AM The Relationship of Immunity and Reproduction in Dairy Cows — **Dr. David Hurley,** University of Georgia

10:00AM Impacts of Heat Stress on Immune Function of Cattle — **Dr. Robert Collier,** University of Arizona

10:50AM Inflammatory Responses to Sub-Acute Ruminal Acidosis — **Dr. Tanya Gressley**, University of Delaware

11:45AM Buffet Lunch

Tuesday, February 4, 2014

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

11:45AM Buffet Lunch

1:00PM Welcome — **Dr. Geoff Dahl**, University of Florida

1:10PM Etiology and Prevention of Fatty Liver and Ketosis in Dairy Cattle — **Dr. Don Beitz,** Iowa State University

1:50PM Applied Protein Nutrition of Ruminants: Current Status and Future Directions — **Dr. Fred Owens**, DuPont Pioneer

2:30PM Vitamin D Metabolism in Dairy Cattle and Implications for Dietary Requirements — **Dr. Corwin Nelson,** University of Florida

3:10PM Refreshment Break

3:40PM Mechanisms of Volatile Fatty Acid Absorption and Metabolism and Maintenance of a Stable Rumen Environment — **Dr. Greg Penner**, University Saskatchewan

4:20PM Strategies to Improve Rumen Microbial Efficiency — **Dr. Timothy Hackman**, University of Florida

5:00PM Welcome Reception

Wednesday, February 5, 2014

6:30AM Continental Breakfast

8:00AM Characteristics of Feed Efficiency and Use for Selection of Heifers and Cows — **Dr. Phillip Lancaster,** University of Florida

8:40AM Use of Corn Co-Products in Beef Cattle Diets — **Dr. James MacDonald**, University of Nebraska

9:20AM Forage Management and Methods to Improve Nutrient Intake in Grazing Cattle — **Dr. Flávio A.P. Santos**, University of São Paulo

10:00AM Refreshment Break

10:30AM Amino Acid Requirements and Post-Absorptive Metabolism in Cattle: Implications for Ration Formulation — **Dr. Hélène Lapierre***,* AgriFood Canada

11:10AM Feed Efficiency and Sustainability of the Cattle Industry — **Dr. Frank Mitloehner**, University of California Davis

11:50AM Ruminant Nutrition Symposium Adjourn

Additional copies of these proceedings are available at $15 per copy. Make checks payable to: Florida Ruminant Nutrition Symposium.

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Symposium Speakers

Guest

Don Beitz, Iowa State University, Ames, IA
Robert Collier, University of Arizona, Tucson, AZ
Tanya Gressley, University of Delaware, Newark, DE
David Hurley, University of Georgia, Athens, GA
Hélène Lapierre, AgriFood Canada, Sherbrooke, Quebec, Canada
James MacDonald, University of Nebraska, Lincoln, NE
Frank Mitloehner, University of California, Davis, CA
Fred Owens, Dupont Pioneer, Johnston, IA
Greg Penner, University of Saskatchewan, Saskatoon, Canada
Flávio A.P. Santos, University of São Paulo, Brazil

University of Florida
Department of Animal Sciences

Timothy Hackman, Assistant Professor
Phillip Lancaster, Assistant Professor
Corwin Nelson, Assistant Professor

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Charles Staples, Dept. of Animal Sciences, University of Florida, Gainesville
David M. Waagner, Elanco Animal Health, Valdosta, GA

25th Annual Florida Ruminant Nutrition Symposium

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Title and Presenter ***Page***

Dr. David J. Hurley is a professor in the Food Animal Health and Management Program at the University of Georgia. He did his BA studies in the University of Wisconsin system in the area of chemistry and biological chemistry. His PhD studies were at the Pennsylvania State University in microbiology. He has worked in animal health research since the mid-1980's, first in the Department of Veterinary Science at the Pennsylvania State University, then at the South Dakota State University, and currently while at University of Georgia. He has served as a consultant and collaborator with many companies in animal health and was co-founder of Rural Technologies, Inc. in Brookings South Dakota. His research has focused on cattle, swine and horses, with a special interest in immune development and regulation. He has worked on the mammary gland, mammary inflammation and immunity and immune transfer to the neonate for about 25 years. His work included methods to assess the immune outcomes of vaccination and the role of inflammatory activation in the progress of disease. He has developed tools and models to study inflammation that impacts production systems including fairly simple ELISA based measurements that assess the level of occult inflammation in the reproductive tract of dairy cows and the relationship of that inflammation to pregnancy success.

Dr. Robert Collier is a professor and former head of the Department of Animal Sciences at the University of Arizona. He received his BS degree in Zoology from Eastern Illinois University in 1969, served in the Army Medical Corps, and then obtained his MSc degree in Zoology from Eastern Illinois University in 1973 and his PhD in Dairy Science from the University of Illinois in 1976. After his PhD, Dr. Collier was a NIH post-doctorate fellow at Michigan State University in the laboratory of Dr. Allen Tucker. He was a faculty member in the Department of Dairy Science at the University of Florida from 1976 to 1985, and then moved to Monsanto Company where he was the Dairy Research Director until 2009. His areas of expertise include environmental and lactation physiology, endocrinology and molecular biology. Currently Dr. Collier teaches undergraduate courses and conducts research in the fields of environmental physiology with a focus on the dairy industry.

Dr. Tanya F. Gressley is an Associate Professor in the Department of Animal and Food Sciences at the University of Delaware. She obtained her BSc in 1997 and the MSc degree in 1999 in Animal Sciences from the University of Maryland. She then moved to the University of Wisconsin in Madison where she received her PhD in 2005. Dr. Gressley teaches dairy production and lactation physiology and her research program focuses on multiple aspects of dairy cattle nutrition as they relate to health and productivity.

Dr. Donald C. Beitz earned B.S. and M.S. degrees in agricultural science in 1962 and dairy science in 1963 from the University of Illinois and a Ph.D. degree from Michigan State University in 1967 with majors in dairy nutrition and biochemistry. Dr. Beitz began his professional career at Iowa State University with an appointment in the Departments of Animal Science and of Biochemistry, Biophysics, and Molecular Biology. He is a Charles F. Curtiss Distinguished Professor of Agriculture. Teaching responsibilities include biochemistry courses for veterinary, undergraduate, and graduate students. Dr. Beitz's research activities relate to practical problems of animal agriculture and human nutrition and focused on milk fever, ketosis, cholesterol regulation by diet, dietary and genetic control of milk and meat composition, mitochondrial DNA and milk production, and beef tenderness as influenced by vitamin D. Dr. Beitz has published more than 263 peer-reviewed manuscripts, 319 abstracts, and 9 patents. Honors included local and national awards for teaching, research, and advising excellence. Moreover, he is a Fellow of ADSA, ASAS, ASN, Iowa Academy of Science, and AAAS. He has served as president of ADSA, CAST, and FASS. Dr. Beitz has had a fulfilling career as a researcher, teacher, and advisor at Iowa State University and serving agricultural societies.

Dr. Fred Owens grew up on a farm in Wisconsin and did his graduate work at the University of Minnesota. He spent 6 years in the Animal Science Department at the University of Illinois before joining the faculty at Oklahoma State University. At Oklahoma State, Dr. Owens conducted research on rumen function, metabolic disorders, feed intake, feed additives, and growth and development. During his 24 years at Oklahoma State, he was Editor-in-Chief for the Journal of Animal Science and won the Morrison Award from the American Society of Animal Science. In 1998, Dr. Owens retired from Oklahoma State to start a new career with DuPont Specialty Grains that was folded into Pioneer Hi-Bred when DuPont purchased Pioneer. As Senior Ruminant Research Nutritionist at Pioneer Hi-Bred International, he is involved with developing and testing cereal crops and oilseeds that have been or can be altered to improve both nutrient availability and quality of milk and meat produced by ruminants.

Dr. Corwin Nelson is an Assistant Professor of Physiology in the Department of Animal Sciences at the University of Florida. Dr. Nelson was born and raised on a dairy farm in East Central Minnesota. After a year of the Farm and Industry Short Course at the University of Wisconsin-Madison, and a couple years of farming, he enrolled at the University of Minnesota-Duluth and earned his BSc in Biochemistry in 2006. He continued on to Iowa State University to study under Dr. Don Beitz and Dr. John Lippolis at the USDA National Animal Disease Center. He received his PhD in Biochemistry and Immunobiology in 2010 for his studies on vitamin D signaling in the bovine immune system. Upon completion of his PhD, Dr. Nelson returned to the University of Wisconsin-Madison as a postdoctorate fellow with Dr. Colleen Hayes in the Department of Biochemistry to study the role of vitamin D in prevention and treatment of multiple sclerosis, an autoimmune disorder. In 2013, he joined the faculty in the Department of Animal Sciences at the University of Florida. His research continues to focus on the regulation and the influence of vitamin D metabolism on the immune system with an emphasis on mastitis in dairy cattle. Dr. Nelson and his family are enjoying the warm Florida weather after many long, cold winters in the Upper Midwest.

Dr. Greg Penner joined the Department of Animal and Poultry Science at the University of Saskatchewan as an Assistant Professor (2009) after obtaining his BSA (2004) and M.Sc. (2004) degrees from the same University, and his PhD from the University of Alberta (2009). His research program focuses on understanding the regulation of absorptive and barrier function of the gastrointestinal tract in ruminants. Notable accomplishments include the development of 2 indwelling pH measurement systems that have been adopted by the research community worldwide. Dr. Penner has a well-funded research program, has published 40 peer-reviewed papers, provided over 25 invited presentations, and in 2012, he received the Canadian Society of Animal Science Young Scientist Award.

Dr. Timothy Hackmann is Assistant Professor of Gastrointestinal Microbiology in the Department of Animal Sciences at University of Florida. He received his BS in Biological Sciences from University of Missouri in 2005, MS in Animal Sciences from University of Missouri in 2008, and PhD in Nutrition from The Ohio State University in 2013. Dr. Hackmann's research attempts to improve efficiency of rumen microbial growth. Specifically, his program characterizes how rumen microbes respond to excess energy, with the goals of 1) eliminating wasteful microbes and 2) improving predictions of diet formulation software.

Dr. Phillip Lancaster was born and raised on a family livestock and crop farm in west central Illinois. He earned his BS degree in Agricultural Science from Western Illinois University in 1999, the MSc degree in Animal Science in 2004 from the University of Missouri, and the PhD degree in Animal Science with an emphasis on ruminant nutrition in 2008 from Texas A&M University. His dissertation research focused on characterization of feed efficiency traits and relationships with body composition and reproductive traits in Brangus heifers. Dr. Lancaster has published several abstracts, research reports, and journal articles on feed efficiency in beef cattle. Following graduation he accepted a position as a postdoctoral fellow in the Department of Animal Science at Oklahoma State University where his research focused on intramuscular adipose tissue development in growing-finishing beef cattle. In 2013, he joined the Range Cattle Research and Education Center at University of Florida as assistant professor, where one of his primary research efforts is feed efficiency in mature beef cattle.

Dr. Jim MacDonald is an Associate Professor of Animal Science at the University of Nebraska-Lincoln. Before moving to Nebraska, he was the project director of the beef cattle research program at the Texas AgriLife Research and Extension Center in Amarillo from 2006 to 2012. While in Texas, he worked extensively on incorporating distiller's grains into beef production systems in the Southern Plains. Current areas of interest include incorporating alternative feeds into beef diets, improving yearling stocker production systems, integrated cropping and livestock systems, improving the management of newly received calves, and investigating dietary effects on beef quality. Dr. MacDonald was raised on a cow-calf and diversified cropping operation in central North Dakota. He received his BSc in Animal Sciences from North Dakota State University, and his MSc and PhD from the University of Nebraska. He lives with his wife and two children outside of Lincoln.

Dr. Flávio A.P. Santos is a Professor of Ruminant Nutrition and current chair of the Department of Animal Sciences in the School of Agriculture (ESALQ) at the University of São Paulo, Brazil. He obtained his MSc degree in Animal Nutrition and Forage Conservation from the University of São Paulo in 1991 and the PhD degree in Ruminant Nutrition in the Department of Animal Sciences at the University of Arizona in 1996. His research focuses on the areas of protein metabolism of dairy cattle, grain processing and starch utilization by ruminants, grain supplementation of cattle grazing tropical pastures, and feedlot nutrition. Dr. Santos has supervised 8 PhD and 31 MSc students, and published 72 scientific papers in the areas of ruminant nutrition and forage conservation and utilization.

Hélène Lapierre is a research scientist working for Agriculture and Agri-Food Canada at the Dairy and Swine Research and Development Centre in Sherbrooke, Québec, Canada. She is also adjunct professor at Université Laval, QC. She obtained her BSc and MSc in Animal Science form Université Laval, Quebec; her PhD in Physiology from Université de Sherbrooke, Quebec; and completed a post-doctorate at the USDA Research Centre in Beltsville, Maryland with Drs. C.K. Reynolds and H.F. Tyrrell. Her research aims to improve the efficiency of nitrogen utilization through a better knowledge of intermediary metabolism of nitrogenous compounds. The outcomes of her research target a reduction in feeding costs for dairy producers and a reduction in dietary nitrogen losses to the environment.

Dr. Frank Mitloehner is a Professor and Air Quality Extension Specialist in the Department of Animal Science at the University of California Davis. Dr. Mitloehner received his MS degree in Animal Science and Agricultural Engineering from the University of Leipzig, Germany, in 1996 and his PhD degree in Animal Science from Texas Technical University in 2000. In 2002 he joined the faculty in the Department of Animal Science at the University of California-Davis. Dr. Mitloehner has generated and published data that are rapidly changing how livestock facilities in California and throughout the US are regulated. The focus of his research are in the areas of air quality as it relates to livestock production, especially quantification of ammonia, dust, and odor emissions in dairies, beef feedlots, and poultry operations. The main objective is to help establishing environmentally benign livestock systems. An additional area of research is in environmental physiology, focusing on effects of air emissions on animal health and welfare.

The Relationship of Immunity and Reproduction in Dairy Cows

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Introduction

Reproduction and immunity are complex processes that involve interactive physiological processes and share some common elements. Dairy cow reproduction has several critical checkpoints where interactions with the immune system are necessary and dysregulation of the immune response can lead to reproductive failure. Understanding these processes is one critical component in building a resolution to dairy reproductive problems. Management of dairy cattle affects both reproduction and immunity. Nutrition is a key management tool in optimizing both reproduction and immune function. Feeding sufficient amounts of calories, fats and protein, and elements that properly stimulate the gut-associated immune tissues have been shown to positively impact dairy cow reproduction and health.

Under current management models, most dairy cows spend only two lactations in the herd. Problems in establishing pregnancy or mastitis are leading reasons that cows are culled from the milking herd. Inflammation associated with mastitis has been associated with failure to establish pregnancy (Hanson et al., 2004; Schrick et al., 2001). It appears that inflammatory problems in other tissues or systemically and the reproductive tract may be linked.

Innate Immunity

Barriers that are part of innate immunity have been recognized as critical and nonspecific protection of the internal structures of the body for a very long time. Break or remove the skin, or damage an area of mucus membrane and problems quickly follow. The value of physical barriers in innate defense has never been questioned. As we came to understand that the world is full of microscopic creatures, we recognized that the surface of the physical barriers of the body was coated with chemical compounds that controlled the growth of microbes. The skin is dry and covered with organic acids and specific oils to encourage the growth of some microbes and block the growth of most others. The internal parts of animal bodies that interact with the outside are covered in mucus that traps microbes, and systems (such as cilia or muscular waves) to move the mucus that remove the trapped microbes. These physical barriers are a first line of defense.

The mucus-covered surfaces that form the interface to the outside world for the eyes, respiratory tract, gastrointestinal tract, and reproductive tract of animals also form

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an effective barrier. Mucus is an effective trap for many microbes. Mucus and ciliated cells in the respiratory tract sweep invading microbes up and out of the airway so that they are expelled from the mouth or swallowed. In the gastrointestinal tract, waves of smooth muscle contraction move the contents through the system and carry away many pathogens. Further, antimicrobial peptides, sugar-binding proteins (called lectins) that recognize structures on microbes lead to the aggregation of invaders. Then, enzymes and acids inhibit the growth of these pathogens. Proteins that control critical nutrients and minerals are also secreted into the mucus. In addition, in the gastrointestinal tract the pH changes markedly as materials pass through. This leads to the death of many microbes along the way. Acidic compounds are also secreted into the most exterior portions of the female reproductive tract. These "select" which microbes will grow and which will have a tough time growing.

Together these barrier functions form the truly non-specific portion of innate immunity. The measures are broadly applied to keep microbes from becoming invaders and all comers are treated exactly the same. There is little in the way of "effector activity" in this part of the system (save the enzymes and anti-microbial peptides that are secreted), and none of these elements appear to be secreted as a result of sensing the presence of microbial organisms on the skin or in mucus. However, these barriers do not function alone.

Since the late 1980's, we have been rediscovering other aspects of the power and importance of innate immunity as that part of immunity that can sense and respond to foreign invaders. Much of that research has been focused on the discovery of the receptors that recognize conserved structures of "pathogens" (in reality essentially all microbes) and how they signal within cells to initiate the complex of inflammatory responses in the host. This research has been the subject of many reviews. However, three of these are my favorites. Beutler (2004) is the most complete, Janeway and Medzhitov (2002) has the best coverage of innate immunity across all organisms and how strongly they are conserved, and Medzhitov and Janeway (2000) has the most compact review. These reviews focus primarily on the receptors that sense dangerous, foreign invaders, and set of core, common signaling pathways involved in activation of the innate immune system. They provide far less discussion of the cells and factors that carry out the effector function of removing invading microbes.

The innate immune response can be divided into two major activities. First, common features of microbes from the environment must be recognized as having entered the body of the animal. This is the sensing component of innate immunity. Sensing can occur at the cellular or molecular level. The second component is the action arm that allows for binding, targeting, or killing the microbial invaders. Again, cells or molecules of the innate immune system can carry out these functions of innate immunity. The innate immune system uses a fixed array of sensors for dangerous and foreign invaders. When they are encountered, the effector arm of the response becomes active. These processes are connected by the signaling network that has been recently described in great detail leading to the production of cytokines, lipid

mediators, release of antimicrobial proteins, and activation of effector cells and molecules.

The innate immune system is represented across both the animal and plant kingdom. Most of the sensor elements are highly conserved. These sensor receptors take a similar form across all species. The definition of self and foreign, which has been recognized as the basis for immune protection, is most clearly defined in the innate response. The activities of the innate response are ready at birth, constantly primed to act upon encounter with dangerous and foreign invaders, and approach the problem of these invaders using the same array of sensors and responses repeatedly without significant change. The innate response is biologically programmed. It is based on the presence of a few hundred receptors that signal through a limited family of intracellular and extracellular molecular pathways leading to the activation of a common set of "killing systems" that clear the invaders from the body. In general, these immediate response systems are highly effective and up to the task of keeping the body safely in balance. In mammals, including food animals, this innate immune response is backed up by the slower, but more narrowly focused adaptive immune response. The two are connected in ways that will be discussed.

A number of families of sensor molecules have been uncovered since we started to look closely for them about 20 years ago. The most completely studied is the family of Toll-like receptors (known as the TLR family). This family of foreign invader and cell damage sensing molecules is highly conserved across all animals. In food animals, nine different TLR molecules have been demonstrated. These molecules are distributed on the cell surface or on internal membranes. They recognize foreign invader signature molecules (such as cell wall components, hydrophobic protein like bacterial flagellin, and microbial patterns of DNA and RNA processing) both at the cell surface and internally. Further, they can recognize common patterns of cellular damage in the extra-cellular environment.

In addition to the TLR family, a number of other families of sensor molecules have been described, but are only well characterized in mice or humans. Far less is known about their analogs in cows, goats, sheep, horses, or pigs. We may learn more about these in the next few years and they may add to our ability to detect the pathogenesis of disease.

The families of sensors for microbial components are specifically gram negative and gram positive cell wall structures (TLR4 and TLR2 [with TLR1 or TLR6 as partner], respectively), the hydrophobic domains of bacterial flagella (TLR5), double stranded RNA (TLR3), unmethylated DNA (TLR9), nucleic acid folds (TLR7 and 8), lipid domains (C3 cleavage structures), and glycosylation patterns (MBL) that provide a context for signaling of danger and the initiation of a cascade of inflammatory events. The signaling that follows activation of these receptors initiates one of two core pathways called MyD88 or TRIF. The pathways both lead to activation of gene activating nuclear proteins through the Nf-kB nuclear activator protein. From this gene activation, the cascade of inflammatory events unfolds leading to production of cytokines, chemokines,

prostaglandins, vasoactive mediators, and other processes that lead to the signs of disease.

In addition to recognizing products of invading microbes directly, there are also sensors that detect cellular damage. One of the primary triggers of cell damage recognition is the family of heat shock proteins (referred to as HSP). Members of this family interact with several of the extracellular sensors on the cell surface to initiate the inflammatory cascade or enhance its development. These proteins that move other proteins around inside cells and aid in their proper folding should never be seen outside of host cells. This is a sure signal of damage.

The innate immune system has a wide variety of effector activities. These activities are mediated either by cells that become activated to remove and kill invaders, or by molecules that can either form complexes to “bind up” invaders or kill them directly. These effectors have hard-wired functions. That is they approach all invaders with the same set of tools and attempt to remove and kill the invaders in the same, preprogrammed way without regard to the specific invader encountered.

Cellular effectors of the innate immune system include macrophages, neutrophils, eosinophils, basophils, and mast cells. Once they have invaders or damaged components inside the cell, in walled off compartments, these cells release radicals, enzymes, and other killing molecules into the compartments containing the invader. This effectively breaks down the invader into pieces that are not dangerous to the host. In addition, macrophages and neutrophils may encounter invaders (often groups of invaders attached to a surface) that cannot be readily taken up. At this point, these cells release their killing molecules into the extra-cellular space and attempt to kill the invaders where they are. Often, this release of killing power causes damage to cells of the host and this damage is perceived as part of the symptoms of disease. All the cells of the innate immune system also make and release compounds that change the flow of blood and allow fluid, protein, and often more cells to enter the invaded and damaged tissue. This leads to the swelling, redness, and pain perceived at the site of infection. In addition to the cells associated with innate immune function, epithelial cells also utilize many of the same sensors to initiate and regulate the local responses to invaders and damage (Akira et al., 2006; Schaefer et al., 2005) These changes are often seen as signs of the disease.

The molecular effectors of the innate immune system provide recognition of invaders in the extra-cellular space. There are many molecules that play a role in this recognition, but the proteins of the complement system and the mannose binding lectin (MBL) family of sugar-binding proteins play particularly important roles. The complement protein known as C3 is particularly sensitive to the surface of microbial and enveloped viral invaders. This protein is cleaved into two parts when it encounters the invader, C3b and C3a. The C3b leads to the activation of a cascade of protein activations that can lead to the direct killing of the invader by “boring holes” in the lipid membrane of the invader. The C3a recruits the cells of the innate immune system from the circulation, particularly those that become macrophages and neutrophils. The C3a

also activate these cells. The MLB recognizes the common pattern of sugar addition to the surface components of invaders. The MLB binds to terminal mannose sugars on those structures and leads to C3 breakdown. Again, C3b can lead to direct killing of the invader and C3a to recruitment and activation of cells that provide effector activity for the innate immune system.

Cells that have been activated by invaders and damage also produce a large number of cytokines and chemokines. Cytokines are hormones that trigger the regulated set of inflammatory activation events that follow signaling by TLR receptors (and their relatives) sensing invaders and damage. Chemokines are a subgroup of cytokines that primarily attract effector cells of the innate immune system to the site of invasion and damage. Cytokines have many jobs in fighting invaders. Some of these cytokines, such as tumor necrosis factor alpha (TNF) and interleukin 1 beta (IL-1), travel from the site of invasion to the brain to regulate body temperature. The result is fever, a major sign of disease. In another context, cytokines enhance or suppress cellular metabolism to help fight or starve the invader. The cytokines and chemokines produced after an encounter with an invader will often play a large role in how the symptoms of the disease the invader has caused is recognized in the host. They affect the timing, severity, and localization of symptoms and offer clues to the nature of the disease.

In addition to the cytokine protein messengers, the body also utilizes a family of lipid mediators. The members of this family that receive the most attention in recognizing and treating food animal disease are the prostaglandins and leukotrienes. These mediators are involved in pain and fever. Thus, they play a role in disease processes we can both see and modulate.

The cascade of events that arise from activation of innate immunity caused by an invader is closely linked to the signs of the disease the invader causes. Signs induced by innate immunity include fever, local swelling, redness, warmth, and pain. Pus is also a sign of the infiltration of neutrophils (and macrophages) into tissue that has been invaded. The cytokines produced often lead to the animal going off feed, becoming “depressed”, or having a change in social behavior. The signs we count on to recognize infectious diseases generally have their source in the innate immune response to invasion.

Innate immunity plays one more critical role in the ecology of disease. It provides important connections to the adaptive immune response. The next essay will describe the adaptive immune response in more detail, but it is all about recognizing many different unique molecular components of invaders and building a rapid response network to block their invasion in the future. The process is based on sampling of invaders in both the extra-cellular and intra-cellular environment of the host and presenting those samples to the cells of the adaptive immune response under a set of rules that indicate a dangerous threat.

The sampling and presentation of the invaders is done by a class of cells that arise from monocytes entering the tissues and become antigen-presenting cells. The

most potent and common antigen-presenting cells are called dendritic cells. These cells differentiate from monocytes that enter the tissue from the blood and seek evidence of invaders. They sample the components of the invaders and present molecular pieces of the invader on their surface by placing them into the groove of either of two proteins. The protein utilized for processed extra-cellular invaders is called major histocompatibility complex (MHC) protein class II, or MHC II for short. The protein utilized when the invader is attacking from within host cells is called MHC class I, or MHC I for short. One of these quality control proteins containing a sample of invader is a necessary part of getting the host to respond to individual molecules from the invader.

In addition, the host requires that antigen-presenting cells provide signals of danger and damage to allow for activation of adaptive immunity. These signals are produced and expressed on the antigen-presenting cell surface as part of the inflammatory cascade. So, if the invader is causing inflammation and is present in large enough numbers to be effectively sampled, the antigen-presenting cells will become loaded with sample and danger signal, then migrate to where the cells of the adaptive immune system are waiting.

Adaptive Immunity

Mammalian food animals have very well developed and complex adaptive immune systems. These systems are based on the function of a family of specialized cells called lymphocytes. Lymphocytes come in two large families, B cells (derived from bone marrow in mammals) and T cells (named for their source in the Thymus). Each of the families has a number of members that have different activation requirements and functional activities. However, all the lymphocytes develop their specificity by gene rearrangement (reviewed in any immunology textbook if you want to know how). The B cells develop their specificity by rearrangement of the antigen-binding domain of the heavy and light chains of antibody (antibody structure and function will be covered below). The B cells then provide specific effector targeting by exchanging heavy chain "constant" domains to make antibodies of different "classes." The T cells attain their molecular specificity by rearrangement of their T cell antigen receptor (TCR) genes. There are two types of rearranged TCR that are produced, those with alpha and beta chains, and those with gamma and delta chains. Both are well represented in the circulation and tissues of the cow, goat, sheep, and pig, but alpha and beta chain TCR bearing T cells are much more common in the horse, human, and rodent.

The adaptive immune system is capable of being modulated to become faster, better, and stronger in response to a specific invader with repeated exposure. We would like to use the enhanced capacity to help us manage the cost and suffering associated with diseases in food animals. The adaptive immune response is the basis of our programs aimed at biological control of disease, primarily vaccination. Vaccination is an exploitation of the biology of the adaptive immune response to the benefit of the animal and the producer.

The B cells use antibody bound to their surface as their antigen (parts of a foreign invader) receptor. This antibody "sees" antigen in 3-D. Binding of surface

antibody activates the B cell and causes it to divide, so there are more B cells that see that specific molecularly defined antigen, and after several rounds of division, some of the B cells become antibody factories to make more antibody. The B cells can recognize a wide variety of antigens, as a typical mammal is capable of making about 10^{18} different gene rearrangement combinations.

The basic structure that allows antibody to function is the complex of four peptide chains, two “light chains” and two “heavy chains” to form the unit structure. Each light chain is composed of a variable (the gene rearranged part) and a constant portion. The light chain is smaller than the heavy chain (about 25K da vs. 50K da, respectively). The heavy chain is similar in construction, except it has three constant portions in each molecule. The variable part of the light and heavy chain “overlay” each other forming the antigen-binding site of the antibody. The first constant part of the heavy chain and the constant part of the light chain interact to make the complex stable. Finally, the distal two constant domains of the heavy chain interact to form the function-determining portion of the antibody molecule. The heavy and light chains and the two heavy chains are further bound together by the formation of disulfide bonds. The more disulfide bonds in the antibody structure the more rigid the antibody molecule, and the fewer places it can go.

Antibody comes in classes that are associated with where in the body they function and how they interact with cells and molecules that remove invaders. The class of antibody found at the highest concentration in healthy individuals is IgG. It is composed of a one unit antibody structure. It is often represented by several subclasses (such as IgG1 and IgG2, but they can have unique names as they do in the horse) that differ in the placement and number of disulfide bonds. This regulates how effectively the antibody enters the tissue or gets to the mucosal surfaces and how well it activates cells (like macrophages and neutrophils) to function, and how well it interacts with complement. The IgM is the first antibody made after activation of “first timer” B cells. It is composed of five unit antibodies. It is very large and rigid, almost flat. It is found in the circulation, but it is not easy for it to get out of the blood. It is good at activating complement-mediated killing of invaders and very good at encouraging cells to take up invaders from the circulation. Two other types of antibody are often observed, IgA composed of two unit antibody structures joined end to end, and IgE that has an extra constant domain on the function-determining end that is associated with fighting parasites and allergies. The IgA is very important at blocking the entry of invaders on mucosal surfaces. The IgA has a modified chemical structure that allows it to be freely transported across epithelial cell barriers and is found on mucosal surfaces in large quantities. It is capable of binding four antigen molecules at once and functions primarily by binding up invaders so they do not get into the body. The IgE is a special antibody molecule. It generally functions when bound to mast cells or eosinophils. When antigen binds, it triggers those cells to release large quantities of preformed vasoactive compounds and leads to the symptoms you may have experienced as allergy.

T cells have antigen receptors on their surface that recognize a small piece of peptide and the MHC antigen that is wrapped around it on the surface of an antigen-

presenting cell. Both the piece of antigen and the one, specific MHC protein is required for recognition of antigen. Further, T cells demand proof of a dangerous context. When an antigen presenting cells encounters evidence of invaders, the cells become activated. With activation, these cells make new copies of proteins that are expressed on their surfaces that indicate that they have faced "danger." These surface proteins, when in the presence of the right piece of antigen and MHC protein, give "permission" to T cells to become activated and begin to divide. Thus, just like B cells, T cells are selected and expanded to provide a better, stronger, and faster immune response in the body of the host. The typical mammal can make about 10^{21} rearrangements of TCR genes.

The T cells come in two major types. One type functions to manage adaptive immunity by making the right combination of cytokines and surface proteins to assure that enough expansion of B cells and T cells occurs to protect the body, and when the time is right, that antibody is produced and killing activity by T cells is armed. These are called "helper cells." The other type produces less cytokine, but can be armed to kill cells that express the right piece of antigen in the right MHC protein indicating that an invader is active inside that host cell. These are called killer T cells.

Because adaptive immune responses are so diverse and complex, the body cannot control these processes in an ad hoc fashion. Therefore, specific tissues, like lymph nodes and the spleen, are organized as adaptive immunity screening and production facilities. Antigen-presenting cells coming from the tissues of the body home to their friendly, local lymph node (or to the spleen from the blood) to report on the invaders present and the danger found. There they migrate among the waiting B cells and T cells until they find those that recognize their antigen "message" about the invaders in the tissue. As the lymphocytes are packed close together in these organized screening and production centers, the process is pretty efficient.

Once a good match between the antigen and lymphocytes is found, the process of lymphocyte activation and division is started. The division phase of this process is often referred to as clonal expansion. Each lymphocyte that encounters a proper match is encouraged to divide and make identical copies. The level of adaptive immune response and its speed are based on the number of cells that recognize the antigen properly and respond when called. Thus, the effectiveness of B cells and T cells at protecting the body is based on experiencing antigen and expanding the clones. Thus, the larger the number of responding lymphocytes that there are in the lymphoid tissues of the body, the faster and stronger the responses to invasion is mounted. The activation of B cells is a complex process that requires antigen-specific signaling in the B cell and support by cytokines and growth factors from other cells. Similarly, T cell activation is a complex process. The T cell activation requires activation by a piece of antigen in the framework of an MHC molecule, permission to act from an antigen-presenting cell confirming danger, and "help" from other T cells. It shares many processes with B cell activation.

So, a major difference between the innate immune response and the adaptive response is how quickly the response occurs. Innate immunity begins within seconds

and is often apparent in hours. The adaptive response occurs in days (about 7 days until the first antibody is measurable in serum after a first time exposure, and 3 days after a later exposure). The innate immune response deals with the invasion and its immediate consequences, but the adaptive response is responsible for the rigor of the response and assurance that the invader is completely removed and neutralized. Innate immune responses can become chronic. They can be sustained by serial recruitment of innate immune cells and release of inflammatory factors to do damage or change local physiology over a considerable period of time.

Another difference between innate immunity and adaptive immunity is that the innate immune response brings the same tools and players to the challenge of invasion every time. The first time response is no different in character or nature than the 50th. The adaptive immune response gets better at responding with each invasion. The number of responsive cells is increased and the time required to respond decreases each time the body encounters an invader. The process of more focused and rapid response is called immune memory. This enhancement with exposure is what we exploit in the production of vaccines. We provide evidence of invasion without the disease consequences to the host to make it better able to deal with the natural invader later.

Common Elements of Immune and Reproductive Function

The establishment and success of pregnancy is a complex process. It appears from studies performed in many species that local immune and inflammatory processes, which are often reflected by changes in systemic activities that can be monitored in the circulation, have a major impact on “fertility” in the female. Prior to fertilization, inflammatory and immune activities in the reproductive tract alter the interaction between egg and sperm. Changes in the viscosity and physical/chemical composition of uterine mucus can alter sperm penetration and survival. Increased inflammatory cell activation can create a hostile environment for sperm, reducing the duration of their viability, and damaging their membranes so that they are less capable of fertilization. Similarly, the fertilized ovum faces challenges to its survival and in its interaction with the lining of the uterus when a strong pro-inflammatory response is occurring.

Immune cells, proteins controlling lymphocyte interaction and products of inflammatory activation are all part of the development of a functional ovum, organization of the primary follicle and the corpus luteum. The development of an ovum and primary follicle requires the expression of the lymphocyte marker Thy-1 on the surface of specific epithelial cells in the ovary, the interaction of these cells with CD8 positive T cells, CD14 and MHC class II antigen-bearing macrophages and monocytes, a loss of the MHC class I antigen by cells in the developing follicle, and interaction with immunoglobulin molecules (Bukovsky et al., 2005). Further interaction with immune cells and a requirement for the chemokine IL-8 have been documented for the proper development and vascularization of the corpus luteum. Neutrophils must infiltrate the epithelial layer to allow for proper functional development of the bovine corpus luteum

and they appear to be the source of angiogenic factors required for maturation of the corpus luteum.

In contrast, the fertilized ovum once it implants in the wall of the uterus requires that the mother go through three sequential periods of immune interaction. First, a pro-inflammatory phase which causes systemic effects on appetite and homeostasis. In this phase, the placental membranes develop and become vascularized appearing to the body as an open wound. The pro-inflammatory responses provide the cytokine context for the enhanced vascularization and promote the complex matrix of tissue formation to foster the development of the placenta and fetus in close contact with the maternal tissues of the uterus. Next during the second trimester, there is a period of immune neutrality and of rapid fetal growth. The mother suppresses cell-mediated immune activity. The condition is stable and the mother does not recognize a new threat. The systemic inflammatory responses have shifted to an anti-inflammatory profile. Finally, as birth approaches, the mother mounts a stronger and stronger pro-inflammatory response that triggers expulsion of the fetus at term.

Alterations of these necessary immunological relationships by infection or chronic immune disruption yield fertility problems. Occult infections with bacteria lead to a change in the level of inflammatory activity that throws the developing fertilized ovum out of sync with the uterus and leads to failure of implantation (Weiss et al., 2009). Many of these interactions are regulated by innate immune receptors that trigger pro-inflammatory responses, like the Toll-like receptors. This continuing pro-inflammatory state is deliberately mimicked by the function of the intra-uterine device for birth control.

The dairy cow has been selectively bred for the production of milk, but in that process, the efficacy of her reproduction has been largely ignored. The problem is multifaceted. It includes energy partitioning problems involved in supporting the volume of milk produced relative to all other essential physiological functions, the depletion of body stores of minerals that can impact inflammatory regulation, and management programs directed at the narrow aim of milk production per lactation cycle (rather than life-time milk yield).

Each of these management choices has led to potential problems in a critical event in profitable milk production, establishing pregnancy and delivering a calf. It appears that it is the rule, rather than the exception, that more than one service is required to obtain a successful pregnancy in dairy cows. This situation has both practical and economic consequences to the industry.

Inflammatory processes have been recognized as both a necessary component of the development of pregnancy and as significant impediments to the development and success of pregnancy (Weiss et al., 2009). Inflammatory processes secondary to acute and occult infections play a significant role in infertility. In unpublished studies in my lab, we found that 30% of dairy cattle examined had occult colonization of the uterus based on recovery of bacteria from uterine flush fluid.

Inflammation also releases mediators that function to up and down regulate inflammatory processes in the form of cytokines, prostaglandins, and chemokines. Several of these mediators play important roles in regulating the response of reproductive cells to leutinizing hormone (LH), in steriodogenesis and steroid conversion, and in the progression of ovum development and release, implantation, and fetal-placental interactions.

The expression and level of the cytokines, IL-1 beta, TNF alpha, and IL-6 (the classic pro-inflammatory triad) modulate aromatase activity regulating the production of estradiol, progestone, and progesterone. They also impact COX-2 activity and the production of PGE and PGF, which are important to the production and release of the ovum. Local, resident macrophages in the reproductive tract modulate the cytokine environment and favor the production of IL-1 alpha, TGF-beta, and IL-10 that modulate LH activity and promote steroid conversion under control of cells within the reproductive tract. Infiltrating inflammatory cells, monocytes, macrophages, neutrophils, eosinophils, and activated lymphocytes produce high levels of cytokines that disrupt regulation of these processes.

The establishment of pregnancy by implantation of the embryo into the uterine wall and establishment of the placenta requires interaction with immune cells, particularly a specific class of NK cells and resident macrophages. This is a pro-inflammatory process and results in systemic inflammatory symptoms. Relatively quickly, the interaction of the placenta with the developing fetus causes a shift in the immune relationship to a somewhat suppressive environment to protect the fetal graft from rejection. Finally, late in pregnancy, a return to a more pro-inflammatory environment serves to trigger events to expel the fetal graft in the birth process (Mor, 2008).

Modern genetic selection, implemented by artificial insemination and complemented by improved management, has dramatically increased the milk yield per lactation in dairy cattle. Over the last decades, yields have increased from 3000 L/cow per year in the 1940s to a current estimate of 9500 L/cow per year. Over the same period, these increases have allowed for reduction in the total number of animals by 85% while maintaining production of 1.5 fold more total milk (Powell and Norman, 2006). The negative effect that increased milk production had on reproductive success was caused by both genetic and metabolic factors (Gilbert et al., 2005). Large-scale analysis of dairy herd fertility in the United States revealed that conception rates declined by more than 30% in Jersey and Holstein cows between 1975 and the beginning of the current century (Powell and Norman, 2006). As gestation is a prerequisite for lactation, a direct implication of this low fertility rate is the requirement for larger herd numbers to maintain the same number of cows in lactation. This is indicated by a heifer replacement rate of infertile cows that can exceed 35% of the herd per year.

Further, systemic inflammation associated with acute disease elsewhere in the cow, such as the mammary gland, have also been shown to be negatively correlated

with the success of pregnancy (Moore et al., 2005; Chebel et al., 2004; Moore et al., 1991; Hansen et al., 2004). Difficulties in human fertility may have parallels in the problems we see in the dairy cow. A large number of cases of human infertility are related to chronic infection, the development of anti-sperm antibody related to chronic inflammation in the reproductive tract of women, and the effects of the inflammatory process yielding altered levels of pro-inflammatory cytokines (particularly IL-1 beta, IL-6, and TNF-alpha) (Weiss et al., 2009). While inflammation alone is not likely the whole cause of infertility in dairy cows, it may in fact represent an important common "sign" of the problem.

In cattle, inflammation problems are fueled by problems in nutrition, housing, and management. These result in part from the huge partitioning of energy that has been focused intentionally on milk production as a mono-focal goal. This narrow focus leads to the development of an "incubator state" in the confinement dairy resulting in more frequent and substantial exposure to pathogens that initiate sub-acute infections with inflammatory consequences on overall health, including having a likely impact on fertility (Garnsworthy, 2004). Therefore, it appears that inflammation is a consequence and possibly a component in the multi-factorial problem of poor fertility in dairy cows.

It is imperative that remediation of low fertility be addressed as a strategy to decrease the number of dairy cows required to maintain and increase milk production in the US and world-wide. As subfertility is a complex, multi-gene, and multi-factorial condition, understanding and mitigation of its causes requires a multidisciplinary approach (Royal et al., 2002).

Parturition Associated Changes in the Dairy Cow

Very significant changes in the dairy cow occur during the period from two weeks before to three weeks after birth. These changes appear to be fueled by significant negative energy balance and the inability of the cow to eat enough to meet all the energy demands of the growing calf and the initiation of milk production (Goff and Horst, 1997). The changes observed include a reduction in neutrophil function, increased incidence of intra-mammary infections, reduced adaptive immune function, reduction in rumen efficiency, spikes in cortisol levels at calving, a shift in progesterone and estrogen levels, and a reduction in circulating calcium.

These changes are essentially universal in the dairy cow and in the normal cow they are transient. However, failure to rapidly resolve these problems leads to chronic colonization of the mammary gland and new intra-mammary infections with systemic inflammatory effects (Hansen et al., 2004), occult colonization of the reproductive tract with increased indicators of inflammatory mediators (Hurley et al. unpublished), and changes in the normal profile of production of mediators and hormones involved in fertility.

There is some hope that at least some of the inflammatory problems can be modulated in dairy cows based on recent research. Long-term (six months of age to

parturition) or short-term (60 day) feeding of Omnigen-AF® to dairy heifers offered evidence of better managed and primed inflammatory activity (Ryman et al., 2013, Nace et al., unpublished). In these studies, we observed enhanced phagocytic activity, better controlled radical production, and stabilized expression of CD62L and CD11c. In addition, in the first study we observed fewer IMI and greater milk production.

Conclusions

Fertility in the dairy cow is linked with many functions shared with the immune system. Common mediators play roles in the function of both immunity and the female reproductive function. Further, there is a direct role for cells of the innate immune system in oogenesis, vascular development of the corpus luteum, and in regulation of embryo implantation in the cow. Enhanced inflammation associated with local infections appears to have a negative effect on pregnancy development. This has been demonstrated clearly by a link between mastitis and poor conception. Nutritional challenges appear to play a significant role in the problems associated with parturition. Our research indicates that modulation of innate immune function may be possible by use of oral immune stimulants. It is time to consider and examine the function of oral immune stimulants that strengthen the regulatory activity by priming innate immune function by action in the gut-associated immune tissue and loading competent innate immune cells into the circulation of dairy cows that would otherwise be significantly compromised as a method for control of the role of inflammation in fertility.

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SESSION NOTES

Evaluation of OmniGen-AF® in Lactating Heat-stressed Holstein Cows

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Introduction

There is now a strong scientific consensus that human-induced climate change is occurring (CCSP,2008). Projections are that this warming will continue throughout the 21st century with global average temperature rising an additional 1.1 to 5.4ºC (CCSP 2008). These changes will have large and measurable impacts on dairy cattle worldwide (Klinedinst et al., 1993) through a variety of routes including changes in food availability and quality, changes in pest and pathogen populations, alteration in immunity and both direct and indirect impacts on animal performance such as growth, reproduction, and lactation.

Heat stress (HS) is a health and economic issue in every dairy-producing area of the world. The economic impact of HS on American animal agriculture is over $2 billion annually and the dairy industry is one of the most susceptible. Conservative estimates have the impact at $900 million/year, in the dairy industry alone (St. Pierre et al., 2003).

Production, reproduction, and animal health are all impaired by hyperthermia. The physiological and production responses to heat stress are well documented, but not fully understood. During HS, respiration rate and body temperature increase while feed intake, milk yield, and reproduction decrease.

Milk synthesis decreases are partially linked to reduced feed intake. There are additional losses in milk yield that are associated with metabolic changes (Rhoads et al., 2009). Specifically, metabolism of lactating dairy cows is shifted towards increased peripheral utilization of glucose. Countering the negative production effects of heat stress on lactating dairy cows requires improving feed intake and preventing the shift in energy metabolism associated with acclimation to thermal stress.

Immune function and health are also reduced with HS. The severity and occasion of disease are increased when immune and inflammatory responses are impaired (Sordillo, 2013). Impaired immune function during thermal stress may be associated with altered production rate of cortisol. Cortisol levels in heat stressed cows increase with exposure to heat within two hours (Christison and Johnson, 1972). After the first 12 hours of heat, cortisol levels return to normal.

The ruminal environment also can be altered during thermal stress. Increased respiration rates can cause respiratory alkalosis, ruminal acidosis, and eventually

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metabolic acidosis (Sanchez et al., 1994). Oxidative stress can be increased with heat stress (Ilanqovan et al., 2006) and can impair the heat shock response and increase cell damage and death (Adachi et al., 2009).

Experimental Objectives and Methods

The purpose of this study was to evaluate the effects of feeding OmniGen-AF to lactating dairy cattle subjected to HS. Specifically, we tested whether OmniGen-AF would improve measures of immune function in heat stressed dairy cows.

The study consisted of two phases: 1) the commercial dairy and 2) the controlled environmental chambers. During the commercial dairy phase, multiparous lactating Holstein cows (n = 30) were balanced by days in milk (DIM), milk production, and parity (91 ± 5.9 DIM, 36.2 ± 2.5 kg/d, and 3.1 ± 1.4). Cows were separated into one of two groups. The control group received the base TMR with no supplement. The treatment group was fed the base diet plus 56 g/head per day of OmniGen-AF mixed into the TMR. Daily milk production was measured. The commercial dairy phase lasted for 45 days. The dairy portion also was used to meet the manufacture's recommended 45 d feeding for OmniGen-AF to function.

After the commercial dairy phase was complete, 12 cows (6 control and 6 treatment) were housed in the environmentally controlled rooms at the University of Arizona, Agricultural Research Center (ARC). Cows continued within the same treatment group in the ARC and the commercial dairy phases.

The ARC portion lasted for 21 days (Figure 1). The diurnal cycle during thermoneutral (TN) and recovery maintained a temperature humidity index (THI) < 68. During HS, the THI was greater than 68 for 16 hours/day. Temperatures mimicked ambient temperatures at a southwest United States dairy during summer heat and TN conditions.

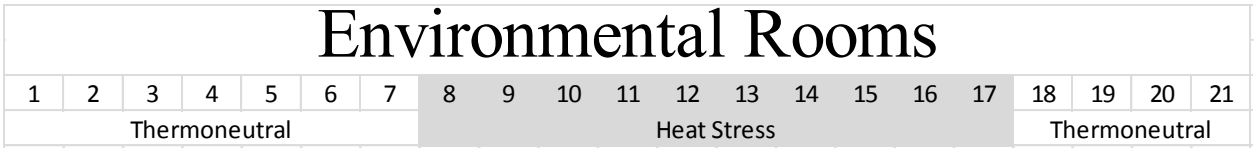

Figure 1. Environmental conditions in the environmental rooms during the 21-day study.

Fresh feed was provided twice daily and cows were individually fed. Control animals received base TMR and OmnigGen-AF cows received 56 g/head per day, split between two meals. Cows were subjected to 7 days of TN conditions, 10 days of HS, and 4 days of recovery (TN). Feed intake, milk production, and milk composition were measured daily. Rectal temperatures and respiration rates were recorded 3 times per day (600, 1400, and 1800 h).

Blood samples were collected by venipuncture from the tail (coccygeal) vein on days 1, 21, and 45 of the commercial dairy phase and on days 7, 8, 14, 17, and 18 in the controlled environmental chambers. Samples were collected 6 times per day (0400, 0800, 1200, 1600, 2000, and 2400 h) on days 7, 8, 17, and 18, and once per day on day 14 (0800 h). Blood was collected in Vacutainer (BD Vacutainer, Franklin Lakes, NJ) tubes containing sodium heparin for plasma, and in sterile blank tubes for serum.

Statistical analyses were performed using the PROC MIXED procedure (version 9.3, SAS Institute, Cary, NC). Cow was the experimental unit (ARC phase). Data are presented as least square means with significance declared with a *P* value ≤ 0.05.

Experimental Results

Commercial Farm Phase

There were no initial differences in milk yield (control = 38.6 kg/day and treatment = 38.6 kg/day) at the start of the commercial dairy phase of the study. There was a numerical advantage to feeding OmniGen-AF (Figure 2) of 1.5 kg of milk/day, but this was not significant (control = 36.8 kg/day and treatment = 38.3 kg/day).

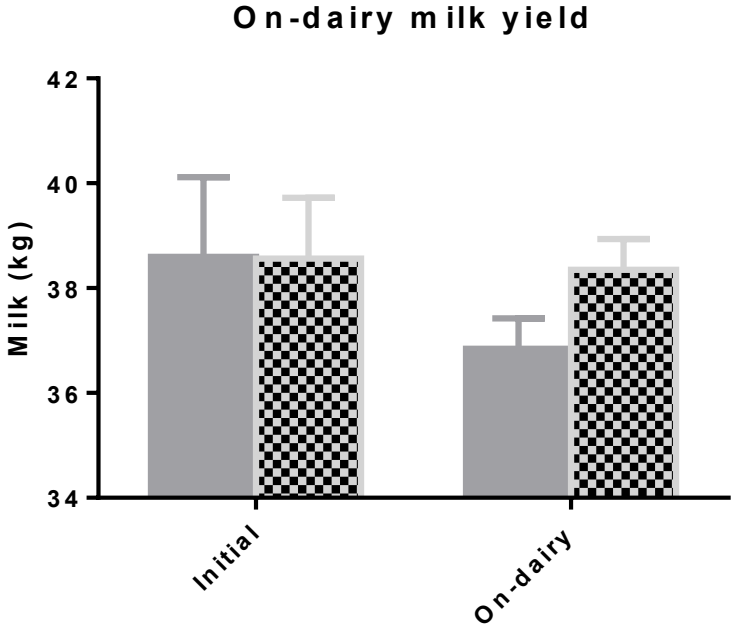

Figure 2. Effect of dietary OmniGen-AF on milk yield under commercial farm conditions.

