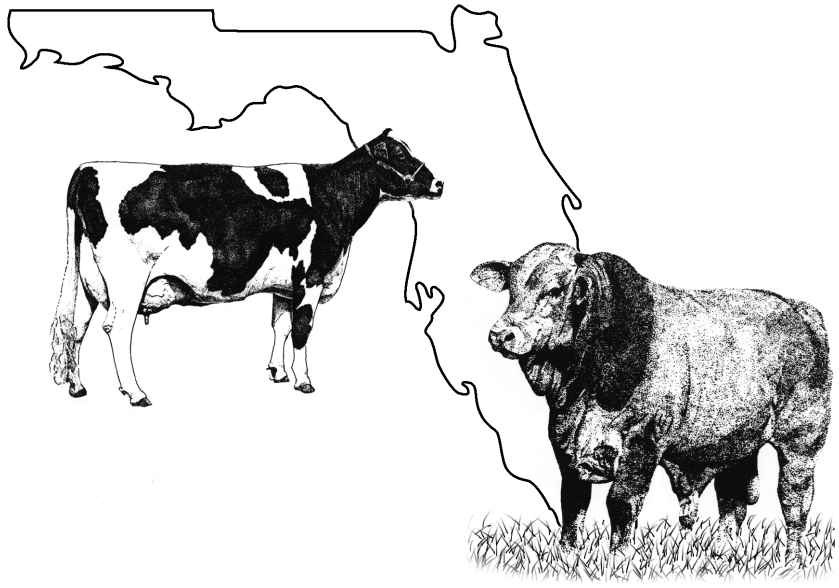
2015 Florida Ruminant Nutrition Symposium

26th Annual Meeting



February 2 - 4, 2015
Best Western Gateway Grand
Gainesville, Florida

PROCEEDINGS

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2015

26th ANNUAL FLORIDA RUMINANT NUTRITION SYMPOSIUM

February 2 - 4, 2015
Best Western Gateway Grand Hotel
Gainesville, Florida

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2015 FLORIDA RUMINANT NUTRITION SYMPOSIUM
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University of Florida, IFAS

February 2, 2015
Balchem Mini Symposium – "Peripartum Metabolism and Health in Dairy Cows"

1:00 – 5:15 PM

1:00-1:10 **Dr. Clay Zimmerman**, Balchem Animal Health and Nutrition: "