Environmental Chamber Phase

There was a period effect on milk yield ($P < 0.01$) during the environmental room (ARC) phase associated with a decline in milk yield in both groups during HS. Milk yield at the ARC ($P < 0.23$) did not differ between control and OmniGen-AF fed groups (Figure 3), however, there was a numerical advantage (1.1 kg/day) for cows fed OmniGen-AF during HS ($P < 0.26$) which was similar to the pattern in milk yield noted during the on-farm phase.

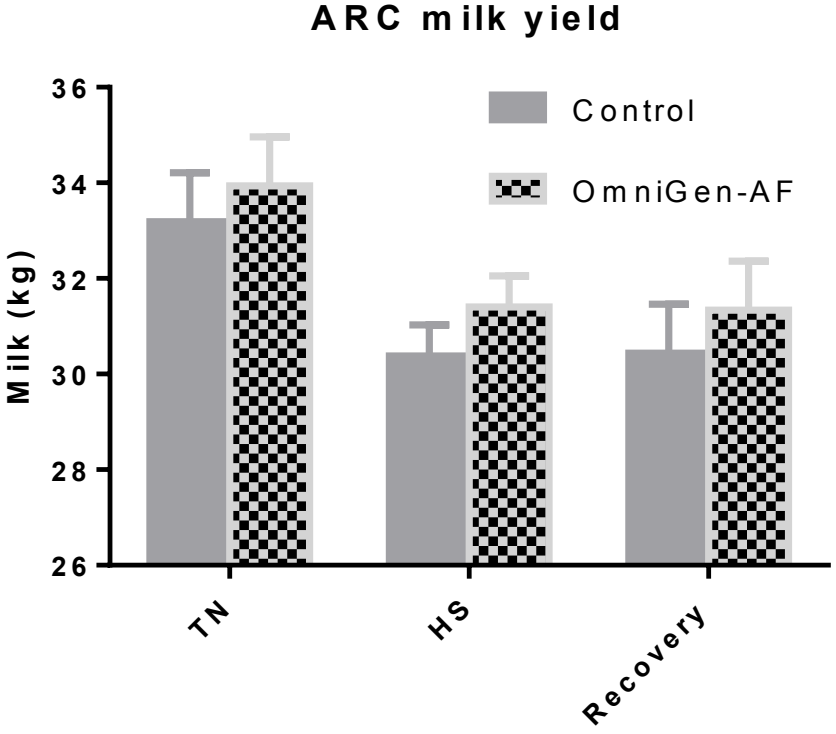

Figure 3. Effect of dietary OmniGen-AF on milk yield during thermoneutral (TN), heat stress (HS), and recovery period conditions in environmental chambers.

Feed intake was not measured during the on-farm phase of this study but was measured during the ARC phase. Feed intakes in the two groups did not differ during TN but was higher during HS in OmniGen-AF-fed cows (46.8 kg/d and 42.9 kg/d, $P < 0.01$, Figure 4; Table 1).

Milk protein (%) and fat (%) were lower in OmniGen-AF-fed cows (Table 1) during HS but not during TN. There was no difference in FCM or protein yield between treatments. Cows fed OmniGen-AF displayed decreased SCC compared to control cows (59.4 and 26.3 × 1000, $P < 0.03$; Table 1) with the greatest difference during the recovery period (Figure 5). There was a spike in SCC around day 5 (TN) and during recovery around day 17 (Figure 5).

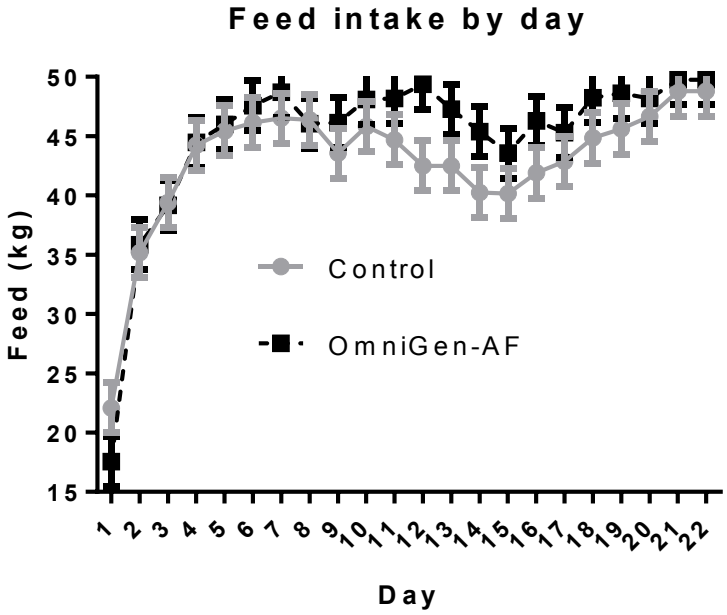

Figure 4. Effect of dietary OmniGen-AF on feed intake by lactating cows exposed to thermoneutral and heat stress conditions in environmental chambers.

Table 1. Effects of OmniGen-AF supplementation in heat-stressed lactating dairy cows

	Control			OmniGen-AF				
Item	TN	HS	Recovery	TN	HS	Recovery	SEM	*P*-value
Feed intake (kg)	46.1	42.9	47.5	47.1	46.8*	49.1	1.04	0.01
Milk yield (kg)	33.1	30.3	30.4	33.9	31.4	31.3	1.02	0.23
Fat (%)	4.03	4.22	4.16	3.94	3.82	3.83	0.22	0.04
FCM (kg/ d)	35.0	33.7	33.7	34.7	32.8	32.6	1.45	0.39
Protein (%)	2.95	2.98*	2.86	2.95	2.86	2.79	0.07	0.15
Protein (kg)	0.98	0.89	0.90	1.00	0.93	0.92	0.30	0.13
Lactose (%)	4.87	4.85	4.99	4.89	4.78	4.96	0.08	0.61
SCC	20.3	23.9	59.4*	19.6	22.9	26.3	9.12	0.03

* = *P*-value ≤ 0.05 and indicates the higher value

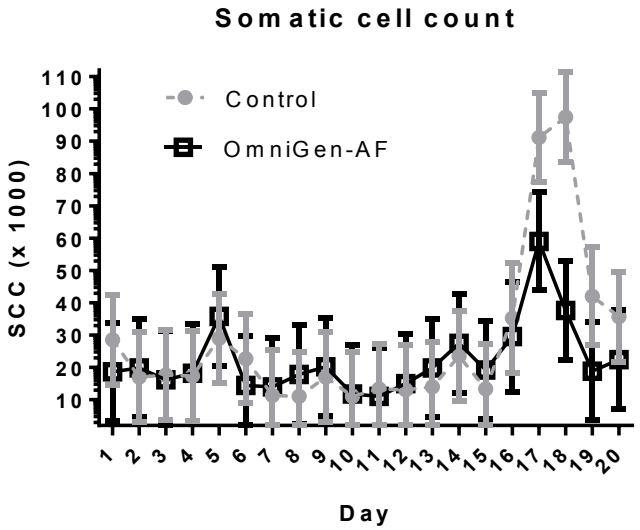

Figure 5. Effect of dietary OmniGen-AF on somatic cell content in milk of lactating Holstein cows subjected to thermoneutral and heat stress conditions in environmental chambers.

Respiration rate and rectal temperatures did not differ between treatments during TN; however, during HS, OmniGen-AF reduced respiration rate, (Table 2, $P < 0.01$) in both environments at 1400 h and in HS animals at 1800 h when environmental heat load was greatest. Rectal temperatures were lower in cows fed Omnigen-AF at 1400 and 1800 h compared to controls when environmental heat loads were maximal.

Table 2. Effects of OmniGen-AF supplementation and environment on respiration rate and rectal temperature in lactating dairy cows

	Control			OmniGen-AF				
Item	**TN**	**HS**	**Recovery**	**TN**	**HS**	**Recovery**	**SEM**	***P*-value**
Resp/ min								
600	**26.9**	**31.9**	**28.3**	**26.6**	**30.4**	**27.9**	**1.40**	**0.40**
1400	**34.3**	**63.1***	**35.3**	**30.1**	**58.3**	**35.5**	**2.99**	**0.20**
1800	**34.9***	**60.8***	**32.1**	**29.5**	**52.4**	**29.7**	**2.62**	**0.01**
Rectal Temp (°C)								
600	**38.2**	**38.0**	**37.9**	**38.2**	**38.1**	**38.1**	**0.05**	**0.26**
1400	**38.0**	**38.7***	**38.0**	**38.1**	**38.5**	**38.1**	**0.09**	**0.77**
1800	**38.2**	**39.1***	**38.2**	**38.2**	**38.8**	**38.3**	**0.08**	**0.25**

* $P \leq 0.05$ and indicates the higher value.

Hormones in plasma are important as potential indicators of the physiological status of a cow and reflect the physiological compensations a cow undergoes at various stages of lactation and exposure to HS. Serum cortisol levels were highest on day 8 (first day of HS, Figure 6). This is in agreement with prior reports that acute but not chronic HS is associated with increases in circulating cortisol concentrations (Christian and Johnson, 1972, Wise et al., 1988). OmniGen-AF treated cows had significantly lower serum cortisol on day 8 (0.8372 vs. 0.4838 µg/dL for control and OmniGen-AF respectively, $P < 0.006$) and did not differ on other days. This suggests that Omnigen may reduce impact of acute stress on the cortisol response in lactating dairy cows.

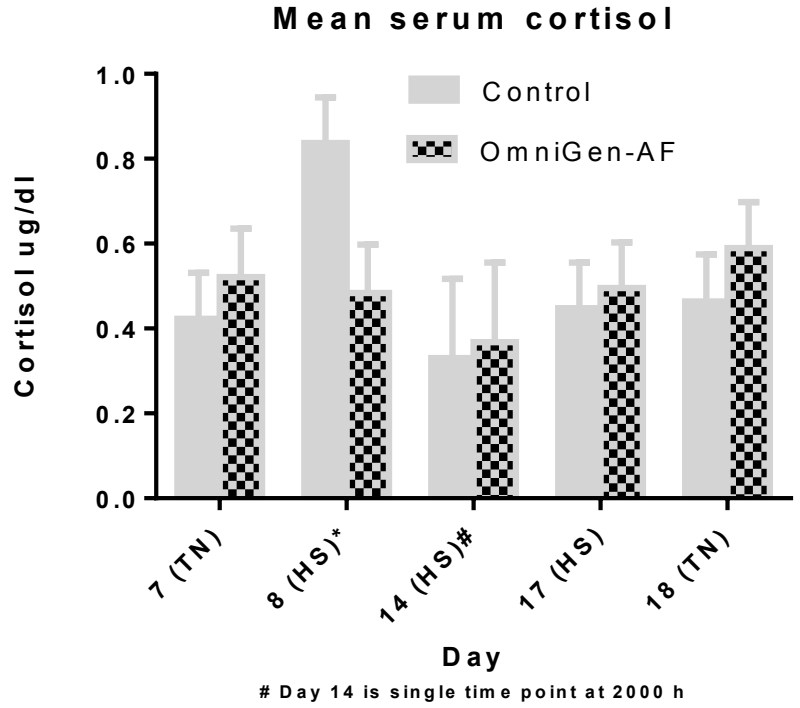

Figure 6. Effect of environment and Omnigen-AF on serum cortisol concentrations in lactating dairy cows housed in environmental chambers.

Serum insulin and plasma glucose levels (Figures 7 and 8) were not different between groups ($P = 0.8248$ and 0.945). Serum insulin concentrations in both groups rose during the latter part of the HS period and during the recovery period. The reason for this pattern is unknown.

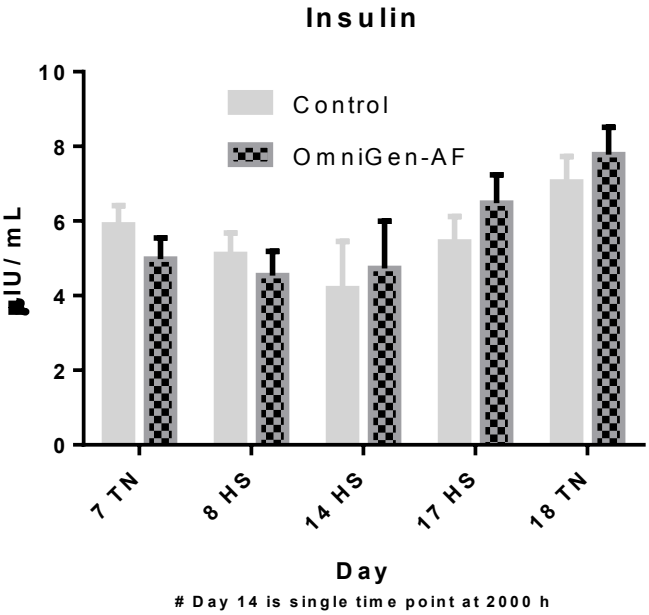

Figure 7. Effect of Omnigen-AF and environment on serum insulin concentrations in lactating dairy cows housed in environmental chambers.

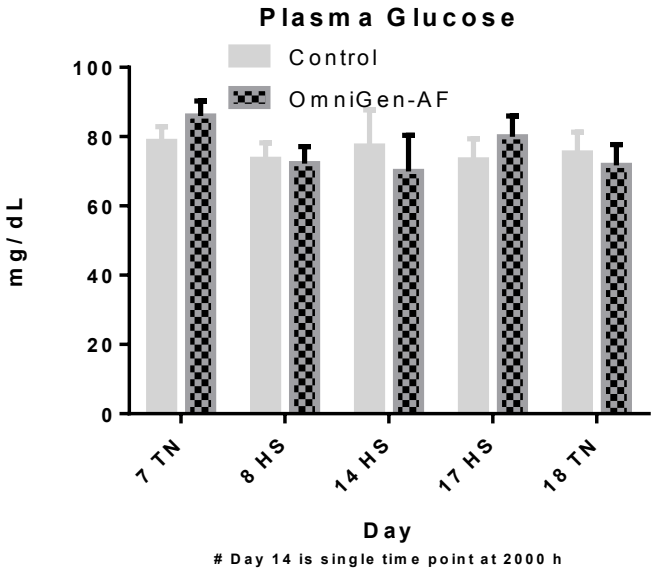

Figure 8. Effect of Omnigen-AF and environment on plasma glucose concentrations of dairy cows housed in environmental chambers.

The immune function of cattle on this study was evaluated by looking at the expression of the interleukin-8 receptor (Figure 9) and expression of Regulated on Activation, Normal T Expressed and Secreted (RANTES) protein (Figure 10) which is a member of the interleukin-8 family of cytokines.

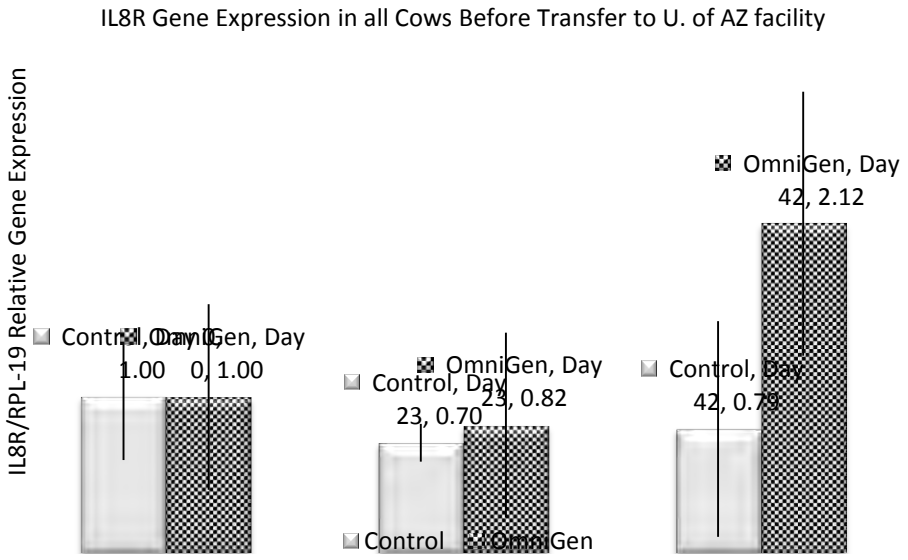

Figure 9. Effect of dietary Omnigen-AF on IL8R receptor gene expression in leukocytes in lactating dairy cows housed in environmental chambers.

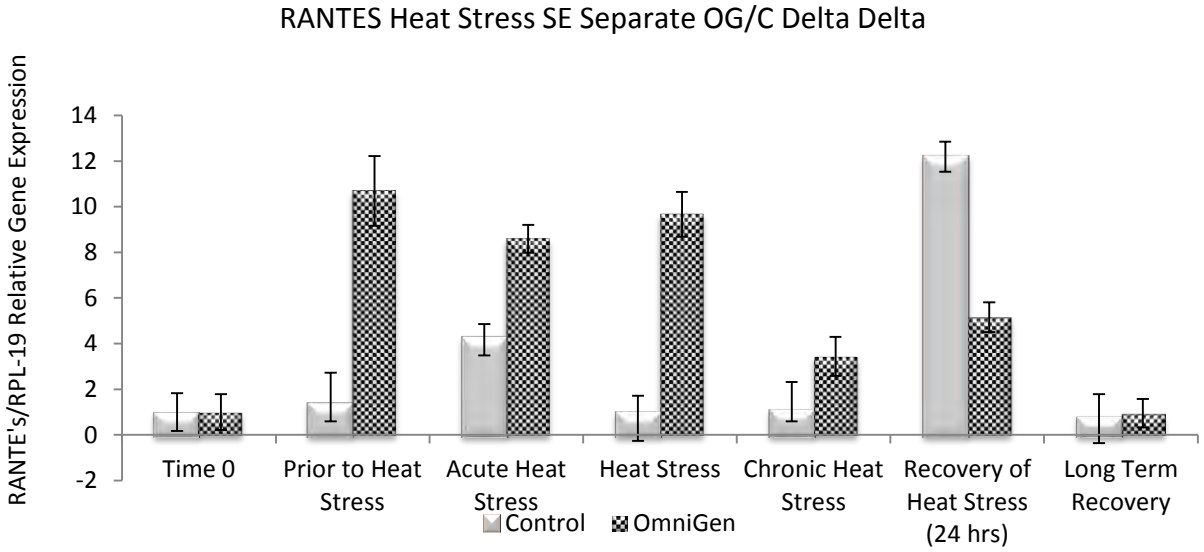

Figure 10. Effect of Omnigen-AF and environment on RANTES gene expression in lactating dairy cows housed in environmental chambers.

Summary

Heat stress exposure was mild to moderate in this study. The threshold for heat stress in lactating dairy cows is a THI > 68, respiration rates > 60 bpm, and rectal temperatures > 38.5°C (Zimbleman et al., 2009). OmniGen-AF reduced impact of thermal stress on stress of lactating dairy cows. Cows fed Omnigen-AF had reduced rectal temperatures and respiration rates during periods of peak thermal load. Respiration rates in treated cows did not exceed 60 bpm and mean rectal temperatures were 0.2 to 0.3°C cooler. OmniGen-AF fed cows displayed higher feed intakes during HS as well. Cows fed OmniGen-AF also displayed a lower cortisol spike on the first day of heat stress.

Milk yield decreased with heat stress in both control animals and the OmniGen-AF fed animals. However, feed intake was unchanged in cows fed Omnigen-AF and milk yields were numerically higher. Changes in SCC were consistent between groups. Cows fed OmniGen-AF displayed decreased SCC compared to control cows with the greatest difference during the recovery period.

Serum cortisol levels were similar to previous findings (Christison and Johnson, 1972) and increased within the first day of heat exposure. The animals in the ARC had higher cortisol levels compared to published levels, but the confinement and changes in surrounding from the dairy to the ARC may account for some of the changes.

Cytokine (RANTES) gene expression was higher in cows fed Omnigen-AF during the HS portion of the study but not during recovery. The elevated cytokine gene expression may be associated with improved immune function in cows fed Omnigen-AF.

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SESSION NOTES

Inflammatory Responses to Sub-Acute Ruminal Acidosis

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Introduction

Sub-acute ruminal acidosis (**SARA**) can occur as a consequence of feeding high energy rations to dairy cattle. During SARA, the rate of rumen short-chain fatty acid (**SCFA**) production exceeds SCFA absorption and results in an unhealthy depression of rumen pH. Definitions of SARA, derived primarily from experiments using ruminally cannulated animals, vary somewhat but typically require rumen pH to be below a certain threshold (5.5, 5.6, or 5.8) for a certain duration of time (Krause and Oetzel, 2006; Oetzel, 2007; Radostits et al., 2007). Zebeli and Metzler-Zebeli (2012) recently proposed that SARA be defined as rumen pH below 5.8 for 6 or more hours per day based on meta-analyses indicating that this threshold resulted in both a decrease in fiber digestibility and an increase in plasma levels of acute phase proteins.

At the level of the rumen, causes of SARA can broadly be classified as management, environmental, and animal factors, which reduce ruminal buffering capacity or increase ruminal SCFA accumulation. As reviewed by Stone (2004), buffering capacity can be increased by increasing dietary forage content and optimizing particle size to increase chewing and saliva flow, by addition of external buffers or alkalinizing agents to the ration, and by increasing the dietary cation anion difference of the ration. Buffering capacity can be reduced in response to heat stress or as a result of decreased chewing, for example due to feed sorting. The rate of SCFA production and the risk for SARA can be increased in response to increased dietary proportion of grain, increased fermentability of grains or forages, increased feed intake, and management factors that lead to larger and less frequent meals. It has also been proposed that cows might be at greatest risk for SARA immediately postpartum due to diminished size and absorptive capacity of rumen papillae following feeding of lower energy density diets during the dry period (Stone, 2004). However, Penner et al. (2007) were unable to reduce postpartum SARA by increasing concentrate feeding prepartum.

Consequences of SARA include feed intake depression, fluctuations in feed intake, reduced diet digestibility, reduced milk yield, reduced milk fat percent, gastrointestinal damage, liver abscesses, and lameness (Krause and Oetzel, 2006; Radostits et al., 2007; Plaizier et al., 2008). Injury to the gastrointestinal lining followed by localized or systemic inflammation appears to mediate many of these negative effects.

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Sub-Acute Ruminal Acidosis Effects on the Rumen and Hindgut

During SARA, ruminal accumulation of SCFA reduces rumen pH and causes a shift in rumen microflora (Zebeli and Metzler-Zebeli, 2012). Fiber and total carbohydrate digestion are reduced as a consequence of this shift, resulting in a loss of energy, and reduced body condition is sometimes noted without a concurrent reduction in intake (Hall, 2002; Kleen et al., 2003; Dijkstra et al., 2012). Khafipour et al. (2009b) evaluated changes in rumen fluid bacterial populations following experimental SARA challenges. Of the changes in bacterial population following a SARA challenge with wheat-barley pellets, the increase in *Escherichia coli* was positively associated with the severity of SARA symptoms, leading them to conclude that increases in *E. coli* may be important to the etiology of SARA. In another study, Mohammed et al. (2012) evaluated the population structures of rumen fluid bacteria both pre- and postpartum and associated those with the severity of SARA. They found that the magnitude of the population shift between prepartum and postpartum was independent of SARA susceptibility. Finally, Chen et al. (2011) found that the structure of the bacterial community adhered to the rumen epithelium changed when beef heifers were switched from a predominantly grass hay diet to a predominantly barley grain diet. Of interest, *Treponema*, *Ruminobacter*, and *Lachnospiraceae* species were found during high grain feeding. Shifts in rumen bacterial communities in response to SARA are believed to be a key first step in the negative impacts of SARA on animal performance.

Concurrent with shifts in microbial populations, there is also an increase in rumen concentrations of potentially toxic and inflammatory compounds during SARA. One that has received the most attention is endotoxin or lipopolysaccharide (**LPS**). The LPS is a component of gram negative bacterial cell walls, and presence of LPS within the body elicits an inflammatory response by mammalian cells. When animals are challenged with a SARA-inducing ration, the availability of fermentable carbohydrates initially results in logarithmic growth of bacteria, which is later followed by massive bacterial lysis in response to reduced availability of substrates, reduced rumen pH, and accumulation of fermentation end products (Zebeli and Metzler-Zebeli, 2012). Free LPS accumulates both during rapid growth and during bacterial lysis, resulting in increased rumen concentrations of LPS during SARA (Li et al., 2012). During an acute acidosis challenge in cows, rumen fluid collected following the challenge had increased endotoxin activity and became increasingly toxic when injected into mice (Nagaraja et al., 1978). These results led the authors to conclude that the effects of acidosis were mediated by systemic effects of rumen endotoxin. In addition, rumen concentrations of LPS were found to be negatively correlated with milk fat percentage and yield when cows were fed increasing levels of barley grain (Zebeli and Ametaj, 2009). Although rumen accumulation of LPS during SARA may be important for subsequent inflammatory responses, the immunoreactive properties of LPS differ among bacterial species. Khafipour et al. (2009b) propose that although rumen LPS increases in both grain-induced SARA and alfalfa pellet-induced SARA, inflammation is observed only in response to grain-induced SARA due to an increase in *E. coli* LPS. Other potentially harmful compounds produced during SARA include biogenic amines and ethanol (Ametaj et al., 2010). Ethanolamine is a biogenic amine that not only has potentially

harmful effects on the host but has also been shown to enhance growth and virulence factor production by pathogenic bacteria (Saleem et al., 2012; Zebeli and Metzler-Zebeli, 2012). Histamine is another biogenic amine produced during SARA and its potential role during the inflammatory response to SARA will be discussed in more detail later in this review.

The rumen epithelium serves as a selective barrier, allowing for absorption of SCFA while preventing entry and colonization by bacteria. Systemic effects of SARA are dependent upon a breach in this barrier. Structurally the rumen epithelium consists of four layers, the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale (Figure 1). In the healthy rumen, bacteria are loosely associated only with the stratum corneum. Tight junction proteins that regulate the permeability barrier are expressed most heavily in the stratum granulosum and to some extent in the stratum spinosum (Graham and Simmons, 2005). Connections among the stratum granulosum, stratum spinosum, and stratum basale allow for the transport of SCFA from the rumen contents to the basal lamina (Graham and Simmons, 2005). The permeability barrier function of the rumen responds to changes in the animal or the rumen. For example, permeability is increased during oxidative stress or heat stress (Mani et al., 2012). Increased permeability may also be an adaptive response to higher grain diets to allow for increased uptake of SCFA (Zebeli and Metzler-Zebeli, 2012). Studies using isolated sections of rumen have also demonstrated increased permeability in response to acidification or hyperosmolality (Aschenbach and Gabel, 2000; Schweigel et al., 2005; Emmanuel et al., 2007; Penner et al., 2010).

In addition to its role as a selective barrier, the rumen epithelium helps direct immune function through its interactions with mucosa-associated lymphoid tissue (**MALT**). These microstructures are found throughout the digestive mucosa and consist of clusters of white blood cells including innate lymphoid cells and mast cells (Pearson et al., 2012; Kurashima et al., 2013). In a healthy animal, commensal bacteria are bound to mucous lining the mucosa instead of directly to the mucosa, and epithelial cells are able to communicate the composition of the microflora to MALT cells through various receptors such as toll-like receptor pathways (Taschuk and Griebel, 2012). Mucosa-associated lymphoid tissue cells respond to this signaling by regulating their production of cytokines that then activate or suppress other immune cells. During homeostasis, MALT cells are usually hyporesponsive, and proteins and enzymes produced by these cells help to maintain tight barrier function and regulate epithelial cell growth and differentiation (Mani et al., 2012; Kurashima et al., 2013). In response to a challenge, MALT cell signaling can induce a variety of responses including production of bactericidal proteases and antimicrobial peptides, recruitment of neutrophils, promotion of B cell differentiation to IgA producing plasma cells, and activation of T cells (Pearson et al., 2012; Kurashima et al., 2013). Signaling by MALT cells is also important for regulating division and differentiation of mucosal epithelium to allow for tissue repair. Altered communication between epithelial cells and MALT cells, as well as increased MALT cell activation are associated with gut inflammatory disease in animals and humans (Kurashima et al., 2013).

Downstream inflammatory effects of SARA are dependent on a breach in the permeability barrier of the rumen wall, causing Oetzel (2007) to conclude that rumenitis (inflammation of the rumen wall) is the fundamental lesion of SARA. During SARA, some combination of increased osmolality, reduced pH, increased bacterial toxins such as LPS, and increased biogenic amines leads to rumenitis. A study using isolated rumen and colon tissue from steers demonstrated that LPS and decreased pH acted synergistically to disrupt epithelial barrier function (Emmanuel et al., 2007). Once the epithelium has been breached, MALT cells respond by triggering local inflammation and altering cytokine production; this in turn further increases permeability and allows for colonization of papillae and increased entry of bacteria and toxins into the papillae which can enhance the inflammatory response (Mani et al., 2012; Kurashima et al., 2013). When cows were switched from a 0% grain ration to a 65% grain ration, the rumen epithelium underwent dramatic changes including visible papillae lesions, decreased tight junctions, sloughing of the stratum corneum, and presence of bacteria in the stratum granulosum and stratum spinosum (Steele et al., 2011). Khafipour et al. (2011) found increased RNA levels of virulence and adhesion factors in *E. coli* isolated during grain-induced SARA, indicating that SARA may increase the potential for pathogenic organisms to take advantage of a breach in epithelial integrity and colonize papillae.

Concurrent with local inflammation in the papillae are changes in epithelial cell cycle, adhesion protein expression, and SCFA absorption. We recently evaluated the transcriptome of rumen papillae 30 h following a SARA challenge and found 172 genes that were differently expressed (Mackey, 2013). Of those genes, one pathway that was unregulated by SARA was homophilic cell adhesion through increased expression of four protocadherin beta genes (Figure 2). Others evaluating rumen tissue from cows fed high forage or high concentrate diets have found dramatic differences in gene expression, including differences in genes for adhesion proteins and cell cycle regulation (Taniguchi et al., 2010; Steele et al., 2011). Injury to the rumen epithelium and changes to the cell cycle in response to SARA can result in parakeratosis or hyperkeratosis (Penner et al., 2011). Increased exposure of the lower epithelial layers to bacteria and toxins as a result of parakeratosis can further increase rumenitis and lead to the formation of microabscesses (Kleen et al., 2003). Both parakeratosis and hyperkeratosis can reduce SCFA absorption which may explain why SARA can become increasingly severe with repeated challenges (Dohme et al., 2008; Plaizier et al., 2008). Reduced rumen motility as a consequence of SARA can also decrease SCFA absorption. Differences in SARA absorption also impact SARA susceptibility, and those animals with greater rates of SCFA absorption are more resistant to a SARA challenge (Penner et al., 2009).

Events that occur in the rumen during SARA are mirrored in the large intestine. An increase in intestinal carbohydrate fermentation typically occurs concurrent with SARA and leads to increased concentrations of SCFA and LPS, a reduction in pH, and damage to the intestinal mucosa (Bissell, 2002; Dijkstra et al., 2012; Li et al., 2012). Fecal indicators of SARA include diarrhea, frothy feces, increased particle size in feces, and presence of mucin casts in feces (Hall, 2002). Because the intestinal epithelium is

composed of only a single layer of epithelial cells, it has been proposed that systemic inflammatory effects of SARA might be due to passage of bacteria or toxins through the intestinal mucosa (Oetzel, 2003). In fact, Khafipour et al. (2009a) found that the timing of the presence of LPS in the blood following a SARA challenge suggested LPS entered the circulation via the intestines instead of the rumen.

Systemic Effects of Sub-acute Ruminal Acidosis

If bacteria or toxins escape from the mucosa, they will typically be delivered to the liver via the portal blood supply. If live bacteria that manage to exit or bypass the liver, they can cause chronic inflammatory diseases in response to SARA such as pneumonia, endocarditis, pyelonephritis, and arthritis (Oetzel, 2007). Bacteria can also colonize the liver and form abscesses. *Fusobacterium necrophorum* is the primary agent isolated from liver abscesses in feedlot cattle, and the liver infection is secondary to infection of the rumen wall (Nagaraja and Chengappa, 1998). This normal inhabitant of the rumen increases in number in response to high grain diets and can opportunistically colonize a rumen wall that has been damaged by parakeratosis or rumenitis in response to SARA (Tadepalli et al., 2009). Bacterial products and toxins entering the liver can affect liver function as well. Haubro Andersen et al. (1994) found that during acute acidosis endotoxin was found in portal and hepatic veins even though it was not detected in the systemic circulation. Increased toxin flow to the liver can result in damage, and Bobe et al. (2004) noted that SARA can increase the likelihood of fatty liver which can further impair liver function.

One clear response of the liver to grain-induced SARA is production of acute phase proteins that can modify immune function and generate a systemic inflammatory response. The main bovine acute phase proteins are serum amyloid A, haptoglobin, LPS-binding protein, and α-1 acid glycoprotein, and they function to stimulate tissue repair, remove harmful compounds, isolate infectious agents, and prevent further damage (Zebeli and Metzler-Zebeli, 2012). Plaizier et al. (2008) summarized results from multiple SARA challenge studies and proposed that LPS, inflammatory amines, or other products of bacteria that reach the liver stimulate release of acute phase proteins from the liver and generate a systemic inflammatory response. Thus, systemic inflammation does not appear to be dependent on bacterial compounds reaching the general circulation. In addition to their release by the liver, mRNA expression of acute phase proteins has also been detected in the gastric mucosa, indicating that the mucosa may contribute directly to this inflammatory response as well (Dilda et al., 2012).

Studies have also been aimed at evaluating why grain-based SARA challenges induce an increase in circulating acute phase proteins while alfalfa-based SARA challenges fail to do so. In a study using cows with ruminal and cecal cannulas, Li et al. (2012) found that although rumen concentrations of LPS increased in response to both types of challenges, cecal concentrations of LPS only increased in response to the grain-based challenge. They propose that translocation of LPS from the large intestine to the liver of grain-challenged animals might account for the increase in acute phase

proteins. However, using challenge models that bypassed the rumen, we and others have been unable to generate similar increases in plasma acute phase proteins as found in response to high grain diets, perhaps due to the short-term nature of those challenges (Bissell, 2002; Mainardi et al., 2011). Khafipour et al. (2009b) found that of the microbiome shifts in response to SARA, rumen *E. coli* abundance, which increased only in response to grain-based SARA challenges, was most strongly associated with concentration of acute phase proteins in the blood. These results suggest that differences in bacterial products reaching the liver in response to dietary changes can differentially impact acute phase protein production. Khafipour et al. (2009a) also suggested that increased LPS binding protein concentrations in the blood are a direct indicator of LPS translocation from the rumen to the liver. As data on acute phase protein response to SARA continues to mount, it is becoming clear that direct passage of LPS or other bacterial products to the general circulation may not be necessary for the systemic inflammatory response to SARA. Instead, immune modulation at the level of the liver or even the gut mucosa seems to be sufficient to drive systemic inflammation.

Laminitis and lameness are consequences of SARA and it is likely that similar mechanisms to those driving systemic inflammatory responses to SARA also mediate hoof damage. In response to rumen acidosis, vasoactive substances including LPS and biogenic amines can be absorbed across the gut mucosa. Damage to the gut wall and entry of bacterial products can drive formation of endogenous vasoactive products including cytokines and prostaglandins. The primary effect of these exogenous and endogenous compounds is dilation of arterioles and constriction of venules which at the level of the gut can enhance inflammation and increase entry of toxins (Shearer, 2011). In the corium of the hoof, these vascular changes result in inflammation, hemorrhage, death of cells, activation of matrix metalloproteinases, and disruption of growth factor signaling (Shearer, 2011). Altered cell growth, cell damage, reduced oxygen and nutrient flow, and reduction of intercellular adhesion can cause sinkage of the pedal bone, damage to the corium, pain, and lesions (Nocek, 1997; Goff, 2006). Histamine that is absorbed from the gut or produced endogenously during inflammation has been proposed to play a key role in development of laminitis. In a study using bulls, Takahashi and Young (1981) demonstrated that grain overload and histamine injection to the digital artery acted synergistically to induce laminitis. As reviewed by Katz and Bailey (2012), equine laminitis resulting from starch overload occurs via a similar mechanism to that proposed in ruminants. A loss of barrier function in the gut allows for influx of bacterial products including LPS and amines into the portal circulation. The resulting inflammatory changes in liver and leukocytes, with or without systemic entry of toxins, is proposed to cause laminitis through vascular changes in the hoof, apoptosis, oxidative injury, and enzymatic degradation of the basement membrane (Katz and Bailey, 2012).

Conclusions

Sub-acute ruminal acidosis impairs cow performance and health. Rumenitis is the initial insult of SARA and results in inflammatory and immune activation which

reduces energy available to support production, allows for transfer of bacterial products across the gut epithelium, and can damage tissues including the liver and hoof. Risks of SARA can be reduced by following feeding recommendations including maintaining adequate particle size and physically effective fiber and avoiding excesses of fermentable carbohydrates (Stone, 2004). In their meta-analysis, Zebeli et al. (2012) additionally concluded that rations should contain at least 39% NDF and no more than 44% concentrate to reduce the risk of systemic inflammation; however they caution that their conclusions were derived from diets based on barley and wheat grain and thresholds might change for more slowly fermented corn-based rations. Although SARA is difficult to diagnose directly, feces can be monitored for signs of SARA (Hall, 2002). Inclusion of feed supplements such as linseed oil or fish oil that contain high levels of omega-3 fatty acids may help to reduce the inflammatory response and tissue damage that can result from feeding high carbohydrate diets (Mani et al., 2012). Other dietary supplements such as biotin and zinc have the potential to strengthen epithelium to prevent tissue injury from SARA (Goff, 2006). Finally, as we continue to increase our understanding of pathologic bacteria that contribute to SARA-induced tissue damage, there may be potential to develop management strategies to reduce the competitive ability of those organisms. For example, Gill et al. (2000) found that vaccination against *Streptococcus bovis* reduced the severity of response to an acute acidosis challenge in sheep, and future development of vaccines against pathologic bacteria associated with SARA might be beneficial. Sub-acute ruminal acidosis will likely continue to be a problem for the dairy industry as high energy diets are required to support high levels of milk production. Careful attention to nutritional management and development of new SARA mitigation strategies may help to reduce its impact in the future.

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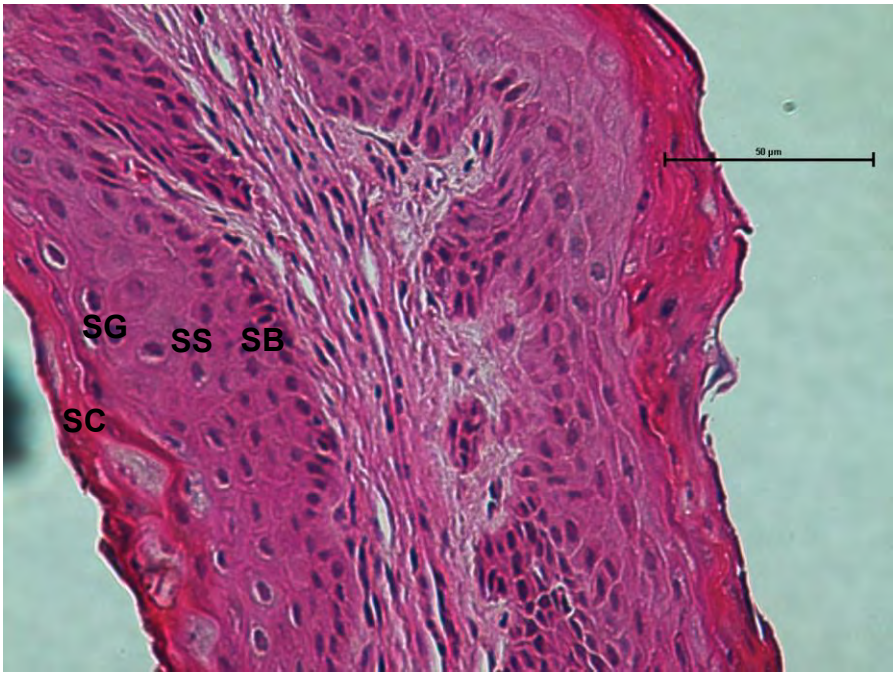
A



B

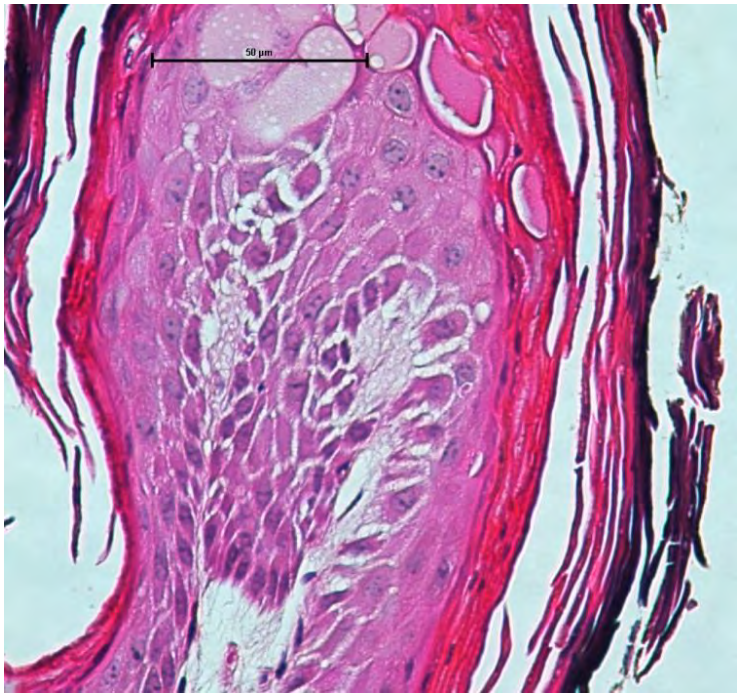


Figure 1. A. Cross-section of a rumen papilla showing the stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS), and stratum basale (SB). **B.** Damaged papilla showing separation of stratum corneum.

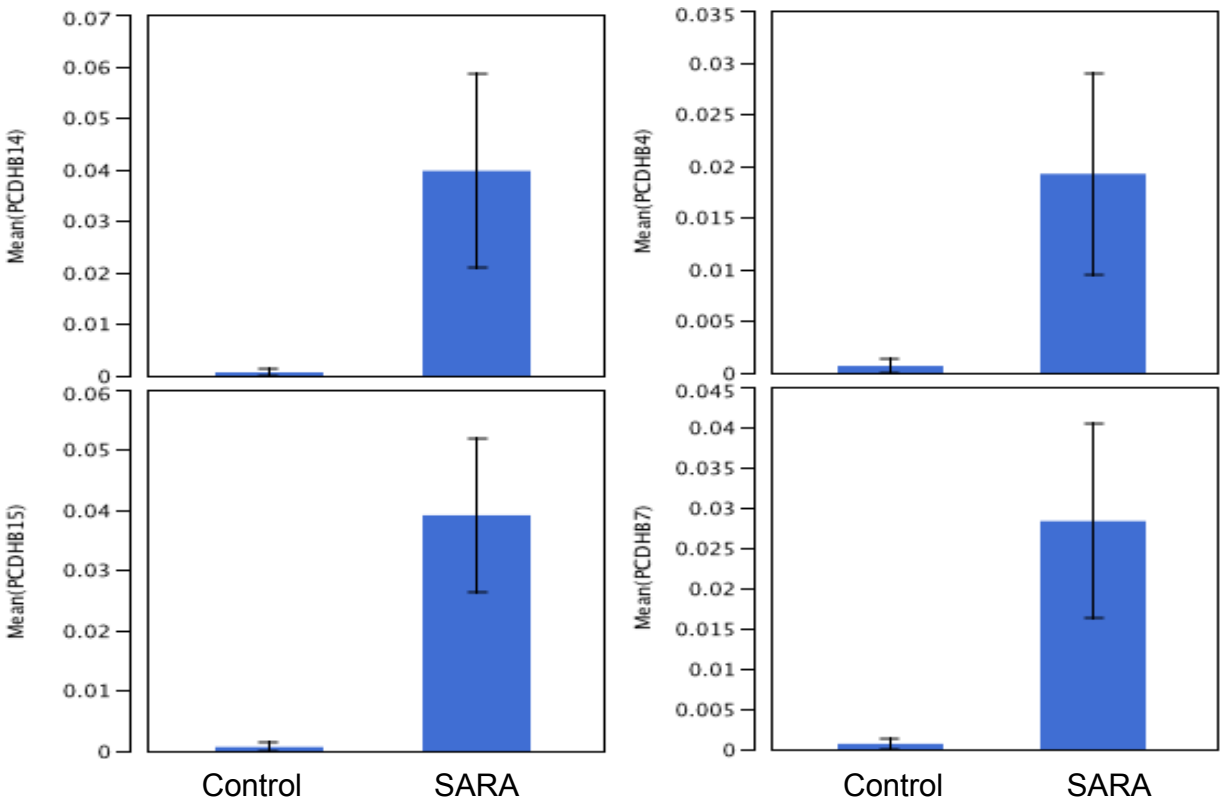


Figure 2. Expression levels of protocadherin beta (PCDHB) genes 4, 7, 14, and 15 at 30 hours following a SARA challenge. These homophilic cell adhesion genes (PCDHB14: top left, PCDHB15: bottom left, PCDHB4: top right, and PCDHB7: bottom right) were greater in SARA-challenged cows (right bars) than control cows (left bars).

SESSION NOTES

Etiology and Prevention of Fatty Liver and Ketosis in Dairy Cattle

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Introduction

According to the most recent National Animal Health Monitoring System for dairy cattle (National Animal Health Monitoring System, 2008), leading causes of morbidity in dairy cattle are clinical mastitis, lameness, infertility, retained placenta, milk fever, reproductive problems, and displaced abomasum. Of cows removed from herds, about 53% leave for one or more of the above reasons. Additionally, the rate of mortality of cows in U.S. dairy herds is nearly 6%, with 43% of these related to periparturient health issues, and likely a large portion of those classified as "unknown" (25%) occurring as a result of complications from the above. Overall, 16.2% of the cows that are permanently removed from a dairy herd are removed before 50 days in milk (**DIM**). These cows represent losses before the most profitable period of lactation. The relationship of the above disorders to excess prepartal body condition score (**BCS**) has been documented by numerous researchers and extensively reviewed (Bewley and Schultz, 2008). Briefly, cows with excessive body condition at calving, or excessive weight loss after calving, demonstrate overall decreased reproductive performance and increased likelihood of dystocia, retained placenta, metritis, milk fever, cystic ovaries, lameness, and mastitis as well as metabolic disorders, fatty liver, and ketosis. An epidemiological study by Gillund et al. (2001) found 20% of cows in surveyed herds to experience ketosis, and cows with BCS > 3.5 at calving to be 2.5 times more likely to become ketotic. Cows that became ketotic lost more body weight (**BW**) during early lactation, and the likelihood of conceiving at first insemination was decreased by 37%. Thus, the relationship of BCS, fatty liver, and ketosis has enormous economic impact on health and reproductive performance of dairy cows.

Fatty liver (i.e., hepatic lipidosis) is recognized as a major metabolic disease of dairy cows. We define *fatty liver* as the percentage of triacylglycerol (**TAG**) that causes detrimental effects to the health, well-being, productivity, or reproductive success of a cow (Bobe et al., 2004). On the basis of its effects, we categorize fatty liver into three classes: *clinical fatty liver* as 10% TAG and greater (wet weight basis), *moderate fatty liver* as between 5 and 10% TAG, *mild fatty liver* as between 1 to 5%, and *normal liver* as < 1% TAG; however, these TAG percentages are not absolute. As many as 50% of the cows in many dairy herds has fatty liver (Gonzalez et al., 2011). Fatty liver exacerbates the outcome of other metabolic diseases, in particular displaced abomasum and ketosis, because fatty liver decreases glucose availability for peripheral tissues (Veenhuizen et al., 1991). Furthermore, similar to excess body condition, increased liver TAG concentrations are associated with increased incidences and

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severity of laminitis, mastitis, milk fever, retained placenta, and metritis (Herdt, 1991). In the long-term, increased TAG concentrations are associated with decreased reproductive success and milk production in dairy cows. Given these facts, it is clear that a preventive for fatty liver would improve health, well-being, productivity, reproduction, and average lifetime of cows, which would result in major savings for U.S. dairy farmers.

Hypothesis for Our Research

On the basis of previous findings, we hypothesized that glucagon will prevent accumulation of TAG in liver of early lactation dairy cows by increasing glucose and lipid availability for peripheral tissues and by decreasing lipolysis in adipose tissue, thereby counteracting the effects of proinflammatory cytokines, in particular tumor necrosis factor α (**TNFα**). This hypothesis is based on results that demonstrate that fatty liver is preceded by and highly correlated with ($r = 0.96$) increased prepartal plasma TNFα concentrations and that fatty liver can be prevented by glucagon injection (Nafikov et al., 2006).

Research Goal and Summary

Veterinarians and herd managers have expressed a strong need for "tools" to prevent fatty liver and the array of prepartal diseases and disorders. Our *long-term goal* is to develop an effective and practical on-farm preventive for fatty liver that will decrease the incidence and severity of fatty liver and other periparturient diseases, thereby improving health, well-being, productivity, reproduction, and average lifetime of dairy cows. We expect future research to lead to such a tool. Our previous research has helped define the etiology and metabolic physiological consequences of ketosis and fatty liver (Hippen et al., 1999; Nafikov et al., 2006) and has led to an improved nutritional protocol that causes fatty liver for experimentation and to the development of glucagon as a treatment/preventive of clinical fatty liver. Glucagon has no known harmful effects on cattle, and it is a relatively small peptide that can be produced by recombinant or chemical means, which makes it economically feasible as a slow-release subcutaneous implant for use in the dairy industry.

In summary, our research has shown that subcutaneous injections of glucagon prepartally prevents fatty liver by increasing glucose and lipid availability for peripheral tissues through increasing hepatic gluconeogenesis and lipoprotein secretion and decreasing adipocyte lipolysis, thereby counteracting the detrimental effects of inflammatory factors such as TNFα. Application of our results is expected to decrease the incidence of fatty liver and related periparturient diseases and to increase the health, productivity, reproduction, lifetime, and well-being of dairy cows, which will improve the profitability of dairying in the U.S. Practical implementation of the validated approach will require from us further development of (1) noninvasive techniques, such as ultrasonography (Bobe et al., 2008; Weijers et al., 2012), for diagnosis of fatty liver and (2) a slow-release formulation for the delivery of glucagon (similar to commercial bovine somatotropin) for on-farm application.

Pathology of Fatty Liver

The pathology of bovine fatty liver (i.e., hepatic lipidosis) is well known. Up to 50% of all dairy cows accumulate excessive TAG in cells of liver and other organs (i.e., kidney and skeletal and cardiac muscles) during the peripartal and early postpartal period (Gerloff et al., 1986). If fatty liver is left untreated, cows often display signs of hepatic encephalopathy; symptoms progress from loss of appetite to general depression, to ataxia, to recumbency, to coma, and finally to death of the cow because of failure of affected organs. In fatty liver, accumulation of TAG in parenchymal cytoplasm is accompanied by disturbances in hepatic structure, including fatty cysts in liver parenchyma, increased cell volume, compression of sinusoids, decreased volume of rough endoplasmic reticulum, and mitochondrial damage, and by disturbances in metabolism, including decreased gluconeogenesis, because of decreased phosphoenolpyruvate carboxykinase (**PEPCK**) synthesis and activity, ketogenesis, ureagenesis, and lipoprotein synthesis and secretion, and increased lipid peroxidation (Bobe et al., 2004). In cows with hepatic steatosis, damages in hepatic structure progress into liver necrosis and cirrhosis, and metabolic disturbances progress into 'steep' decreases in hepatic gluconeogenesis, oxidative processes, and ureagenesis, causing toxic concentrations of ammonia (Veenhuizen et al., 1991).

Fatty liver is associated with the duration and severity of other diseases (Gerloff et al., 1986) and decreased milk production and reproductive success. The damaging effects of excessive accumulation of TAG on the structure and metabolism of affected organs together with the increased concentrations of ammonia, ketone bodies, and nonesterified fatty acids (**NEFA**) in plasma and the decreased peripheral glucose, lipid, and amino acid availability explain why hepatic TAG concentrations determine the outcome of a myriad of additional disorders (Rehage et al., 1986). For example, cows with clinical fatty liver retained viable bacteria in the mammary gland much longer after experimental mastitis than did cows with slight fatty infiltration of liver (Hill et al., 1985). Cows with fatty livers are leukopenic, with decreases in neutrophils, eosinophils, and lymphocytes (Herdt et al., 1986). In both the peripartal and early postpartal period, fatty liver is associated strongly with ketosis left displaced abomasum, decreased immune response, increased susceptibility for retained placentas and parturient paresis, infectious diseases such as lameness, mastitis, and metritis , and fertility problems (Bobe et al., 2004). Cows that are obese at calving almost invariably have fatty liver and are highly susceptible to a complex of metabolic and infectious diseases known as "fat-cow syndrome". In one high-incidence herd, morbidity was 82% and mortality was 25% (Morrow et al., 1979). Postpartal serum NEFA concentrations are associated with the risk of developing displaced abomasum, clinical ketosis, metritis, and retained placenta during the first 30 days of lactation (Ospina et al., 2010).

Etiology of Fatty Liver

Deposition of TAG in liver is the consequence of mobilization of NEFA from adipose tissue exceeding capabilities of liver for oxidation and secretion of lipids (Gross et al., 2013). Cows with fatty liver have greater adipose stores and mobilize more TAG,

which leads to greater plasma NEFA concentrations, because adipose tissue from cows with fatty liver is less responsive to lipogenic substances and more responsive to lipolytic substances. Furthermore, cows with fatty liver have decreased fatty acid oxidation, hepatic apolipoprotein synthesis and lipid secretion, as indicated by decreased plasma apolipoprotein and lipid concentrations and decreased serum lecithin: cholesterol acyltransferase (**LCAT**) activity (Bobe et al., 2004). Besides disturbances in lipid metabolism, cows with fatty liver also have disturbances in glucose metabolism: Cows with fatty liver are either hyperinsulinemic-hyperglycemic or hypoinsulinemic-hypoglycemic (Holtenius, 1991), because either peripheral glucose uptake is decreased, indicating insulin resistance, or insulin and glucagon secretion and, therefore, hepatic gluconeogenesis are decreased. Furthermore, plasma amino acids are decreased. In summary, the availability of glucose, amino acids, and lipids for peripheral tissues is decreased in cows with fatty liver.

The metabolic effects in the etiology of fatty liver may in part be explained by direct or indirect actions of TNFα, a proinflammatory cytokine synthesized by macrophages, lymphocytes, and primarily adipose tissue (Bradford et al., 2009; Trevisi et al., 2012). Infections, trauma, and also parturition induce TNFα secretion, which mediates inflammatory responses that use great amounts of glucose, amino acids, and lipids. Tumor necrosis factor α induces the secretion of acute phase proteins from liver such as haptoglobin and serum amyloid A (**SAA**) and causes cell apoptosis and necrosis. Tumor necrosis factor α injections increased lipolysis, evidenced by increased plasma NEFA concentrations, decreased lipoprotein secretion, and decreased plasma lipid concentrations and induced a biphasic short-term hyperinsulinemic-hyperglycemic and long-term hypoinsulinemic-hypoglycemic response and insulin resistance in dairy cattle (Holtenius, 1991). The main supporting evidence was revealed when Ohtsuka et al. (2001) linked fatty liver in dairy cows with elevated serum TNFα concentrations and Bradford et al. (2009) showed that the cytokine promoted TAG deposition in liver. Cell culture and *in vivo* rat studies support the link between fatty liver and TNFα, because TNFα induces hepatic lipidosis, increases lipolysis in adipocytes, inhibits pancreatic insulin and glucagon secretion and glucose utilization, induces cell apoptosis and necrosis, decreases hepatic gluconeogenesis, lipoprotein synthesis, and ketogenesis, increases hepatic lipid peroxidation, and interferes with insulin and glucagon signal transduction. These metabolic actions of TNFα have stimulated our research team to conclude that TNFα is a major factor in the etiology of bovine fatty liver.

Tumor necrosis factor α production is increased in adipocytes from obese individuals and seems to contribute to basal lipolysis in obesity (Bradford et al., 2009). Moreover, TNFα-mediated increase in lipolysis seems mediated by changes in perilipin concentrations. Intracellular TAG of adipocytes is hydrolyzed primarily by adipose triglyceride lipase (desnutrin, **ATGL**) to diacylglycerol (**DAG**; Ducharme and Bickel, 2008). The DAG is hydrolyzed primarily by hormone-sensitive lipase (**HSL**) to monoacylglycerol, which then is hydrolyzed by another lipase to glycerol and another fatty acid. Perilipin in the phosphorylated form (protein kinase A-catalyzed synthesis) facilitates HSL interaction with the lipid droplet. Preliminary data from a colleague (Koltes and Spurlock, 2011) suggest that increased phosphorylation and not concentration of perilipin in bovine

adipose tissue is associated positively with NEFA mobilization from adipose tissue. Therefore, perilipin phosphorylation seems to be a major contributor to increased NEFA mobilization for fatty liver. We believe glucagon counteracts these actions.

Glucagon

Glucagon is a 29 amino acid long hyperglycemic hormone secreted from alpha cells of the pancreas and is highly conserved between species. Glucagon increases plasma glucose by stimulating hepatic gluconeogenesis, glycogenolysis, amino acid uptake, and ureagenesis (Bobe et al., 2009). Glucagon increases lipolysis *in vitro* but not *in vivo* because of its hyperinsulinemic and hyperglycemic effects. Glucagon decreases hepatic TAG synthesis, increases TAG oxidation in bovine liver, and increases lipoprotein synthesis. Effects of glucagon on metabolic pathways are mediated by cAMP, which binds to nuclear factors to increase mRNA expression and stability of key enzymes of metabolic pathways such as pyruvate carboxylase (**PC**) and phosphoenol pyruvate carboxykinase (**PEPCK**). Infusions of glucagon have increased k-casein and as-casein and decreased αs1-casein and α-lactalbumin in milk of dairy cows. Importantly, we have shown that intravenous infusions of glucagon for 14 d beginning at 2 d postpartum prevent fatty liver (Nafikov et al., 2006).

Rationale to Study the Role of Glucagon on Fatty Liver

To summarize our research that led us to test the hypothesis that glucagon would control fatty liver development and/or treatment, we determined that cows in early lactation are approximately 500 g/d deficient in glucose. On the basis of those results, we developed a preventive for fatty liver consisting of intraduodenal infusion of 500 g glucose per day for 14 d; we discontinued this approach because several cows showed signs of hyperexcitability during the last days of glucose infusion. We switched our research emphasis to use glucagon to increase blood glucose concentrations. Glucagon injections of 0.5 mg gave significantly increased plasma glucose concentrations for 2 h, which led us to suggest that administration of glucagon might be beneficial in alleviating or preventing the subsequent development of clinical ketosis/fatty liver by supplying more blood glucose (Veenhuizen et al., 1991. Because glucagon has a physiological half-life of 5 min, we found that continuous, 14-d infusions would cure cows of fatty liver (Hippen et al., 1999). We then developed the more practical preventive for fatty liver by using subcutaneous injections of glucagon every 8 h for 14 d starting on d 2 postpartum (Nafikov et al., 2006). The glucagon injections actually led to an acute decrease in plasma NEFA concentrations, which suggest antilipolytic effects (via perilipin phosphorylation?) in adipose tissue.

Prevention of Fatty Liver

We determined that intervention with glucagon as a treatment/prevention of fatty liver is most effective within 14 days after parturition. The results demonstrated that subcutaneous injections of glucagon of 7.5 and 15 mg/d starting at 2 d postpartum are

sufficient for fatty liver prevention (Figure 1); however, some cows developed fatty liver already at d 2 postpartum.

We have confirmed our previous results (Osman et al., 2008) showing that prepartally and subcutaneously injected glucagon will decrease markedly the accumulation of lipid in the liver of the postparturient dairy cow. Daily administration of the same amount (15 mg/day) of glucagon for several days prepartally in a limited number of cows was effective in preventing fatty liver during the early postparturient period. Figure 2 summarizes our proposed mechanism of action of glucagon on prevention and alleviation of fatty liver in dairy cows by subcutaneous administration of glucagon.

Summary of Our Research Contributions

Our research group has studied ketosis and fatty liver in ruminants for over 40 years. We developed techniques and models to investigate the effects of ketosis and fatty liver. The results of our work has:

- improved technique for collection of hepatic tissue and showed that liver biopsies provide representative samples of liver (Hippen et al., 1999).
- developed and improved laboratory techniques to study metabolic effects of fatty liver in vitro in bovine liver and adipose tissue (Veenhuizen et al., 1991).
- established and improved a nutritional model to induce fatty liver (Hippen et al., 1999).
- developed the first reliable method to diagnose fatty liver by using ultrasonography with a 3.5 MHz probe, which enables us to categorize normal and fatty liver cows with an accuracy > 90% (Bobe et al., 2008).
- demonstrated that glucagon administration treats (Hippen et al., 1999) and prevents (Nafikov et al., 2006) fatty liver in post-parturient dairy cows.

We helped elucidate the etiology and pathology of fatty liver as summarized in our review article (Bobe et al., 2004). We proved that fatty liver is preceded by hormonal imbalances as indicated by decreased plasma insulin and glucagon concentrations and increased growth hormone concentrations, which increase lipolysis in adipose tissue. During fatty liver, pancreatic insulin and glucagon secretion and peripheral glucose uptake are inhibited. We proved that fatty liver is preceded by metabolic disturbances in lipid metabolism as indicated by increased lipolysis in adipocytes, leading to increased plasma NEFA and ketone body concentrations. During fatty liver, the metabolic imbalances worsen as demonstrated by steep increases in adipocyte lipolysis, even stronger in the presence of lipolytic hormones, and increased plasma and liver NEFA and ketone concentrations, despite decreased hepatic fatty acid ketogenesis and oxidation. We also demonstrated that fatty liver is preceded by metabolic disturbances in glucose metabolism, as indicated by decreased plasma insulin, glucagon, and glucose concentrations and decreased liver glycogen concentrations. During fatty liver, the metabolic imbalances worsen because hepatic ketogenesis, oxidative processes, and gluconeogenesis from different gluconeogenic precursors are decreased strongly. The

latter changes are stimulated by decreased concentrations of gluconeogenic precursors, such as plasma amino acids, as well as by decreased PEPCK mRNA synthesis and PEPCK activity, as further indicated by increased hepatic phosphoenol pyruvate concentrations. Pancreatic insulin and glucagon secretion and peripheral glucose uptake seemed inhibited in cows with fatty liver (Veenhuizen, et al., 1991; Hippen et al., 1999; Nafikov et al., 2006; Osman et al., 2008).

To better understand the etiology and pathology of fatty liver, we added a new aspect to our investigations and analyzed samples of 4 cows with fatty liver and 4 normal cows for inflammatory responses. Our results show that lipid accumulation in the liver is preceded by increased prepartal TNFα concentrations in plasma. During lipid accumulation, concentrations of the acute phase proteins haptoglobin and SAA in plasma are increased, indicating an inflammatory response, plasma NEFA concentrations are increased, indicating increased lipolysis, and plasma glucose and lactate concentrations are decreased, indicating a shortage of gluconeogenic precursors. During the progression of fatty liver, plasma NEFA concentrations remained elevated, indicating increased lipolysis, and plasma cholesterol concentrations are decreased, indicating decreased liver lipid secretion. The correlation between liver lipid and plasma cholesterol on d 6 postpartum was r = -0.86. The inflammatory response, as shown by plasma TNFα, haptoglobin, and SAA concentrations, decreases in parallel to the decrease in liver lipid concentration. Concentrations of plasma haptoglobin on d 2 and liver lipid concentration on d 9 postpartum are highly correlated (r = 0.71); concentrations of plasma SAA on d 2 and liver lipid concentration on d 9 postpartum are even more highly correlated (r = 0.86). Even more remarkable is the correlation of 0.96 between the prepartal plasma TNFα concentration and the liver lipid concentration at d 9 postpartum (Ametaj et al.,2005).

Future Research

Our long-term goal is to develop an effective and practical on-farm preventive for fatty liver that will decrease the incidence and severity of fatty liver and other fatty liver-related periparturient diseases, thereby improving health, well-being, productivity, and average lifetime of dairy cows. If fatty liver could be either prevented or controlled in its earliest stages, millions of dollars in losses to U.S. dairy farmers could be avoided. Estimated annual economic losses range from $62,640,000 to $150,000,000 (Bobe et al., 2004). Currently, there is no practical preventive available for fatty liver. In future research, we wish to test the hypothesis that prepartal administration of glucagon will precondition the liver so that postpartum fatty liver development is less likely to occur. The rationale for testing subcutaneous glucagon injections is that (1) early postpartal glucagon injections have been proven to be effective in preventing fatty liver in dairy cows and (2) glucagon administration has proven to be safe for the cows when given on a repeated basis. Because subcutaneous glucagon injections increase gluconeogenesis and decreases lipolysis, we believe that these two processes will, in part, explain a preconditioning of the liver by prepartal glucagon administration to decrease postpartal fatty liver development. Finally, glucagon is a relatively small peptide that can be produced by recombinant or chemical means, making it likely that

glucagon can become economically feasible as a slow-release subcutaneous implant for use in the dairy industry. The rationale for focusing on the effects of glucagon on adipose lipolysis, hepatic gluconeogenesis, and lipoprotein secretion is that results from our laboratory and from refereed literature clearly indicate that a major problem in the etiology of fatty liver and other fatty liver-related periparturient diseases is deficiencies in glucose and lipid availability for animal tissues. Therefore, our future research will test the metabolic processes that have (1) a direct impact on the glucose, amino acids, and lipid availability and (2) are known to be affected by fatty liver. Our rationale for also focusing on TNFα is based on the intriguing fact that infectious and nutrition-related periparturient diseases are associated so strongly (Bradford et al., 2009).

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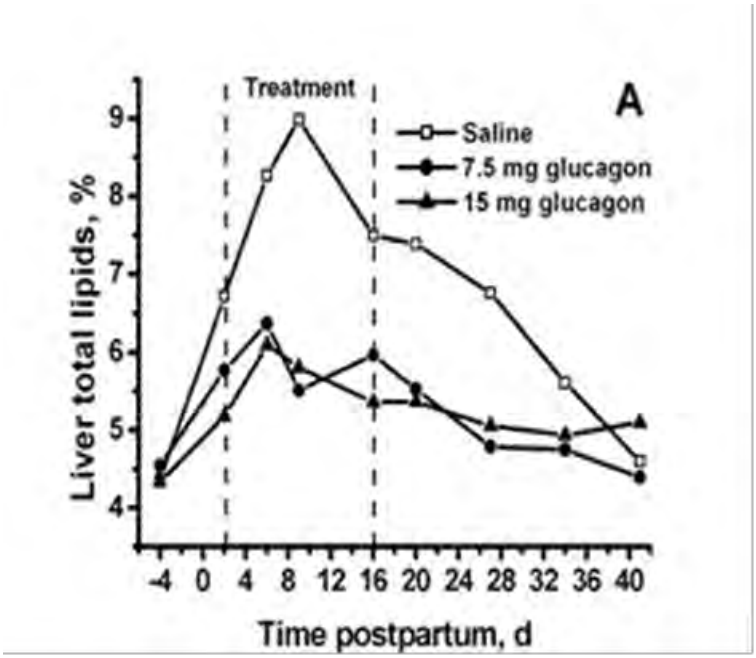

Figure 1. Effect of subcutaneous glucagon injections over 14 days beginning at d 2 (fatty liver prevention study) on liver lipid concentrations (SEM = 0.6) in dairy cows (n=8 in each group). These data were presented in (Nafikov et al., 2006).

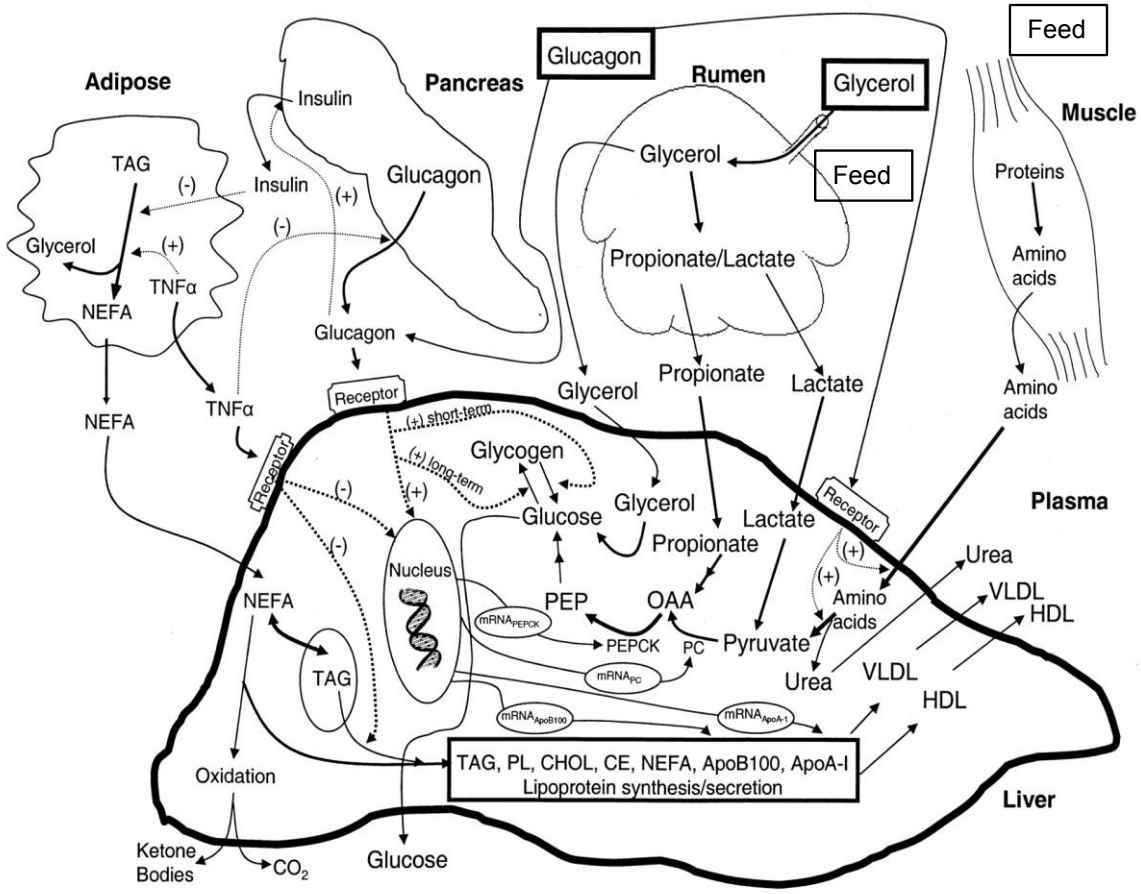

Figure 2. Expected cellular mechanisms by which consecutive subcutaneous injections of glucagon prevent fatty liver in dairy cows. Dashed arrows indicate regulatory functions.

SESSION NOTES

Protein Nutrition of Dairy Cattle – An Overview

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Abstract

Based on performance trials with lactating and feedlot cattle, the maximum milk yield and rate of gain were achieved only when the dietary protein concentration reached 20.6 and 15.2%, respectively. Because these concentrations are considerably greater than those in commercially fed rations, altering the protein source to supply a larger quantity of specific amino acids or supplying post-ruminal amino acids has been proposed to improve productivity. But which specific amino acids are inadequate for maximum production and to what degree has proven difficult to assess and production responses to amino acid supplementation have proven variable and inconsistent (Robinson, 2010). Considering the wide diversity in composition and in processing of both energy and protein sources being fed to ruminants, variability in production responses should not be surprising. Presumably, this variability can be because 1) amino acid requirements for ruminants are not defined adequately and 2) the supply and availability of amino acids from both microbes and dietary protein that escapes ruminal fermentation are not predicted precisely. Comparison of measured values with those predicted by NRC equations for the intestinal supply of microbial protein and ruminal escape of dietary protein revealed lack of both precision and accuracy. In addition to increasing the supply of amino acids for ruminants, an elevated protein concentration has also consistently increased feed intake and diet digestibility. Whether intake is increased due to effects of an increased supply of limiting amino acids or some indirect response associated with altered digestion or physiology is not clear. Through increasing water consumption and ruminal turnover, additional dietary protein could indirectly increase the efficiency of microbial protein production and ruminal escape of dietary protein sources. Although field tests sometimes detect performance responses to supplementation with specific sources of amino acids, extrapolation of results to other dietary or production conditions must await further refinement in models that predict metabolizable protein as well as specific amino acid requirements for both production and maintenance.

Introduction

Systems for evaluating the requirements for dietary protein for growth and lactation of ruminants have evolved during the last 50 years from simply measuring performance responses to various dietary crude protein concentrations to quantitative estimates of the small intestinal supply of protein (metabolizable amino acids) and estimates of amino acid requirements for production and maintenance. This evolution

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reflects a deepening understanding of microbial protein synthesis in the rumen and the amino acid composition of ruminant products. In contrast, estimates of amino acid requirements for maintenance have remained elusive.

Our comprehension of amino acid (and protein) metabolism and requirements for animals and humans is based on knowledge gained from thousands of studies conducted with non-ruminants as discussed in detail by the World Health Organization (2007). Among 26 amino acids, 8 to 12 are considered "essential" to include in the diet for various non-ruminant species. The non-essential amino acids can be synthesized by body tissues at a rate sufficient to meet the need for production, but to achieve maximum rates of production, additional amounts of the essential amino acids must be supplied in the diet. Thanks to ruminal bacteria, ruminants have an internal supply of essential amino acids, which are manufactured by microbes within the rumen if and when these microbes have an adequate supply of energy, ammonia and/or peptides, and sulfur. Although the supply of essential amino acids from ruminal microbes is sufficient for survival and low rates of production, dietary supplementation with essential amino acids is required to achieve the high rates of gain by young growing animals or of milk production achieved commercially.

Briefly, when an essential amino acid is limiting, adding it stepwise to the diet for a non-ruminant will increase growth rate, protein retention, other indicators of protein status (even immune responsiveness) until some response plateau is reached (Figure 1). The break between the upslope and the plateau represents the point when the amount of this essential amino acid first fully meets the requirement for that amino acid for the specific response being measured and thereby is considered to be the dietary requirement for the amino acid being studied. (In fact, this break-point simply represents the point where some other amino acid, nutrient, or energy places a ceiling on the response by the animal or bird.) Intake of an individual amino acid beyond this plateau may increase performance slightly due to hormonal (e.g., growth hormone) or antibacterial effects. However, intake of a large excess of single essential amino acids relative to other amino acids typically depresses performance due to antagonisms among amino acids, an imbalance, or toxicity, each of which can depress energy intake and performance. Note that a diet devoid of the essential amino acid may result in a negative response (e.g., weight loss). This indicates that essential amino acids are used both for maintenance and production. Only after maintenance needs are met can an amino acid be used for production.

When supply of an amino acid exceeds the amount required, the excess must be degraded. Surplus amounts of an amino acid will elevate its concentration in blood plasma and increase its rate of oxidation by liver, kidney, or muscle tissue. Consequently, plasma concentrations and rates of amino acid oxidation can and are used as indicators of the adequacy of individual essential amino acids. Conversely, when one amino acid is deficient, the supply of all other amino acid exceeds the amount needed and those excesses must be oxidized. Consequently, the rate of oxidation of a check amino acid will decrease as a limiting amino acid is added to the diet until the requirement for the limiting amino acid is met. Within a group of animals or birds given

free choice access to feed, the break point will differ among individuals due to differences in genetics, in amino acid metabolism or oxidation, or in feed intake. Therefore, when measured within a group of animals, the break point is less abrupt than illustrated in Figure 1 but instead is curved so requirements usually are assessed through curvilinear regression analysis.

Dietary protein requirements for maximum production can be assessed in a similar fashion as for individual amino acids. Again, the break point will vary with various factors (level of production, differences in animal or bird size or feed intake that can influence maintenance requirements, or with differences in the concentration of the second limiting nutrient in the diet), so f estimates of required amounts of either amino acids or protein calculated across studies become less precise.

Protein Requirement Models and Systems

The Classical Protein Requirement Model

Traditionally, protein requirements for maximum production of ruminants were assessed and developed by the dose-response measurements discussed above. Performance or production was measured for groups of animals fed a single diet supplemented with various concentrations of protein. When milk production or rate of gain increased when additional protein was provided, the lower protein concentration was considered to be inadequate. Conversely, when removal of protein from a diet failed to reduce animal productivity, the supply of protein provided by the diet was considered to be above the protein requirement already. Various mathematical and statistical models (e.g., curvilinear or broken line regression procedures) have been employed to determine the break point or the requirement. Such an approach can be used in the field by livestock producers for assessing whether or not the protein requirement is being met for a group of animals with a given genetic merit when being fed a specific diet. Unfortunately, small changes in production may not be easily or rapidly detected, particularly with growing or non-lactating ruminants.

If the cost of supplemental sources of dietary protein is high, maximum productivity may not be the most economical level for production. In contrast, when low-cost feeds rich in protein (e.g., distillers' grains) are fed, least cost diets may contain much more protein than required and lowering the protein concentration of the diet may increase cost of the diet (Stewart et al., 2012).

To estimate crude protein requirements of lactating dairy cows, data from multiple experiments must be compiled and analyzed. Consequently, data from 25 lactation trials involving 75 different diets fed to a total of 1151 cows were assembled in the current study. Most of these trials were published after the 2001 NRC Nutrient Requirements for Dairy Cattle publication had been compiled. These trials included both longer-term feeding studies and studies where diets were rotated among cows over time in cross-over or Latin square experiments. Results were plotted and the significance of relationships was assessed by determining the significance of linear or

linear plus quadratic regressions weighted by the number of animals fed each diet and adjusted for the mean effect of trial by including trial as a covariate. Although DM intake and milk yield were reported for cows in all the trials included in this data set, other measurements (apparent digestibility, urinary and fecal excretion of nitrogen (**N**), N retention, milk components, milk urea, and blood urea) were reported in only some of the trials. 'Days in milk' (**DIM**) when these trials began ranged from 1 to 238 days with a mean of 85 days. The fat-corrected milk yield for each individual diet is shown in Figure 2. The points connected by a line are from the same trial, and the best fitting regression line is plotted.

Milk yield increased at a decreasing rate as the dietary concentration of protein was increased both in this summary and in a summary of earlier studies compiled by NRC (2001). Considering the wide diversity in milk yield and days in milk among these trials, it seems surprising that 20.6% dietary crude protein was required to achieve the maximum milk yield. This concentration is considerably higher than fed to most commercial herds. However, this level is lower than the NRC (2001) estimate that 23% dietary crude protein is needed for maximum milk yield! Within individual basal diets (separate lines within Figure 2), yield responses usually were greater (steeper slope) when milk production was greater. Based on averages from these 2 equations, a decrease in dietary protein from 18 to 17, 16, and 15% of diet DM (sequentially reducing the daily dietary protein supply by about 0.5 kg per step) should be expected to decrease daily fat-corrected milk yield by an average of 1.4, 3.0, and 5.0 kg/d below milk yield with an 18% CP diet. Small changes in production may prove difficult to detect (and may be statistically nonsignificant) in single studies or in on-farm trials, but these decreases should be of concern when evaluating recommendations that protein concentrations should be reduced merely to decrease N excretion and improve the efficiency of converting dietary protein to milk protein. A deficiency of protein should have less impact on production in later lactation than early in lactation. Therefore, adjusting dietary protein concentration based on milk production and DIM should help to conserve protein while minimizing adverse effects of a protein deficiency on milk yields.

Concentrations of dietary protein in commercial diets typically are greater for cows early than late in lactation, when the source of forage is alfalfa rather than corn silage, and when supplemental protein sources have a low cost. In contrast, concerns about waste management and disposal often leads producers to lower the protein content of their diets, to switch to protein sources with a high content of essential amino acids or that resist ruminal degradation (e.g., fish meal; bypass soy products) and to attempt to substitute commercial sources of rumen escape amino acids for some of their supplemental dietary protein.

Although dietary concentrations of energy and other nutrients were maintained within each of these trials, intake of dry matter was not limited. In all cases, cows had free-choice access to feed. Generally, dry matter intake increased in a curvilinear fashion similar to milk yield (Figure 3). Similar intake responses were apparent in trials summarized for finishing feedlot cattle with intake of dry matter by feedlot cattle increasing linearly ($P = 0.08$) as dietary crude protein concentration was increased

(Owens et al., 2014). Precisely how and why dietary protein concentration should impact dry matter intake is uncertain. Certainly, with low protein intakes, a deficiency of ruminal ammonia will decrease rate of NDF digestion in the rumen and increases in rumen NDF fill will depress feed intake of cattle fed high NDF diets. In contrast, additional dietary protein, through increasing water intake and rumen passage rate of both solids and liquid, would be expected to increase flow of protein to the small intestine both through increasing efficiency of microbial growth in the rumen and by increasing ruminal escape of dietary protein. Similarly, increases in rumination and chewing time should increase flow of liquids and solids from the rumen. Because dietary protein and NDF concentrations typically are negatively related, maintaining a consistent flow of protein to the small intestine across diets, through retarding ruminal outflow of less digestible forage, should have given ruminants an evolutionary advantage that remains evident in commercial cattle today (Owens et al., 2014).

Concentration of milk protein, often used as an index of protein adequacy, often increases in response to supplementation with ruminally protected amino acids. On that basis, one would expect milk protein concentration to increase when supplemental dietary protein is provided and milk production increases. No such response was detected (Figure 4) in these trials. Instead, milk protein concentration often decreased when very high-protein diets were fed. In a trial by Leonardi et al. (2003), concentration of milk protein decreased when dietary protein was increased even though supplementation with rumen-escape methionine increased the concentration of protein in milk within both their high-and their lower-protein diet groups. Precisely why milk protein concentration may respond to supplemental rumen escape methionine but not to supplemental protein that will increase milk yield is uncertain.

Other changes detected with higher dietary crude protein concentrations included linear increases in milk efficiency (yield/DM intake; $P < 0.03$), in plasma and milk urea concentrations, in milk fat concentration ($P < 0.03$ but peaking at 19.4% crude protein, in crude protein digestibility that increased linearly and quadratically, in dry matter digestibility that peaked at 17.4% protein, in NDF digestibility with both linear and quadratic responses and a peak at 16.9% CP, and in daily intake of digestible DM that peaked at 18.4% CP.

Reducing the environmental footprint of milk production is of increasing public concern. Feeding less dietary protein will improve the efficiency with which dietary N is converted to milk protein (Figure 5). Within each of these trials, adding protein to the diet decreased extent to which dietary N was converted to milk N. However, milk N efficiency continued to increase even when diets contained such a low protein level that milk yield was depressed (Figure 2). This reduction in milk N efficiency is due largely to an increased excretion of urinary N by cows fed higher protein diets although excretion of N in both urine and feces linearly increased ($P < 0.03$) as dietary protein concentration increased. However, the magnitude of the increase in excretion was greater for urine (115%) than for feces (15%) when dietary protein was increased from 14 to 20% of DM.

Protein expenditures by lactating dairy cows at various stages of lactation are compiled and illustrated in Figure 6. Early in lactation, secreted milk protein accounts for about 60% of the total N output by the cow whereas late in lactation, milk will account for less than 40% of total protein expended. Other protein expenses by the cow that involve body maintenance include N lost as metabolic fecal N and endogenous (inevitable) urinary N, as well as scurf (skin, hair, hoof tissue). Very little protein is deposited daily within the developing fetus until very late in pregnancy.

Early in lactation, cows lose weight as energy reserves are raided to meet the energy demand for lactation. The extent to which tissue protein reserves are mobilized during this period has been debated. In trials where N retention was monitored at various stages of lactation, a negative N balance (body N loss) was apparent early in lactation even when very high amounts of protein were fed (circled values in Figure 7). This would imply that, as with energy, providing an excess of dietary protein early in lactation does not prevent the increase in mobilization of tissue protein reserves driven by hormonal changes early in lactation. Indeed, mobilization of protein reserves early in lactation should supply amino acids for synthesis of milk protein and reduce the need for dietary protein.

Because milk protein yield decreases later in lactation, the need for dietary protein decreases. In 2000, Wu and Satter, based on milk production responses with their diets and the levels of milk production their cows achieved, concluded that 17% dietary protein was adequate for maximum milk production during the first 7 weeks of lactation, possibly due to protein sparing by mobilization of tissue protein reserves. A higher concentration of protein (19%) was required from 7 to 16 weeks of lactation (Figure 7), but thereafter 17% protein was adequate to 30 weeks and 16% CP was adequate after 30 wk of lactation. Note that except for the first period, protein requirements decreased in parallel with milk protein yields. However, even late in lactation when production was reasonably low, Kalscheur et al. (1999) concluded that milk yield often increased when more supplemental CP was provided. However, when dietary crude protein concentration dropped below 16% of DM, NDF digestibility declined and this may have caused a reduction in energy intake. Nevertheless, altering dietary protein concentration in an attempt to match the need for N with specific segments of lactation rather than targeting a single CP for an entire lactation is a logical and effective approach both to avoid waste of N and to maximize productivity.

Metabolizable Protein Models

Since initially proposed by Burroughs et al. for growing cattle (1974) and lactating dairy cows (1975), the metabolizable protein concept has stimulated development of sophisticated models of protein metabolism that often are used to explain production responses and to formulate diets. These models all divide the N needs for ruminants into two fractions, one for growth of and fermentation by ruminal microorganisms, and a second to meet the animal's need for essential amino acids for both maintenance and production as illustrated in Figure 6. The general scheme behind these models and the interchange among these compartments is diagrammed in Figure 8. Central to all

models is an estimate of the supply of protein to the small intestine. Small intestinal protein supply (metabolizable protein) equals the sum of 1) microbial crude protein synthesized in the rumen that is flushed or sluiced to the small intestine and 2) dietary protein that escapes ruminal digestion. Numerous interchanges among pools are apparent, but transfer coefficients and the magnitude that external factors may alter these coefficients are not well understood. If amino acid supply has an impact on energy intake, as implicated in Figure 3, simulating the effect of protein supplementation on production becomes quite involved. Efficacy of any model must be compared with other models that predict productivity, but for any model that has intermediate points that can be measured, values at those intermediate points must be accurate if the model is working properly. To evaluate efficacy of the metabolizable protein model described by NRC (2000) that employs most of the same coefficients employed in other models, the precision with which this model predicted microbial protein supply to the small intestine as well as the amounts of dietary escape protein and of total protein reaching the small intestine was examined.

For this evaluation, a data set was compiled consisting of published data from 21 trials with 37 diets supplemented with various levels or sources of protein for a total of 117 different diet-protein combinations. Small intestinal supply of microbial protein, total protein, and by difference, ruminal escape dietary protein had been measured with growing-finishing steers in each of these trials. Based on the reported feed intake and feed composition, the NRC (2000) model was used to predict microbial protein flow and the amount of undegraded dietary protein that should reach the small intestine. Accuracy with which this model predicted the microbial protein supply and the amount of dietary protein that escaped ruminal digestion were plotted and regression coefficients were calculated.

Microbial Protein Supply

The quantity of microbial protein produced in the rumen that reaches the small intestine typically is limited by the amount of energy available from fermentation of organic matter within the rumen. Because the amount of organic matter fermented in the rumen from various feeds is not readily measured, some proxy for ruminally digested organic matter is used. Typically the proxy for ruminal organic matter digestion is feed digestibility (e.g., total digestible nutrients, **TDN**). This in turn is multiplied by dry matter intake to give an estimate of the total amount of energy available for synthesis of microbial protein. This in turn results in linear estimates of predicted yield of microbial protein from equations as illustrated from equations advanced by NRC (2000) for beef cattle, by NRC (2001) for dairy cattle, by Burroughs et al. (1975), and by Valadares Filho et al. (2010) as shown in Figure 9. Although estimates from NRC (1985) for beef cattle and values from Clark et al. (1992) are based on concentrate and roughage intake and therefore are tied as closely to TDN intake, regression estimates also are provided from those sources. Measured microbial protein flows to the small intestine measured in individual metabolism trials also shown in Figure 9 can be compared with the values predicted from these models. Measured microbial N flow to the small intestine was considerably less than predicted by any of the published equations even

though microbial protein supply increased linearly as intake of digestible energy increased. Imprecision in prediction of published models causes one to question whether the published models provide valid and realistic predictions of the supply of microbial protein to the small intestine.

Most metabolizable protein models use TDN as an estimate of the quantity of energy available for ruminal microbes to grow. But the extent to which TDN or dietary organic matter is fermented within the rumen varies drastically with grain and forage processing procedures and with the ruminal residence time that ruminal microbes have to digest feeds. As an example, Firkins et al. (2001) reported that the extent to which lactating cows digest dietary starch in the rumen ranges from only 53% for dry rolled corn to 88% for high-moisture ground corn grain. That a constant proportion of TDN from various feeds will be fermented within the rumen is an erroneous assumption. Furthermore, the primary source of energy for microbial growth is fermented carbohydrate. Consequently, ruminally fermented carbohydrate would seem preferable even to organic matter for predicting yield of microbial protein. Because it seems unlikely that tabular values can accurately predict the extent of ruminal digestion of carbohydrate from a totally mixed ration or an individual feedstuff processed in a specific fashion, other screening methods to appraise the availability of energy for ruminal microbes (e.g., gas production measurements; Fermentrics) may prove useful to refine estimates for specific feeds and feed processing methods.

The extent to which dietary organic matter had been digested within the rumen was measured in these trials. This was compared with the TDN content of each diet that had been calculated from diet composition and tabular TDN values for feeds from NRC (2000). The plot relating ruminal digestion of organic matter to TDN content of the diet is shown in Figure 10. Obviously, within this data set, extent of digestion of organic matter within the rumen, presumably the primary factor that limits yield of microbial mass in current metabolizable protein models, was not reliably predicted by TDN content of the diet. Considering that microbial yield increased with intake of TDN in these studies (Figure 8) but that TDN was not related to extent of organic matter fermented in the rumen, one could conclude that this relationship is driven by intake of ruminally fermented organic matter (Figure 11), not intake of TDN (Figure 11). Whether the remaining variability within this regression is due to analytical errors in analysis or reflects the fact that additional factors (e.g., rate of passage) are altering efficiency of conversion of digested organic matter into microbial protein remains uncertain.

That level of intake is a major factor driving the supply of microbial protein should not come as a surprise. Higher energy intakes permit a higher percentage of the available energy to be used for growth (with less expended for maintenance) for ruminal microbes just like for growing or lactating cattle. Consequently, ruminal residence time or dilution rate as well as the extent to which dietary organic matter is fermented within the rumen would seem preferable to TDN intake as a predictor of microbial N flow to the small intestine.

Rumen Escape Protein

The amount of dietary protein that escapes ruminal fermentation was calculated from the amount of each individual ingredient supplied to the cattle, the protein concentration of each feed component, and the fraction that is proposed to escape ruminal fermentation based on tabular values of undegraded intake protein (**UIP** or 100-Degraded intake protein, **DIP**) specified in tables from NRC (2000). Such rumen escape values presumably were estimated largely from estimates of protein solubility or the extent of enzymatic or microbial degradation of the protein from various feeds. The relationship between the measured amounts of dietary protein reaching the small intestine versus these predicted values are shown in Figure 12. Results force one to question the validity of UIP values for individual feeds. For refining such estimates, those feeds contributing the highest proportion of protein to the diet should be of primary concern. Effects of processing of grains and forages on their ruminal degradation rate and ruminal residence time also need to be included.

Metabolizable Protein Supply

Separation of microbial protein from total protein flow to the small intestine relies on some marker of microbial protein. For the animal, total flow of protein to the intestine from microbes plus feed is the factor of primary importance. Metabolizable protein flow to the duodenum according to most metabolizable protein models is calculated as 64% of microbial protein (based on the assumption that 80% of microbial protein consists of amino acids and true digestion in the small intestine is 80%) plus 80% of dietary protein that escapes ruminal fermentation (small intestinal true digestibility). Such estimates are compared with measured duodenal flow of microbial plus feed protein to the small intestine with cattle fed these 117 diets in Figure 13. Microbial protein synthesis from TDN intake was imprecisely predicted (R^2 = 0.48) estimating that the amount of microbial protein reaching the small intestine was 130 $\pm$ 27% of observed values (ranging from 74 to 224%) and ruminal escape of dietary protein was imprecisely predicted (R^2 = 0.41) estimating that the amount of rumen escape protein reaching the small intestine (Figure 12) was 113 $\pm$ 84% of observed values (ranging from 0 to 549%). Nevertheless, the predicted metabolizable protein supply still tended to increase as measured metabolizable protein supply increased. Predicted metabolizable protein on the average was 113 ± 24% of observed metabolizable energy (ranging from 60 to 203%) as shown in Figure 13. This would indicate that total protein flow to the small intestine was predicted more precisely than flow of its components, microbial and rumen-escape protein. Yet, all metabolizable protein models are based on the concept that microbial and rumen escape protein shall be predicted separately and combined in order to estimate the total metabolizable protein supply for producing ruminants. Based on that premise, efforts to improve precision of estimating these two components are needed if performance is to be predicted reliably so that diets can be formulated correctly based on such estimates.

Conclusions

Based on its imprecision in reliably predicting either microbial protein or rumen escape protein flow to the small intestine, the utility of the metabolizable protein model that was tested must be questioned. Because other metabolizable protein models are based on similar inputs and equations, their validity also seems questionable. High variability forces one to question whether current models are simulating ruminal metabolism correctly and properly. If duodenal supply of protein, a measurement that can be readily checked, is not predicted reliably by current prediction models, it seems useless to compare this supply with some additional estimate of the postruminal need for protein or individual amino acids for production plus maintenance of producing ruminants because these measurements that cannot be readily quantified. The fact that a model may predict whether milk yield will decrease or increase with a given dietary change when other factors (e.g., energy intake or digestibility) change does not prove that a model is either accurate or precise. Current models may lack important components, transfer coefficients may be inaccurate, or modulation of coefficients by recognized or unrecognized factors may be underestimated. Improvements might be achieved by substituting ruminally fermented carbohydrate for TDN intake and by altering estimates of both microbial protein yield and ruminal escape of dietary protein based on ruminal residence time. Yet, even if duodenal flows can be predicted reliably, deficiencies still cannot be predicted reliably from metabolizable protein supply alone because the amounts of individual essential amino acids required for both maintenance and production of ruminants still must be quantified with a high degree of precision.

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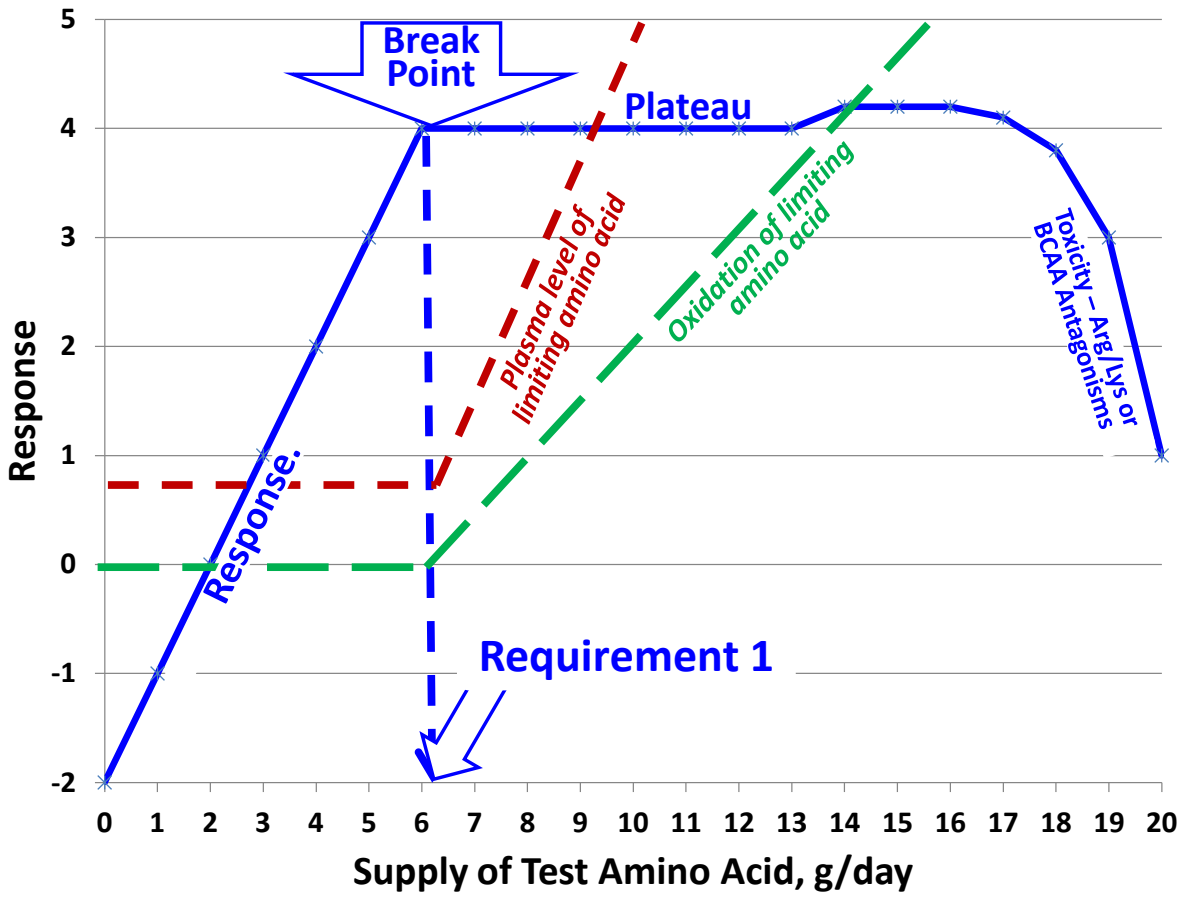

Figure 1. Classical physiological responses (e.g., growth rate) to supplementation with a limiting essential amino acid. BCAA = Branched chain amino acids; Arg = argininie; Lys = Lysine.

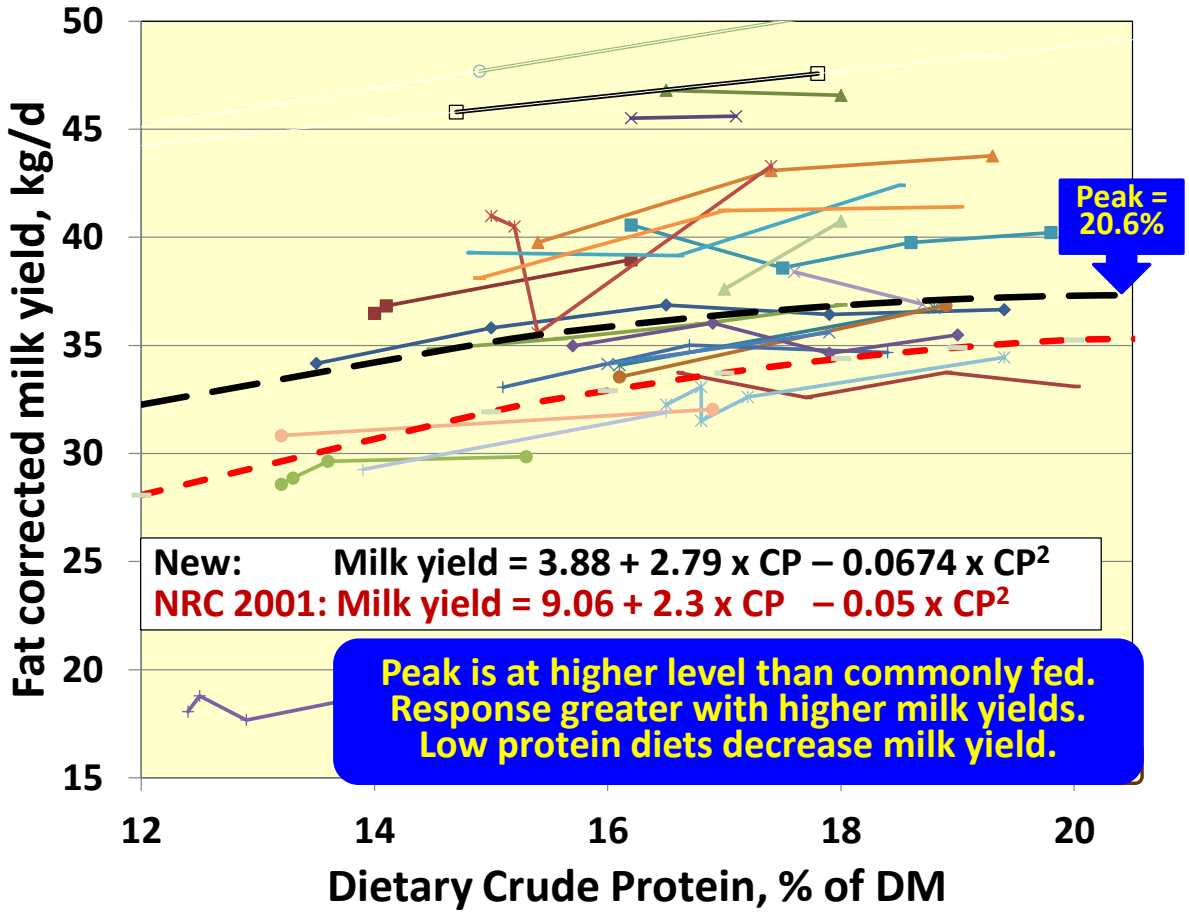

Figure 2. Fat-corrected milk yield by lactating cows fed diets with different crude protein concentrations. Lines connect milk yields from individual studies. The upper dashed dark line is the best fitting regression among these trials whereas the lower dashed curve is the NRC (2001) relationship.

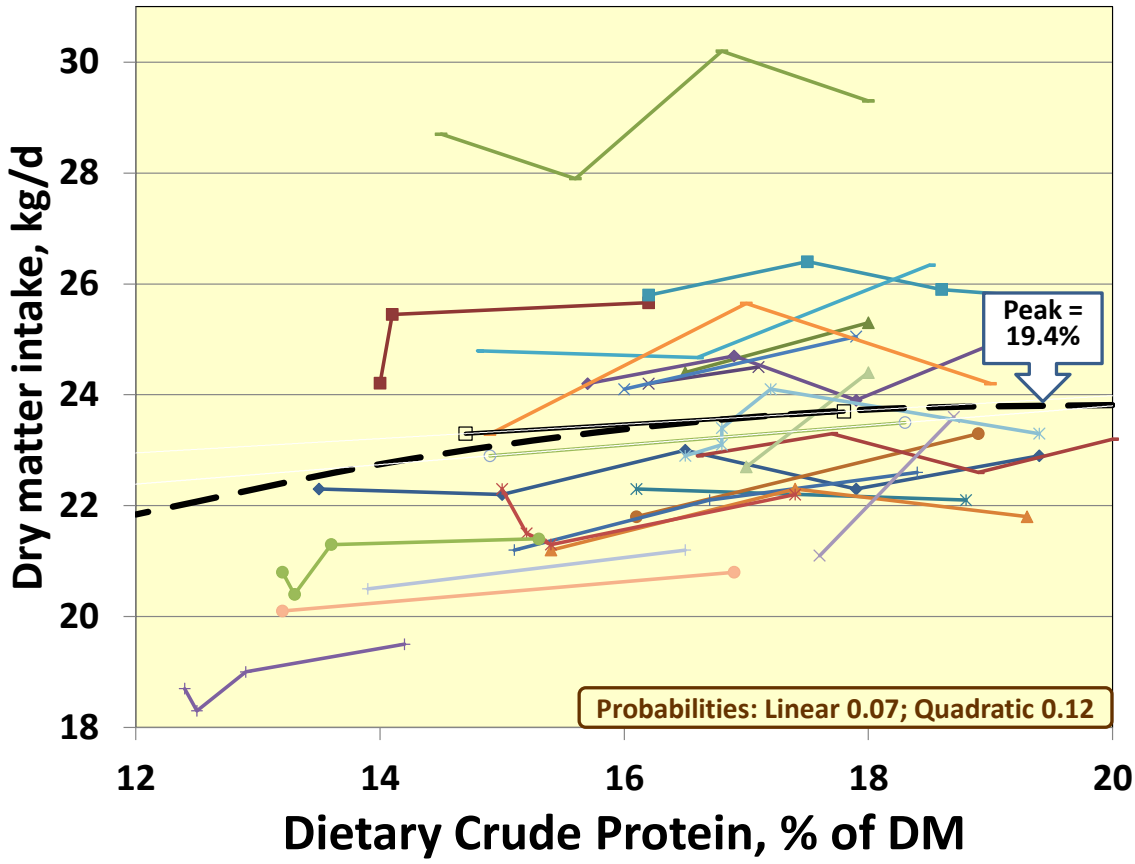

Figure 3. Dry matter intakes by lactating cows fed diets that differed in crude protein concentration.

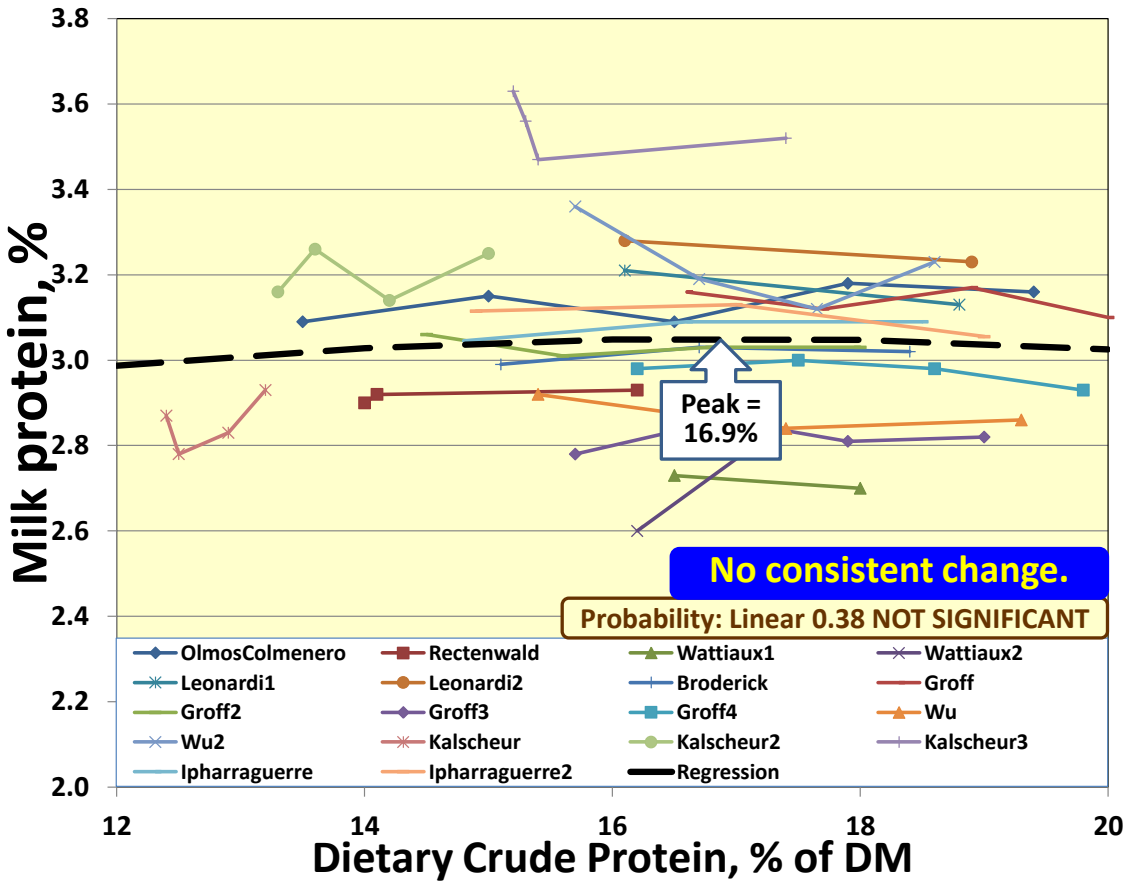

Figure 4. Milk protein concentration responses to varying dietary crude protein concentrations.

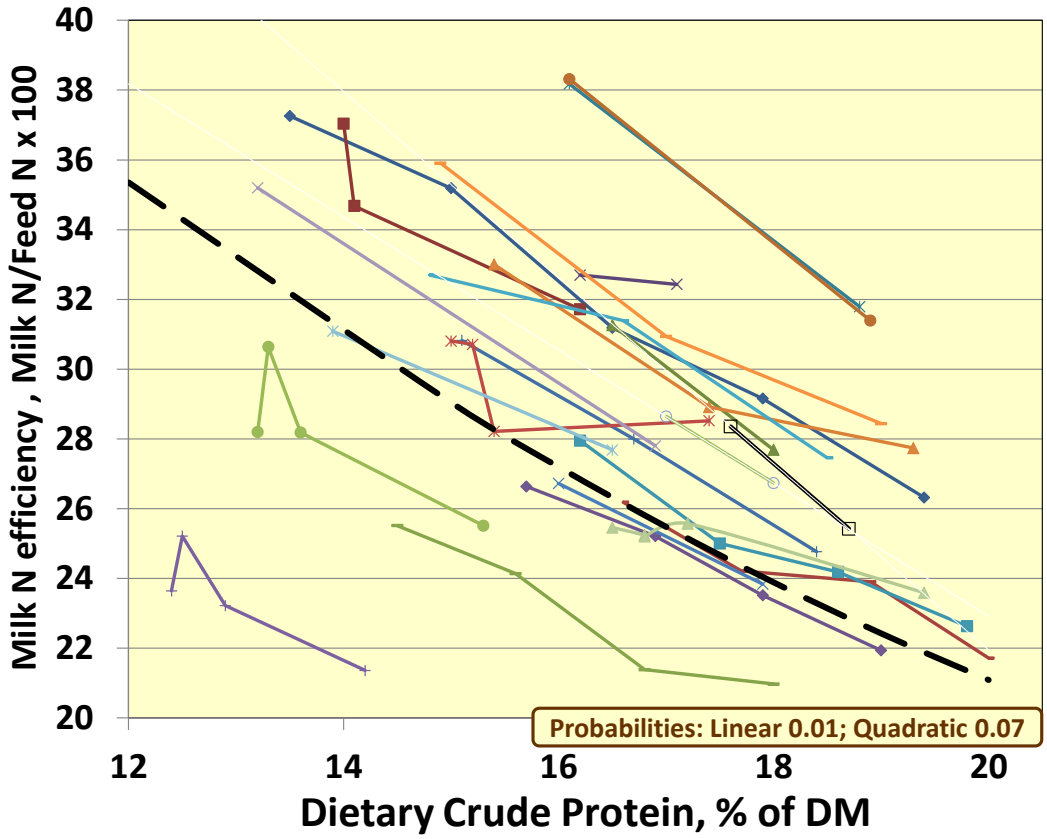

Figure 5. Milk N efficiency of lactating cows fed diets with various crude protein concentrations.

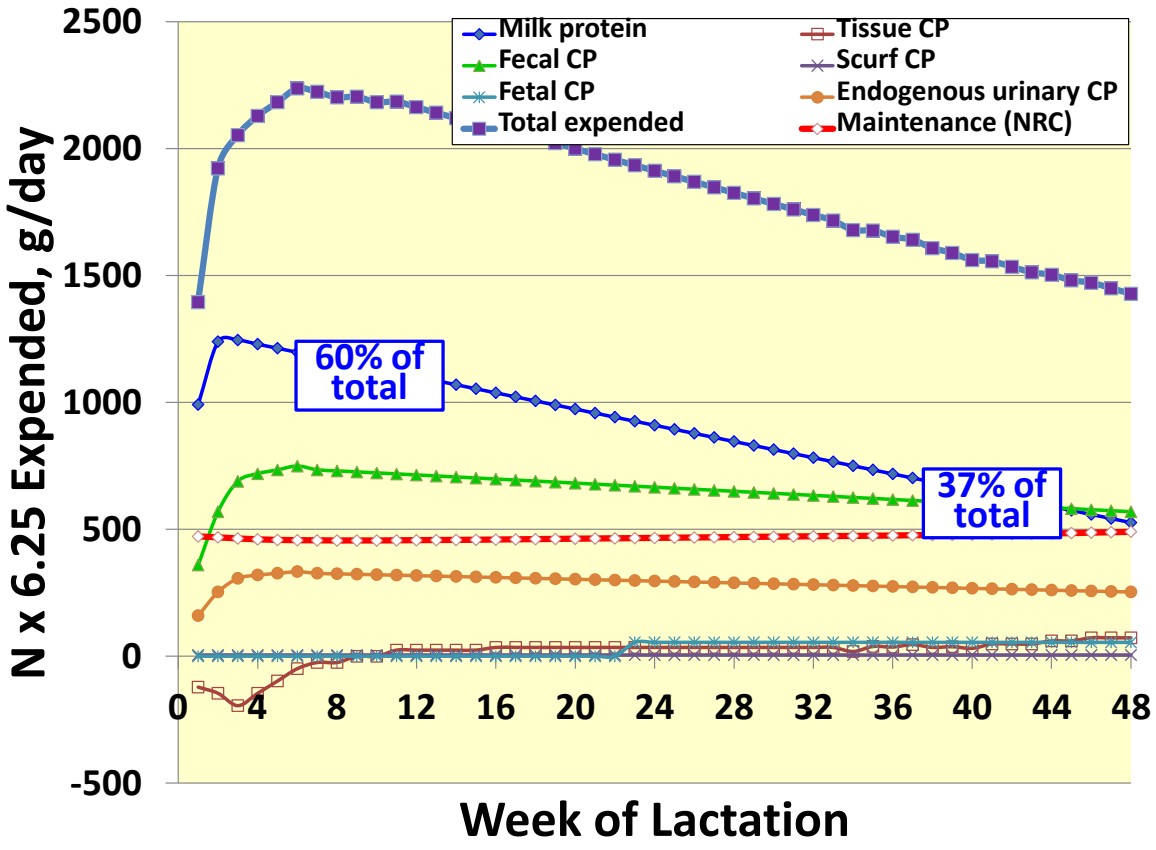

Figure 6. Daily protein expenses by lactating dairy cows at various weeks of lactation.

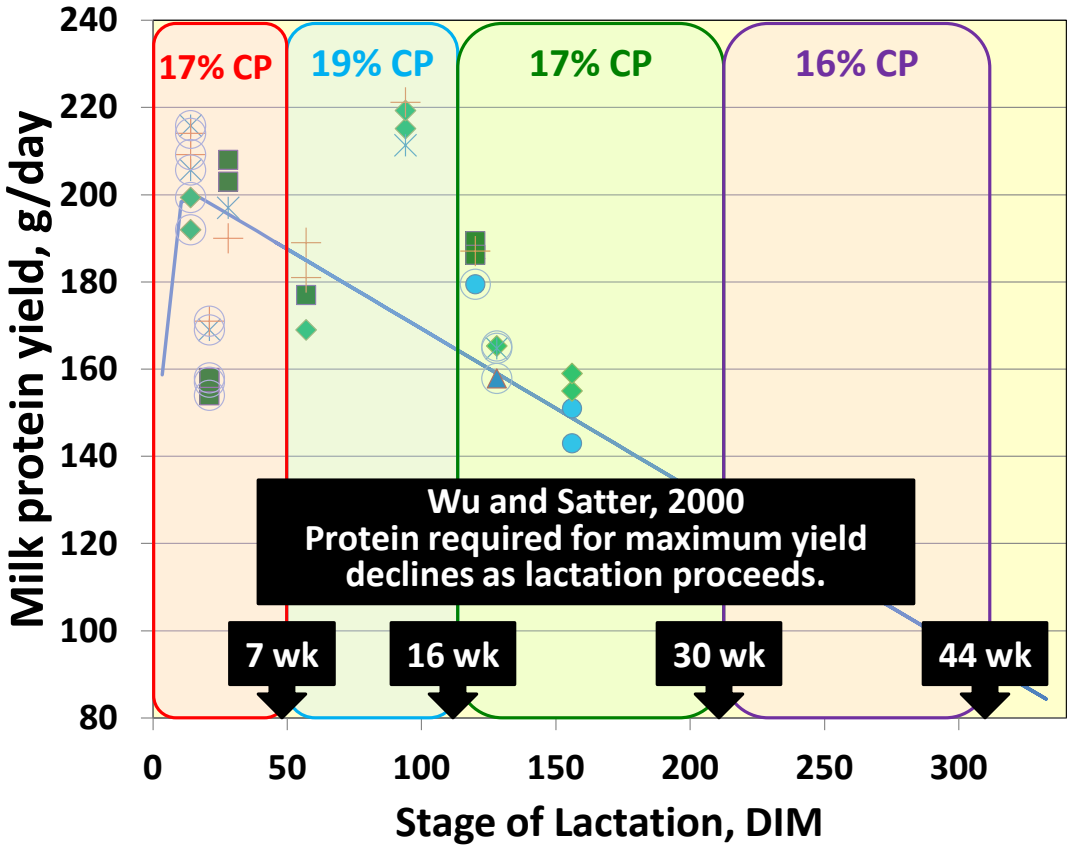

Figure 7. Milk protein yields, responses to protein supplementation (with negative nitrogen balance values being circled) and estimates of dietary protein requirements at various stages of lactation of dairy cows from Wu and Satter (2000).

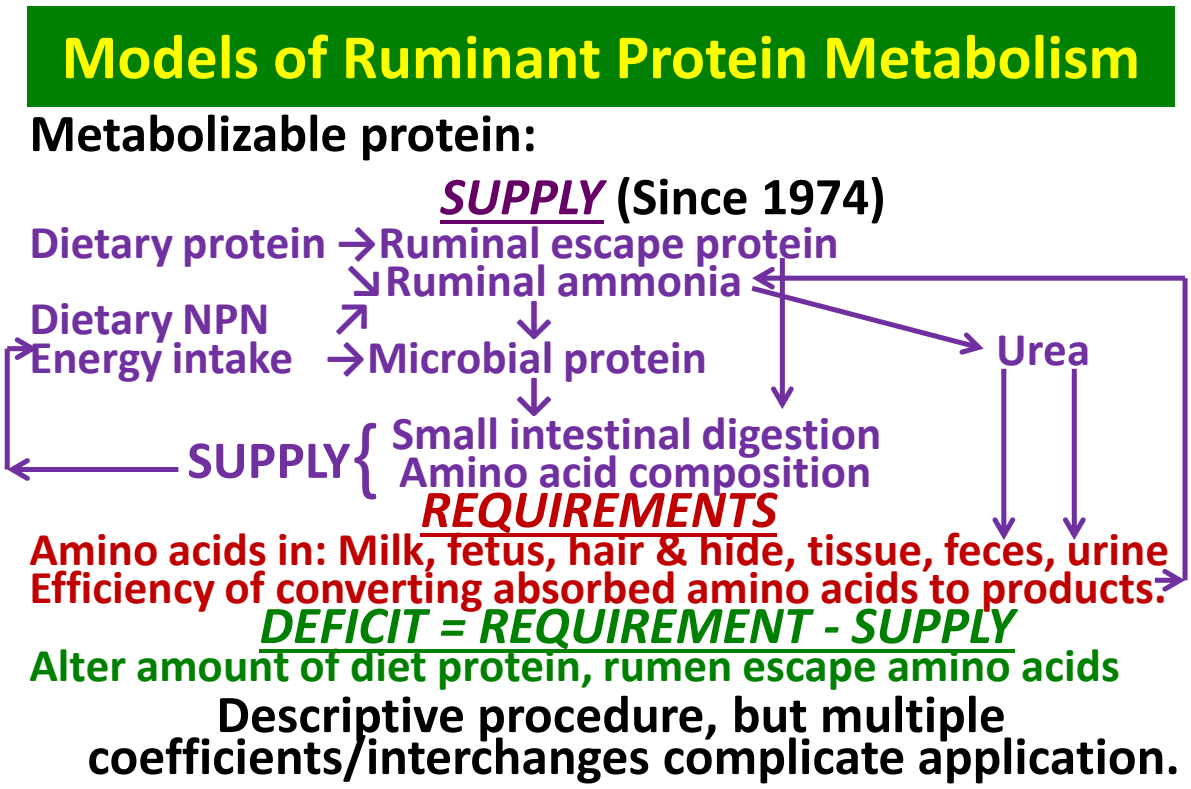

Figure 8. Schematic of typical modeling approaches to estimate protein requirements of ruminants.

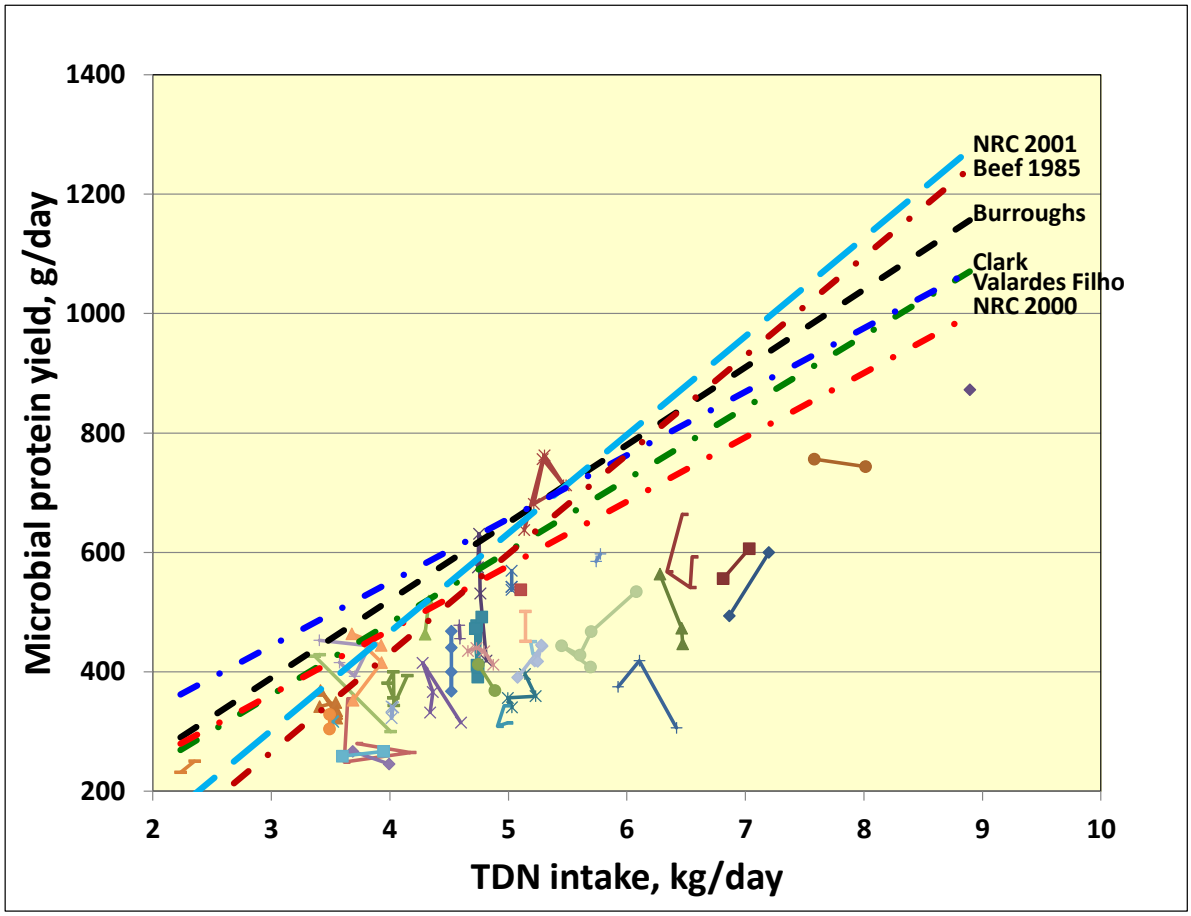

Figure 9. Microbial N yield from published equations compared with measured values from 117 diets fed in metabolism trials for cattle consuming various amounts of total digestible nutrients.

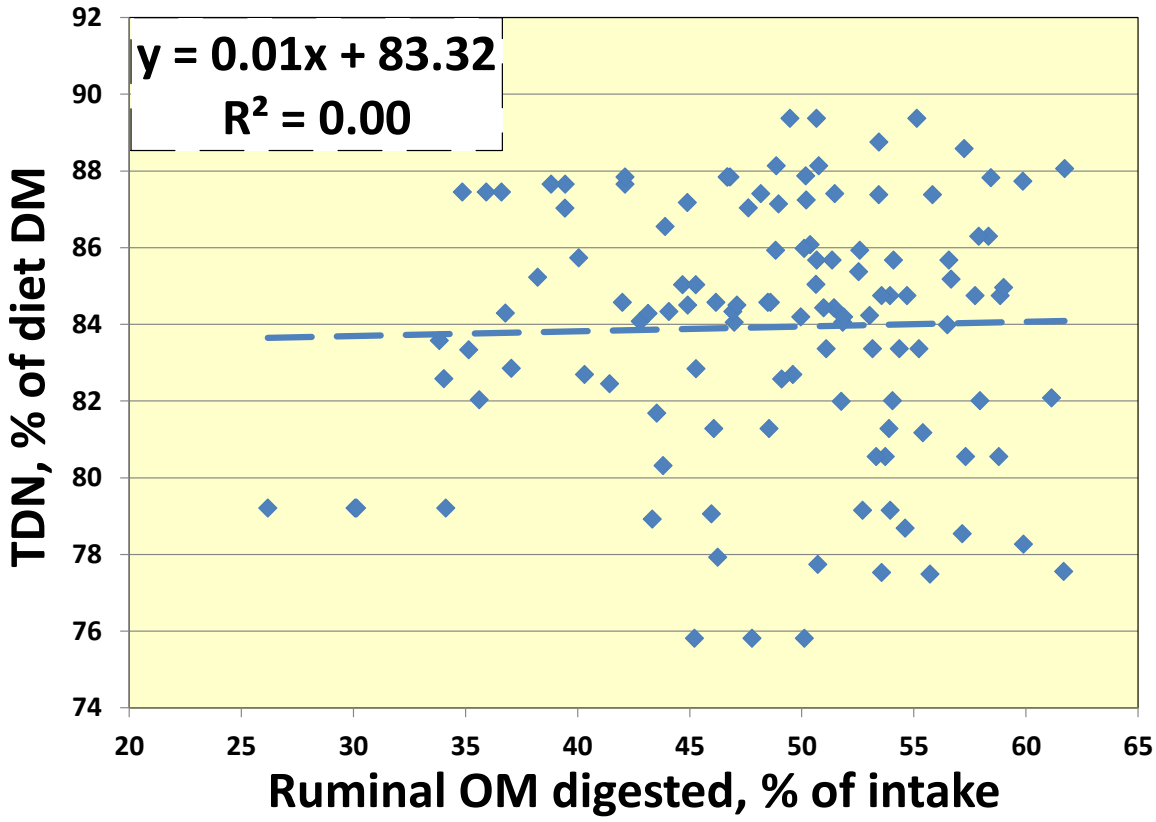

Figure 10. Extent of ruminal digestion of dietary organic matter for diets that differed in total digestible nutrient content among 117 diets from metabolism studies.

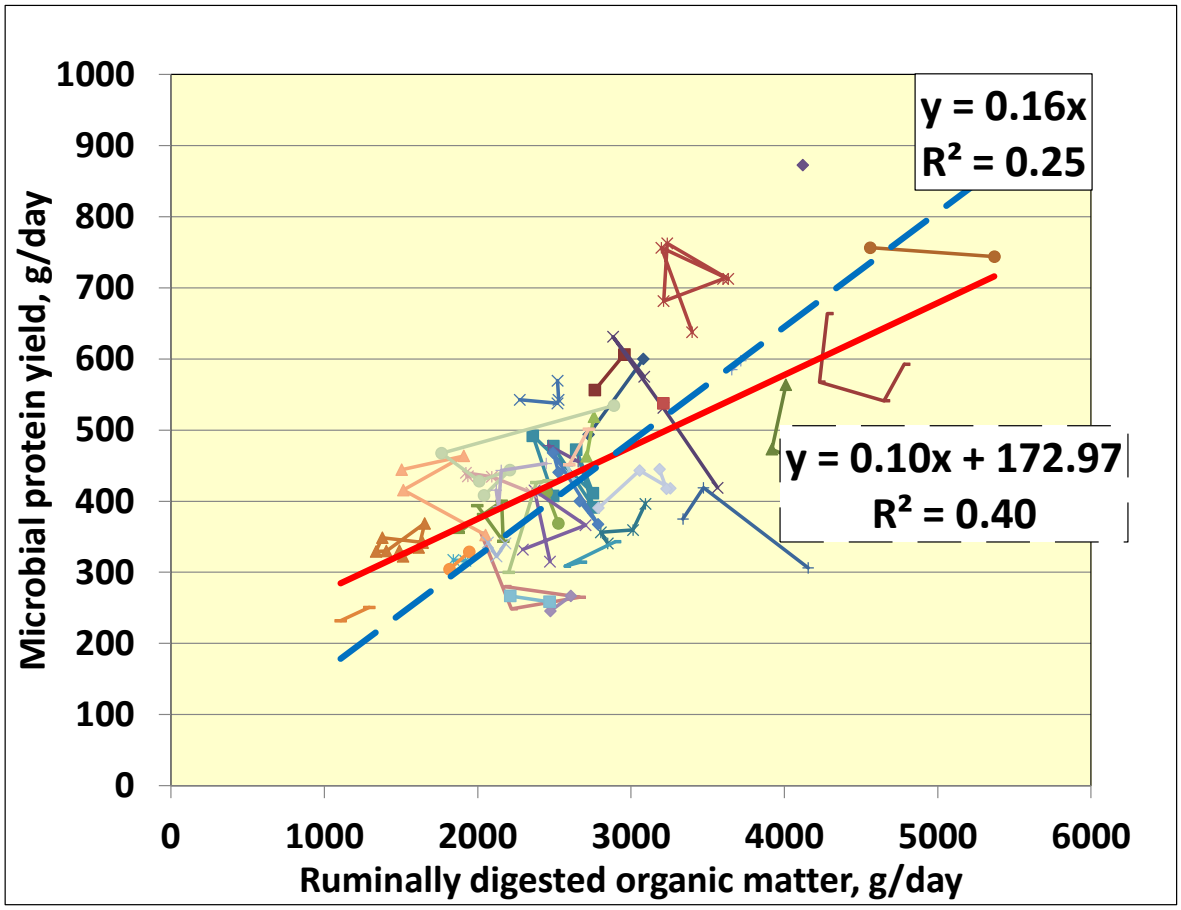

Figure 11. Relationship of microbial N yield to the amount of organic matter digested within the rumen.

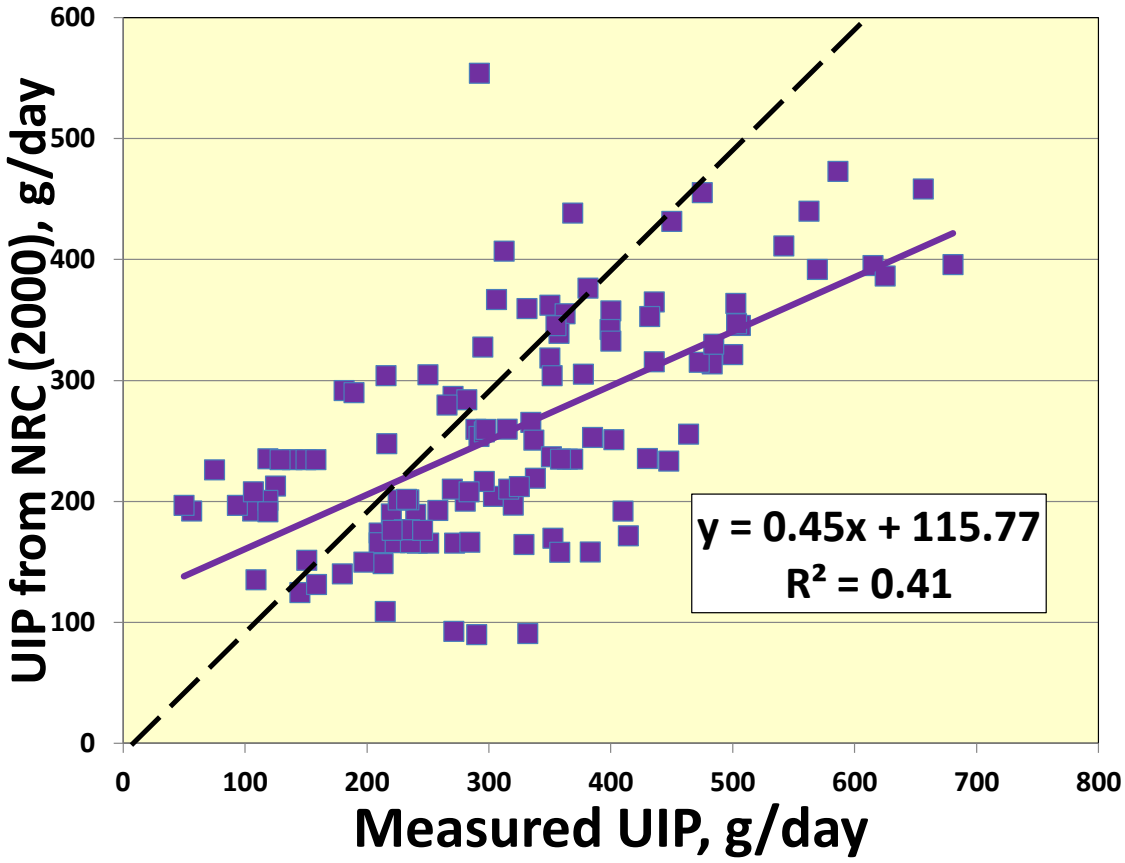

Figure 12. Relationship between undegraded dietary protein intake (UIP) predicted from NRC (2000) equations and flow of undegraded dietary protein to the small intestine.

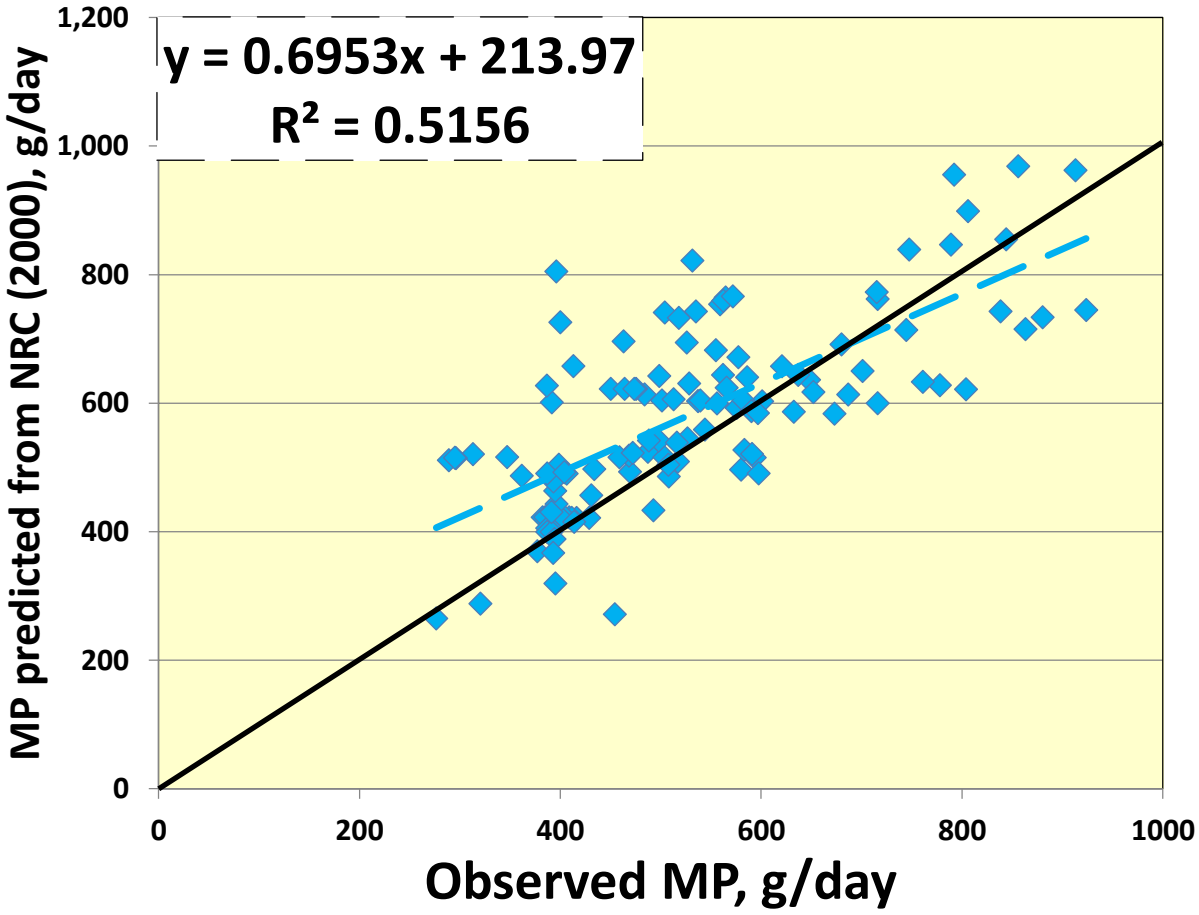

Figure 13. Relationship between metabolizable protein (MP) flow to the small intestine predicted from NRC (2000) equations and measured flow of metabolizable protein to the small intestine of cattle.

SESSION NOTES

Vitamin D Metabolism in Dairy Cattle and Implications for Dietary Requirements

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Introduction

Vitamin D was originally discovered nearly a century ago as a factor in butterfat that prevented rickets (McCollum et al., 1922). In the years to follow it was also found to be synthesized in the skin exposed to sunlight and to be critically involved in calcium homeostasis. The role of vitamin D in calcium homeostasis initiated research on its use for milk fever prevention in dairy cattle, and that research has largely contributed to the minimization of milk fever (Horst et al., 2005). The solution for milk fever, however, was not simply to ensure dairy cattle were supplied with sufficient vitamin D. The reason being, vitamin D itself does not have biological activity. It must first be metabolized in the animal to 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$), and the 1,25$(OH)_2D_3$ then activates a receptor within the cell that controls gene expression (Haussler et al., 2013). Knowledge of how vitamin D metabolism is regulated and how it affected physiological functions was key to the solution of milk fever (Horst et al., 2005). Likewise, understanding the dynamics of the vitamin D pathway is critical for solving the issue of subclinical hypocalcemia that is still prevalent in dairy herds today.

Besides its contribution to bone formation and maintaining the calcium balance, vitamin D also contributes to several other physiological processes critical for dairy cattle production and well-being. Long-before the discovery that vitamin D prevented rickets, sunlight and cod liver oil were prescribed as a therapy for rickets; both are sources of vitamin D. Those same therapies were also prescribed for tuberculosis. As it turns out, the receptor for vitamin D is present in activated immune cells and controls several immune responses (Hewison, 2010). In cattle, 1,25$(OH)_2D_3$ strongly enhances production of nitric oxide and β-defensin antimicrobial peptides, molecules that are toxic to bacteria (Nelson et al., 2012). Sufficient evidence indicates vitamin D also contributes to reproductive performance and mammary development of cattle (Kemmis et al., 2006; Panda et al., 2001; Ward et al., 1971). Thus, determination of vitamin D requirements for dairy cattle must consider more than its contribution to maintaining calcium balance.

In the classical vitamin D endocrine system, the concentration of 1,25$(OH)_2D_3$ in blood is controlled in the kidneys in response to calcium phosphate needs of the body (Horst et al., 2005). The circulating 1,25$(OH)_2D_3$, in turn, acts on target tissues such as the bones, kidneys, and intestines to control the flow of calcium. However, vitamin D

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metabolism is regulated in an intracrine and paracrine manner for many of the non-calcemic functions of vitamin D (Hewison, 2010). For example, in the immune system $1,25(OH)_2D_3$ is produced in activated macrophages, and acts in the macrophage and surrounding cells to influence immunity (Hewison, 2010; Nelson et al., 2010b). Regulation of $1,25(OH)_2D_3$ synthesis in the immune system is, for the most part, independent of that in the endocrine system. The dynamics of vitamin D metabolism in each system differ, and as a consequence, the requirements of each system for vitamin D also may differ.

For dairy cattle nutrition, the goal is to supply the animal with an amount of vitamin D_3 that achieves a serum 25-hydroxyvitamin D (25(OH)D) concentration that supports the multiple outcomes of vitamin D. The 7th edition of Nutrient Requirements of Dairy Cattle published in 2001 recommends 21,000 IU of vitamin D_3 per day for lactating Holstein cows (NRC, 2001). In a limited survey of current practices, however, most cows receive 1.5 to 2.5 times that amount, and had serum $25(OH)D_3$ concentrations between 60 and 70 ng/mL. Based on all available evidence, that range is adequate for maintaining the calcium balance in dairy cattle. Is that range optimal for immunity, reproduction, or the transition period? Do calves and beef cattle receive adequate amounts of vitamin D_3? Future work should consider those questions along with further exploration of factors that affect vitamin D metabolism in cattle.

Vitamin D Metabolic Pathway

There are two forms of vitamin D, vitamin D_2 and vitamin D_3. Metabolites of both forms are found in plasma of cattle (Horst and Littledike, 1982). Vitamin D_2 is derived from ergosterol in plants and vitamin D_3 is derived from 7-dehydrocholesterol in animals. Vitamin D_2 and vitamin D_3 metabolism occurs through the same pathway in cattle, with exceptions in digestion in the rumen and side chain catabolism (Horst et al., 1994). Both forms contribute to the overall signaling events of vitamin D, but vitamin D_3 is the predominant form in cattle (Horst and Littledike, 1982). The metabolic pathway of vitamin D_3 is shown in **Figure 1**. Vitamin D is hydroxylated to 25-hydroxyvitamin D (25(OH)D) in the liver by cytochrome P450 enzymes. The enzymes CYP2R1, CYP27A1, and CYP3A4 have demonstrated 25-hydroxylase activity in mammals (Jones et al., 2014). The CYP2J2 gene in cattle is correlated with 25(OH)D, implicating that CYP2J2 catalyzes 25-hydroxylation of vitamin D in cattle as well (Casas et al., 2013).

Conversion of vitamin D_3 to $25(OH)D_3$ is not tightly regulated; so most vitamin D_3 that is acquired in the diet or synthesized in the skin is quickly converted to $25(OH)D_3$ (Horst et al., 1994). The $25(OH)D_3$ is the most abundant vitamin D metabolite in plasma of cattle, and is relatively stable over time (Sommerfeldt et al., 1983). Consequently, the concentration of $25(OH)D_3$ in plasma serves as a suitable marker of vitamin D status. Normal serum concentrations of 25(OH)D [$25(OH)D_2$ and $25(OH)D_3$] for cattle are typically defined as 20 to 50 ng/mL (Horst et al., 1994). Most mid-lactation dairy cattle in a recent survey of several Midwest dairies were supplemented with 30 to 50 KIU of vitamin D_3 and had serum concentrations between 40 to 100 ng of 25(OH)D/mL regardless of time in sun or season of sample collection (Lippolis 2012, unpublished).

The $25(OH)D_3$ metabolite serves as the precursor to the biologically active metabolite, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). The conversion is catalyzed by the 25-hydroxyvitamin D 1α-hydroxylase (1α-OHase/CYP27B1), a mitochondrial cytochrome P450 enzyme that is tightly regulated. The concentration of $1,25(OH)_2D_3$ in blood is tightly regulated and typically ranges from 5 to 20 pg/mL in serum of cattle, but is elevated to > 300 pg/mL during severe hypocalcemia (Horst et al., 1994). The biological function of $1,25(OH)_2D_3$ is to regulate gene expression by activating the vitamin D receptor (**VDR**) . The VDR is a nuclear hormone receptor that forms a heterodimer with the retinoid X receptor (**RXR**). The DNA binding domains of the VDR/RXR heterodimer recognize DNA sequences, known as vitamin D response elements (**VDRE**), in the promoter regions of vitamin D responsive genes (Haussler et al., 2013). The human and murine genomes are predicted to have nearly 1,000 genes with potential VDRE (Wang et al., 2005). Regulation of each gene would depend on the presence of the VDR and accessibility of the promoter, but the wide distribution of VDRE does suggest that $1,25(OH)_2D_3$ has multitude of effects throughout the body.

Both $25(OH)D_3$ and $1,25(OH)_2D_3$ are substrates for CYP24A1. The CYP24A1, or 24-hydroxylase (24-OHase), is a cytochrome P450 enzyme that adds a hydroxyl group at the 24 position of both $25(OH)D_3$ and $1,25(OH)_2D_3$ (Horst et al., 1994). The expression of 24-OHase is under control of multiple VDRE and as such is highly responsive to increases in $1,25(OH)_2D_3$ concentrations in most cells that have the VDR (Haussler et al., 2013). The 24-hydroxyvitamin D metabolites are inactive, so 24-OHase serves as a feedback regulator of $1,25(OH)_2D_3$ synthesis (Reinhardt and Horst, 1989). The 24-hydroxyvitamin D metabolites undergo further side chain oxidation in the kidney to eventually form more polar metabolites, which are excreted in the bile (Horst et al., 1994).

Nearly all vitamin D metabolites in serum are bound by the vitamin D binding protein (**DBP**). The DBP is a member of the albumin family of serum proteins and is produced in the liver (Haddad, 1995). It has multiple functions besides vitamin D binding, including actin binding, macrophage activation, and fatty acid transport (Speeckaert et al., 2006). The DBP is very abundant in serum and has a high affinity for vitamin D metabolites. As a result, over 99.9% of $25(OH)D_3$ and 99% of $1,25(OH)_2D_3$ in serum are bound by DBP (White and Cooke, 2000). The DBP has not been studied in cattle, but its concentration may contribute to $25(OH)D_3$ and $1,25(OH)_2D_3$ concentrations in serum and greatly impact function of the vitamin D system.

Targets of the Vitamin D Receptor

As noted above the biological activity of vitamin D is carried out by the activation of the VDR with $1,25(OH)_2D_3$. Several of the genes upregulated by the activated VDR are shown in **Figure 2.** The activated VDR serves to regulate transcription of genes under control of accessible VDREs. Classical targets of the VDR in the kidneys and intestines are genes that code for calcium transport and calcium binding proteins; examples are calbindin-D(9k), calbindin-D(28k), and TRPV6 (Haussler et al., 2013).

The $1,25(OH)_2D_3$ also increases osteocyte RANKL and fibroblast growth factor 23 (**FGF23**) production and promotes both bone resorption and mineralization (Haussler et al., 2013). In bovine monocytes, $1,25(OH)_2D_3$ enhances iNOS, RANTES, and several β-defensin genes (Nelson et al., 2012). It also dampens the antigen-specific IFN-γ and IL-17 responses of T cells (Nelson et al., 2011). Mammary epithelial cell proliferation also is inhibited by $1,25(OH)_2D_3$, likely through cell cycle regulators p21 and p27 (Welsh, 2007). In bovine mammary epithelial cells, $1,25(OH)_2D_3$ upregulates β-defensin 4 gene expression, but down regulates several other of the β-defensins (Merriman and Nelson, unpublished).

The transcriptional response is proportional to the concentration of $1,25(OH)_2D_3$ and VDR in the cell. The concentration of $1,25(OH)_2D_3$ required to elicit a response depends on the abundance of the VDR and accessible VDREs in the target gene (Haussler et al., 2013). For instance, CYP24A1, one of the most responsive vitamin D target genes, responds to picomolar concentrations of $1,25(OH)_2D_3$ in the kidneys and intestinal epithelial cells. In contrast, genes such as iNOS or RANTES in bovine monocytes require nanomolar concentrations of $1,25(OH)_2D_3$ to elicit a meaningful response (Nelson et al., 2011; Nelson et al., 2010b). That contrast is a key difference between the vitamin D endocrine system, that maintains blood calcium and phosphorous, and the intracrine and paracrine mechanism in the immune system. The calcium binding and transport genes in the intestines and kidneys, and consequently blood calcium, are influenced by the concentration of $1,25(OH)_2D_3$ circulating in the blood. That blood concentration normally ranges from 20 to 50 pg/mL (50 to 125 pM) in cattle, and reaches 100 to 200 pg/mL in serum of cows post-partum during periods of hypocalcemia (Horst et al., 1994). The vitamin D responsive genes of the immune system, in contrast, are not influenced by circulating $1,25(OH)_2D_3$. The mechanisms that influence circulating and localized $1,25(OH)_2D_3$ are considered next.

Regulation of Renal Vitamin D Metabolism

The concentration of $1,25(OH)_2D_3$ in blood is primarily determined by renal expression of 1α-OHase (synthesis) and 24-OHase (degradation). Those enzymes are tightly regulated in response to parathyroid hormone (**PTH**), FGF-23, and $1,25(OH)_2D_3$ at a ratio that keeps circulating $1,25(OH)_2D_3$ at a concentration that maintains blood concentrations of calcium and phosphate (Haussler et al., 2013; Horst et al., 2005). If blood calcium decreases, calcium sensing receptors in the parathyroid gland stimulate PTH production. The PTH subsequently elevates renal 1α-OHase expression and inhibits renal 24-OHase. In contrast, FGF-23 inhibits renal 1α-OHase expression and stimulates 24-OHase expression (Haussler et al., 2013). The FGF-23 is produced by bone cells in response to $1,25(OH)_2D_3$ and phosphorous levels. It suppresses renal sodium-phosphate co-transporters to decrease phosphate reabsorption. Finally, $1,25(OH)_2D_3$ directly represses renal 1α-OHase and stimulates 24-OHase to regulate its own concentration in a feed-back manner.

The ratio of 1α-OHase:24-OHase in the kidneys is critical in the transition dairy cow (Horst et al., 2005). The higher the 1α-OHase:24-OHase ratio the better suited is

the cow to increase circulating $1,25(OH)_2D_3$. Conditions that promote PTH production and PTH receptor signaling are expected to increase the 1α-OHase:24-OHase ratio. Greater PTH sensitivity is achieved through feeding a diet low in dietary cation-anion difference (**DCAD**) (Horst et al., 2005). The acidic conditions achieved with a low DCAD diet alter the conformation of renal PTH receptors slightly to make them more sensitive (Goff and Horst, 2003). In theory, keeping the FGF-23 concentration low also will increase the 1α-OHase:24-OHase ratio. The FGF-23 was recently discovered and so far has not been studied in cattle, but limiting excess intake of phosphorus is expected to inhibit FGF-23 production.

The $25(OH)D_3$ concentration also affects levels of 1α-OHase and 24-OHase in the kidneys. If the $25(OH)D_3$ concentration is low, the body compensates by producing more PTH (Lips, 2004), thereby stimulating 1α-OHase and depressing 24-OHase. Under normal conditions in humans, PTH rises to compensate for serum $25(OH)D_3$ concentrations < 30 ng/mL (Vieth et al., 2003). Conversely, as $25(OH)D_3$ concentrations rise, less 1α-OHase and more 24-OHase are required to keep circulating $1,25(OH)_2D_3$ in the correct balance (Engstrom et al., 1984). Consequently, circulating $1,25(OH)_2D_3$ does not correlate with the $25(OH)D_3$ concentration.

Extra-renal Vitamin D Metabolism

In contrast to the genes related to calcium and phosphate balance, vitamin D responsive genes in the immune system are controlled by locally produced $1,25(OH)_2D_3$ (Nelson et al., 2010a; Nelson et al., 2010b). Macrophages are major sources of the $1,25(OH)_2D_3$ that controls vitamin D-mediated immune responses. The 1α-OHase is stimulated in bovine macrophages via toll-like receptor (**TLR**) recognition of pathogen associated molecular patterns such as lipopolysaccharide, peptidoglycan, and mycobacterial lipopeptides. The macrophage 1α-OHase enables conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, and subsequently activation of vitamin D-mediated immune responses. The response of genes *in vitro* such as iNOS, RANTES, and β-defensins is correlated with the concentration of $25(OH)D_3$. That correlation is in contrast with the vitamin D endocrine system, where calcium and phosphate do not correlate with $25(OH)D_3$.

The 1α-OHase is expressed in the udder during mastitis in dairy cattle (Nelson et al., 2010a). The majority of 1α-OHase in the infected mammary gland is present in the $CD14^+$ cells (macrophages) secreted in the milk. Induction of 1α-OHase in the udder in response to bacterial infection enables conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ as indicated by upregulation of 24-OHase in the mammary gland. Normally milk $25(OH)D_3$ is < 5ng/mL (McDermott et al., 1985), but intramammary administration of 100 µg of $25(OH)D_3$ inhibited mastitis in dairy cattle (Lippolis et al., 2011). The effects of intrammamary $25(OH)D_3$ presumably occurred via 1α-OHase conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, and $1,25(OH)_2D_3$-mediated induction of nitric oxide and β-defensin production. Meanwhile, the intramammary $25(OH)D_3$ infusion did not affect serum $25(OH)D_3$ or $1,25(OH)_2D_3$ concentrations. In addition, circulating $1,25(OH)_2D_3$ does not

increase during mastitis, indicating that vitamin D signaling is limited to the infected mammary gland.

In addition to stimulation of 1α-OHase, TLR ligands are potent inhibitors of 24-OHase expression in bovine macrophages (Nelson et al., 2010b). In a freshly isolated, resting bovine monocyte, 10 nM of $1,25(OH)_2D_3$ upregulates 24-OHase expression ~50 to 100 fold. However, if the monocytes are stimulated with LPS, the upregulation of 24-OHase by $1,25(OH)_2D_3$ is < 10 fold greater than resting monocytes. The pathogen induced inhibition of 24-OHase seemingly allows for unchecked $1,25(OH)_2D_3$ synthesis in the macrophage. Unchecked production of $1,25(OH)_2D_3$ is a key difference between vitamin D metabolism in the immune system and vitamin D metabolism in the kidneys; the local concentration of $1,25(OH)_2D_3$ is not tightly controlled like the circulating concentration.

Altogether, expression of $1,25(OH)_2D_3$-regulated genes in immune cells is determined by abundance of 1α-OHase, 24-OHase, and $25(OH)D_3$. The strength of the pathogen derived signal (i.e. TLR or IFN-γ) contributes to macrophage 1α-OHase and 24-OHase. The magnitude of vitamin D-regulated responses, such as nitric oxide and β-defensins, will be insufficient if the $25(OH)D_3$ concentration is insufficient. The threshold for $25(OH)D_3$ required to support vitamin D mediated immunity in cattle has not been determined. Epidemiological data from the human population suggests there is a correlation between serum $25(OH)D_3$ and immune function, and that concentrations < 32 ng/mL of serum are insufficient for immunity (Adams et al., 2007).

Besides immune cells, mammary epithelial cells and the placenta are additional sources of $1,25(OH)_2D_3$ synthesis that have significance for dairy cattle. In mice, the 1α-OHase is expressed in mammary tissue during mammary development and involution (Welsh, 2004). Cultured bovine mammary epithelial cells also express the 1α-OHase and respond to $25(OH)D_3$ treatment. The placenta produces enough $1,25(OH)_2D_3$ to affect the circulating pool of $1,25(OH)_2D_3$. Circulating $1,25(OH)_2D_3$ also increases with estrogen therapy in women. However, the function of $1,25(OH)_2D_3$ in pregnancy and reproductive physiology in cattle is unknown. In any case, vitamin D supplementation improved reproductive performance in dairy cattle (Ward et al., 1971), and circulating $1,25(OH)_2D_3$ is elevated during pregnancy (O'Brien et al., 2014). Consequently, reproductive physiology also should be considered in regard to vitamin D metabolism.

Nutritional Implications

Because vitamin D_3 can be synthesized in sun-exposed skin and its biological activity is regulated via tightly regulated processes, a clear dose response to vitamin D supplementation will not occur if the appropriate conditions are not met. As a consequence defining dietary vitamin D_3 requirements has been difficult. Rather than focusing strictly on effects of vitamin D_3 supplementation on a given outcome, emphasis should be placed on identifying serum 25(OH)D concentrations that support the various outcomes of vitamin D metabolism.

The serum concentrations of 25(OH)D required for calcium maintenance in cattle have been studied in depth. Under normal circumstances in calves and lactating cows, serum 25(OH)D concentrations of 20 to 100 ng/mL support a normal calcium and phosphate balance. At the onset of lactation, cows would presumably benefit from having higher serum $25(OH)D_3$ concentrations to support the urgent need for renal $1,25(OH)_2D_3$ synthesis. However, plasma $1,25(OH)_2D_3$ was not greater (~300 vs. 400 pg/mL) in the hours and days postpartum in cows with ~ 175 ng of $25(OH)D_3$/mL of serum compared to cows having ~40 ng of $25(OH)D_3$/mL of serum (Wilkens et al., 2012). Furthermore, cows in that study with the higher $25(OH)D_3$ had lower ionized and total calcium than cows with normal serum $25(OH)D_3$ when not fed a low DCAD diet. There is likely a saturation point of the renal 1α-OHase for $25(OH)D_3$ during the post-partum period. Future experiments should aim to determine the maximum serum $25(OH)D_3$ that benefits the transition cow. Meanwhile, serum $25(OH)D_3$ concentrations over 100 ng/mL serum do not seem to provide the transition cow much benefit compared to concentrations between 20 and 50 ng/mL as regards blood calcium.

The optimal $25(OH)D_3$ concentration for immunity has not been determined for cattle yet. The effects of $25(OH)D_3$ concentration on macrophage host defense responses *in vitro* suggest a linear benefit to at least 100 ng/mL (Nelson et al., 2010b). Calves with ~175 ng of $25(OH)D_3$/mL of serum, however, did not fair any better than calves with ~30 ng of $25(OH)D_3$/mL of serum in regards to severity of experimental respiratory syncytial virus (**RSV**) infection (Sacco et al., 2012). That study does not indicate whether there is a maximal benefit somewhere in between that range, and clearly further work is needed on the relationship between serum $25(OH)D_3$ and infectious disease outcome in cattle. Insufficient vitamin D conceivably impairs immunity, so until more data is available, serum $25(OH)D_3$ concentrations of at least 30 ng/mL are recommended to support immune function in cattle.

The justification for dietary vitamin D recommendations in the 7th edition of Nutrient Requirements of Dairy Cattle (NRC, 2001) cited a study by Ward et al. (1971) that found cows receiving 300,000 IU of vitamin D_3/week by oral bolus reached estrus 16 days earlier post-partum and conception 37 days earlier than non-treated cows. Serum $25(OH)D_3$ data was not available in that study. The NRC (2001) also cites a study (Hibbs and Conrad, 1983) that milk production and feed intake were greatest for cows supplemented with 40,000 IU of vitamin D_2/d than cows receiving no vitamin D or 80,000 IU of vitamin D_2/day. However, vitamin D_2 was much less effective in raising total serum 25(OH)D [$25(OH)D_2$ and $25(OH)D_3$] than vitamin D_3 in a recent study (Hymoller and Jensen, 2011), so an equivalent amount of vitamin D_3 may not have the same effect on milk production.

Overall, the ideal serum $25(OH)D_3$ concentration for cattle likely lies between 40 and 80 ng/mL. Targeting a lower range, below 40 ng/mL, may result in some animals with serum $25(OH)D_3$ concentrations below 20 ng/mL based on variation observed within dairy herds. Based upon the available data, there appears to be no benefit in exceeding 100 ng/mL. A limited survey of dairy herds in the Midwest indicated producers supply lactating Holstein cows with 30,000 to 50,000 IU of vitamin D_3/d

(Lippolis 2012, unpublished). The average $25(OH)D_3$ concentration of 320 serum samples collected from 100 to 250 DIM over a course of 18 months from those herds was 70 ng/mL. Ninety percent of those samples were between 40 and 100 ng/mL. A significant correlation was not detected between serum $25(OH)D_3$ and dietary vitamin D_3, time outside during the day, or month (March, June, September, or December) of collection in those samples. A conclusion on the effects of those factors on serum $25(OH)D_3$ cannot be made, but in another study serum $25(OH)_3$ did not differ between lactating cows fed 10,000 or 50,000 IU of vitamin D_3/d (McDermott et al., 1985). Therefore, supplying cows with 50,000 IU compared to 30,000 IU/d, or the NRC recommended 21,000 IU/d, may not provide a significant advantage. Regardless, supplying cows with 20,000 to 50,000 IU of vitamin D_3/d should result in serum $25(OH)D_3$ concentrations between 40 and 80 ng/mL.

Calves and beef cattle presumably require serum $25(OH)D_3$ concentrations between 40 and 80 ng/mL as well. Calves housed indoors and fed milk replacer supplying 1700, 11,000, or 17,900 IU of vitamin D_3/kg of diet had approximately 30, 90, and 180 ng/mL of serum $25(OH)D_3$, respectively (Nonnecke et al., 2010; Sacco et al., 2012). Close attention should be paid to vitamin D status of calves just receiving cow's milk because serum $25(OH)D_3$ of calves fed whole milk or colostrum declined from 20 ng/mL to < 10 ng/mL in just 7 days (Rajaraman et al., 1997). So, in limited sun conditions, calves should be supplied with at least 2,000 IU/kg of diet DM, but no more than 11,000 IU/kg of diet DM in order to achieve serum $25(OH)D_3$ concentrations of 40 to 80 ng/mL.

The NRC recommendations for beef cattle are 275 IU/kg of diet (NRC, 2000), and for beef cattle in the southern US (below 35°N) that amount should be adequate (Webb et al., 1988). However, beef cattle in the northern states during the winter months, or in conditions with limited sun, may require additional supplementation to keep serum $25(OH)D_3$ above 20 ng/mL (Hymoller et al., 2009). Feedlot steers supplied with the NRC recommended amount of vitamin D_3 had on average ~20 ng/mL of serum $25(OH)D_3$ (Pickworth et al., 2012). Seventy days after removal of supplemental vitamin D and only incidental sun exposure, serum concentration of $25(OH)D_3$ of those steers dropped below 10 ng/mL. Steers in those same conditions supplied with 1,860 IU of vitamin D_3/kg of diet (~15,000 IU/d or 50 IU/kg of BW) for 70 days had on average 67 ng/mL of serum $25(OH)D_3$. In light of that data, beef cattle may require 15 to 50 IU/kg of BW, depending on environmental conditions, to keep serum $25(OH)D_3$ above 30 ng/mL.

Conclusions

Vitamin D contributes to more than calcium and bone formation in cattle. The active vitamin D hormone also contributes to immune, reproductive, and mammary physiology. Multiple tissues and factors also contribute to vitamin D activity. Regulation of renal vitamin D metabolism is fairly well understood, but the contribution of FGF-23 in cattle requires further consideration as to its influence on circulating concentrations of $1,25(OH)_2D_3$. Immune cells utilize $25(OH)D_3$ independent of the kidneys, but the

optimal 25(OH)D_3 concentration for immune function has yet to be determined. Similarly, optimal serum 25(OH)D_3 concentrations for reproduction and lactation have not been determined, even though vitamin D has been shown to affect both. According to available data, moderate serum 25(OH)D_3 concentrations that range from 40 to 80 ng/mL are ideal for cattle. As a general rule of thumb if sun exposure is limited, daily supplemental feeding 30 to 50 IU of vitamin D_3/kg of BW should achieve that range for cattle.

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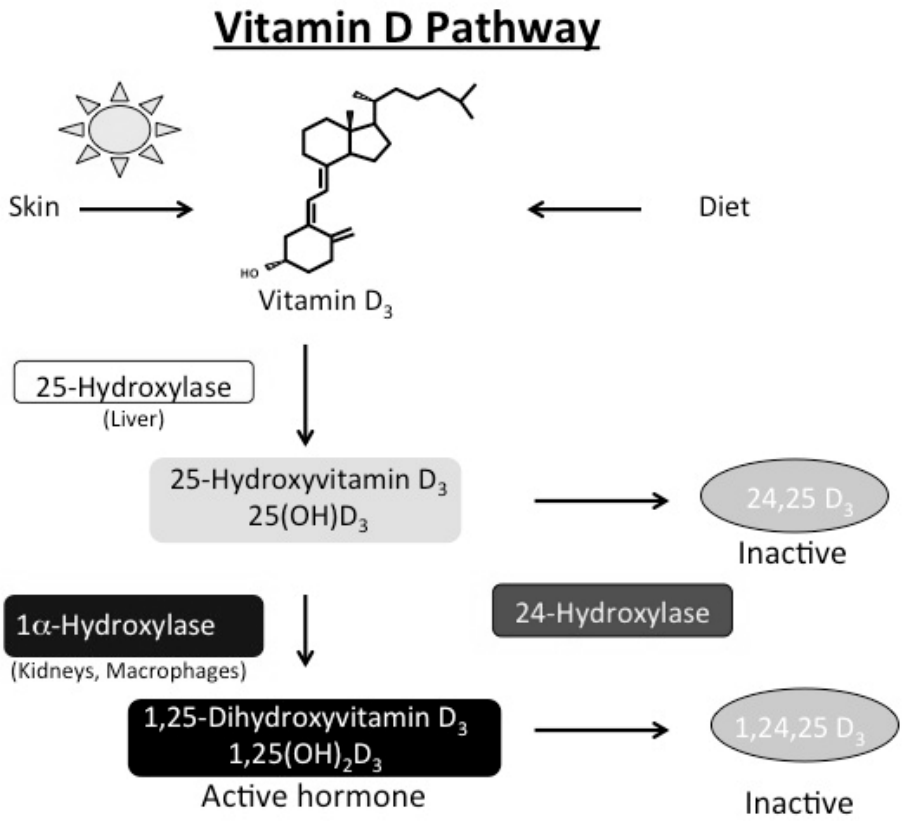

Figure 1. General vitamin D metabolic pathway. Vitamin D_3 is acquired in the skin from photoconversion of 7-dehydrocholesterol, or through dietary supplementation. Vitamin D_3 is readily converted to 25-hydroxyvitamin D_3 (25(OH)D_3). The 25(OH)D_3 is activated to 1,25-dihydroxyvitamin D_3 by the 1α-Hydroxylase, a tightly regulated enzyme expressed in kidneys and macrophages in cattle. The 1,25(OH)$_2D_3$ activates the VDR as shown in Figure 2, and also induces it own catabolism via the 24-hydroxylase. The 24-hydroxylated vitamin D metabolites are further metabolized and excreted in the bile.

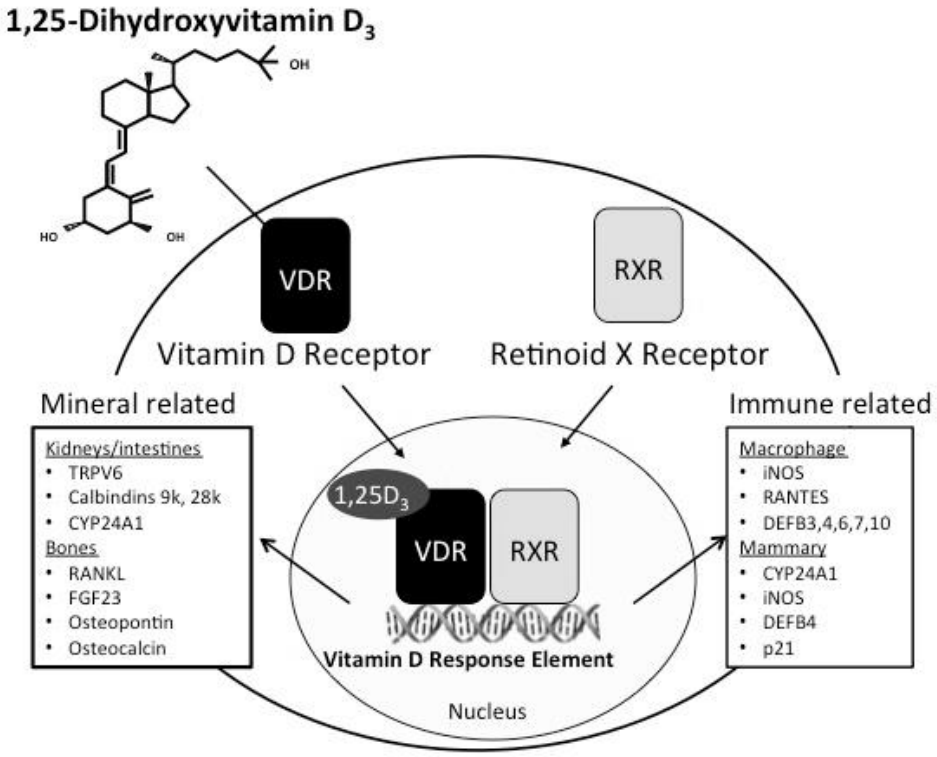

Figure 2. Molecular actions of 1,25-dihydroxyvitamin D_3. The vitamin D receptor (VDR) is the receptor for $1,25(OH)_2D_3$, and once activated it joins with the retinoid X receptor to activate genes with an accessible vitamin D response element. CYP24A1, or 24-hydroxylase, is induced in most cells that have the VDR. Examples of calcium related genes regulated by the VDR are TRPV6 and Calbindins D9k and D28k. In the bone the VDR induces RANKL (bone resorption), FGF23 (decreased renal phosphate reabsorption), osteopontin, and osteocalcin (bone mineralization). The VDR enhances iNOS and DEFB4 (antimicrobial) in macrophages and mammary epithelial cells, and DEFB3,4,6,7,&10 (defensin antimicrobial peptides) in macrophages. The VDR also decreases mammary epithelial cell proliferation via upregulaiton of p21.

SESSION NOTES

Mechanisms of Volatile Fatty Acid Absorption and Metabolism and Maintenance of a Stable Rumen Environment

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Introduction

As a primary energy source for ruminants, volatile fatty acids (**VFA**) have been estimated to provide up to 75% of the total metabolizable energy (Bergman, 1990). Thus, it is not surprising that diets promoting fermentation and greater production of VFA also promote greater levels of productivity (e.g. milk production) than less fermentable diets (Kolver and de Veth, 2002; Oba and Allen, 2003a,b). However, as a weak acid, VFA will dissociate in the rumen releasing a proton thereby decreasing ruminal pH under most circumstances. Thus, when production of VFA exceeds the ability to neutralize the protons, ruminal pH decreases and ruminal acidosis can occur.

Ruminal acidosis occurs in dairy (Penner et al., 2007; Penner and Oba, 2009b), and beef cattle (Bevans et al., 2005; Weirrenga et al., 2010). However, the most common form of ruminal acidosis in dairy cattle is thought to be sub-acute ruminal acidosis (SARA) whereas, lactic acidosis or acute is thought to be the primary form in beef cattle fed high-concentrate diets (Schwaiger et al., 2013a,b). Sub-acute ruminal acidosis (pH < 5.8) is caused by a rapid rate of VFA production while, for acute acidosis (pH < 5.2), the pH depression is often associated with an increase in lactic acid (Owens et al., 1998). While thresholds are used to characterize ruminal acidosis, the actual pH value that induces damage to the ruminal epithelium, alters the microbial community structure and activity, and results in depressed feed intake likely varies among animals.

Many studies have investigated strategies to reduce the risk for ruminal acidosis, but it should be recognized that low pH does not always result in reduced performance. In a meta-analysis, Kolver and de Veth (2002) reported strong negative relationships between ruminal pH and microbial N flow to the small intestine and milk production. Oba and Allen (2003a,b) reported that cows fed high starch (32% DM; corn based diets) had a mean ruminal pH that was 0.16 units below cows fed the low starch diets, but also had greater milk production (38.6 vs. 33.9 kg/d; respectively). It is not surprising that these results occur when considering that greater VFA production, and hence greater energy availability, corresponds with low ruminal pH (Allen, 1997). Thus, understanding how cattle can regulate ruminal pH is perhaps more desirable that avoiding low ruminal pH.

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Regulation of Ruminal pH

Regulation of ruminal pH is complex and involves aspects affecting VFA production and removal of acid from the rumen. While the author acknowledges that many factors can influence ruminal pH based on VFA production (e.g. rate of fermentation, meal size and frequency, and fermentation pathway), this paper will focus on strategies that remove acid from the rumen. Most previous studies have investigated dietary strategies to promote chewing activity (Allen, 1997). Chewing increases the saliva production rate and could greatly increase the supply of bicarbonate to the rumen. In fact, it is estimated that saliva contains 126 mEq/L of bicarbonate and may contribute to approximately 30% of the total ruminal buffering capacity (Allen, 1997). However, for cattle fed low physically effective fibre (peNDF) diets such as for finishing cattle, it could be expected that the salivary contribution is much lower. The other major contributor to the regulation of ruminal pH is VFA absorption (Allen, 1997; Penner et al., 2009a; Aschenbach et al., 2011), which has been estimated to account for up to 53% of the ruminal buffering capacity (Gäbel et al., 1991; Allen, 1997). This paper will describe the current understanding of the mechanisms involved in VFA absorption and how absorption helps to stabilize ruminal pH.

Mechanisms of VFA Absorption and Implications on Ruminal pH

Mechanisms of VFA Absorption

While many textbooks supported the concept that VFA absorption occurs via passive diffusion, pioneering work (Danielli et al., 1945; Masson and Phillipson, 1951; Ash and Dobson, 1963) clearly showed that VFA were absorbed across the ruminal wall and that absorption was associated with the appearance of bicarbonate and carbon dioxide in the buffer solution. Unfortunately those authors were not able to confirm the mechanisms for VFA absorption, they did provide the first evidence that VFA absorption was linked to ruminal pH. Subsequently, Dijkstra et al. (1993) reported that the rate of VFA absorption increased with decreasing pH and that the increase in absorption was linear to the reduction in pH. Given the lack of saturation in the absorption rate with increasing concentration of VFA and an increase for the absorption of VFA with lower pH, absorption had to a large extent been thought to occur via passive diffusion (Dijkstra et al., 2003; López et al., 2003; Graham et al., 2007). It is important to recognize that as pH declines, the proportion of VFA that would be in the undissociated state increases and that only undissociated VFA are permeable across the lipid bilayer of cells (Walter and Gutknecht, 1986; Gäbel et al., 2002). Thus, a reduction in pH would increase the proportion of undissociated VFA that could then freely diffuse across the rumen epithelium.

While evidence seemed to support the theory of passive diffusion, there are numerous theoretical constraints. Firstly, the proportion of VFA in the undissociated state is low under normal pH conditions in the rumen. Volatile fatty acids have a pKa of approximately 4.8. Even with pH values of 5.8, more than 90% of the VFA would be in the dissociated state and thus only a small fraction would be lipophilic and it could be

expected that the rate of VFA absorption would proceed slowly. Although the relative proportion of undissociated VFA would be low, it was suggested that there was an acidic pH microclimate on the luminal side of the ruminal epithelia (Graham and Simmons, 2005). This microclimate could then favor VFA to be in the undissociated state adjacent to the epithelium thereby promoting absorption. However, the pH on the surface of the epithelia has been reported to be basic with values ranging between 7.47 and 7.68 depending on the incubation conditions (Leonhard-Marek et al., 2006). Limitations to the model of exclusive passive diffusion also extend to differences for the lipophilicity of individual VFA. Lipophilicity from greatest to least is butyric acid > propionic acid > acetic acid (Walter and Gutknecht, 1986), and as such it could be expected that the rates of absorption would be greatest for butyrate > propionate > acetate when corrected for concentration in the rumen. However, similar fractional absorption rates have been reported among VFA in vitro (Aschenbach et al., 2009) and when differences are found (Dijkstra et al., 1993; López et al., 2003), they are not consistent with the increase that would be predicted based on lipophilicity (e.g. butyric acid is 14 times greater than acetic acid; Walter and Gutknecht, 1986). Moreover, a recent study showed that although the concentration of VFA increased from 10 to 50 m*M*, the rates of acetate and butyrate absorption only increased by 2.1 and 2.4 times for acetate and butyrate, respectively (Schurmann, 2013).

Numerous studies have been conducted to determine the mechanisms for VFA absorption across the rumen epithelium. A model showing the current understanding of the mechanisms involved in VFA absorption and how the absorption of VFA contributes to the stabilization of ruminal pH is depicted in Figure 1. The predominant mechanisms include; 1) VFA^-/HCO_3^- anion exchange, 2) passive diffusion, 3) nitrate-sensitive VFA absorption, 4) proton-coupled VFA^- transport, and 5) electrogenic VFA transport. While these are the major absorption mechanisms, other processes such as Na/H exchange, and bicarbonate import into the cell are required to enable the maintenance of intracellular pH and to promote VFA absorption.

Pathway of VFA Absorption and the Removal of Protons from the Rumen

Few studies have aimed to determine the relative proportion of the various VFA transport processes. However, based on the available data it appears that for acetate the proportion accounted for by bicarbonate-dependent transport, nitrate-sensitive transport, and passive diffusion are 42 to 57%, 0 to 14%, and 29 to 59% respectively (Penner et al., 2009a; Schurmann, 2013). For butyrate, the proportion accounted for by bicarbonate-dependent transport, nitrate-sensitive transport, and passive diffusion are 24 to 46, 0 to 4, and 25 to 76%, respectively (Penner et al., 2009a; Schurmann, 2013).

In the rumen, the vast majority of the VFA will be in the dissociated state (**VFA^-**). Absorption of VFA^- occurs in exchange for HCO_3^- in an electro-neutral process that is mediated by a number of potential anion exchangers (Bilk et al., 2005; Aschenbach et al., 2009; Penner et al., 2009b). This mechanism provides a source of bicarbonate to the ruminal environment where it can neutralize a proton via the carbonic anhydrase reaction producing carbon dioxide and water. Driving forces for bicarbonate-dependent

transport include the concentration of ruminal VFA and ruminal pH. In fact, the bicarbonate-dependent VFA absorption increases with increasing luminal VFA concentration and with decreasing ruminal pH (Aschenbach et al., 2009). The bicarbonate facilitating this transport does not seem to occur in the cytosol, but rather is transported from arterial circulation into the cell (Sehested et al., 1999; Aschenbach et al., 2009). There are several bicarbonate transporters including anion exchangers on the basolateral (blood-facing) side that may also help to export VFA^- out of the cell and into arterial blood. Thus, it appears that this transport process is crucial in terms of helping to regulate ruminal pH (Penner et al., 2009a) and exporting VFA to be metabolized by other tissues.

Passive diffusion of VFA seems to occur, but it should be acknowledged that the contribution of passive diffusion towards VFA absorption declines as more transport processes are defined. When VFA are absorbed via passive diffusion, 1 proton is removed from the ruminal contents; however, upon appearance in the cytosol, VFA will rapidly dissociate. The proton released then needs to be expelled from the cell in order to maintain intracellular pH and tissue integrity. Transporters involved in the regulation of intracellular pH include the sodium/hydrogen exchangers **(NHE)** that export protons back to the lumen or into extra-cellular spaces. In addition to NHE, the monocarboxylate transporter **(MCT)** has been shown to be localized on the basolateral membrane (blood facing; Graham and Simmons, 2007) and can facilitate the removal of a proton along with metabolic end-products of VFA metabolism such as ketone bodies and lactate (Müller et al., 2002; Kirat et al., 2006). Thus, the direction of proton export has major implications for whether passive diffusion contributes to the stabilization of ruminal pH. For example, if the proton is exported back into the rumen contents as a strategy to maintain intracellular pH, there would be no net proton removal from the rumen and therefore ruminal pH would not be affected. Interestingly, the expression and activity of NHE in ruminal epithelia increase when highly fermentable diets are fed (Etschmann et al., 2009; Yang et al., 2009; Schurmann, 2013). However, due to the complexity of the transport mechanisms involved and the regulation of their activity, it is very difficult to quantify or predict the proportion of protons recycled back to the lumen relative to those that account for permanent removal from the ruminal contents. That said, it is clear that under some circumstances passive diffusion does contribute to the removal of protons from the rumen (Penner et al., 2009a).

In addition, it is now known that there is a nitrate-sensitive transport pathway for VFA. This process occurs both in the presence and absence of bicarbonate (Aschenbach et al., 2009), but currently the transporters involved are not known. Recent unpublished work (K. Wood, J.R. Aschenbach, F. Stumpff, and G.B. Penner) has shown a clear inhibitory effect with increasing concentrations of nitrate for acetate but no effect for butyrate. Future studies are required to improve our understanding of this transport mechanism and its regulation.

Finally, electrogenic VFA^- transport has been documented (Stumpff et al., 2009; Georgi et al., 2013). This transport process is thought to be mediated by maxi-anion channels but the total contribution to VFA transport is not currently known.

Evidence Linking VFA Absorption to the Stabilization of Ruminal pH

Early studies (Masson and Phillipson, 1951; Dobson and Ash, 1963; Gäbel et al., 1991) had suggested that VFA absorption could be one mechanism for the stabilization of rumen pH. While not an intended outcome, Dijkstra et al. (1993) reported that when artificial buffers were placed in the evacuated and washed rumen of dairy cows, pH increased to 7.1, 8.0, 8.2, and 8.2 from initial pH values of 4.5, 5.4, 6.3, and 7.2, respectively. Several reviews (Allen, 1997; Gäbel et al., 2002; Aschenbach et al., 2011) have suggested that VFA absorption should help to stabilize ruminal pH when evaluating the mechanisms involved in VFA transport. Despite the suggestions, the hypothesis was not proven correct until recently.

The first evidence supporting the pH stabilizing effect of VFA absorption was provided by Resende Júnior et al. (2006). In that study moderate (r^2 = 0.43) positive correlations between the fractional rate of VFA clearance and ruminal pH were observed suggesting that greater rates of VFA clearance corresponded to improved ruminal pH. Resende Júnior et al. (2006) further evaluated whether the effect on pH was due to absorption of VFA across the rumen wall or the passage of VFA out of the rumen finding that both mechanisms were positively related to ruminal pH. In another study Penner et al. (2009b), reported negative associations between the expression of a number of genes involved in VFA metabolism and the severity of ruminal acidosis for dairy cows fed a diet containing 64% concentrate. While these studies (Resende Júnior et al., 2006; Penner et al., 2009b; Schlau et al., 2012) showed relationships between ruminal pH or the severity of ruminal acidosis and the absorption of VFA or indicators for intra-epithelial metabolism of VFA, they cannot prove that VFA absorption improves ruminal pH nor can they elucidate how the pathway of VFA and type of VFA affect ruminal pH.

Penner et al. (2009a) conducted a study to determine the relationship between the uptake of VFA and the severity of ruminal acidosis. In that study, ruminal acidosis was induced in 17 lambs using an oral glucose drench (5 g glucose/kg BW). Based on the ruminal pH response over 3 hours after the drench, lambs were assigned to 1 of 2 classifications; non-responders (**NR**; the 7 lambs that had the least ruminal pH reduction) or responders (**RES**; the 7 lambs that had the greatest reduction in ruminal pH following the challenge). To evaluate the relationship between ruminal pH and VFA absorption, the rumen epithelium was collected and the uptake of acetate and butyrate was measured ex vivo. Results from the NR and RES lambs were compared to a group that was not exposed to an acidotic challenge (**SHAM**). Ruminal pH differed between sheep classified as NR (67.8 min), RES (153 min) and SHAM (1.1 min) as did the uptake of acetate and butyrate. It is important to note that we assumed that the acidotic challenge imposed did not compromise the ruminal epithelium as acetate and butyrate uptake did not differ between the RES and SHAM treatments. Interestingly, we found that epithelia from NR sheep had a greater rate of total acetate and butyrate uptake than RES indicating that the improved ruminal pH response could be attributed to greater capability for VFA uptake. In addition, retrospective correlation analysis showed that acetate and butyrate uptake was also positively related to the mean pH prior to the

acidotic challenge. This is the only study (Penner et al., 2009a) that has provided comprehensive data demonstrating that the rate of acetate and butyrate uptake has a substantial effect on ruminal pH homeostasis.

As mentioned above, the pathway of VFA absorption may play a role in the stabilization of ruminal pH. In addition to total uptake, Penner et al. (2009a) also reported that the main mechanisms facilitating acetate and butyrate uptake were different between NR and RES. For acetate, the bicarbonate-dependent and bicarbonate-independent nitrate-sensitive transport was greater for NR than RES. As mentioned above, with the bicarbonate-dependent transport, bicarbonate secretion and acetate absorption are coupled. Interestingly, for butyrate, bicarbonate-independent (passive diffusion) uptake was higher for NR than RES. Collectively these data indicate that the pathway of VFA absorption may differ based upon the type of VFA and thus the relative contribution towards the stabilization of ruminal pH may also differ. For example, acetate is not as lipophilic as butyrate and thus protein-mediated pathways contribute substantially towards its uptake. This is important as the bicarbonate-dependent pathway would also provide bicarbonate to buffer the rumen contents (Aschenbach et al., 2009). In contrast, butyrate has a greater potential for diffusional uptake (Walter and Gutknecht, 1986). Thus, factors promoting a concentration gradient between the rumen, cytosol, and blood should promote absorption (Gäbel et al., 2002). The suggestion that intracellular metabolism enhances butyrate absorption is in alignment with Gäbel et al. (2001) and previously reported negative correlations between the expression of genes involved in butyrate metabolism and the severity of ruminal acidosis (Penner et al., 2009b). Furthermore, we found that NR sheep had greater serum β-hydroxybutyric acid **(**BHBA (**BHBA**; a metabolite of butyrate metabolism) that RES sheep after the 180 min acidotic challenge (Penner et al., 2009a). The increase in serum BHBA may also indicate that for butyrate, metabolism to ketone bodies and export from the cell via MCT may help to regulate ruminal pH.

A clear limitation with our understanding of VFA transport is that most studies have only evaluated acetate and butyrate transport thereby omitting propionate and longer chain VFA. Based on lipophilicity (Walter and Gutknecht, 1986) and the extent of metabolism by the rumen epithelium, it is expected that propionate transport would be intermediate to acetate and butyrate and thus have a high reliance on bicarbonate-dependent transport (Aschenbach et al., 2009). Based on the current data available it is evident that VFA absorption helps to stabilize ruminal pH and that the relative effect of individual mechanisms (passive diffusion, bicarbonate-dependent transport) for absorption differs based on the VFA (acetate, propionate, and butyrate) absorbed.

Nutritional Modulation of VFA Transport

Given the importance of VFA transport towards meeting the energy requirement and stabilization of ruminal pH, several studies have investigated whether dietary or feeding management can modulate the response. Interestingly, past studies have clearly demonstrated that feeding management can both positively and negatively influence VFA absorption.

Feed Restriction and Feed Deprivation Decrease VFA Absorption

The vast majority of current research has focused on rumen epithelial adaptation from an anabolic perspective, however, in times of scarcity or in response to a nutritional insult, the adaptive response certainly includes regression. In fact, the long-term changes induced by a low plane of nutrition have been shown to decrease gut mass and reduce O_2 consumption by visceral tissue, and reduce VFA absorption (Doreau et al., 1997). Understanding how the ruminal epithelium responds to reductions in VFA production due to a transient exposure to feed restriction and, more importantly, the timeline required for the epithelium to return to the pre-restriction function is needed to develop feeding strategies and mitigate disorders associated with digestive upset.

Albeit unintentional and generally short in duration, beef and dairy cattle are exposed to periods of feed restriction or complete feed deprivation. Examples include during weaning, transportation, prior to and immediately after parturition, immediately following digestive upset, while experiencing heat stress, and in association with metabolic disorders and infection. Gäbel et al. (1993) demonstrated that 48-h of complete fed reduced VFA, Na^+, Ca^{2+}, and Mg^{2+} absorption by approximately 40 to 60%. It is important to note that these changes were likely due to a reduction in the functional capacity and blood flow rather than changes induced by epithelial surface area. More recently, the effect of the severity of short-term feed restriction, rather than complete feed deprivation, has been investigated (Zhang et al., 2013a). In this study, 18 heifers were fed ad libitum and then allocated feed equating to 75, 50, or 25% of their ad libitum DMI for a period of 5 d. A 5-d feed restriction period, regardless of the severity, tended (P = 0.09) to decrease total VFA absorption and decreased acetate absorption. Additionally, heifers restricted to 50 and 25% of ad libitum intake tended (P = 0.07) to have lower rates for total VFA and acetate absorption compared to those restricted to 75% of ad libitum intake. It does not appear that shifting the dietary forage-to-concentrate ratio will mitigate this effect despite expected changes in fermentability and ruminal retention time (Albornoz et al., 2013a). For example, when cattle were restricted to 25% of their ad libitum intake for 5 d, the total VFA absorption rate decreased by 120 mmol/h relative to baseline measurements and did not differ between heifers fed a diet consisting of 92% forage vs. those fed 60% forage (Albornoz et al., 2013a). Thus, it appears that reductions in ruminal epithelial function occur rapidly in response to lower energy intake.

A rapid reduction in ruminal epithelial function may be a compensatory mechanism to reduce energy expenditure by ruminal tissue (Zhang et al., 2013a) during periods of low energy intake. However, given the transient nature of low feed intake under conventional feeding systems, a rapid increase in epithelial function corresponding to increased energy intake would be desirable. Zhang et al. (2013b) provided heifers ad libitum access to feed, without changes in the diet composition, after a 5-d period of feed restriction. That study reported two important findings: 1) return to ad libitum feeding without dietary change induced ruminal acidosis, and 2) that time to recover absorptive function increased with increasing severity of feed restriction. In fact, heifers restricted to 25% of their ad libitum intake required 3 week for VFA absorption

rates to recover, whereas those restricted to 75% of their ad libitum intake recovered within 1 wk. The delayed recovery response suggests that at least a portion of the response is mediated by the epithelia and not solely due to changes in blood flow. Interestingly, the recovery response appears to be hastened when cattle are fed greater proportions of concentrate prior to dietary restriction and greater proportions of forage after feed restriction (Albornoz et al., 2013b). Future work is needed to develop strategies that can be used to mitigate a reduction in epithelial function in response to transient nutritional challenges and to accelerate the rate of recovery in response to feed restriction or feed deprivation.

Ruminal Acidosis Compromises VFA Absorption

Providing adequate time for dietary adaptation has been recommended as a strategy to reduce the risk for ruminal acidosis. It is evident that repeated exposure to sub-acute ruminal acidosis or a single exposure to acute ruminal acidosis may also negatively affect VFA absorption. Dohme et al. (2008) reported that the response to repeated ruminal acidosis inductions increased in severity with each consecutive challenge despite the cows consuming less grain during consecutive challenges. While there may be a number of reasons behind this response, a decrease in VFA absorption is highly plausible because previous studies have shown that at similar pH values (< 5.4) epithelial damage was induced (Steele et al., 2009) and ion transport was impaired (Gaebel et al., 1987, Gaebel et al., 1988; Gaebel et al., 1989). That said, it is not clear whether adaptation reduces the risk for ruminal acidosis. In a recent study, we compared whether cattle fed a high-grain diet (81% barley grain, 10% vitamin and mineral supplement, 9% barley silage) for 34 d were more resistant to ruminal acidosis than cattle fed the same diet but for only 8 d (Schwaiger et al., 2013a,b). Ruminal acidosis was induced by restricting feed intake on the d before the challenge and the challenge itself included an intraruminal infusion of ground barley grain. There were no differences observed for the risk or severity of ruminal acidosis between short-adapted and long-adapted cattle. However, we did observe that ruminal pH recovered more rapidly in long-adapted cattle than short-adapted cattle. Interestingly, long-adapted cattle also had greater lactate absorption than short-adapted cattle immediately following the challenge.

While the total VFA absorption rate was not different between the short- and long-adapted cattle, it was very clear that induction of ruminal acidosis decreased VFA absorption (Schwaiger et al., 2013a,b) when measured 2 d following induction of ruminal acidosis but not when measured 9 d after the induction of ruminal acidosis (Figure 2). Moreover, there appears to be a compensatory shift in ruminal buffering strategies such that absorption is reduced following a bout of ruminal acidosis while at the same time, saliva production increases (Figure 2). Thus, it appears that ruminal acidosis impairs VFA absorption but the recovery following a bout of ruminal acidosis may be rapid and that cattle may increase salivary buffer supply to compensate for the reduction in VFA absorption. The negative effect of severely low ruminal pH on VFA absorption is supported by previous work in vivo (Krehbiel et al., 1995) and in vitro (Wilson et al., 2012).

Understanding Adaptation of the Rumen Epithelium and VFA Transport in Response to an Increase in Diet Fermentability

In conventional production systems cattle are exposed to abrupt dietary change. Examples include Holstein calves at weaning, beef cattle upon arrival at a feedlot, and immediately following calving for transition dairy cattle. Interestingly, all of the above-mentioned situations have also been suggested as periods with high risk for ruminal acidosis. Previous literature had suggested that 4 to 8 weeks were required for maximal increases in the absorptive surface area of the ruminal epithelium (Dirksen et al., 1985; Bannink et al., 2008); however, more recent evidence has suggested that changes in the functional activity precede increases in surface area (Etschmann et al., 2006).

Schurmann (2013) used 25 Holstein calves to determine the rate of epithelial adaptation when exposed to an abrupt dietary change focusing on the absorptive surface area of the ruminal epithelium and the rate and pathway of VFA absorption. In that study, calves were fed a diet containing 92% grass hay and 8% mineral and vitamin pellet and then abruptly changed to a diet containing 50% grass hay, 42% ground barley grain, and 8% mineral and vitamin pellet. Calves were fed the latter diet for 0, 3, 7, 14, and 21 d before they were killed to measure VFA transport rate and the pathway of transport. Ruminal pH responded quadratically with an initial decrease until d 7 and then increasing to d 21. This finding supports previous work by Steele et al. (2011) when dairy cattle were abruptly exposed to a diet change. Moreover, effective surface area of the ruminal papillae was not affected by treatment indicating that changes in absorption rates for acetate and butyrate could be attributed to functional changes in the epithelial cells rather than increased size or abundance of cells. For acetate, the rate of transport increased by 18% within 7 d of feeding the 50% forage diet whereas, butyrate increased (27%) linearly from d 0 to d 21. While it was hypothesized that the increase would primarily be due protein mediated transport processes such as anion exchange, the only pathway affected was passive diffusion. This indicates that changes in epithelial cell permeability may be one strategy to facilitate a rapid increase in VFA absorption; however, this approach does not necessarily correspond to an improvement in ruminal pH.

Conclusions

Short-chain fatty acid absorption clearly helps to stabilize ruminal pH by either removing protons with passive diffusion or by the secretion of bicarbonate with anion exchange mechanisms. Interestingly, the relative contribution of individual pathways of VFA absorption differ based on the type of VFA absorbed and moreover, the contribution of salivary bicarbonate and epithelial buffering towards stabilization of ruminal pH appear to be affected by ruminal pH itself. A number of factors such as feed deprivation and feed restriction and ruminal acidosis negatively affect VFA absorption and thereby increase the risk for ruminal acidosis. Future research is needed to determine how nutritional management can be used to enhance the absorptive function of the ruminal epithelia in effort to mitigate ruminal acidosis and improve nutrient delivery.

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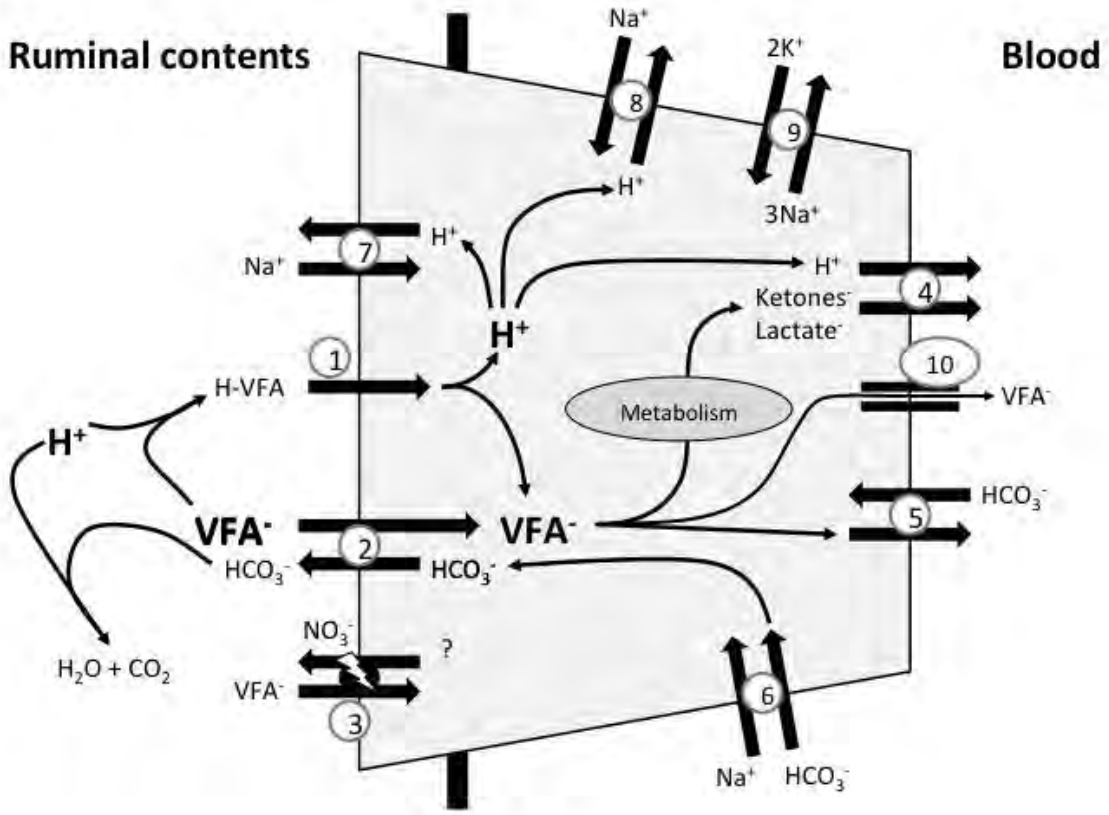

Figure 1. Partial model depicting the current understanding for SCFA absorption in relation to the stabilization of ruminal pH. 1) Diffusional absorption of SCFA facilitates the removal of a proton associated with the SCFA. This proton will rapidly dissociate in the cytosol where it can be exported by sodium/hydrogen exchanges (7, 8) or coupled with metabolites of SCFA (e.g. ketone bodies and lactate) via the monocarboxylate transporter (4). Dissociated SCFA can be absorbed in an anion exchange mechanism thereby providing a source of bicarbonate to the ruminal contents (2). This bicarbonate can then neutralize a proton through the carbonic anhydrase reaction thereby stabilizing ruminal pH. The bicarbonate supply to the epithelia is derived from blood (5, 6). The VFA can also be absorbed via a nitrate sensitive pathway (3) and can be exported into blood via a voltage-gated channel (10). Note, the model does not show the structural complexity of the ruminal epithelia including the number of strata and cells within strata. Adapted from Aschenbach et al. (2011).

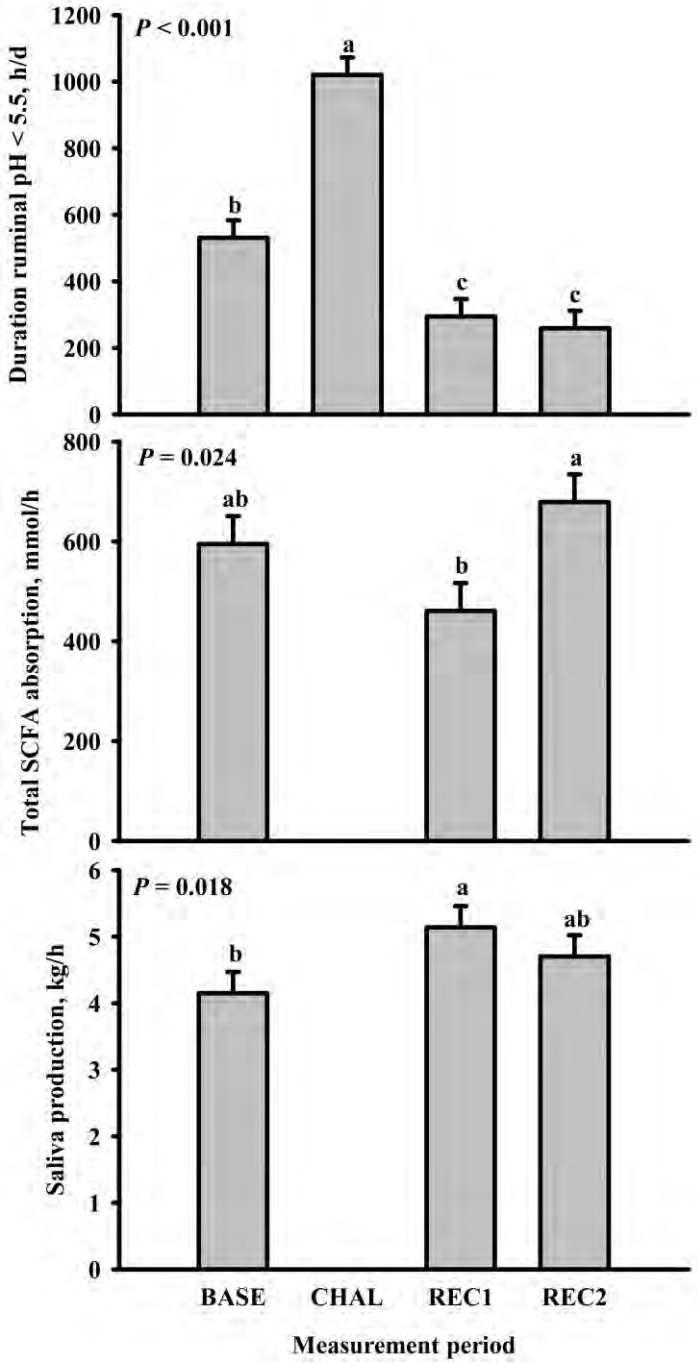

Figure 2. Changes in ruminal pH (panel A) in response to inducing ruminal acidosis and the corresponding changes in VFA absorption across the temporarily isolated reticulo-rumen (panel B), and saliva production (panel C) in beef heifers fed a high-concentrate diet.

SESSION NOTES

Strategies to Improve Rumen Microbial Efficiency

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Introduction

Though hidden from view, the rumen and its microbes hold a central role in feeding of cattle. During ruminal fermentation, microbes break down fiber and other feed components and produce volatile fatty acids (**VFA**). In the process, microbes generate adenosine-tri-phosphate (**ATP**, energy) for themselves, then harness part of this ATP to produce microbial protein. The VFA so produced meet up to 70% of the animal's energy needs (Bergman, 1990), and microbial protein meets 60 to 85% or more of protein needs (Storm et al., 1983).

Microbial fermentation may be essential to cattle, but microbes charge a fee for their services. Microbial metabolism causes 4% of gross energy to be lost as heat (Czerkawski, 1986), and methane production from methanogens causes an additional 2 to 12% loss of gross energy (Johnson and Johnson, 1995). Feeding more concentrate and fat can help curtail these losses (Russell, 2007a, Hristov et al., 2013), but this practice may not always be economical. Few other strategies have been developed that are successful in the long term, and these losses seem to be unavoidable in a healthy fermentation.

Even if we cannot reduce the energetic fee that microbes collect, we may be able to make microbes spend those fees more efficiently. At present, microbes are not particularly efficient with the energy they harvest from fermentation, directing as little as 1/3 ATP towards synthesis of protein. By increasing ATP directed to protein synthesis, we could increase production of microbial protein, gaining more value out of the energy we feed.

The Rumen Microbial Ecosystem at Glance

Hundreds of trillions of microbes can be found in a single, 20-gallon rumen of an average cow. Bacteria represent probably more than 98% of all cells (Lin et al., 1997), but owing to their small size, they may account for barely over half of the microbial biomass (see below). About 200 species of bacteria have been cultured in the lab (Mackie et al., 2002), but molecular techniques (sequencing of the 16s rRNA gene) suggest may be thousands more uncultured "species" exist (Kim et al., 2011). Collectively, these microbes form a complex consortium to degrade fiber, protein, starch and other components of feed.

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In addition to bacteria, the rumen teems with protozoa, fungi, methanogens, and viruses. Protozoa are a fraction of a percent (~0.01%) of total cells in the rumen (Lin et al., 1997). They are much larger than bacteria - some protozoa can be seen with the naked eye - and thus contribute around 5 to 40% of the microbial biomass despite their low numbers (Williams and Coleman, 1992, Sylvester et al., 2005). They degrade feed components much the same as bacteria, except they also engulf bacteria as a food source (for growth factors) (Williams and Coleman, 1992).

Fungi also represent a fraction of a percent of total cells and contribute up to 8% of the biomass (Orpin, 1984). By breaking apart plant tissues with their powerful rhizoid cell structures, they help degrade recalcitrant fiber (Dehority, 2003).

Methanogens have earned infamy for producing methane. Though methanogens in a typical cow rumen collectively produce up to 500 liters per day (Johnson and Johnson, 1995), they account for only perhaps 2% of total microbial cells (Lin et al., 1997). In producing methane, methanogens remove hydrogen that would otherwise inhibit a normal fermentation (Russell, 2002).

Viruses, which are not true organisms, infect and can lyse bacteria. Nearly 1/4 of rumen bacteria harbor a virus, though viruses usually exist in a dormant (lysogenic) state (Klieve et al., 1989). Viruses do not contribute directly to degradation of feed.

Sources of Waste

For more than 40 years, we have recognized that microbes grow (synthesize microbial protein) with far from perfect efficiency (Stouthamer, 1973). For mixed rumen microbes in vivo, actual growth efficiency ranges from only 1/3 to 2/3 of the theoretical maximum (calculated from biochemical pathways) (Table 1). That is, microbes spend as little as 1/3 of ATP on growth. This phenomenon is not restricted to microbes growing in the cow, as the efficiencies are roughly as low in vitro for mixed and pure cultures of bacteria (Table 1).

For microbes both in the cow and lab, ATP not spent on growth is instead directed towards functions such as maintenance, energy storage, and energy spilling (Figure 1). Maintenance encompasses “housekeeping” required for the cell to simply stay alive, such as maintaining crucial ion balances across the cell membrane (Russell and Cook, 1995). Energy storage refers to energy preservation in the form of glycogen and other compounds synthesized during period of energy excess (Preiss and Romeo, 1989). Energy spilling refers to energy dissipated as heat when ATP exceeds needs for growth, maintenance, and storage (Russell, 2007b). Simply put, it is burning energy for the sake of burning energy. It can be likened to water flowing over the brim of an overfilled bucket (Figure 2).

Maintenance

Because it is required for cell survival, maintenance is an unavoidable source of waste. Despite its requirement by microbes, it is indeed wasteful from the cow's perspective because its net product is heat (no microbial protein).

Energy directed towards maintenance accounts for a large proportion of the energy expended when growth rates are low. At low growth rate of 5%/h, it accounts for more than 30% total energy expended. At high growth rate of 20%/h, by comparison, it accounts for only 10% (Russell, 2007a). This may be explained by analogy to a company's profit, overhead, and sales: when profits (growth) are low, overhead (maintenance) makes up a large proportion of sales (energy expended) (Russell, 2007a).

Growth rate increases with digesta passage rate in the rumen. For reasons explained above, increasing passage rate might seem a good strategy to decrease the relative impact of maintenance. Such a strategy for reducing waste may have unintended consequences, however, because increasing passage rate, such as by grinding forage, decreases digestibility (Van Soest, 1994).

Energy Storage and Spilling

Energy storage occurs when an excess of energy exists (Fig. 1, 2). Although stored energy can be later mobilized for growth (Wilkinson, 1959), storage is still somewhat wasteful because ATP is irreversibly spent to synthesize glycogen. This waste represents 20 to 50% of the available ATP in glucose, given 1 net ATP is spent on glycogen synthesis (Stouthamer, 1973) and between 2 to 5 ATP are available from glucose fermentation (Russell, 2002).

While small to moderate excesses of energy can be stored, large excesses of energy can also be simply burned off as heat through energy spilling (Fig. 1, 2). The function of spilling is not known, but it may give a microbe a growth advantage over its competitors (Russell, 2007a). Regardless of its function to the microbe, spilling may not be strictly required for its survival. Spilling produces only heat (no protein), and thus may be considered particularly wasteful. When mixed rumen microbes were given a large excess of energy (20 mM glucose), nearly 40% of heat was from spilling alone (Hackmann et al., 2013a).

Avoiding Energy Excess

Proper ration formulation is needed to avoid carbohydrate excess and thus reduce waste through spilling and storage. Carbohydrate excess occurs when rumen-degradable protein (**RDP**) is low, thereby limiting synthesis of microbial protein. The Dairy NRC (2001) reports RDP requirements between 9.5 to 11.3% of diet dry matter for lactating cattle and 8.6 to 10.8% for heifers.

A shortfall in RDP arises most commonly for feedlot-type rations with high inclusion of concentrate (Russell, 1998), but it can still occur in dairy rations with corn silage as the sole source of forage (VandeHaar, 2005). If ration formulation software or a nutritionist indicates a shortfall of RDP in a ration, this can be corrected by increasing the inclusion of high-RDP ingredients (such as soybean meal and urea). Alternatively, the carbohydrate concentration of the ration should be decreased by substituting it with fat (which is energy-rich but cannot be fermented).

Though adequate RDP may reduce energy spilling and storage, it may not completely eliminate them. Some energy storage occurs even when RDP is apparently adequate: glycogen can be detected in microbes when cattle are given a lactation diet with adequate RDP (Hackmann et al., 2013b). Additionally, mixed rumen bacteria still spilled energy in vitro when adequate RDP was given in the form of non-protein nitrogen (**NPN**) from ammonia. Bacteria grew more slowly with NPN than with true protein, and spilling resulted (Van Kessel and Russell, 1996). This finding has been corroborated in vivo, where microbial efficiency has been increased 36% by partially replacing urea (NPN) with casein (true protein) (Hume, 1970).

Wasteful Microbes

Some microbes may be inherently more wasteful than others and thus are targets for elimination from the rumen. *Streptococcus bovis* is a conspicuously wasteful microbe, though it was first recognized for causing acidosis (Russell, 2002). When cattle are abruptly switched from high-forage to high-concentrate diet, *S. bovis* feasts on the abundant carbohydrate (starch and sugars) available in the rumen. If left unchecked, it can hijack the rumen by 1) rapidly fermenting carbohydrate to lactate, 2) growing at a rate unmatched by any other rumen bacterium, and 3) decreasing rumen pH to 4.5 or lower, which few microbes besides acid-tolerant lactobacilli can survive. The result is acute rumen acidosis.

For the same reason it causes acidosis, *S. bovis* excels at spilling energy. Cells rapidly ferment carbohydrate to lactate whether they can grow or not. Because *S. bovis* lacks ability to store energy, spilling inevitably occurs when growth is limited (Figures 1 and 2). The mechanism of spilling is by a futile cycle of protons, in which protons are pumped out of the cell only to return later, with net production of heat (Russell, 2007b).

Although it proliferates best under the abrupt switch to concentrate as mentioned above, *S. bovis* is still present in low abundance regardless of the diet, even in high-forage diets (Russell, 2002). Because it is a fixture in the rumen and conspicuously wasteful, *S. bovis* is a natural target for reduction.

Reducing *Streptococcus bovis*

A few strategies exist for reducing *S. bovis*, most of which were developed originally to ameliorate acidosis. The antibiotic virginiamycin decreased *S. bovis* counts 10-fold or more in vivo and prevented uncontrollable accumulation of lactic acid (Coe et

al., 1999). Monensin plus tylosin were less effective. Antibiotics kill bacteria other than *S. bovis* (Nagaraja and Taylor, 1987), however, and their net effect on microbial efficiency remains unknown.

Live yeast (*Saccharomyces cerevisiae*) compete with *S. bovis* for carbohydrate, at least *in vitro* (Chaucheyras et al., 1996). However, how extensive this ruminal competition really is remains unclear (Russell, 2002)

Other strategies, still experimental, involve administering 1) antibodies, which bind to and inhibit bacteria, 2) vaccines, which stimulate production of antibodies, 3) bacteriophages, which are viruses that infect and kill bacteria, and 4) bacteriocins, which are antibiotic-like compounds produced naturally by rumen bacteria. Antibodies reduced *S. bovis* counts about 4-fold in vivo as long as they were fed (DiLorenzo et al., 2008). A vaccine reduced *S. bovis* counts by less than half, but it did protect against acidosis for the majority of animals (Shu et al., 1999). However, the efficacy of this strategy has not been tested long term (more than a week or two after the last booster).

Bacteriophages and bacteriocins can sometimes be effective in vitro, but success in animal trials is yet to be firmly demonstrated. One problem is the existence of *S. bovis* stains that are or become resistant. *Streptococcus bovis* was initially inhibited by the bacteriocin, nisin, in vitro, but resistant cells soon developed and grew as fast as untreated ones (Russell and Mantovani, 2002). Strains of *S. bovis* from the rumen were sensitive to one bacteriophage for 40 d, but afterwards resistant strains developed spontaneously (Iverson and Millis, 1977).

Identifying Other Wasteful Microbes

Streptococcus bovis is unlikely to be the only wasteful microbe. We have observed that populations of rumen microbes can spill energy even when lactate is not produced (Hackmann et al., 2013a). Because lactate production is a fingerprint of rapid fermentation and energy spilling by *Streptococcus bovis* (Russell and Strobel, 1990), other microbes must be responsible for this waste. Further, *S. bovis* does not store energy, but populations of rumen microbes can store large amounts of energy (Hackmann et al., 2013a). If we focus on energy spilling by *S. bovis* alone, we ignore microbes wasting energy through storage. Research is underway to identify those wasteful microbes, and strategies to eliminate these microbes could then follow.

Microbial Turnover

The strategy to increase microbial efficiency emphasized most by this review is increasing direction of ATP towards microbial growth. Another strategy is to decrease turnover of microbial protein to ammonia, as this effectively “undoes” microbial growth. As much as 50% of microbial protein turns over in the rumen (Wells and Russell, 1996, Oldick et al., 2000).

Protozoa promote turnover by engulfing bacteria. Although protozoa incorporate some of engulfed bacterial protein into their own cells, they break down and release some as ammonia into the rumen milieu (Williams and Coleman, 1992). Accordingly, removing protozoa from the rumen decreases turnover, increasing microbial efficiency by an average of 58% (Williams and Coleman, 1992). Removing all protozoa is not a practical strategy, however, as animals spontaneously re-acquire protozoa unless the animal is isolated.

Viruses also cause bacterial lysis and turnover. However, the majority of viruses exist in a dormant (lysogenic) state in the bacterial cell, and their lytic activity is probably low (Klieve et al., 1989). Bacteria, protozoa, and probably other microbes also autolyse (die spontaneously) (Williams and Coleman, 1992, Wells and Russell, 1996). This autolysis is not necessarily a response to starvation (Wells and Russell, 1996), and it is unclear how to reduce it.

Conclusions

Rumen microbes do not grow with perfect efficiency, but spend considerable ATP on non-growth functions (maintenance, energy storage, and energy spilling). Because they generate heat instead of microbial protein, these non-growth functions are wasteful to the cow. Waste can be reduced by including adequate dietary RDP to avoid excesses of energy. *Streptococcus bovis* is a conspicuously wasteful microbe because of its propensity to spill energy. Anti-bacterial agents, including antibiotics, antibodies, and vaccines, are effective in reducing *S. bovis* counts, though it is not clear if they increase microbial efficiency in turn. Improving efficiency will depend on identifying other wasteful microbes and further developing strategies to manipulate the microbial population.

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Table 1. Efficiency of rumen microbial growth

	Efficiency	
Organism	g microbial DM mol ATP^{-1*}	% of theoretical maximum[#]
Mixed rumen microbes, in vivo	11 to 21	34 to 66
Mixed rumen bacteria, in vitro	7.5 to 16.7	23 to 52
Pure cultures, in vitro	10 to 25	31 to 78

[*]From Russell and Wallace (1997).
[#]32 g (g microbial DM mol ATP)$^{-1}$; from Stouthamer (1973).

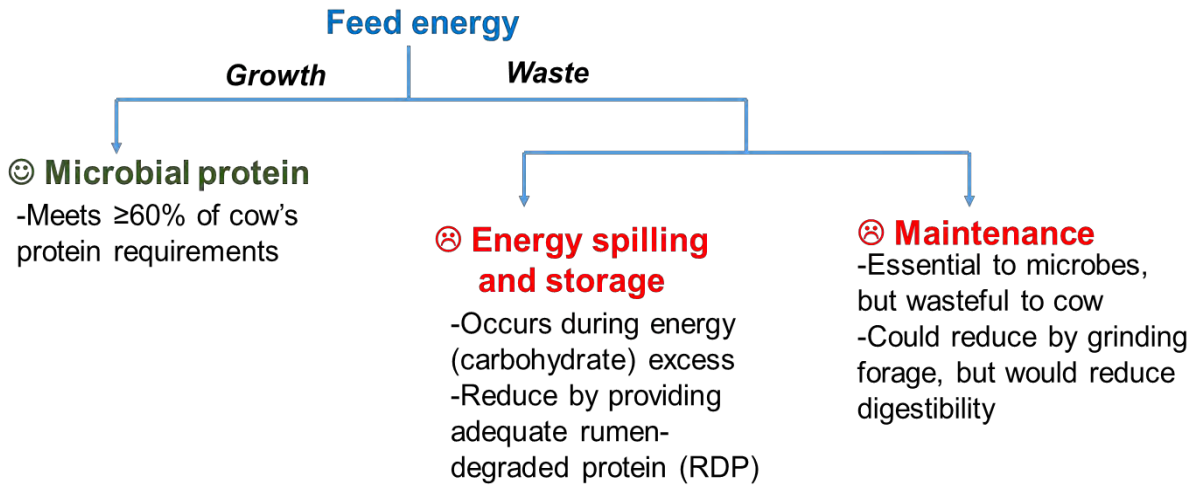

Figure 1. Use of feed energy by rumen microbes. As little as 1/3 energy that microbes harvest will be used for production of microbial protein (growth), and the rest is wasted on energy spilling, energy storage, and maintenance.

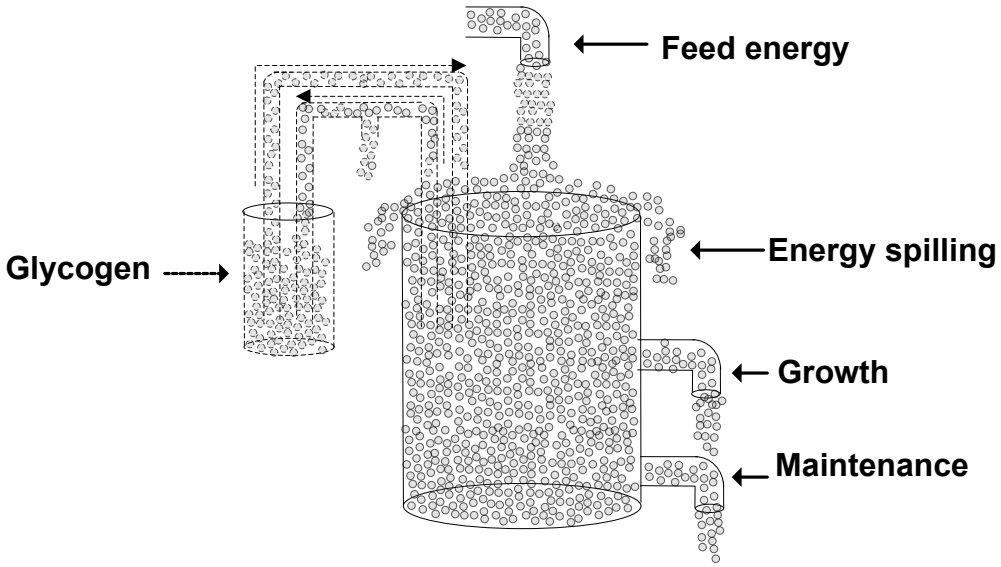

Figure 2. Bucket model of energy spilling. The large bucket represents the main pool of ATP-equivalents available to cell functions (maintenance, growth, energy storage, energy spilling). The smaller bucket represents the pool of ATP-equivalents in glycogen, which can be stored from and mobilized to the main pool by pumps. Modified from Russell (2002).

SESSION NOTES

Characteristics of Feed Efficiency and Use for Selection of Heifers and Cows

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Introduction

A significant challenge facing the world today is the expected 34% increase in the human population by 2050, which will require 70% more food from existing natural and land resources (FAO, 2009). Thus, the efficiency of natural resource use must be improved to meet the food security goals for a growing population while protecting the environment (FAO, 2009). Due to their ability to utilize cellulose for production of meat, cattle can be used to produce human food from land that cannot be used to grow food crops. However, cattle have a low conversion rate of feed to meat. Therefore, considerable research has focused on nutrient use efficiency or feed efficiency in cattle.

Typically, feed conversion ratio (**FCR**; i.e., feed to gain) has been used as the measure of feed efficiency in beef cattle. However, more efficient animals can either have lower feed intake for the same gain or faster gain for the same feed intake indicating that selection pressure may not be consistently placed on either feed intake or rate of gain making genetic improvement difficult. Feed conversion ratio is strongly negatively correlated with growth rate such that selection for faster growth rate will improve FCR (Bailey et al., 1971; Irgang et al., 1985; Mrode et al., 1990). However, other studies (Thompson et al., 1985; Aaron et al., 1986; Herd et al., 1991) reported no improvement in FCR between genetic lines selected for fast or slow growth rate. Thus, selection for faster growth rates may not improve feed efficiency, but will significantly increase mature weight (Herd et al., 1991; Archer et al., 1997; Herd et al., 1997), thereby increasing feed required to maintain the cow herd. Given that the cow herd accounts for 65% of feed required to produce a pound of beef, selection for improved feed efficiency based on FCR or growth rate may be detrimental to feed efficiency of beef production.

Recently, considerable research has focused on evaluating residual feed intake (**RFI**), also referred to as net feed intake or net feed efficiency, as a trait for use in selection programs. Koch et al. (1963) suggested RFI as an alternative trait to FCR that is independent of growth rate and mature weight, and will not result in greater maintenance requirements of the cow herd. Residual feed intake is calculated as actual feed intake minus expected feed intake based on growth rate and body weight; animals that consume less than expected have low RFI and are more efficient. The use of RFI

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to measure feed efficiency identifies animals that consume less feed for the same growth rate and body weight, putting selection pressure directly on feed intake. Therefore, consistent genetic improvement in feed efficiency can be made using RFI.

Relationships of RFI with production traits

Average correlation coefficients of RFI with other production traits from several published studies (Arthur et al., 1997; Herd and Bishop, 2000; Arthur et al., 2001a; Arthur et al., 2001b; Basarab et al., 2003; Nkrumah et al., 2004; Brown, 2005; Nkrumah et al., 2007; Lancaster et al., 2009a; Lancaster et al., 2009b) are presented in Table 1. As expected, RFI is not correlated (phenotypically independent) with body weight and average daily gain indicating selection for improved RFI (low RFI) will not result in increased mature weight. However, RFI is positively correlated with feed intake and FCR indicating that animals with low RFI consume less feed and are more efficient based on FCR. Thus, selection for lower RFI will improve feed efficiency by reducing feed intake for the same growth rate and body weight.

The impact of selection for low RFI on carcass merit has been studied extensively. From the published studies mentioned above, RFI was not correlated with ribeye area, but was positively correlated with rib fat thickness in all studies. Additionally, RFI is positively correlated with marbling score or ultrasound intramuscular fat in some studies but not others. The correlations of RFI with rib fat thickness and marbling score are very weak relationships indicating that the correlated response in rib fat thickness and marbling score to selection for low RFI is expected to be small. However, Herd et al. (2003) and Arthur et al. (2005) reported less rib fat thickness in finished steers and mature cows, respectively, from a low RFI selection line compared with a high RFI selection line. McDonagh et al. (2001) and Baker et al. (2006) reported no difference in Warner-Bratzler shear force values between low and high RFI steers, but Baker et al. (2006) found lower sensory panel scores for juiciness and off-flavor in low RFI steers. Residual feed intake can be calculated to be independent of carcass fat by including measurement of 12^{th}-rib fat thickness in the regression model to calculate expected feed intake (Basarab et al., 2003; Lancaster et al., 2009a; Lancaster et al., 2009b). Given these relationships, RFI should be computed using ultrasound estimates of 12^{th}-rib fat thickness to reduce any correlated responses in carcass fat, but no studies have evaluated the impact on beef sensory characteristics.

Relationships of RFI with Reproductive Performance

Body condition score or body fat is known to impact reproductive performance and pregnancy rates in beef cows; cows of low body condition with less body fat have lower pregnancy rates and longer postpartum interval. Given this relationship, selecting cattle with low RFI and the correlated decrease in body fat could negatively impact reproductive performance in heifers and mature beef cows. Although, previous studies (Lancaster, 2008; Basarab et al., 2011; Shaffer et al., 2011) have reported no difference in age at puberty between low and high RFI heifers, even though differences in body fatness were evident. Lancaster (2008) reported no difference in pregnancy, calving or

weaning rate, but Basarab et al. (2011) found that low RFI heifers had lower pregnancy and calving rates. However, when RFI was adjusted for rib fat thickness the difference in calving rate between low and high RFI heifers was eliminated (75.5 vs. 81.5%; *P* = 0.31), but when the rib fat adjusted RFI was used, low RFI heifers were older and heavier at puberty than high RFI heifers indicating that including rib fat thickness in the calculation of RFI may negatively impact attainment of puberty in heifers (Basarab et al., 2011).

Only 2 studies have evaluated reproductive performance in mature cows (Table 3). Arthur et al. (2005) found no difference in pregnancy, calving, or weaning rate between cows from the low and high RFI selection lines. These authors did report a tendency for cows from the low RFI selection line to calve 5 days later in the year indicating that these cows were bred later in the breeding season compared with cows from the high RFI selection line. There was no difference in milk yield or pounds of calf weaned per cow exposed between cows from the low and high RFI selection lines (Arthur et al., 2005). Basarab et al. (2007) evaluated the effect of RFI on cow productivity retrospectively by evaluating performance of dams of steers with low and high RFI; RFI was not measured on the cow. These authors also found no difference in pregnancy, calving, or weaning rate between dams based on RFI of their steer calves. Similar to Arthur et al. (2005), Basarab et al. (2007) found that dams of steers with low RFI calved 4 days later in the year than dams of steers with high RFI, but these authors found no difference in calving interval indicating that the dams of steers with low RFI were bred later in the breeding season as heifers and continually bred later in the breeding season in subsequent years. There was no difference in pound of calf weaned per pound of cow body weight between dams of steers with low and high RFI (Basarab et al., 2007). Additionally, Crowley et al. (2011) found a negative genetic correlation (-0.29) between RFI and age at first calving again indicating selection for improved RFI may result in heifers that conceive later in the breeding season. Collectively, these results suggest that selection for more efficient cattle using RFI may negatively impact age at puberty and pregnancy rates of heifers, but have little impact on reproductive performance and productivity of mature cows.

Relationships of RFI Measured as Growing and Mature Cattle

Selection for improved RFI typically occurs in young growing bulls measured shortly after weaning in performance test stations utilizing high energy diets. However, mature cows consume a low energy diet and produce milk rather than deposit nutrients into tissue. This raises a significant question: “Will selection of young growing bulls with superior RFI fed high energy diets translate to improved feed efficiency in mature cows consuming low energy diets?”

Several studies have evaluated the relationship between RFI measured in growing and finishing phases in cattle fed a moderate energy growing diet followed by a high energy finishing diet. Crews et al. (2003) and Durunna et al. (2011b) reported genetic correlations of 0.55 and 0.50, respectively, between RFI measured in the same steers fed a growing diet then a finishing diet. Brown (2005) reported a phenotypic

correlation of 0.47 between the same steers fed a growing versus finishing diet. These data suggest that either diet or stage of maturity impacts feed efficiency.

Arthur et al. (2001b) found a strong genetic correlation (0.75) between RFI measured in weanling bulls and again as yearling bulls fed the same diet. Archer et al. (2002) reported a strong genetic correlation of 0.98 between RFI measured in growing heifers and again as non-pregnant, non-lactating 3 yr-old cows fed the same diet. Durunna et al. (2011a) found that the Spearman rank correlation between RFI measured in steers fed a growing diet then a finishing diet was 0.33 compared with 0.44 and 0.42 between RFI measured in steers fed either the growing or finishing diet, respectively, for the entire study. These studies indicate that both stage of maturity and diet impact ranking of cattle for RFI suggesting that selection for improved RFI in young growing bulls fed high energy diets may not translate to improved efficiency in mature cows fed low energy diets.

To further evaluate the relationship between RFI in young growing cattle compared with mature cattle, several studies have compared RFI in growing heifers and again as mature cows. Nieuwhof et al. (1992) reported a genetic correlation of 0.58 between RFI measured in growing heifers and again in lactating first-calf heifers fed similar diets, but the phenotypic correlation was not different from zero (0.07; Table 4). Similarly, Archer et al. (2002) reported a very strong genetic correlation (0.98) between RFI measured in growing heifers and again as 3-yr-old non-pregnant, non-lactating cows fed the same diet, but the phenotypic correlation of 0.40 was much lower. These results suggest that RFI measured at different stages of maturity is genetically the same trait, but that expression of that genetic potential may be altered.

Other studies have determined the phenotypic relationship between RFI in growing heifers and mature cows. Arthur et al. (1999) and Herd et al. (2006) found phenotypic correlations of 0.36 and 0.39 between RFI measured in growing heifers and again as 3-yr-old non-pregnant, non-lactating cows fed the same diet (Table 4). Hafla et al. (2013) reported a similar correlation (0.42) between RFI measured in growing heifers and again as pregnant, non-lactating first or second calf heifers fed a different diet. Adcock et al. (2011) found a slightly lower correlation (0.30) when cows were non-pregnant, but lactating and fed the same diet. However, Black et al. (2013) reported no significant relationship (0.13) between RFI measured in growing heifers and again as 3-yr-old non-pregnant, lactating cows fed a different diet. Collectively, these results suggest that different physiological state (growing versus pregnant or lactating) and diet can combine to drastically reduce the relationship with feed efficiency of growing cattle, which indicates that selection for improved RFI in young growing bulls fed high energy diets will most likely result in minimal improvement in feed efficiency of mature lactating cows.

Energy Efficiency Index: A Trait to Measure Feed Efficiency of Mature Cows

As outlined above, selection of cattle with superior RFI measured as growing cattle is not likely to significantly improve feed efficiency of the mature cow. Thus, RFI

may be better suited to selection of cattle for improved feed efficiency in the feedlot rather than the breeding herd.

Efficiency of the mature cow is complex. Feed has to be used for lactation and conceptus growth, as well as body fat and muscle reserves. The cow must have the ability to use nutrients from feed and body reserves to produce milk early in lactation, then shift nutrients toward conceptus growth and rebuilding body reserves later in the production cycle. Thus, the efficient cow must have the genetic ability to efficiently produce milk in the mammary gland, transport nutrients across the placenta, and synthesize/breakdown lipids and protein in fat and muscle. Moreover, she must do all this with the feed resources available. Ferrell and Jenkins (1985) indicated that the most efficient cows in one environment are not necessarily the most efficient cows in another environment. For example, a cow with genetic ability to efficiently produce milk when high quality forage is available will struggle to maintain body condition and rebreed when only low quality forage is available. This stresses the importance of measuring efficiency of the cow in the conditions in which she will have to perform (i.e., on the ranch).

Recently, Tedeschi et al. (2006) developed a model to calculate an energy efficiency index (**EEI**) for beef cows on the ranch based on body condition score and body weight of cows, weaning weight of calves, and forage nutritive value. The amount of metabolizable energy required per pound of weaned calf, EEI, can be calculated for each cow in the herd. The use of EEI to evaluate efficiency of mature cows has some advantages compared with RFI. First, this calculation does not require measurement of feed intake making it less difficult to evaluate efficiency of mature beef cows and allowing evaluation on the ranch. Additionally, the calculation is based on energy metabolism of the mature cow taking into account lactation, conceptus growth, and body reserves rather than that of growing animals as with RFI. However, one potential disadvantage is that estimating the metabolizable energy required rather than actually measuring feed intake may lead to erroneous classification of efficient and inefficient cows.

Currently, there is little data evaluating EEI. Bourg (2011) found that EEI was negatively correlated with milk expected progeny difference (**EPD**) of cows indicating that the most efficient cows produced more milk. However, EEI was not correlated with weaning weight EPD, average daily gain EPD, hot carcass weight EPD, ribeye area EPD, marbling EDP, or residual feed intake EPD. These results suggests that more efficient cows could be selected based on EEI without correlated responses in growth, body weight, or carcass merit of offspring. Energy efficiency index was not related to RFI EPD, which suggests that these traits are not measuring the same biological processes and supports the idea that RFI measured in growing animals does not translate to improved energy metabolism and efficiency of mature beef cows.

Conclusions

Residual feed intake is a measure of feed efficiency that is independent of growth rate and mature body weight indicating that selection for improved residual feed intake would not negatively impact mature cow size and maintenance requirements of the cow herd. Limited research indicates that selection for improved residual feed intake would have minimal impact on cow productivity. However, residual feed intake is weakly correlated with body fat content such that more efficient cattle are leaner. Additionally, more efficient heifers as measured by residual feed intake may achieve puberty at older age and conceive later in the breeding season as heifers, which may be related to differences in body fat content. Additional research is needed on the relationship of residual feed intake with female reproductive performance.

Several studies have reported a weak relationship between residual feed intake measured in growing cattle and again as mature cows. This suggests that even though selection for residual feed intake will not impact cow productivity, it likely will not result in much improvement in feed efficiency of the mature cow either. This is most likely due to the differences in energy metabolism between growing cattle and mature lactating cows. Energy efficiency index is based on energy metabolism of mature beef cows and allows evaluation of the beef cow in her production environment. Very little data is available evaluating energy efficiency index in beef cows, but energy efficiency index shows potential as a trait to identify efficient beef cows.

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Table 1. Average correlations of residual feed intake with production traits in growing beef cattle from published studies.[1]

Trait	r_p[2]	No. of studies[3]
Body weight	0.00	0 of 10
Average daily gain	-0.01	0 of 10
Feed intake	0.65	9 of 9
Feed conversion ratio	0.55	9 of 9
Ribeye area	0.00	0 of 10
12th-rib fat thickness	0.21	7 of 8
Marbling score or IMF%	0.12	2 of 6

[1] Arthur et al., 1997; Herd and Bishop, 2000; Arthur et al., 2001a; Arthur et al., 2001b; Basarab et al., 2003; Nkrumah et al., 2004; Brown, 2005; Nkrumah et al., 2007; Lancaster et al., 2009a; Lancaster et al., 2009b.
[2] r_p = phenotypic correlation coefficient.
[3] Number of studies where correlation coefficient is different from zero ($P < 0.05$).

Table 2. Reproductive performance of beef heifers with low and high residual feed intake.

	Lancaster, 2008		Shaffer et al., 2011		Basarab et al., 2011	
Trait	Low RFI	High RFI	Low RFI	High RFI	Low RFI	High RFI
Age at puberty, d	279	271	425	411	353	347
Pregnancy rate, %	89	79	62	66	77[a]	86[b]
Calving rate, %	81	78			73[a]	84[b]

[ab] Means within a row and study with different superscripts differ ($P < 0.10$).

Table 3. Reproductive performance and productivity of mature beef cows with low and high residual feed intake.

	Arthur et al., 2005		Basarab et al., 2007	
Trait	Low RFI	High RFI	Low RFI	High RFI
Pregnancy rate, %	90.5	90.2	95.6	96.0
Calving rate, %	89.2	88.3	84.9	86.3
Weaning rate, %	81.5	80.2	81.5	82.3
Calving date, Julian day	215[a]	210[b]	92[a]	88[b]

[ab] Means within a row and study with different superscripts differ ($P < 0.10$).

Table 4. Relationships of residual feed intake measured in growing and mature cattle.

Study	Cattle Description		r_p[1]
	Age 1	Age 2	
Nieuwhof et al., 1992	Growing dairy heifer	Non-pregnant, lactating 1st-calf heifer fed similar diet	0.07
Arthur et al., 1999	Growing heifer	Non-pregnant, non-lactating cow fed same diet	0.36*
Archer et al., 2002	Growing heifer	Non-pregnant, non-lactating cow fed same diet	0.40*
Herd et al., 2006	Growing heifer	Non-pregnant, non-lactating cow fed same diet	0.39*
Adcock et al., 2011	Growing heifer	Non-pregnant, lactating cow fed same diet	0.30*
Black et al., 2013	Growing heifer	Non-pregnant, lactating cow fed different diet	0.13
Hafla et al., 2013	Growing heifer	Pregnant, non-lactating 1st or 2nd-calf heifer fed different diet	0.42*

[1] r_p = phenotypic correlation coefficient.
* Correlation coefficient is different from zero ($P < 0.05$).

SESSION NOTES

Use of Corn Co-Products in Beef Cattle Diets

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Introduction

The ethanol industry has expanded at an amazing rate since the turn of the century. From 2000 to 2011, the US ethanol industry grew from 1.6 billion gallons to 13.9 billion gallons of ethanol produced (Renewable Fuels Association, 2013a). The renewable fuels standard mandates that should there be 20.5 billion gallons of ethanol produced annually in the US by 2015 (Renewable Fuels Association, 2013b) with no more than 15 billion coming from traditional dry milling of corn. The byproduct of ethanol production from fermentation of corn starch (distillers grains; **DG**) have become an important livestock feed because ethanol production now uses nearly 30% of US corn production (National Corn Growers Association, 2012). The nutrient composition of DG may make them a good fit in high forage diets or as a supplement to grazing cattle because they provide protein, energy, and minerals as well as serve as a forage substitute. However, producers considering using these byproducts must be aware of specific risks associated with their use that require special attention, but can be easily managed. Specifically, high sulfur levels may cause polioencephalomalacia or potentially tie up other minerals required by the animal.

Byproducts of Dry Milling

Distillers grains (**DG**) are a byproduct of the dry milling industry. The dry milling process is described elsewhere (Stock et al., 1999), but consists of converting starch from cereal grains into an alcohol through fermentation by yeast. After removal of the alcohol by distillation, the remaining residue, called whole stillage, may be centrifuged or pressed to separate course particles from fine particles and liquid. The course particles are DG and may be fed as wet DG or dry DG (**DDG**), which may affect animal performance (Ham et al., 1994). The fine particles and liquid is called thin stillage, is often evaporated to condensed distillers solubles, and may be marketed separately or a portion may be added back to the DG. Thus, there are a variety of products marketed under the name of DG and there compositions are dependent upon the amount of solubles added back to the DG and other processes of the plant in which the DG are produced. This can lead to substantial variability in the nutrient composition of DG by plants (Table 1; Holt and Pritchard, 2004; Spiehs et al., 2002). However, some generalizations can be made. By weight, roughly one third of the original grain dry matter (**DM**) is converted to ethanol, another third is lost as carbon-dioxide, and one third remains as DG. Therefore, the nutrient composition of DG is approximately three times the nutrients found in the cereal grain from which it was produced.

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Distillers Grain as a Source of Protein and Energy

The nutrient composition of DDG may make them a good fit in high-forage diets or as a supplement to grazing cattle. One potential advantage to using DDG in forage diets is that most of the starch has been removed. Negative associative effects of starch on fiber digestion have long been noted and some have suggested supplementing highly digestible fiber as a means of providing energy without negatively affecting forage digestion (Horn and McCollum, 1987). Using DDG may allow for energy supplementation without decreasing forage digestibility. Griffin et al. (2012) summarized 13 pasture grazing studies and seven confinement-fed studies that supplemented varying amounts of DG to growing cattle. In pasture supplementation studies, each 1% BW supplementation increased average daily gain (**ADG**) by 0.69 lb (i.e. a slope of 0.69) when supplement was provided up to 1.2% BW. The response in confinement situations was quadratic with diminishing returns with increasing amounts of DG supplementation. The response to improved ADG due to DDG supplementation appears to be somewhat consistent with high-quality forages. We supplemented DDG to 600-lb heifers grazing summer range in the Texas Panhandle. Heifers were supplemented three times weekly at a level that was equivalent to 0.5% BW per day. Supplementation of DG resulted in a 14% improvement in ADG and a slope of 0.56 (MacDonald, unpublished data), comparable to the slope of 0.69 in the summary by Griffin et al. (2012). The response to DDG supplementation is likely due to providing a combination of protein and energy. Distillers grains are a good source of undegradable intake protein (**UIP**). They contain approximately 30% of CP, of which 54% (Firkins et al., 1984) to 66% (Ham et al., 1994) bypasses rumen degradation which may improve the efficiency of protein use in high-fiber diets (Horn and Beeson, 1969; Waller et al., 1980; DeHaan et al., 1982). In addition to providing protein, the fat in DG can serve as an energy source. It is difficult to separate effects of DG on animal performance due to protein or energy. We previously supplemented similar amounts of UIP from DDG or corn gluten meal to heifers grazing high quality bromegrass pastures (Figure 1; MacDonald et al., 2007). The gains for cattle supplemented with corn gluten meal were 39% of those supplemented with DDG across three levels of supplementation. Therefore, the response to DDG was not solely due to meeting a UIP deficiency. A third supplementation strategy in this study was to supplement corn oil equivalent to the amount of fat found in distillers grains (data not shown). There was no performance response to supplementing corn oil demonstrating that energy was not the first limiting nutrient for these heifers. As one might expect, there appears to be an additive effect of supplying protein and energy together in one supplement. A recent unpublished analysis by our group comparing the energy value of DG to corn in forage diets fed to confined calves suggests DG has an energy value of 137% and 136% the value of corn when fed at 15 and 30% of the diet, respectively.

As may be expected, the response to DG supplementation to low-quality forage diets appears greater than to high-quality forage diets. However, the response curve is also strikingly consistent across forage types. Steer calves grazing dormant native range were provided DDG thrice weekly at levels equivalent to 0, 0.25, 0.50, or 0.75% BW per day (Jenkins et al., 2009; Figure 2). We observed a large quadratic

improvement in ADG across the three levels of supplementation. Steers receiving no supplement gained 0.59 lb/day whereas steers receiving the highest level of supplement gained 1.75 lb/d. Similarly, Gustad et al. (2006) provided DG supplementation from 0.29% to 1.27% BW to steers grazing corn residue and also observed a quadratic increase in ADG. After correcting for the intercept, the response curves of Jenkins et al. (2009) and Gustad et al. (2006) are nearly identical.

Fat as an energy source in DG may be especially advantageous to cow/calf producers. Proper nutrition to first-calf heifers is especially important because the heifers are still growing. During the last trimester of pregnancy, heifers may not be able to physically consume enough feed to meet their nutrient requirements as well as the requirements of the fetus. Therefore, supplementing an energy dense component to the diet may be beneficial. Distillers grains have been shown to give greater weight gains than soybean hulls in pregnant heifer diets fed at 40% of dietary DM in the last trimester of pregnancy without any effect on body condition score (BCS) and without causing calving difficulties (Engel et al., 2005). Fat may also be advantageous for improving conception rates. Smith et al. (2001) supplemented cows grazing native range with equal amounts of CP from DDG or alfalfa hay with and without cull beans at a level which provided half of the CP. When DDG was supplemented alone, a greater percentage of cows were cycling prior to estrus synchronization compared to when DDG was supplemented with cull beans. It is possible that this response is related to the fat in DDG because plant oils are known to affect ovarian follicular growth, luteal function, and postpartum reproductive performance independent of increased energy intake (Williams and Stanko, 1999). However, cows supplemented with only DDG lost more body condition compared to cows receiving other supplements. Cattle consuming low-quality forages may be limited by a degradable intake protein (**DIP**) deficiency rather than a metabolizable protein deficiency. Therefore, providing UIP which bypasses rumen degradation may not elicit a performance response if DIP is deficient. Therefore, the authors suggested that the greater loss of body condition score due to DDG supplementation compared to alfalfa and cull bean supplementation was due to the high UIP content of DDG that did not meet the DIP deficiency.

To determine if DDG could meet a DIP deficiency, Stalker et al. (2004; Table 2) provided urea at a level that met the predicted DIP deficiency to heifers consuming a low-quality hay supplemented with 3 lb of DDG. Two pieces of evidence from this study suggest the DDG met the DIP deficiency. Animals that are deficient in DIP will experience a reduced rate of fiber digestibility, which slows passage of fiber out of the rumen. This results in reduced animal performance and reduced dry matter intake. No differences in animal performance or dry matter intake were detected. Also, a DIP deficiency will reduce microbial growth in the rumen which should subsequently lead to reduced microbial crude protein flow out of the rumen. Researchers in this study used the ratio of allantoin to creatinine as an indicator of microbial crude protein flow. An increase in the ratio of allantoin to creatinine would indicate an increase in microbial flow. No differences in this ratio were detected. These data indicate that DDG can be used to meet a DIP deficiency while providing metabolizable protein from UIP and energy. Physiologically, this is achievable through recycling of nitrogen to the rumen. It

is unclear why cows receiving DDG lost more body condition compared to other treatments in the study by Smith et al. (2001), but it may not be due to a DIP deficiency.

Forage Intake and Supplementation Frequency

Effects of supplementation with DDG may include improved animal performance and/or reduced forage intake (i.e. forage substitution). Forage substitution may allow for additional animal units to graze a fixed land base and thus is an important consideration when considering a forage supplement. Morris et al. (2005) provided 1.5, 3.0, 4.5, or 6.0 lb of DDG to heifers consuming either high-quality (alfalfa and sorghum silage) or low quality (bromegrass hay) forage. The efficiency of supplementation (pounds of additional gain per pound of supplement) was greater for the low-quality forage than for the high-quality forage diet (data not shown). However, the effects of DDG supplementation on forage intake (Figure 3) were consistent across forage qualities. The slope of the line for both high and low quality forage was approximately 0.40 suggesting every pound of DDG replaces 0.40 lb of forage. More recently, Gillespie et al. (2012) adjusted grazing pressure by assuming DG supplementation at the level of 0.6% of BW replaced 17% of forage intake. No differences in post-grazing forage residues were observed. Estimated reductions in forage intake are now accurate enough to provide recommendations for changing stocking rate when supplementing DG.

Producers providing supplements to grazing cattle will often supplement several times per week, but not daily. To determine if animal performance is similar when cattle are supplemented with DDG daily or multiple times per week, Stalker et al. (2005; Table 3) supplemented heifers consuming low quality grass hay with the equivalent of 3 lb of DM DDG per day. Heifers were provided supplement either 3 times or 6 times per week. Heifers supplemented 6 times per week had greater ADG compared to those supplemented e times per week. This difference in animal performance due to alternate day supplementation has also been reported by Loy et al. (2003) who compared feeding DDG or dry-rolled corn either daily or on alternate days. Cattle supplemented on alternate days consumed less hay on average compared to cattle supplemented daily. Data from a subsequent metabolism study (Loy et al., 2004) substantiates this forage intake response and indicates the effects on forage intake due to alternate day supplementation are independent of negative effects on rumen metabolism. Producers must determine if the added performance from daily supplementation compared to supplementation several times weekly is profitable when added costs and management are considered. Alternatively, if reduced forage intake were desirable, such as in a drought situation, alternate day supplementation may prove beneficial.

Mineral Considerations

Supplementation strategies are often developed to correct a deficiency; phosphorus is thought to be the most deficient nutrient in the world for grazing livestock (Greene, 1999). The phosphorus content of DDG ranges from 0.70% to 1.00% of DM

(Spiehs et al., 2002). Therefore, supplementation strategies which utilize DG may be able to concurrently reduce the need for phosphorus supplementation for grazing cattle.

One issue that requires special attention for producers considering the use of DG is the level of sulfur it contains. High sulfur levels are associated with polioencephalomalacia (**PEM**; Gould et al., 1991) due to the production of hydrogen sulfide gas in the rumen. Hydrogen sulfide gas is produced because sulfur can be reduced to hydrogen sulfide, thereby providing a hydrogen sink in the rumen. The sulfur content of DDG can range from 0.33 to 0.74% (Spiehs et al., 2002; Table 1). Since corn grain contains approximately 0.14% sulfur (NRC, 1996), the sulfur concentration in the DG above is approximately 0.40%. Presumably, this high S concentration is the result of addition of sulfuric acid during the dry milling process. The maximum tolerable level of sulfur is 0.40% of dietary DM (NRC, 1996). Yet nutritionists in the field commonly feed diets containing DG at levels that exceed 0.40% total dietary sulfur without noticeable PEM. Sarturi et al. (2013) recognized that a portion of the sulfur contained in DG is associated with sulfur containing amino acids. Because DG is also high in UIP, a portion of these sulfur containing amino acids may not be available to the rumen microbes and therefore may not contribute to the production of hydrogen sulfide. The concept of rumen available sulfur (**RAS**) explained 65% of the variation in ruminal hydrogen sulfide production compared to 29% of the variation being explained by total dietary sulfur (Sarturi et al., 2013). Therefore, producers need to be aware of all sulfur sources available to their cattle, including mineral supplement, other feedstuffs, and water. Anecdotal evidence of sulfur toxicity is often associated with cases where producers combined byproducts of the corn milling industries such as DG, condensed distillers solubles, steep liquor, or wet or dry corn gluten feed not realizing that all of these byproducts are potentially high in RAS. Other cases of sulfur toxicity have been reported in cases where producers provided one or more of these byproducts without testing their water source, which may be high in RAS. The issue of sulfur toxicity is relatively easy to manage through testing of feedstuffs and water sources. The cost of testing for sulfur is relatively inexpensive relative to the risk of animal loss.

Conclusions

The opportunity to use DG in high-forage diets has been a tremendous asset to producers located in areas where DG are readily available. Distillers grains provides highly digestible fiber, protein, and fat which increases the performance of cattle consuming high-forage diets. The removal of starch during ethanol production may reduce the negative associative effects on forage digestibility associated with cereal grain supplementation. The protein in DG appears to meet the DIP deficiency associated with intake of low-quality forages. Forage intake is reduced when DDG is supplemented, and to a greater extent when the supplement is provided several times per week rather than daily. However, animal performance may also be reduced when supplemented several times a week rather than daily. Concerns with PEM are more easily managed when using the RAS concept instead of total dietary sulfur.

Although we have a great deal of information to provide recommendations about utilizing DG in high-forage diets, more information will likely be needed in the future. Specifically, three changes in the dynamics of renewable fuels will change the products that will be available to us in the future. First, ethanol plants have begun to remove a portion of the fat from the solubles stream and they are selling the oil for biodiesel production. Second, ethanol produced from sorghum grain has been awarded the classification of an advanced biofuel. Therefore, more DG resulting from fermentation of sorghum will likely be available. Finally, cellulosic ethanol production is being scaled up from pilot plants to production-scale plants. The fiber in DG will be an attractive feedstock for cellulosic ethanol production.

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Table 1. Average nutrient values reported for distillers grains from NRC and range of nutrient values across several plants as reported in two studies[a].

Item	NRC[b]	Holt and Pritchard[c]	Spiehs et al.[d]
DM	91.0	89.4-90.9	87.2-90.2
CP	29.5	30.7-33.2	28.7-31.6
Crude Fat	10.3	10.3-14.2	10.2-11.7
NDF	46.0	37.3-48.9	36.7-49.1
Ca	0.32	NR[e]	0.03-0.13
P	0.83	0.66-0.78	0.70-0.99
K	1.07	0.76-1.07	0.69-1.06
Mg	0.33	0.26-0.33	0.25-0.37
S	0.40	0.37-0.69	0.33-0.74

[a]All values are expressed as a percentage on a DM basis.
[b]Taken from: NRC (1996).
[c]Adapted from: Holt and Pritchard (2004).
[d]Adapted from: Spiehs et al. (2002).
[e]Not reported.

Table 2. Performance and allantoin:creatinine ratio in urine of heifers fed diets where 0 or 100% of the NRC predicted degradable intake protein requirement was met with supplemental urea. Adapted from Stalker et al. (2004)

Item	0	100	SEM	P-value
Initial BW, lb	452	449	1	0.10
Final BW, lb	579	585	4	0.38
Daily gain, lb	1.53	1.63	0.05	0.17
Total DM intake, lb/d	11.9	11.6	0.50	0.76
Feed:gain	9.8	9.1	0.50	0.37
Allantoin:creatinine	0.89	0.89	0.04	0.98

Table 3. Performance of heifers fed the daily equivalent of 3 lb (DM) of dried distillers grains either 3 (3×) or 6 (6×) times per week. Adapted from Stalker et al. (2005)

Item	3x	6x	SEM	P-value
Initial BW, lb	426	424	1.22	0.42
Final BW, lb	559	571	1.93	0.005
Daily gain, lb	1.58	1.74	0.031	0.01

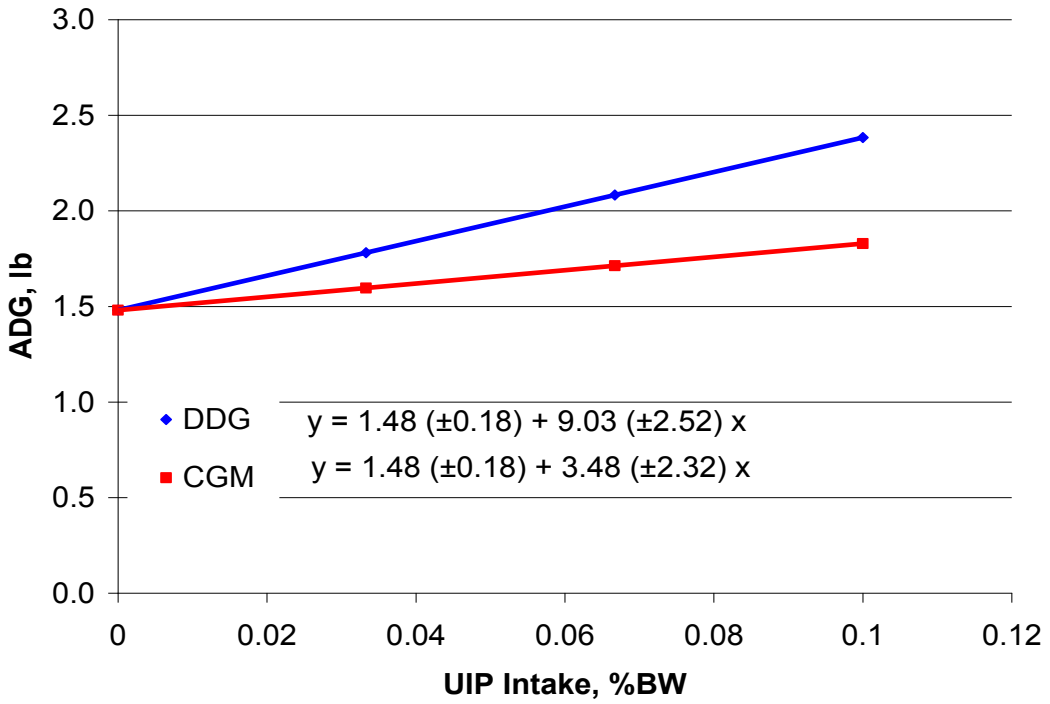

Figure 1. Effect of undegradable intake protein (UIP) intake from dry distillers grains (DDG) or corn gluten meal (CGM) on ADG. DDG slope > 0 (P<0.01). CGM slope > 0 (P=0.14). DDG slope > CGM slope (P=0.10). From MacDonald et al. (2007).

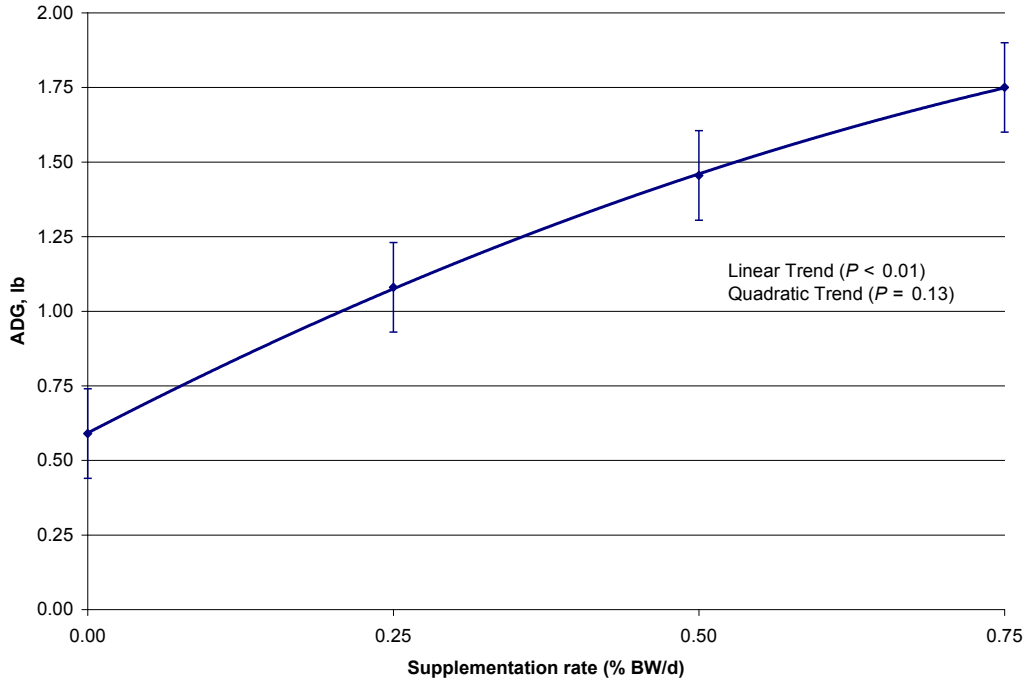

Figure 2. Effects of dry distillers grains supplementation on ADG of calves grazing dormant range (Jenkins et al., 2009).

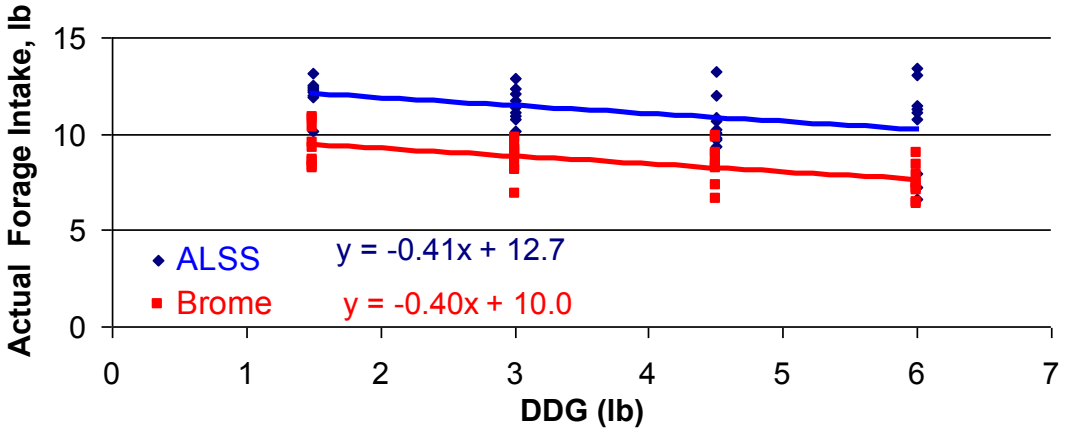

Figure 3. Effects of dry distillers grains (DDG) supplementation on forage intake for heifer calves consuming low quality brome grass hay (Brome) or high quality alfalfa and sorghum silage (ALSS). Adapted from Morris et al. (2005).

SESSION NOTES

Forage Management and Methods to Improve Nutrient Intake in Grazing Cattle

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Introduction

Most of the grassland areas in Brazil are covered with tropical grasses. The majority of the milk (Stock et al, 2011) and about 92% of the beef (Millen et al., 2009) are produced on pasture based systems. The latter authors reported that only 3 to 4 million cattle were finished in feedlots in the year of their survey from a total of approximately 36 million head slaughtered. Despite the great volume of beef and milk produced in Brazil, productivity is still low because, in part, of poor pasture management practices resulting in low stocking rates and poor animal performance. Stocking rate in Brazilian grassland areas averages 0.92 animal units (**AU**)/hectare (**ha**) (1 AU = 450 kg body weight) (IBGE, 2006). This value is considered adequate during the dry winter season, but far from what can be achieved in intensive grazing systems during the hot rainy season, i.e. 6 to 10 AU//ha (Agostinho Neto, 2010; Correia, 2006). Despite the higher stock capacity, the average daily gain (**ADG**) and milk production are lower than the animals' genetic potential, even when tropical grazing systems are managed intensively. The low performance is attributed to the limited energy intake resulting from low grazing efficiency and rumen fill caused by the high fiber content in tropical grasses. Those variables are strongly influenced by pasture management. Therefore, management practices that improve energy intake by grazing cattle, such as improvement in forage quality and availability and/or energy supplementation may be implemented as tools to improve animal performance and productivity in tropical grazing systems.

Forage Intake

Nutritional Value of Tropical Forages

According to Conrad et al. (1964), forage intake is regulated by the capacity of the rumen-reticulum to digest low quality forages, also known as physical effect or rumen fill, or by the feedback caused by nutrients absorbed from high energy diets, also known as physiological effects. For grazing cattle, the physical regulation is the major mechanism controlling forage intake.

Allen (2000) recently reviewed dietary effects on short-term regulation of feed intake in lactating dairy cattle. It was found that the time spent chewing and the

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distention within the gastrointestinal tract were major factors affecting dry matter intake (**DMI**) in cattle. The rumen-reticulum is the major site in the gastrointestinal tract where the distention mechanism occurs. Tension receptors are stimulated by distension of the rumen-reticulum, which is sensed by satiety centers in the brain signaling the end of a meal. Dietary forage neutral detergent fiber (**NDF**) content, physical form of fibrous feed, NDF digestibility, and fiber particle fragility are some of the most critical feed factors that influence intake by inducing rumen distension and fill (Allen, 2000).

The chemical composition of hand plucked or grazing horizon samples of well managed tropical pastures collected during Spring, Summer and Fall are presented in Table 1. It is noted that even in well managed tropical pastures, forage NDF content is high, varying from 54.2 to 66.3%. Recently, Lopes (2011) characterized the nutrient profile and fiber digestibility of the main tropical grasses produced under intensive rotational grazing management in Brazil. One-hundred and six samples of Palisade grass (*Brachiaria brizantha*), Mulato grass (*Brachiaria hibrida*), Bermuda grass (*Cynodon dactylon*), African Bermuda grass (*Cynodon nlemfuensis*), Guinea grass (*Panicum maximum*) and Elephant grass (*Penisetum purpureum*) were used. Tropical grasses presented 14 to 21% crude protein (**CP**) and 60 to 63% NDF. Comparison between mean of *in vitro* NDF digestibility (**IVNDFD**) estimates of tropical grasses by time-point and a standard sample of alfalfa silage indicated that the tropical grasses had higher fiber digestibility than that obtained from alfalfa (Figure 1). The author concluded that tropical forages subjected to intensive grazing management practices can be relatively high in CP content and fiber digestibility (Lopes, 2011). Despite the lower IVNDFD, alfalfa presents less NDF content with more fragile particles. Allen (2000) reported that lactating cows had greater DMI when diets contained alfalfa silage compared with grass silage despite the higher NDF digestibility found in the latter source of forage. The author suggested that alfalfa caused less rumen filling because particles were more fragile and resulted in a decreased retention time in the rumen-reticulum, thereby causing less distention and allowing increased DMI. Romero (2008) evaluated *in situ* DM digestibility of Elephant grass (*Penisetum purpureum*) under intensive rotational grazing management. The author reported that DM effective digestibility was 55.9%. Furthermore, Pacheco Junior (2009) reported that DM effective digestibility of Palisade grass (*Brachiaria brizantha*) was 58.8% and for Mulato grass (*Brachiaria hibrida*) it was 58.7%.

Collectively, these data indicate that total NDF content, particle structure, and digestibility of NDF of tropical forages may all limit forage intake because of rumen fill, which in turn reduces energy intake by grazing cattle.

Sward Structure

The discussion above highlighted the importance of the physiological mechanisms regulating forage intake. However, for grazing cattle, before forage reaches the gastrointestinal tract, it has to be harvested by the animal. Hodgson et al. (1977) reported that the structure of the sward may have a greater impact on forage

DMI in grazing cattle than the physiological mechanisms discussed by Conrad et al (1964) and Allen (2000).

The structure of the sward has a great impact on grazing efficiency in cattle on either temperate grasses (Hodgson et al., 1994) or on tropical grasses (Da Silva and Pedreira, 1996; Carvalho et al., 2001; Da Silva et al., 2009). Shortly after harvesting, the grass initiates its regrowth with intense appearance and growth of leaves and minimal stem elongation. This process continues until the sward reaches a certain height and light starts to become limiting for the lower portions of the sward. That point is when more leaves become senescent and most of the elongation of stems happens. The result is an increased proportion of senescent material, a greater participation of stems and a decreased proportion of green leaves in the sward, altering the grazing efficiency and forage intake (Dórea et al., 2013a,b).

Rotational grazing, based on fixed or pre-established number of days between grazing activities, has been criticized by Da Silva and Corsi (2003). Grass dry matter production is dictated by the genetic potential of the grass variety and by environmental conditions such as temperature, sun light, soil fertility, and water availability. As these conditions vary, grazing interval must vary as well. The ideal start grazing point should be based on plant physiology aspects instead of on fixed grazing intervals.

The concept of an ideal start grazing point originated from studies with temperate grasses using the 95% light interception (LI) criterion by Hodgson (1990), which has been successfully applied to tropical grasses (Da Silva and Nascimento Júnior, 2007). The latter authors reported a number of studies conducted in Brazil with various tropical grasses and a range of canopy height values that correlated to 95% LI. The studies reported by Da Silva and Nascimento Júnior (2007) indicated that the canopy heights correlating to 95% LI vary with time of the year, but a much greater degree of variation was observed between plant species than within species. Canopy heights correlating to 95% LI in various studies with tropical grasses are presented in Table 2.

Morphological composition of plants managed based on 95% LI criterion as the start grazing point presented significantly higher proportion of leaves and less proportion of stems and senescent material compared with plants managed with fixed grazing intervals (Table 3). Carvalho et al., (2001), pointed out that the structure of the sward strongly influences forage intake in grazing cattle, having more effect than the physiological and chemical mechanisms discussed by Conrad et al (1964) and Allen (2000) due to its impact on the forage harvesting process. The impact of the sward structure on forage intake in grazing cattle has been reported by several other authors (Da Silva and Carvalho, 2005; Casagrande, 2010; Vieira, 2011; Paula et al., 2012).

There is scarce literature on animal performance studies in which the 95% LI criterion was compared with fixed grazing interval for tropical grasses. Gimenes et al. (2011) compared two sward heights as the start grazing point, 25 cm (95% LI) and 35 cm (approximately 100% LI) using Nellore yearling bulls grazing Palisade grass (Table 4). In both treatments pastures were grazed down to 15 cm height. Animals grazing the

pastures managed based on 95% LI criterion gained faster and stocking capacity was greater. A relatively small modification on pasture management resulted in 55% increase in total BWG/ha. Voltolini et al., (2010) reported increased milk production, stocking rates and consequently increased production per area when cows grazed pastures of *Elephant grass* cv. Cameroon managed using 95% LI (103 cm canopy height) as the grazing starting point compared with cows grazing paddocks managed with 27 days fixed grazing intervals (Table 5). Post grazing sward heights were 62 and 71 cm for the 95% LI and for the 27 d fixed grazing interval respectively. Carvalho et al. (2001), Da Silva and Carvalho (2005), Casagrande (2010), Vieira (2011) and de Paula et al. (2012) reported greater DMI of cattle grazing pastures with better sward structure. Carvalho (1997) and Pedreira et al. (2005) reported that cattle grazing pastures with same initial forage mass but with different sward structures had different forage intake and performance.

Collectively, these data support the positive responses of beef and dairy cattle to pasture management based on the 95% LI criterion. Applying these concepts result in increased nutrient intake, primarily calories, because more forage is consumed under shorter grazing times that result in less energy expenditure, as reported by Dórea et al. (2013a,b). The greater stocking rate is the result of more efficient grazing process, with less wasted forage material.

In order to maximize forage intake of grazing animals, the ideal point to remove them from paddocks is as important as the entrance height. Mezzalira (2012) demonstrated that forage ingestion rate (g of DM/min) was depressed drastically when cattle was forced to graze down more than 40% of the initial sward height (Figure 2). Therefore, based on the discussion above, combining the 95% LI criterion to start grazing with removal of cattle when 40% of sward initial height is grazed down maximizes forage intake and animal performance. Nevertheless, meat production per area may be maximized with more severe grazing due to greater stocking capacity (Sarmento, 2007). Feeding concentrates high in energy is a tool to increase grazing cattle performance, stocking rate and beef and milk production per area (Correia, 2006, Macedo, 2012), which can minimize the detrimental effects of severe grazing on individual animal performance (Costa, 2007).

Energy Supplementation of Grazing Cattle

Beef Cattle

Multiple experiments have been conducted by our group to address performance of growing beef cattle under intensive grazing of tropical pastures with supplementation. The positive effect of energy supplementation of yearling bulls grazing Palisade grass (12.5% CP and 57% NDF, hand plucked) on rotational system during summer and autumn, using N fertilization, is presented in Table 6. Cattle were supplemented daily with increasing levels of a 20% CP supplement (Correia, 2006). Compared with non-supplemented animals (control group), supplementing at 0.9% of BW, increased ADG by 63%, pasture stocking rate by 36%, and carcass yield per hectare by 91% (Correia,

2006). Dórea (2011) tested multiple feeding schemes (0, 0.3, 0.6 and 0.9% BW as fed) of a supplement containing finely ground corn, mineral mix and monensin (mineral and monensin concentrations varied to supply same amount per treatment) using rumen cannulated Nellore steers grazing Palisade grass (13% CP; 62% NDF, hand plucked) on a rotational system (25 cm entrance height and 15 cm stubble height) (Table 7). The author found that supplementation up to 0.9% of BW had no negative effect on fiber digestibility. Feeding 0.6 or 0.9% BW had little impact on grazing time and forage intake compared with the 0.3% level. Substitution was greater for steers receiving the 0.3% supplement, which might have been caused by shifts in grazing behavior. The energy intake data of the metabolism study corroborates with performance results from the study of Correia (2006) presented in Table 6. Feeding 0.3% had no significant effect on ADG and had a large impact on pasture stocking rate. In conclusion, in intensive rotational grazing systems using tropical forages, it is necessary to supplement more than 0.3% BW of an energy supplement to increase energy intake, ADG and beef production per area.

Agostinho Neto (2010) compared two strategies to supplement energy (ground corn + monensin) to growing cattle grazing Palisade grass (11.9% CP; 66.3% NDF, hand plucked) during autumn, when forage dry matter production is low. Pastures were managed on rotational system based on the 95% LI criterion as the start grazing point. The experiment lasted 149 days. Cattle were fed no supplement (0%), or were supplemented daily with 0.6% BW for the entire experimental period or with 0.3% of BW for the first 65 days, 0.6% for the next 51 days and then 0.9% for the last 33 days of experimental period (Table 8). In general, feeding more supplement by the end of pasture growing season was more beneficial. Energy supplementation helps alleviate depression in animal performance when pastures are grazed down to lower stubble heights. Costa (2007) evaluated the efficacy of energy supplementation (0.6% BW) for cattle grazing Palisade grass pastures (15% CP; 64% NDF) managed on a rotational grazing system using the 95% LI criterion as the start grazing point (25 cm height) and two stubble heights, 15 or 10 cm (Table 9). The author found that the detrimental effects of more severe grazing on individual performance were minimized when cattle were supplemented, while higher stocking rates were achieved.

To better understand the interactions between pasture management practices and energy supplementation on forage and energy intake by grazing cattle, 7 metabolism studies were conducted by our research group. In Exp. 1 (Figures 3, 4, 5, 6 and 7), 8 rumen cannulated Nellore steers grazed Palisade grass managed with 25 vs. 35 cm sward height as the start grazing point, both treatments with 15 cm stubble height, supplemented (0 vs. 0.6% BW as fed) with energy (ground corn + monensin) (Dórea et al., 2013a). Cattle grazing pastures managed based on the 95% LI criterion (25 cm) spent less time grazing and more time resting (Figure 3), had increased biting rate (Figure 5), and consumed more forage dry matter, more total dry matter and more digestible dry matter (Figure 6) compared with cattle grazing pastures managed with 35 cm sward height as the start grazing point. Supplying a feed supplement at 0.6% BW decreased grazing time (Figure 3), but had no effect on biting rate (Figure 5), decreased forage DMI, but increased digestible dry matter intake (Figure 6) compared with no

supplementation. Pasture management (25 vs. 35 cm) increased intake of digestible dry matter by 43% (1.42 vs. 0.99% BW), whereas supplementation at 0.6% of BW increased intake of digestible dry matter by 29.5% (1.36 vs. 1.05% BW) (Figure 6). The reason for these differences is that adoption of the 95% LI criterion increased total DMI from 1.52 to 2.18% BW, whereas supplementation at 0.6% BW caused a nonsignificant increase in total DMI (1.77 up to 1.93% BW). There was an interaction between sward height and supplementation (Figure 4). The decrease in grazing time and, consequently, the substitution of grain for forage was greater for pastures managed at 35 cm height most likely because cattle stopped grazing earlier in pastures with less favorable sward structure. In Experiment 2, the supplementation level was reduced to 0.3% BW (Figures 7, 8 and 9). Most results observed in this study were similar to the those of Experiment 1, except that supplementation affected digestible dry matter intake. Because of the high substitution rate, feeding only 0.3% resulted in decreased forage intake and less total DMI. The extra energy from supplement did not compensate the decreased energy intake from forage. This result is in agreement with previous studies from Correia (2006) and Dórea (2011) (Tables 6 and 7).

According to Carvalho et al. (2009) and Mezzalira (2012), forage dry matter ingestion rate is not decreased until cattle grazes down to 40% of the pre grazing sward height. In Experiments 1 and 2 from Dórea et al. (2013), post grazing height was the same, managed at 15 cm for both pre-grazing heights (25 vs. 35 cm). Raising the post grazing height to 21 cm for Palisade grass managed with 35 cm could theoretically alleviate part of the negative effects on cattle ingestive behavior, because it would represent 40% of the initial height. Preliminary data from Experiments 3, 4, 5 and 6 (Agostinho Neto, unpublished results; Santos, unpublished results) and data from Difante et al. (2009) corroborate this hypothesis.

Dairy Cattle

The main objective of supplementation of grazing dairy cows is to increase DMI and energy intake relative to that achieved with diets based exclusively on grazed forages. In a review, Bargo et al. (2003) stated that in temperate pastures with concentrate supplementation ranging from 0 to 10 kg DM/d, milk responses ranged from 0.60 to 1.45 kg milk/kg concentrate, and overall milk yield increased at 1 kg milk/kg concentrate. Macedo (2012) reviewed studies using supplements fed from 1 to 11 kg DM/d to dairy cows grazing tropical pastures. The author reported that milk yield increased at 1.42 kg milk /kg concentrate.

The addition of fat sources and more extensive processed cereal grains are efficient methods to increase energy density of supplements for dairy cows. Two studies were conducted by our research group to address fat supplementation and steam-flaking of corn with flint endosperm fed to lactating cows grazing tropical grasses. Souza et al. (2013) supplemented early lactation crossbred ½ Holstein ½ Jersey cows grazing elephant grass with 8 kg DM concentrate (ground corn + soybean meal + minerals and vitamins) with the addition or not of 400 g/cow/day of calcium salts of palm oil or of soybean oil from day 15 until 105 postpartum. After that period, the residual effect of

lipid supplementation was evaluated until 280 DIM (Figure 10; Table 10). Calcium salts of palm oil supplementation was effective to increase yields of milk and total solids and had a residual effect on milk and total solids production from day 105 to 280 post calving. Calcium salts of soybean oil depressed DMI and NDF total tract digestibility, caused severe milk fat depression and did not influence yield of total solids throughout the lactation. Batistel et al. (unpublished) supplemented early lactation cows grazing Elephant grass with 9 kg DM concentrate with or without 400 g/cow/day of calcium salts of palm oil associated with two corn processing methods, ground vs. steam-flaking (Table 11). Both fat supplementation and steam-flaking of corn of flint endosperm increased milk yield and these effects were additives. However fat supplementation caused greater increase in milk yield than corn processing. In addition, steam-flaking or corn had a pronounced effect on milk protein content.

Conclusion

Increments in productivity of grazing productions systems can be met with current technology simply by changing management practices such as when animals should be placed or removed from pastures. More favorable sward structure may be reached when the 95% light interception criterion as the start grazing point is adopted, resulting in positive effects on forage intake and energy saving by grazing cattle.

In intensive rotational grazing systems, supplementing the diet of cows with ingredients rich in fermentable carbohydrates increases animal performance because of increased digestible dry matter intake and decreased energy expenditure for grazing activity. Supplementation also buffers the negative effects of more severe grazing on energy intake, which allows increments in pasture stocking rate. However, these benefits are dose dependent.

For dairy cows grazing tropical grasses, the supplementation of calcium salts of palm oil is an effective method to increase intake of digestible energy, in particular during early lactation, which results in beneficial effects on performance that last beyond the period of supplementation.

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Table 1. Chemical composition (% DM) of hand plucked or grazing horizon samples of well managed tropical pastures collected during spring, summer and fall

Forage	CP[1], %	NDF[2], %	Reference
Brachiaria brizantha cv. Marandu	12. 6	57. 4	CORREIA (2006)
Brachiaria brizantha cv. Marandu	13. 6	56. 2	CORREIA (2006)
Brachiaria brizantha cv. Marandu	15. 3	65. 0	COSTA (2007)
Brachiaria brizantha cv. Marandu	15. 4	63. 9	PACHECO, JR. (2009)
Brachiaria brizantha cv. Marandu	11. 9	66. 3	AGOSTINHO NETO (2010)
Brachiaria brizantha cv. Marandu	13. 1	62. 6	DÓREA (2011)
Pennisetum purpureum cv. Cameroon	13. 7	62. 9	MARTINEZ (2004)
Pennisetum purpureum cv. Cameroon	14. 6	65. 1	VOLTOLINI (2006)
Pennisetum purpureum cv. Cameroon	20. 6	63. 2	CARARETO (2007)
Pennisetum purpureum cv. Cameroon	17. 6	64. 4	ROMERO (2008)
Pennisetum purpureum cv. Cameroon	18. 5	61. 4	MARTINEZ (2008)
Pennisetum purpureum cv. Cameroon	18. 5	58. 7	DANÉS (2010)
Pennisetum purpureum cv. Cameroon	15. 5	60. 2	CHAGAS (2011)
Pennisetum purpureum cv. Cameroon	18. 6	54. 4	MACEDO (2012)
Pennisetum purpureum cv. Cameroon	18.3	54.2	SOUZA (2014)

[1] CP = Crude protein; [2]NDF = Neutral Detergent Fiber

Table 2. Suggested sward canopy and stubble heights for pastures managed based on 95% light interception (LI)

Plant spp.	Canopy height (cm)		Reference
	Entrance	Exit	
Mombaça	90	30 to 50	Carnevalliet al., (2006)
Tanzânia	70	30 to 50	Difante, (2005); Barbosa et al., (2007)
Xaraés	30	15 to 20	Pedreira, (2006)
Cameroon	100	40 to 50	Voltolini, (2006); Carareto, (2007) Zeferino, (2006); Sarmento, (2007);
Marandu	25	10 to 15	Costa (2007); Souza Júnior, (2007); Trindade, (2007)
Tifton-85	25	10 to 15	Da Silva and Corsi (2003)
Coastcross	30	10 to 15	Da Silva and Corsi (2003)

Mombaça = *Panicum maximum* cv. Mombaça; Tanzânia = *Panicum maximum* cv. Tanzânia; Xaraés = *Brachiaria brizantha* cv. Xaraés; Cameroon = *Pennisetum purpureum* cv. Cameroon; Marandu = *Brachiaria brizantha* cv. Marandu; Tifton-85 = *Cynodon dactylon* cv. Tifton-85; Coastcross = *Cynodon dactylon* cv. Coastcross

Table 3. Morphological composition of plants managed either based on 95% light interception (LI) criterion or on fixed grazing intervals

Reference	Forage	Grazing frequency	Leaves % DM	Stem % DM	Senescent material % DM
Voltolini et al.(2010)	Cameroon	27 fixed days	48.0	46.0	6.0
Voltolini et al. (2010)	Cameroon	95% LI	53.0	42.0	5.0
Correia (2006)	Marandu	21 fixed days	34	32.7	33.3
Costa (2007)	Marandu	95% LI	56	32	12

Cameroon = *Pennisetum purpureum* cv. Cameroon; Marandu = *Brachiaria brizantha* cv. Marandu

Table 4. Effect of start grazing point (25 vs. 35 cm sward height) on performance of Nellore yearling bulls grazing Palisade grass (Brachiaria Brizantha cv. Marandu)

Sward height	Daily gain, kg	Stocking rate, AU/ha[1]	Kg live weight gain/ha
25 cm	0.957	2.88	621
35 cm	0.769	2.43	401

Gimenes et al. (2011). Average values for summer and fall season.
[1] AU = animal unit (450 kg body weight); ha = hectare

Table 5. Effect of start grazing point (103 cm sward height vs. 27 day of fixed grazing interval) on performance of dairy cows grazing Elephant grass cv. Cameroon

Item[1]	27 d interval	95% LI (height of 103 cm)	P
3.5% FCM, kg/d	14.88	17.65	0.10
Cows/ha	5.1	7.2	0.002
Milk, kg/ha/d	75	114	0.0004

Voltolini et al. (2010).
[1] FCM = fat-corrected milk; ha = hectare.

Table 6. Effect of level of supplementation (% BW) on performance of grazing yearling bulls

	Supplementation level (% BW as fed)					
	0	0.3	0.6	0.9	Intercept a[7]	Slope P[9]
FA,[1] % BW	6.92	6.81	6.52	6.11		
AU/ha[2]	4.50	5.33	5.58	6.12	4.62 (0.26) **	1.70 (0.46) **
Initial BW, kg	223.0	226.0	218.0	219.0	225.45 (3.16)**	-7.31 (5.60) NS
Final BW, kg	287.8	301.1	308.6	320.3	288.50 (4.55)**	35.11 (8.06) **
Daily gain, kg	0.595	0.673	0.810	0.968	0,583 (0.04)**	0.408 (0.07) **
@/ha[3]	18.06	22.50	29.76	34.45	17.72 (0.70)**	18.80 (1.25) **

[1] Forage availability, DM as % of BW
[2] AU = 450 kg BW
[3] @ = 15 kg of carcass
( ) = Error
*Linear (P<0,05)
**Linear (P<0,01) t-Student test
Correia (2006)

Table 7. Effect of level of supplementation on performance of canulated grazing cattle

	Supplementation, % BW[1] as fed						Contrast[2]	
	0	0.3	0.6	0.9	SEM	P	Lin	Quad
DMI, % BW	1.90	1.94	2.10	2.35	0.12	0.01	0.05	ns
Forage DMI, % BW	1.90	1.64	1.55	1.50	0.11	0.02	0.05	ns
TDN[3] intake, % BW	1.10	1.21	1.39	1.62	0.10	0.01	0.05	ns
Substitution, kg/kg	0	1.13	0.74	0.58	0.21	0.02	ns	0.05
Grazing, min/d	441	385	372	363	9.82	0.04	0.05	ns
Rumination, min/d	395	399	358	378	9.38	0.31	-	-
Resting, min/d	441	515	548	551	6.37	0.09	0.05	ns

Dórea (2011).
[1] BW = body weight.
[2] Lin = linear effect of level of supplementation; Quad = quadratic effect of level of supplementation.
[3] TDN = total digestible nutrients.

Table 8. Effect of supplementation strategy on performance of crossbred yearling bulls

Item[2]	Supplementation strategy[1]			SEM	P
	0	0.6%	0.3 - 0.6 - 0.9		
Initial body weight, kg	208.0	207.7	208.4		
Final body weight, kg	283.7	322.4	335.7		
Supplement, kg	0	230.4	220.4		
Daily gain, kg	0.535[c]	0.787[b]	0.867[a]	0.016	0.001
Animal unit/hectare	5.94[b]	7.13[a]	6.90[a]	0.01	0.04
BW gain, kg/hectare	887[c]	1464[a]	1580[a]	276.0	0.009

Agostinho Neto (2010).
[1] Supplement offered daily as % of body weight either as constant % or at increasing %. 0 = no supplementation; 0.6 = 0.6% of BW for the entire experimental period (1490 d); 0.3-0.6-0.9: or supplemented with 0.3% of BW for 65 days, 0.6% for the next 51 days and 0.9% for the last 33 days of experimental period.
[2] Animal unit = 450 kg of body weight (BW).

Table 9. Effect of level of supplementation (0 vs. 0.6% of body weight) for cattle grazing Palisade grass managed with different stubble heights (11 vs. 15 cm)

Item[3]	Treatment[1]				P[2]		
	11 cm 0%	11 cm 0.6%	15 cm 0%	15 cm 0.6%	SH	S	SH x Sup
Daily gain, kg	0.38[d]	0.76[b]	0.51[c]	0.86[a]	0.008	0.001	0.54
Animal unit/ha	7.1[ab]	8.5[a]	5.8[b]	6.1[b]	0.009	0.12	0.36
BW gain kg/ha/d	5.7[b]	11.4[a]	5.6[b]	9.1[a]	0.48	0.02	0.52
Final BW, kg	284[c]	351[a]	306[b]	369[a]	0.04	0.001	0.82

Costa (2007).
[1] Stubble height of 11 vs. 15 cm; Level of supplementation of 0 vs. 0.6% of the body weight.
[2] SH = stubble height; Sup = level of supplementation; SH x Sup = interaction between SH and Sup.
[3] ha = hectare; BW = body weight.

Table 10. Milk yield and composition of grazing cows fed different fat sources

	Treatment[1]				
	Control	CSSO	CSPO	SEM	P
Initial BW, kg	486.5	493.3	496.2	25.2	0.43
Body condition at 75 DIM	2.75^{ab}	2.84^{a}	2.54^{b}	0.08	0.09
Dry matter intake, kg/d	17.9^{ab}	17.2^{b}	18.0^{a}	0.42	0.04
Yield, kg/d					
Milk	6094^{c}	6575^{b}	7328^{a}	28.8	0.001
Fat	234.1^{b}	219.2^{c}	266.6^{a}	5.2	0.001
Crude protein	212.1^{b}	213.5^{b}	232.6^{a}	7.8	0.01
Lactose	274.9^{b}	290.1^{b}	326.2^{a}	8.6	0.03
Total solids	779.4^{b}	784.3^{b}	894.9^{a}	18.4	0.001

Souza et al. (2013).
[1] CSSO = calcium salts of soybean oil; CSPO = calcium salts of palm oil

Table 11. Total milk production and composition of cows according to fat supplementation and method of corn processing

	Ground		Steam-flaked		SEM	P		
	No fat	Fat	No fat	Fat		Corn	Fat	Corn*Fat
Milk yield, kg/d	20.3^{d}	24.0^{b}	22.3^{c}	25.1^{a}	0.35	0.001	0.0001	0.15
Fat, %	3.33^{a}	3.34^{a}	3.26^{ab}	3.18^{b}	0.054	0.01	0.33	0.26
Crude protein, %	3.17^{c}	3.13^{c}	3.46^{a}	3.36^{b}	0.038	0.0001	0.18	0.04
Lactose, %	4.64	4.62	4.61	4.62	0.062	0.68	0.32	0.27

Batistel (unpublished results).

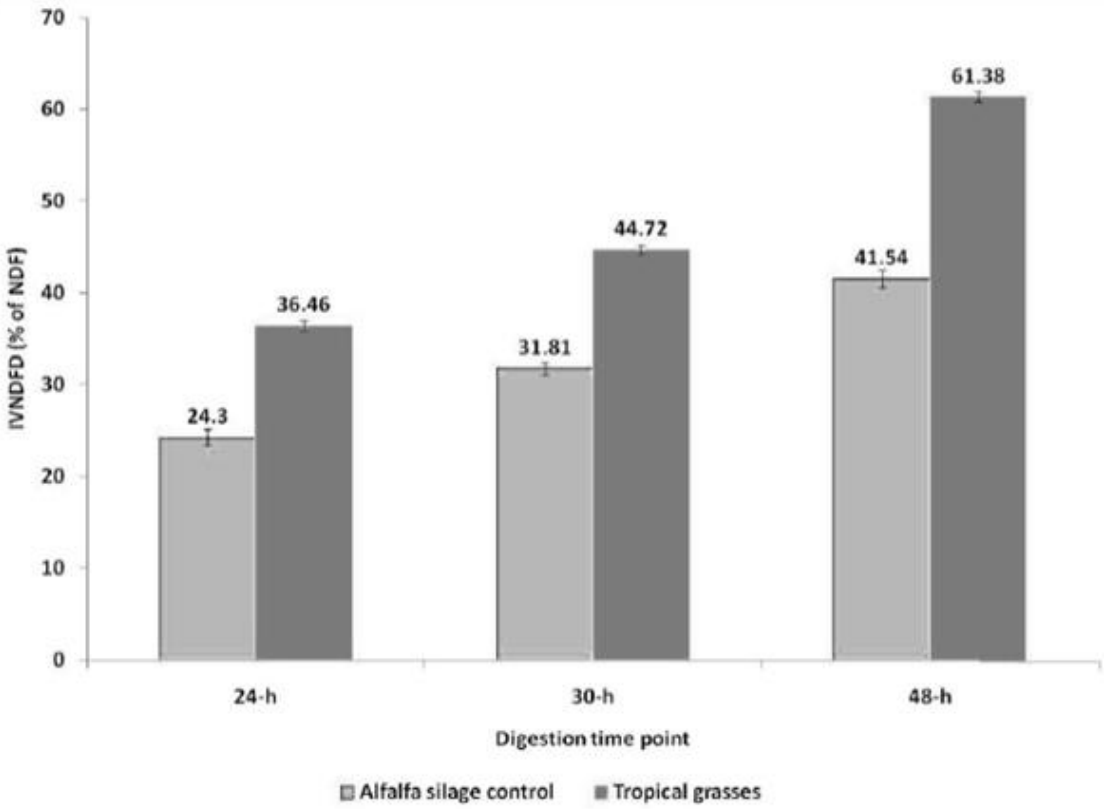

Figure 1. Comparison between mean *in vitro* NDF digestibility (IVNDFD) estimate across 6 tropical grass species and the alfalfa silage standard by time point (Lopes, 2011).

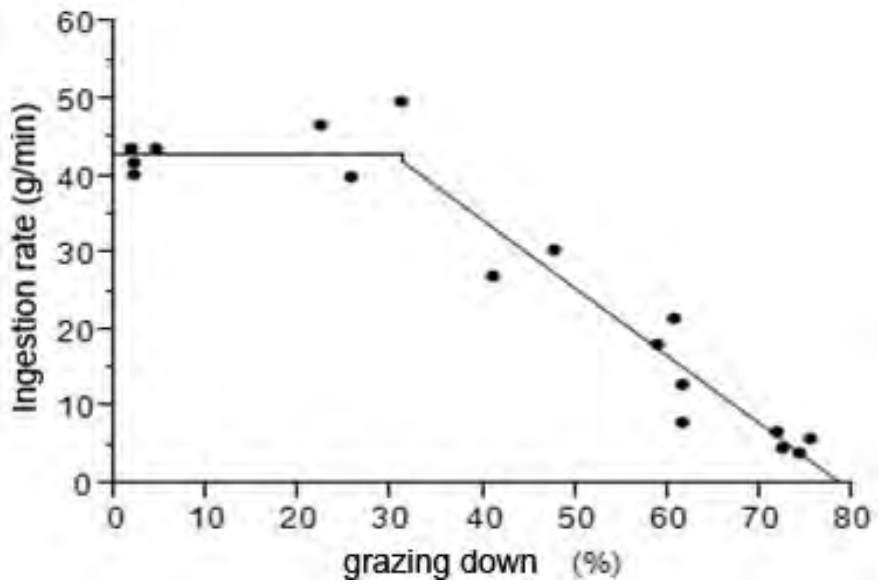

Figure 2. Effect of final grazing point (as % of initial sward height) on forage ingestion rate (Mezzalira, 2012)

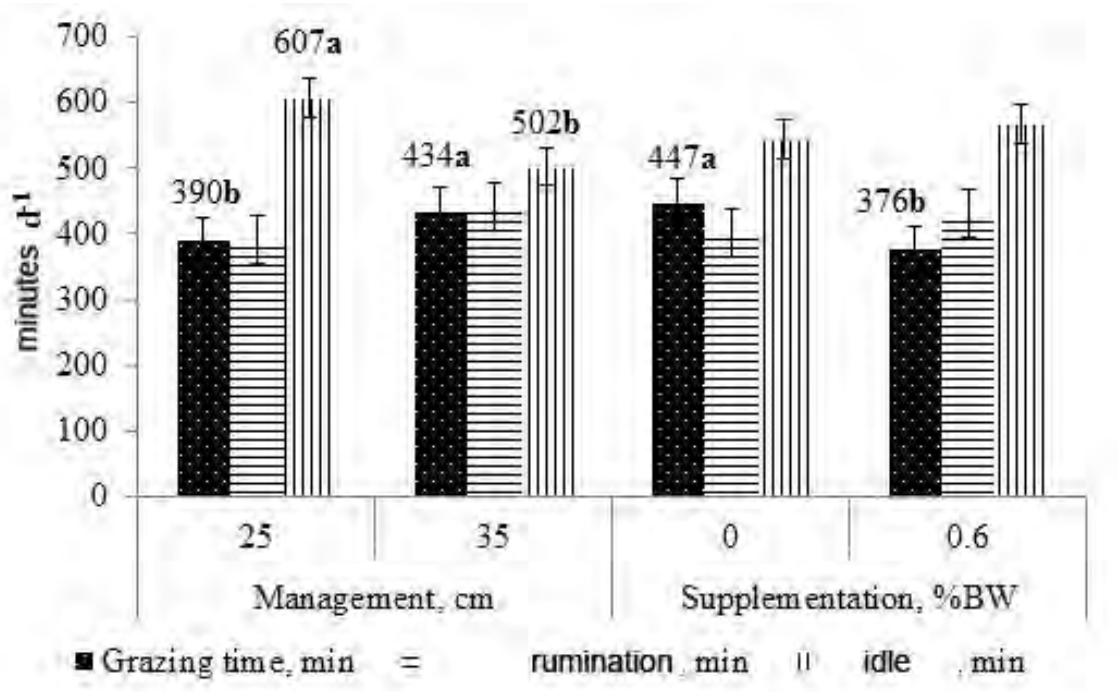

Figure 3. Ingestive behavior of grazing cattle according to management of plant height (25 vs. 35 cm) and level of supplementation 0 vs. 0.6% of body weight). [a,b] Different superscripts differ ($P < 0.05$). (Dórea et al., 2013b).

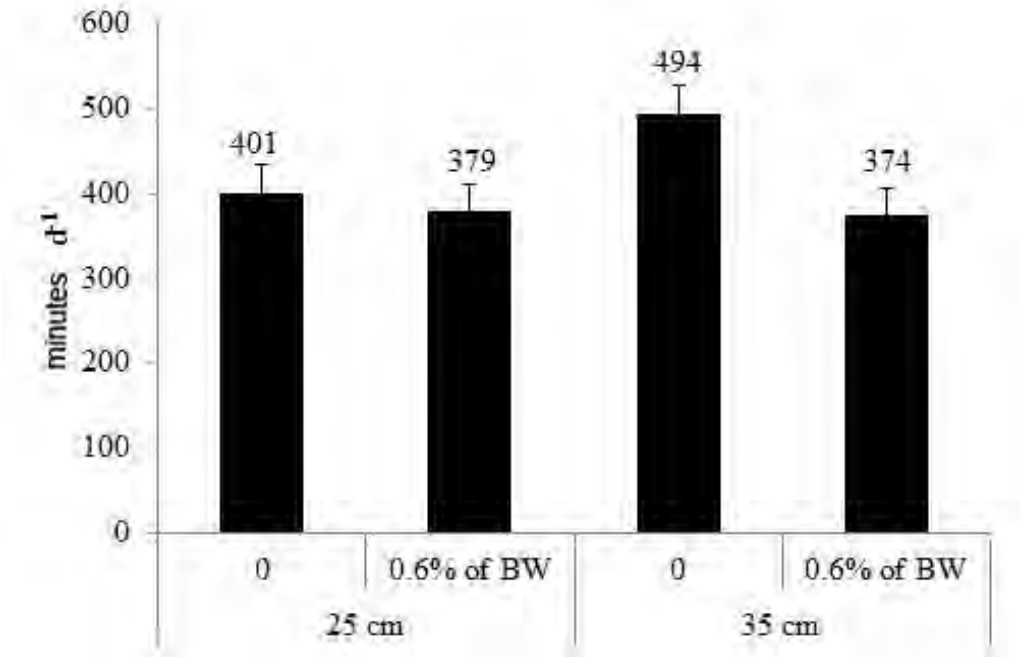

Figure 4. Ingestive behavior of grazing cattle according to plant height and level of supplementation. [a,b] Different superscripts differ (P < 0.05). (Dórea et al., 2013b).

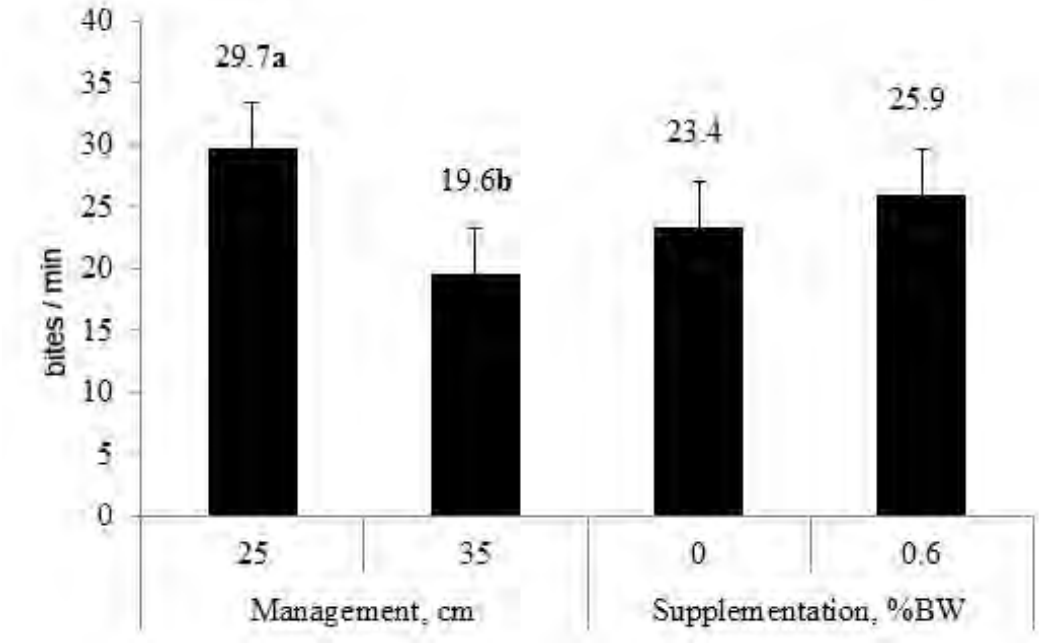

Figure 5. Effects of plant height (25 vs. 35 cm) and level of supplementation (0 vs. 0.6% of BW) on bite rate of grazing cattle. [a,b] Different superscripts differ (P < 0.05). (Dórea et al., 2013b).

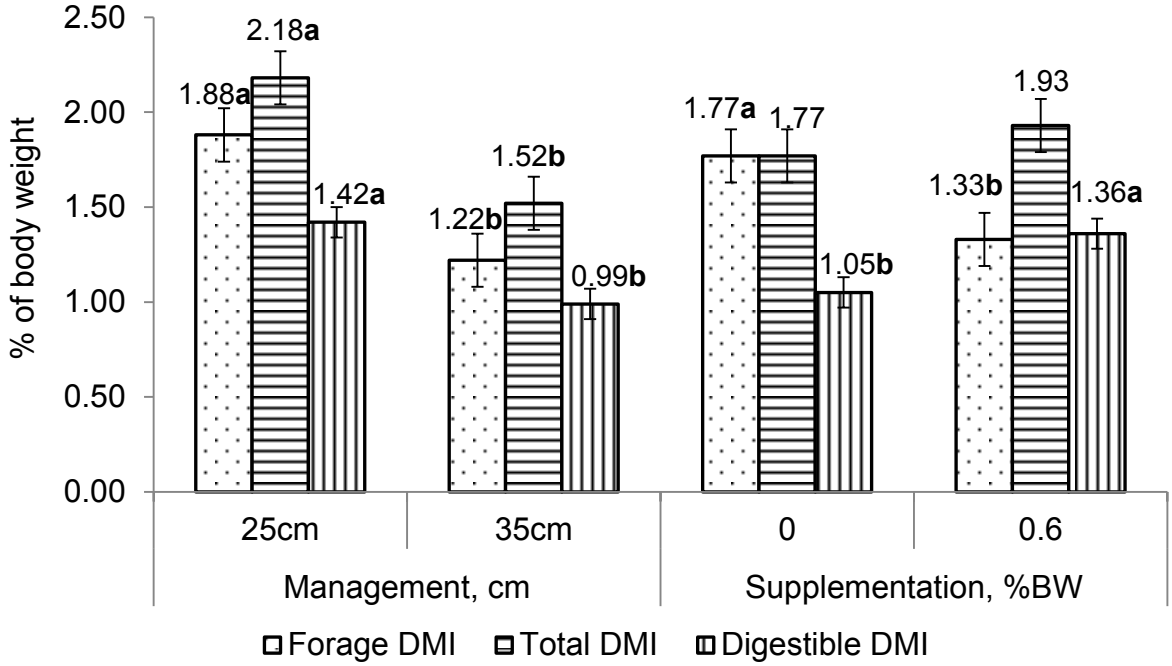

Figure 6. Effects of pasture management (height of 25 vs. 35 cm) and energy supplementation (0 vs. 0.6% of BW) on intake of forage DM, total DM, and calories by grazing cattle. [a,b] Different superscripts differ (P < 0.05). (Dórea et al., 2013b).

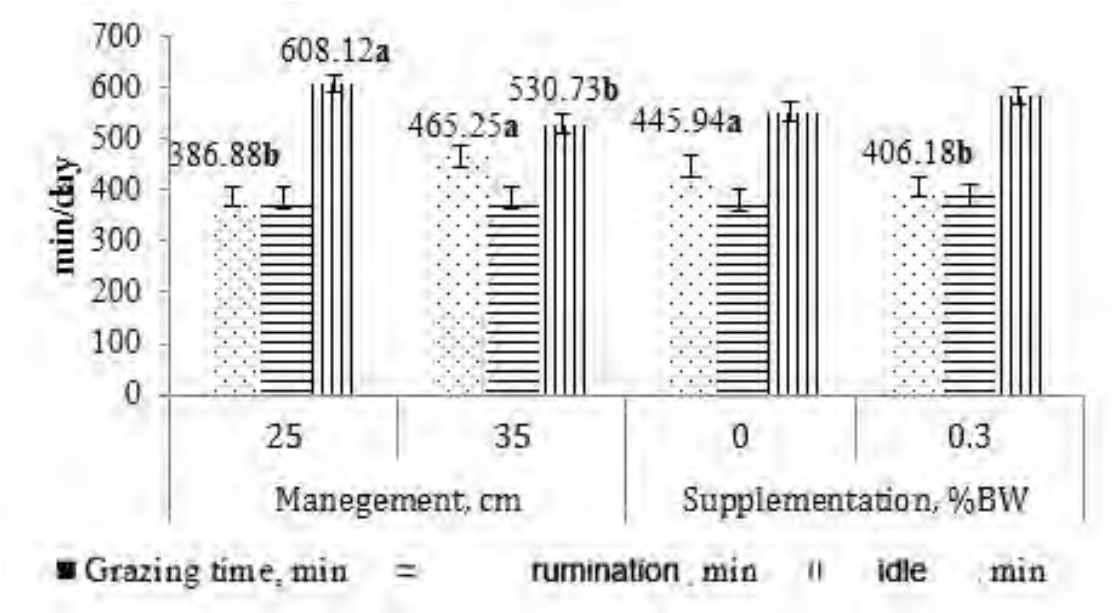

Figure 7. Ingestive behavior of grazing cattle according to plant height (25 vs. 35 cm) and level of supplementation (0 vs. 0.3% of BW). [a,b] Different superscripts differ (P < 0.05). (Dórea et al., 2013a).

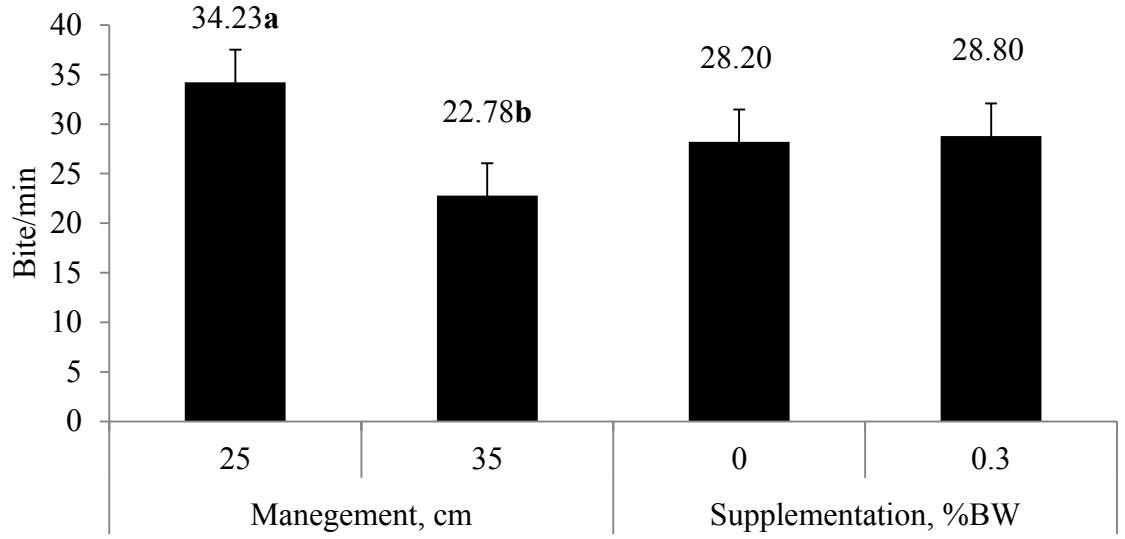

Figure 8. Effect of plant height (25 vs. 35 cm) and level of supplementation (0 vs. 0.3% BW) on biting rate of grazing cattle. [a,b] Different superscripts differ (P < 0.05). (Dórea et al., 2013a).

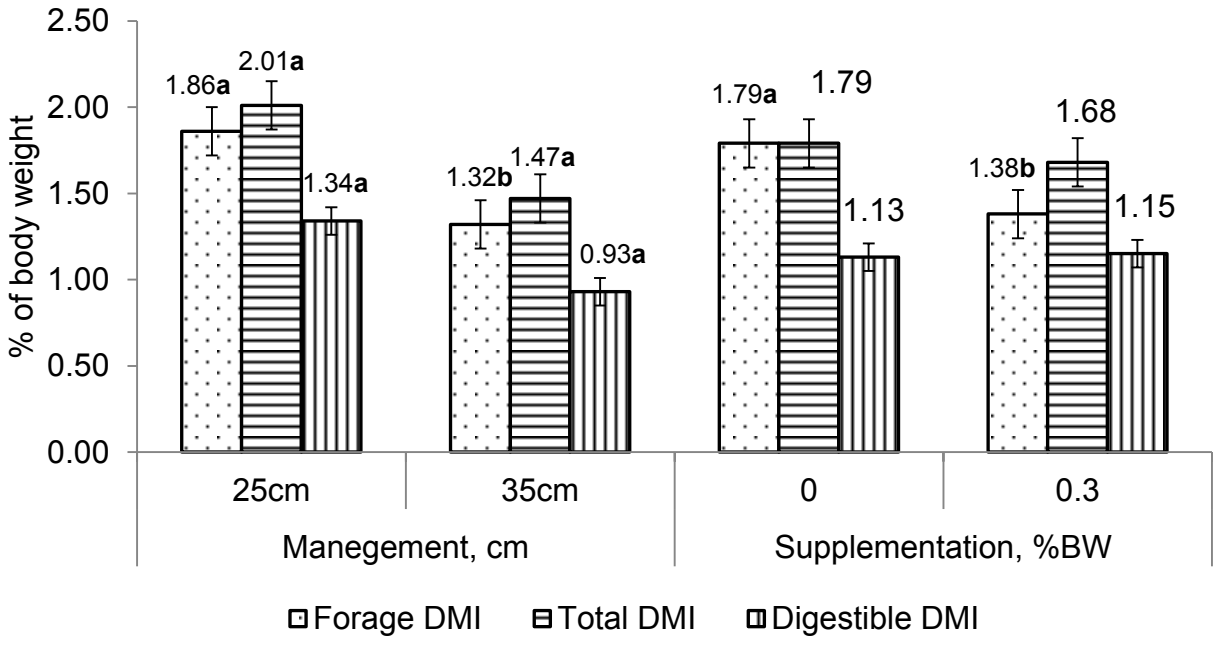

Figure 9. Effect of pasture management (plant height at 25 vs. 35 cm) and energy supplementation (0 vs. 0.3% of body weight per day) on forage dry matter (DM) intake, total DM intake, and digestible DM intake of grazing cattle. [a,b] Different superscripts differ (P < 0.05). (Dórea et al., 2013a).

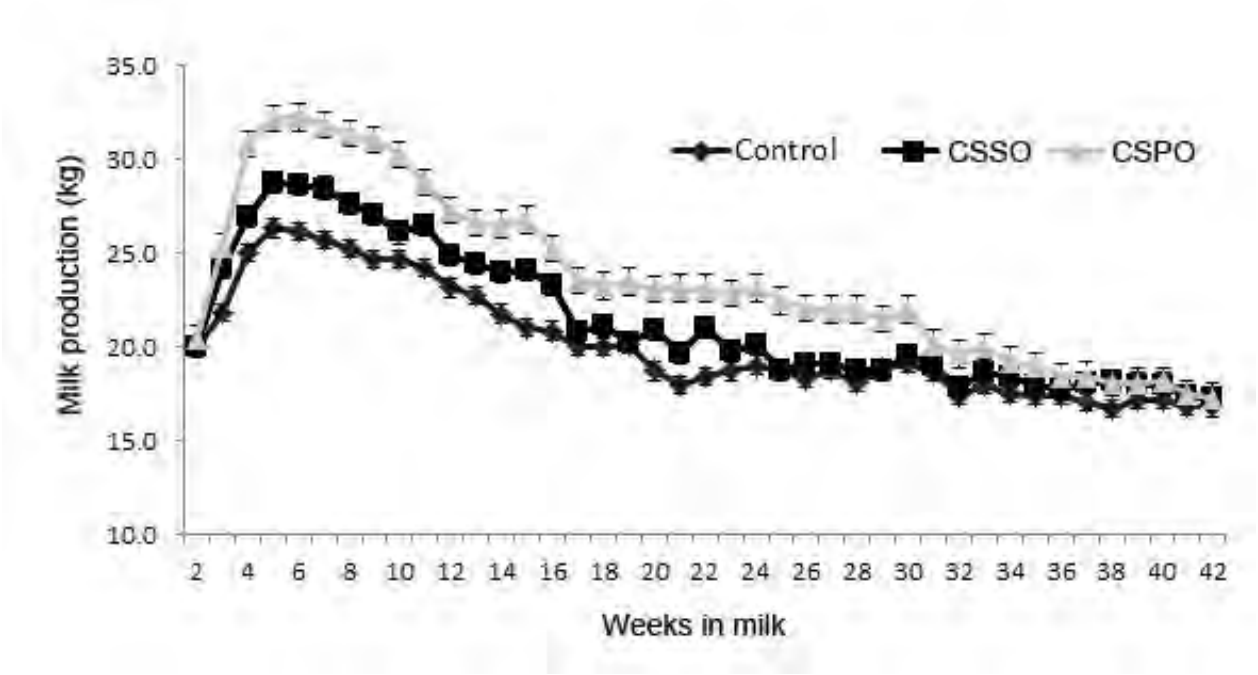

Figure 10. Lactation curves of grazing cows fed no fat (♦), 400g/d of calcium salts of soybean oil (CSSO - ■) or of palm oil (CSPO - ▲) from day 15 to 105 post calving (Souza et al., 2013).

SESSION NOTES

Amino Acid Requirements and Post-Absorptive Metabolism in Cattle: Implications for Ration Formulation

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Introduction

In addition to decreasing feeding costs, improving the efficiency of utilization of N directly addresses the general concern regarding the environmental footprint of animal production. Excretion of N, especially urinary, may become a potential source of water and air pollution, the latter as N_2O, a green-house gas, or as small particulate aerosols having a negative effect on air quality [smog; National Research Council (**NRC**), 2003].

It is now acknowledged that improving the formulation of dairy rations requires accurate estimation of both supply and requirements of metabolizable protein (**MP**), far beyond the sole estimation of crude protein (**CP**). A further step involves the transfer of MP supply and requirement into individual essential amino acids (**AA**). Currently, most of the ration balancing models used in North America [e.g. Amino Cow (Evonik AG Industries, Hanau, Germany); Cornell Net Carbohydrate and Protein System (**CNCPS**, Fox et al., 2004) and the CNCPS-derived Agricultural Modeling and Training Systems (**AMTS**); NRC (2001)] have invested considerable time and effort to develop complex rumen sub-models to improve the predictions of the duodenal flow of proteins that will be digested in the small intestine and available to the cow, defined as MP and associated digestible flows of AA. Although fine-tuning of these models might still be needed to improve predictions with variable feed ingredients, their predictions of duodenal flows of proteins or AA fit quite well with measured values (Pacheco et al., 2012). However, the intensive and regular efforts to improve the predictions of supply over the last decades have not been matched by similar efforts to improve the estimations of requirement. Requirements of MP for maintenance in most ration balancing models are still based on work published almost four decades ago (Swanson, 1977) and both requirement for maintenance and milk protein are calculated using a fixed efficiency of utilization, independent of supply. This simplification of the complex biological event of lactation was necessary as a starting point, because “the knowledge of metabolism of nutrients is not as advanced as the prediction of ruminal fermentation, because of the almost infinite metabolic routes connecting various tissue and metabolic compartments, the multiple interactions, and the sophisticated metabolic regulations that determine the partitioning of absorbed nutrients” (Fox and Tedeschi, 2003). The assumed linear relationship between supply and output arising from the use of a fixed efficiency factor is, however, biologically unrealistic. It is well known that MP-allowable milk is usually overestimated at high protein intakes and underestimated at low protein

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intakes with both NRC (2001) and CNCPS (Fox et al., 2004) ration balancing models. The current review proposes updates on the estimation of the requirement of MP and essential AA for maintenance and milk.

Current Estimations of Requirements

Proteins

This presentation will only deal with requirements for maintenance and lactation, considering mature cows as non-gestating and not changing body weight and composition. Estimation of requirement first requires identification of the proteins and AA that represent a net output, i.e. proteins and AA that will be exported out of the body of the cow. In a second step, the efficiency at which the absorbed protein will be used to support the protein secretion needs to be assigned. Efficiency will be discussed at the end of this section.

Therefore, to begin the first step, definition of the protein required to cover milk protein secretion can be straightforwardly measured as the amount of protein secreted into milk: it currently refers to true protein in NRC (2001) and crude protein in CNCPS (Fox et al., 2004). Unfortunately, the estimation of the protein exported out of the animal to cover requirement for maintenance is not as straightforward as milk protein production. First, the concept of a requirement for maintenance that needs to be fulfilled before milk production occurs does not exist in the lactating dairy cow as the cow will run in negative N balance to support milk production. In addition, estimation of protein requirement for maintenance in the ruminant has always been a challenge due to the inability to feed ruminants a protein-free diet without creating a negative impact on the rumen microflora, a problem exacerbated by the recycling of N into the rumen and associated microbial protein synthesis. In 1977, Swanson made a thorough literature review to estimate “new factors for each of the three loss routes of maintenance nitrogen based only upon appropriate original data from cattle indicative of true maintenance”, these routes being endogenous urinary, scurf and metabolic fecal protein (**MFP**) losses.

Endogenous urinary losses represent the amount of N that would be lost in urine if the animal was consuming a diet adequate in energy but devoid of protein. These losses include creatinine, urea, purine derivatives, nucleic acids, hippuric acid and small quantities of some AA (NRC, 1985). Urinary losses in cattle fed with very low-N diets but adequate energy were used to determine endogenous urinary losses, which were estimated to be 2.75 g CP/kg $BW^{0.50}$ per day (with an efficiency of utilisation of 0.67, this translates to a MP requirement of 4.1 g CP/$kg^{0.50}$). Maintenance requirement for integumental protein include loss and growth of hair, scurf and scales rubbed from the skin surface, along with some N-compounds in skin secretions (Swanson, 1977): requirement were estimated at 0.2 g CP/kg $BW^{0.60}$ per day (MP requirement of 0.3 g CP/$kg^{0.60}$, using the efficiency factor of 0.67).

The last route assessed by Swanson (1977) was MFP, for which it is not easy to have a clear description. For example, the definition of MFP has changed with time, from “a residue of body secretions and tissue incident to movement of food through the gastrointestinal tract” (Swanson, 1977) to the definition of Swanson (1982) adopted in the last NRC (2001) “bacteria and bacterial debris synthesized in the caecum and large intestine, keratinized cells, and a host of other compounds”. The requirement for MFP used by NRC and CNCPS is based on studies of Swanson (1977, 1982). Best correlations were obtained relative to indigestible dry matter (**DM**), but due to uncertainties related to the digestibility of DM, the most recent NRC (2001) has chosen to use DM intake (**DMI**) as the basis to determine MFP (30 g MP/kg DM intake). In addition, the NRC sub-committee estimated that rumen microbial protein (included in the calculation of MFP) should not be included as metabolic loss and, therefore, 50% of the undigested microbial protein is subtracted from this estimation (the other half is assumed to be digested in the hindgut). Therefore, in NRC (2001), the MP requirement for MFP = (DMI (kg) x 30) - 0.50 x ((bacterial MP/0.80) - bacterial MP). The CNCPS currently calculates MFP as 9% of indigestible DM (Fox et al., 2004), as previously estimated by NRC (1989), without any correction for the presence of bacteria. Therefore the CNCPS estimates of MFP are higher than those from the NRC. In addition, NRC (2001) also assumes a requirement for the endogenous flow of protein at the duodenum, but NRC also includes this flow in MP supply.

Table 1 summarizes estimated MP requirement for a “typical” 700 kg cow producing 45 kg milk/d at 3.2% CP (3.0% true protein) and eating 27 kg DM/d with the NRC (2001) and CNCPS (Fox et al., 2004) ration balancing models.

Amino acids

Two different approaches are used to determine the AA requirement. The proportional approach was put forward by NRC (2001) as the sub-committee stated that at that time, “current knowledge (on AA) is too limited to put forth a model that quantifies AA requirement for dairy cattle”. To determine the proportion of each AA needed to maximize milk protein yield or percentage, a dose-response relationship between the % of individual AA in MP supply and milk protein yield or percentage was established, using a broken stick model. The % observed at the breakpoint represents the proportion of this AA in MP supply required to maximize the targeted output. This approach adopted by NRC (2001) yielded recommendations for lysine at 7.08% and 7.24% of MP, and for methionine at 2.35% and 2.38% of MP, for maximal milk protein yield and milk protein concentration, respectively. This is also the approach adopted by the INRA ration balancing model (2007), which states similar recommendations.

On the other hand, the factorial approach cumulates the requirement for individual functions (maintenance and lactation in the current presentation) with a defined AA composition and a defined efficiency of transfer of each digested AA for each function. This is the approach used, for example, by CNCPS (Fox et al., 2004), AMTS and Amino Cow. In addition to the determination of the MP needed to fulfill each function, this approach requires the AA composition of the protein involved in each

function as well as the efficiency with which each digested AA will be used to cover that function. In CNCPS (Fox et al., 2004), milk AA composition is derived from a rather old estimation (Jenness, 1974) whereas the assumed AA composition used for endogenous urinary and MFP is based on that of whole body tissues (Ainslie et al., 1993). For scurf, whole-body tissue AA composition is used (Fox and Tedeschi, 2003), although the AA composition of keratin had been proposed (O'Connor et al., 1993).

Efficiency

In NRC (2001) and CNCPS (Fox et al., 2004), once the "raw" requirement for net protein have been determined for maintenance or lactation, they are translated into a requirement for MP supply, using a single transfer coefficient (0.67 and/or 0.65), except for MFP to which no efficiency factor is applied.

In CNCPS, to each of the maintenance and lactation processes is associated a fixed efficiency of utilization of AA, different for each AA but independent of the supply (Table 2). The efficiencies of utilization of AA for maintenance are largely derived from one article (Evans and Patterson, 1985), averaging 0.85 for all essential AA except the branched-chain AA for which it averaged 0.66. The efficiency of lactation is based on the uptake:output ratio of individual AA across the mammary gland (Fox et al., 2004). Although it is acknowledged that "AA absorbed in excess will be used less efficiently, and those absorbed at levels below requirement will be used with a higher efficiency" (Fox and Tedeschi, 2003), there is no attempt to propose variations in these efficiencies.

Proposed Updates to Requirements

Proteins

As a general statement, protein requirement could be defined as the sum of all the protein needed to support proteins and AA excreted out of the body of the cow. This would include all proteins excreted out of the cow (milk, endogenous fecal protein, scurf), plus N excreted in urine but originating from digested AA (AA-derived compounds plus urea synthesized from AA catabolism). This latter amount represents the difference between 1 (100% efficiency) and the efficiency of utilization of MP or AA. This proposed approach would then eliminate the ambivalence of a so-called "maintenance" requirement which is dependent on DM intake: for example, in our typical cow, can we really refer to maintenance requirement when assessing the MFP of a cow eating 27 kg/day to maintain her high milk production?

Milk protein used to estimate protein requirement should refer to true protein, especially knowing that the proportion of non-protein N can vary with protein supply (e.g. Raggio et al., 2004). In our typical cow, the sum of endogenous urinary plus scurf represents less than 5% of the MP requirement: therefore, the current assumptions can probably be used, at least until the other major contributors to MP requirement are better sorted out. The next version of the French system in preparation, now called

Systali, has however reviewed endogenous urinary requirement and first estimations have almost doubled it. Only the NRC (2001) ration balancing model includes requirement for the flow of endogenous proteins at the duodenum. Based on the definition above, as this flow is not leaving the body of the animal, it should not be included in the requirement per se. Indeed, the fraction that will be digested and re-absorbed does not represent a cost of AA per se for the animal: only the undigested fraction flowing at the ileum and mostly recovered in the feces represents a real cost. However, adequate quantification of the contribution of endogenous protein to the duodenal flow is crucial: this contribution needs to be removed from measured duodenal flow to determine the true net supply, because the endogenous proteins, which may represent up to 15-20% of duodenal CP flow, are not a net contributor to the AA supply (Ouellet et al., 2002, 2007 and 2010).

The largest component of the so-called maintenance requirement is MFP. As discussed previously, this component currently differs substantially between NRC (2001) and CNCPS (Fox et al., 2004, Table 1). Another peculiarity of this component of requirement is that no efficiency of utilization of absorbed protein is applied to it to convert the "exported" protein into requirement. This occurs despite the clear indication given by Swanson (1977): "Furthermore, whichever portion of the fecal N is designated MFN, it is converted to maintenance requirement for protein only when modified by appropriate factors for utilization efficiency of feed proteins". An efficiency factor was not applied as this would have yielded maintenance requirement too high for the MP supplied. This overestimation of MFP is probably due to the methods used in the studies that Swanson (1977) used to obtain that value: for example, included in these estimations of MFP is urea recycled in the rumen and captured by bacteria, which is not per se a requirement on digested proteins. Therefore, we propose to use as an estimation of MFP the ileal flow of endogenous proteins estimated by isotopic dilution in dairy cows (Lapierre et al., 2007; Ouellet et al., 2007). As a first step, these estimations were reported based on DM intake and would average 15.8 g CP/kg DM intake, and using an average proportion of true protein/CP of 0.80 and an efficiency of 0.67 (NRC, 2001), that would yield a requirement of 19 g MP/kg DM intake. Research is currently underway to update these values and determine if DM intake is the major factor affecting the magnitude of MFP.

Amino acids

When using the factorial approach, the proportions for lysine and methionine relative to MP supply proposed by NRC (2001) have been widely used. However, recent analyses conducted by the group of Dr. Schwab clearly indicate that the proportions recommended should be evaluated within each ration balancing model (Whitehouse et al., 2009, 2010a and b). These recommendations can differ substantially between models and also depending of the target, i.e. milk protein yield or milk protein concentration. For example, when assessing the milk protein concentration response, the recommendations for lysine would vary between 6.84% (AMTS) and 7.24% (NRC), depending on the ration balancing model used (Table 3). Similarly, although CNCPS is using a factorial approach, they have estimated, based on their refined feed library

(Higgs et al., 2012) and updated efficiencies of utilization, which will be used in version 6.5, a lysine and methionine requirement of 7.00 and 2.60 of MP, respectively, for maximal milk protein yield (van Amburgh et al., 2013).

When using the factorial approach, two factors need to be known to transfer the protein exported out of the body into AA requirement: the AA composition of this protein and the efficiency with which the absorbed AA will be used. Efficiency will be discussed in the following section. For milk AA composition, we propose an update using the AA composition of the reference protein of each protein family (e.g. different caseins, lactalbumin α, different Ig, etc) most recently detailed in the Journal of Dairy Science (Farrell Jr et al., 2004; Lapierre et al., 2012). This update includes all the proteins secreted in the milk and not only the proteins synthesized within the mammary gland. Milk AA composition should be determined based on true protein and not CP as the non-protein N fraction of milk can vary with protein supply (e.g. Raggio et al., 2004). Therefore, adopting a constant AA composition relative to CP in milk could be misleading. Also, despite the common use of the factor 6.38 for the conversion of milk N into CP concentration, the factor 6.34 would be more appropriate (Karman and van Boekel, 1986; personal calculations). Table 4 presents the proposed update of milk AA composition.

As presented previously, MFP is another major source of proteins exported out of the cow. Proteins from endogenous origin flowing at the ileum and excreted in the feces originate from very different sources. They are either constitutive proteins (sloughed cells) or proteins exported out of the cells where they have been synthesized (saliva, enzymes, mucins; Tamminga et al., 1995): therefore, the AA composition of this protein mixture is a challenge to determine, but it is clear that the composition of empty body which is currently being used is not the most appropriate composition. In dairy cows, there are very few studies assessing either directly (Ørskov et al., 1986) or indirectly (Larsen et al., 2000) the AA composition of endogenous protein at the duodenal level and virtually no studies defining AA composition of intestinal endogenous proteins. However, some data are available in pigs for this gut segment (Jansman et al., 2002) and AA composition of intestinal secretion in pigs are close to the AA composition of duodenal flow of endogenous proteins in ruminants. Therefore, we propose, for now, an average of these values (Table 4), which should be closer to the true AA composition of endogenous secretion than that currently used. Obviously, further work is needed to improve the estimation of the AA composition of this important loss of protein.

Efficiency

Either for protein or for AA, once the exported proteins have been identified and quantified, to calculate the requirement, we need to assess with which efficiency the absorbed protein or AA will be used to support protein synthesis. Although is it widely recognized that absorbed protein is used with a lower efficiency at higher supply (Hanigan et al., 1998), it is a real challenge to integrate this variability in ration balancing models.

At the individual level, essential AA not used for anabolic functions are removed from blood circulation in specific tissues depending on the presence of the enzymes responsible for their catabolism (Lobley and Lapierre, 2003). For example, essential AA from Group 1 (histidine, methionine, phenylalanine + tyrosine) are mainly removed by the liver and very little extraction of excess AA occurs in the mammary gland or in peripheral tissues other than the amount removed to support milk protein secretion and endogenous secretions. At the opposite, for the essential AA of Group 2 (isoleucine, leucine, lysine and valine), little is removed by the liver whereas oxidation (=inefficiency) occurs in the gut, the peripheral tissues and the mammary gland (Lapierre et al., 2012). Given that removal of excess AA does not occur at the site of protein synthesis and exportation but is specifically related to tissues having the enzymes for catabolism, we have proposed that rather than using an efficiency for maintenance different from the efficiency for lactation, we could use a combined efficiency of utilization (Lapierre et al., 2007). In addition, variation of the proportion of absorbed AA removed by the different tissues and recovered in milk greatly depends on AA supply (Doepel et al., 2004; Raggio et al., 2004). Based on these observations, a meta-analysis was conducted in studies where AA supply was increased through infusions, in order to have an assessment of the increased supply independent of any ration balancing model (Doepel et al., 2004). After this first analysis, we calculated a combined efficiency of utilization for AA and MP, presented in Table 5 (Lapierre et al., 2007). The next version of CNCPS 6.5 will adopt the combined efficiencies estimated at 100% of the MP requirement (van Amburgh et al., 2013).

Therefore, the following updates are proposed:

- Milk:
  - AA composition should be based on true protein,
  - An updated AA composition is proposed.

- Metabolic Fecal Protein:
  - represents the endogenous protein losses,
  - MP requirement of 19 g MP/kg DMI, calculated based on the estimation of ileal endogenous flow is proposed,
  - AA composition based on abomasal isolates in cattle and ileal endogenous secretions in pigs is proposed.

- No need to include requirement for endogenous flows at the duodenal level as this protein synthesis can be considered similar to protein synthesis in other tissues (but this digested protein needs to be removed from net supply).

- Efficiency
  - Efficiency of utilization of MP and AA should vary with supply,
  - The mammary uptake:output ratio should not represent the efficiency of lactation,
  - A combined efficiency of AA utilization for both maintenance and lactation is proposed.

Conclusion

The suggested recommendations are far from capturing all the complexities of the digestive and lactation processes, but they are based on the most recent knowledge of dairy cow metabolism and offer an enhanced framework to include this knowledge to improve our estimation of protein and AA requirement using current ration balancing models. Of course, they represent only approximations of complex metabolic pathways operating in dairy cows. Some aspects are not considered, especially interactions such as how changes in amount or type of energy alter outputs and efficiencies. Furthermore, as all the factors are based on empirical observations and equation fits, they do not permit different predictions for cows with different genetic potential, which may alter significantly the response to changes in the ration. Nonetheless, better appreciation of 'true' losses via digestive tract metabolism and the inclusion of a variable coefficient will yield immediate practical benefits. Adoption of such changes should not deter us from developing more mechanistic models, capable of responding to improved genetic selection, animal husbandry and feed processing technology and that can predict both within and between animal responses to changes in nutrient inputs.

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Table 1. Estimation of metabolizable protein (MP) requirement (requirement) for maintenance and milk in dairy cows

Function Ration balancing model[1]	Variables associated with MP requirement			
	Crude protein, g/d	True protein, g/d	Efficiency	MP requirement, g/d
Milk				
NRC	1440	1350	0.67	2015
CNCPS	1440	1339[2]	0.65	2060
Scurf				
NRC	10	ND[3]	0.67	15
CNCPS	10	ND	0.67	15
Endogenous urinary				
NRC	73	ND	0.67	109
CNCPS	73	ND	0.67	109
Metabolic fecal protein				
NRC	631	ND	ND	631
CNCPS	810	ND	ND	810
Duodenal endogenous flow				
NRC[4]	257	128	0.67	191
CNCPS	ND	ND	ND	ND
Total requirement				
NRC				2961
CNCPS				2994

[1]National Research Council (NRC, 2001) and CNCPS (Cornell Net Protein and Carbohydrate System, Fox et al., 2004).
[2]Using a fixed proportion of non-protein N in milk of 7%.
[3]ND: not defined / not used.
[4]Assuming 1436 g of MP from bacterial origin.

Table 2. Coefficients of efficiency for individual amino acids (AA) for maintenance or lactation currently used by CNCPS[1]

Function	AA								
	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Maintenance	0.85	0.85	0.66	0.66	0.85	0.85	0.85	0.85	0.66
Lactation	0.35	0.96	0.66	0.72	0.82	1.00	0.98	0.78	0.62

[1]Adapted from Fox et al., 2004.

Table 3. Optimal proportion (%) of lysine and methionine in metabolizable protein supply to maximize milk protein yield or concentration, according to different ration balancing models

Response to maximize	Ration balancing model	Amino acid	
		Lysine	Methionine
Milk protein yield	Initial NRC[1]	7.08	2.35
	NRC - expanded database[2]	6.95	2.38
	AMTS[2,3]	6.74	2.31
	CPM[2]	7.36	2.44
Milk protein concentration	Initial NRC	7.24	2.38
	NRC - expanded database	6.89	2.23
	AMTS	6.84	2.40
	CPM	7.23	2.40

[1]NRC (2001).
[2]Whitehouse et al., 2010 a and b.
[3]Whitehouse et al., 2009 for Met in AMTS ration balancing model.

Table 4. Proposed amino acid (AA) composition of milk and metabolic fecal protein (MFP)[1]

AA	Milk mg AA / g true protein	MFP mg AA / g CP[2]
Arg	37.4	32.3
His	29.0	20.0
Ile	61.3	31.5
Leu	103.6	40.1
Lys	87.6	42.4
Met	29.9	10.6
Phe	52.2	28.5
Thr	47.0	46.2
Trp	16.2	12.0
Val	69.3	43.9
Ala	35.4	41.7
Asn	42.7	67.6[3]
Asp	37.8	-
Cys	9.0	19.6
Gln	96.5	96.3[3]
Glu	128.8	-
Gly	20.0	122.1
Pro	103.8	68.4
Ser	67.4	51.2
Tyr	58.4	25.3

[1]See text for detail.
[2]Assuming 80% of true protein in MFP crude protein (CP).
[3]For MFP, Asn is the sum of Asn+Asp and Gln is the sum of Gln+Glu.

Table 5. Combined efficiency (maintenance plus lactation) of utilization of amino acids (AA) and metabolizable protein (MP) in relation to their optimal supply

	% of optimal supply			
AA	50%	75%	100%	125%
Arg	0.68	0.63	0.58	0.52
His	0.96	0.85	0.76	0.68
Ile	0.77	0.72	0.67	0.60
Leu	0.74	0.67	0.61	0.55
Lys	0.81	0.75	0.69	0.62
Met	0.85	0.74	0.66	0.59
Phe	0.70	0.63	0.57	0.51
Thr	0.68	0.68	0.66	0.60
Val	0.79	0.72	0.66	0.59
MP	0.72	0.67	0.62	0.56

[1]From the database of Doepel et al. (2004) and adapted from Lapierre et al. (2007); estimated as AA in milk protein plus the net requirement for maintenance (proposition in this paper) divided by net supply of AA.

SESSION NOTES

Feed Efficiency and Sustainability of the Cattle Industry[1]

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Introduction

Key Points

- Production and reproduction efficiencies are strongly related to environmental impacts of the dairy or beef cattle operation. Optimization of efficiencies is the most important tool in environmental impact mitigation.
- In US dairy herds, the current national average age at first calving is 25.2 months. Increasing first-lactation milk yield could improve milk's life-cycle production efficiency and decrease emissions.
- The productive life of dairy Holsteins in the United States born in 2000 decreased by 3.95 months compared with Holstein cows born in 1980. This negative trend needs to be halted to achieve environmental and economic gains.

Production efficiency in the dairy and beef industry can be defined as minimizing the amount of inputs (e.g., feed, fossil fuels) and undesirable outputs (e.g., ammonia, NH_3; greenhouse gases, **GHG**) to produce a given quantity of milk or meat. The present paper will focus on the dairy example. Production efficiency improvements can come from minimizing waste, maximizing a dairy cow's milk production, and maximizing the proportion of her life spent in peak milk production without sacrificing animal health and well-being. To a degree, when milk production per cow is improved, the life-cycle emissions of dairy production decrease per unit of milk (i.e., per kg of 3.5% fat-corrected milk (**FCM**); VandeHaar and St-Pierre; 2006). This is achieved through a dilution of maintenance costs per kilogram of FCM at the level of both the individual cow and the entire US dairy production system. Cows that produce more milk reduce the proportion of total consumed feedstuffs going toward maintenance energy costs (Moe and Tyrell, 1975; Bauman et al., 1985; VandeHaar, 1998). Secondly, more milk per cow can decrease the total lactating herd size needed to produce a given quantity of milk (Capper et al., 2008, 2009). Past improvements demonstrate the ability of production efficiency to decrease the environmental impact per unit of milk. Capper et al. (2009) found that historical advances in genetics, nutrition, and management of dairy farms allowed dairy production in 2007 to emit 43% of the CH_4 and 56% of the N_2O that were emitted in 1944 to produce one billion kilograms of milk. As the following sections

[1] Excerpts from: Contemporary environmental issues: A review of the dairy industry's role in climate change and air quality and the potential of mitigation through improved production efficiency S. E. Place and F. M. Mitloehner Department of Animal Science, University of California, Davis, One Shields Ave., Davis 95616-8521

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demonstrate, more opportunities for improving a dairy's production efficiency exist that could lead to further reductions in emissions per kilogram of FCM.

Heifer Management

Replacement heifers are an important part of the life-cycle emissions of a kilogram of FCM. Before calving, heifers are consuming inputs and producing both GHG and air pollutants without contributing to the production of milk. In the milk-fed stage of a heifer's life, she can efficiently convert consumed energy and protein into lean body tissue without depending on emission-producing rumen microbes. Recent research has found that increasing and altering the nutrients supplied to milk-fed calves can improve growth rates and feed efficiency (Brown et al., 2005; Bascom et al., 2007; Hill et al., 2008). "Intensified" feeding programs for dairy heifers have been shown to lower age at first calving (Raeth-Knight et al. 2009), with no reduction (Van Amburgh et al., 1998) or even an improvement in first-lactation milk yield (Drackley et al., 2007). Both decreasing the current national average age at first calving of 25.2 months (USDA, 2007) and increasing first-lactation milk yield could improve milk's life-cycle production efficiency and decrease emissions per kilogram of FCM.

Colostrum administration is another aspect of heifer management that can affect GHG and air quality emissions per kilogram of FCM. Dairy calves depend on passive immunization from the absorption of antibodies in colostrum to provide adequate immunity during their early life stages (Robison et al., 1988). Failure of passive transfer of immunity leads to increased mortality and morbidity and decreased growth performance (Robison et al., 1988; Beam et al., 2009). Administering the proper quantity of high quality colostrum within the first few hours of life has been shown to improve long-term animal health and first-lactation performance (DeNise et al., 1989; Faber et al., 2005). Beam et al. (2009) estimated that failure of passive transfer occurs in 19.2% of US dairy heifer calves; therefore, decreasing this incidence could substantially decrease death and performance losses and lessen emissions per kilogram of FCM.

Herd Health

Herd-health challenges affect per-unit of-milk emissions by increasing mortality and losses of saleable milk and decreasing reproductive performance and milk production efficiency. Herd health is influenced by many factors, including management, nutrition, the environment, and social stressors. Over the past 25 yr, the dairy industry has steadily shifted its structure toward fewer farms with larger herds and fewer workers per cow. In 2008, 3,350 US dairy farms with 500 or more cows (approximately 5% of total dairy operations) produced 58.5% of the nation's milk with 54.9% of the nation's dairy cows (NASS, 2009). Along with the industry's consolidation, milk production per cow has doubled over the past 25 yr, although it appears that disease incidence has remained stable (LeBlanc et al., 2006). However, the productive life of Holsteins in the United States born in 2000 decreased by 3.95 months compared with Holstein cows born in 1980 (Dechow and Goodling, 2008). Thus, opportunities exist for the dairy

industry to advance production efficiency by improving herd health to simultaneously enhance milk production, reproductive performance, and cow longevity. When dairy cattle transition from a pregnant, non-lactating state to a lactating state, they face a tremendous change in their metabolic requirements (e.g., Ca requirements are estimated to increase 4-fold on the day of parturition; Overton and Waldron, 2004). Consequently, most health concerns arise during the transition period. Approximately 75% of disease occurs within the first month after calving (LeBlanc et al., 2006), and a study of Pennsylvania dairy herds found that 26.2% of dairy culls occur from 21 d before to 60 d after calving (Dechow and Goodling, 2008). Recent research has linked disease incidence and excessive negative energy balances during the transition period with significant decreases in milk yield and reproductive success during the subsequent lactation (Drackley, 1999). Further research into the biology and management of transition cows and the extension of this critical knowledge to commercial herds can enhance the life-cycle efficiency of the US dairy production system. Environmental or social stressors can decrease the production efficiency of the cow and subsequently increase the emissions of each kilogram of milk that she produces. Heat stress has been estimated to cost the dairy industry nearly $1 billion per year in decreased milk production, reproductive performance, and increased death losses (St-Pierre et al., 2003). With regard to social stress, grouping animals according to size and age and minimizing overcrowding can improve DMI, consequentially improving milk production (Grant and Albright, 2001). Improving cow cooling during hot summer months and grouping animals to minimize behavioral stress has been the focus of research to improve farm profitability, but these improvements have the potential to decrease emissions per kilogram of FCM as well.

Mastitis is a herd-health challenge that can affect emissions per kilogram of FCM by decreasing milk production performance and increasing losses of saleable milk. Hospido and Sonesson (2005) analyzed the environmental impact of mastitis using a Life Cycle Analysis (**LCA**) of dairy herds in Galicia, Spain. The authors found that decreasing the clinical mastitis rate from 25 to 18% and the subclinical mastitis rate from 33 to 15% reduced the Global Warming Potential (**GWP**) of a unit of milk by 2.5% (Hospido and Sonesson, 2005) because of increased input-use efficiency, decreased losses of milk production, and a decreased amount of waste milk.

Lameness is a critical herd-health concern that seems to have worsened over the past 25 yr (LeBlanc et al., 2006). Lameness or injury is responsible for approximately 20% of mortalities and 16% of selective culls in mature US dairy cows (USDA, 2007). In addition to decreased survivability, lameness causes decreased milk production (Warnick et al., 2001) and poorer reproductive performance in affected cows (Garbarino et al., 2004). Improved facilities, management, nutrition, and genetics all have the potential to decrease the incidence of lameness (Baird et al., 2009) and decrease emissions per kilogram of FCM.

Nutrition and Feed Production

The nutrition of dairy cattle greatly determines the emissions produced directly by the ruminant animal and its waste. Diet composition can alter rumen fermentation to reduce the amount of CH_4 produced (Ellis et al., 2008) and, as previously discussed, the NH_3 emissions produced from the manure (James et al., 1999; VandeHaar and St-Pierre, 2006). The substrates used by methanogens are byproducts of structural carbohydrate fermentation; thus, high concentrate diets containing more nonstructural carbohydrates can lead to decreased CH_4 emissions (Lana et al., 1998; Ellis et al., 2008). However, diets very high in concentrate (such as those fed to the majority of US beef feedlot cattle) can decrease rumen pH and lead to rumen acidosis (Owens et al., 1998). Furthermore, very high-concentrate diets diminish the principal environmental benefit of dairy cows: their ability to convert cellulose, indigestible to humans and the Earth's most abundant organic molecule, into high-quality proteins for human consumption (Oltjen and Beckett, 1996). Therefore, the CH_4 produced by dairy cattle cannot simply be seen as a gross energy loss and GHG source but is a necessary consequence of transforming inedible fibrous forages and byproducts (e.g., almond hulls, citrus pulp, distillers grains) into food and fiber products fit for human use. Nonetheless, substantial reductions in CH_4 emissions can be achieved without feeding high levels of concentrates by altering the previously mentioned nutritional factors: microbial-altering feed additives, dietary lipids, and forage processing and quality (Johnson and Johnson, 1995). Feed additives, such as the ionophore monensin, can change microbial processes in the rumen to potentially improve feed efficiency and reduce CH_4 emissions (Tedeschi et al., 2003). However, research with monensin has shown conflicting results (Guan et al., 2006; Odongo et al., 2007; Hamilton et al, 2009; Hook et al., 2009), which suggests a need for more in-depth research on its effect on rumen microbial populations and the metabolism of dairy cows. Alternatives to ionophores such as probiotics (e.g., yeast), essential oils, and biologically active plant compounds (e.g., condensed tannins) have shown promise for CH_4 reductions; however, most research to date has been conducted in vitro and more in vivo studies are needed to evaluate the effect of these alternatives on CH_4 and their commercial viability (Calsamiglia et al., 2007; Beauchemin et al., 2009b). Dietary lipids, specifically unsaturated fatty acids, have the potential to act as an alternate H sink in the rumen, thereby reducing the H available to methanogens and the CH_4 produced (Ellis et al., 2008). Additionally, CH_4 reductions from feeding dietary lipids can be attributed to their suppression of fiber-digesting bacteria and toxicity to protozoa closely associated with methanogens (Hristov et al., 2009). Johnson et al. (2002) tested the ability of canola and whole cottonseed to reduce CH_4 and found no difference in emissions when compared with a control diet, whereas other researchers have found crushed canola seed to have a CH_4-suppressing effect (Beauchemin et al., 2009a). The inconsistency of the effect of dietary lipids on CH_4 is due, in part, to the variation in diets, the fatty acid profile, amount and form of the lipid source, and the length of the feeding trial, because the rumen ecosystem may adapt to lipid supplementation (Martin et al., 2008; Beauchemin et al., 2009a). Although lipids do have the potential to reduce CH_4 emissions, consideration must be given to their adverse side effects of reducing DMI or decreasing milk fat when fed at levels over a critical threshold (Giger-Reverdin et al., 2003; Martin et al., 2008). Furthermore, the source and availability of lipids must be

considered, because price will dictate their commercial adoption, and long-distance transport of lipid sources may defeat their emission-reducing potential by increasing fossil fuel combustion.

Forage quality and management can affect both air quality and GHG emissions per kilogram of FCM. Fermented feeds are a major source of Volatile Organic Compounds (**VOC**) (Alanis et al., 2008) and require substantial fossil fuel inputs during their production (de Boer, 2003; Schils et al., 2007); therefore, minimizing dry matter loss throughout the production, storage, and feeding of these feedstuffs will decrease the air quality and climate change impact of each kilogram of feed. Higher quality forages, produced by ideal crop production, harvesting, and preservation practices, maximize DMI and milk production (Oba and Allen, 1999). Additionally, forages with higher digestibility and higher rates of passage out of the rumen have the potential to reduce enteric CH_4 emissions for each unit of feed consumed (Johnson and Johnson, 1995).

So-called precision feeding that closely matches the nutrients needed by the dairy cow for maintenance, growth, lactation, and gestation to the supplied dietary nutrients can minimize the environmental impact of the cow's excreta (Tylutki et al., 2008). Precision feeding requires nutritional models with sufficient accuracy and a level of management that can reduce the feeding system's variation (Wang et al., 2000). By constantly monitoring the dry matter and nutrient composition of feedstuffs, dairy producers can avoid expensive overfeeding and minimize nutrient excretion that can lead to emissions. The potential reduction in NH_3 emissions by more tightly managing the crude protein content of the diet to match the animal's needs is substantial because most of the N fed over requirements is excreted as urinary urea-N. Castillo et al. (2001) found that cows with intakes of 419 g of N/d had similar milk production as cows consuming 516 g of N/d; however, 74% of the extra 94 g of N/d was excreted as urinary urea-N, which could be lost to the environment as NH_3 emissions. Moreover, a precision feeding strategy decreases the amount of refusals, which may become waste on a dairy or be fed to other production groups (e.g., lactating cow refusals fed to heifers) that have dissimilar nutrient needs, thereby increasing the likelihood for higher nutrient excretion (St-Pierre and Thraen, 1999). Additionally, closely monitoring and ensuring the correct nutrition of individual groups of animals can minimize the risk of other nutritionally influenced diseases and conditions, such as ketosis, lameness, and prolonged anestrous (Lucy, 2001; Roche, 2006). Overall, managing feed and feeding programs to minimize waste while maximizing milk production can improve farm profitability and decrease the life-cycle emissions per kilogram of FCM.

Reproduction

Perhaps not as apparent as nutrition, reproductive performance greatly affects emissions per kilogram of FCM. Dairy cows that have extended calving intervals because of conception failure spend more time out of peak milk when feed conversion into milk is most efficient. The total productive lifetime of many dairy cows is determined by reproductive performance, because reproductive problems are responsible for 26.3%

of the selective culls in the United States (USDA, 2007). Over the past 30 yr, the reproductive performance and productive lifetime of dairy cattle have substantially decreased while milk production has increased (Lucy, 2001; Dechow and Goodling, 2008). The negative effect per kilogram of FCM emissions caused by declining reproductive efficiency has likely been offset by increases in milk production per cow. However, restoring reproductive performance in combination with increased milk yield would further reduce emissions per kilogram of FCM. Garnsworthy (2004) modeled the environmental impact of reproductive performance and milk production in the United Kingdom. The model found that both higher milk yield and improved reproductive performance (better estrus detection and conception rates) contributed to reduced CH_4 and NH_3 emissions because of the smaller lactating and replacement herd population required to meet UK production quotas (Garnsworthy, 2004). The cause of the decline in reproductive efficiency of dairy cattle is multifaceted and is not completely understood currently (Ingvartsen et al., 2003), because reproductive success is influenced by nutrition, genetics, health disorders during transition, management, and the environment (Lucy, 2001). The level of reproductive success across all US herds is variable by region, breed, and management (Norman et al., 2009), suggesting that improvements are achievable. Encouragingly, recent data show that the long-term trend of decreasing reproductive performance and survivability may be slowing or reversing (Hare et al., 2006; Norman et al., 2009). Extensive research in dairy cattle reproduction is needed to identify the factors impeding fertility and to further develop strategies to improve reproduction on commercial herds. Wide adoption of these successful reproductive strategies could potentially lengthen the productive life of the US dairy cow and lower emissions per kilogram of FCM.

Sexed semen is a reproductive technology that has the potential to both help and hurt the impact of the dairy industry on air quality and climate change per kilogram of FCM. If used selectively, sexed semen can increase the rate of genetic gain in dairy cattle, allowing advantageous traits to become ubiquitous in the entire dairy cattle population (De Vries et al., 2008). Furthermore, on average, heifer calves are smaller than bull calves and cause fewer dystocias, which may allow for earlier breeding of heifers, and fewer mortalities and health problems (Weigel, 2004). However, if all animals are bred with sexed semen (or even all heifers), the replacement population for the US dairy herd will increase in size. To keep the total population of dairy cattle at a level that does not create an oversupply of milk, the lactating cow cull rate must increase. Again, this can be advantageous, because poor performing animals and those with poor genetic merit would likely be culled, but in the context of environmental impact per kilogram of FCM, the widespread use of sexed semen could increase emissions per kilogram of FCM by shortening the total productive lifetime of dairy cows. Furthermore, a larger replacement herd size means more nonproductive emissions for each kilogram of FCM produced.

Overall, this paper shows that some of the most important gains that can be achieved in mitigation of dairy environmental impacts are tightly connected to efficiencies around feeds and feeding as well as reproductive management.

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SESSION NOTES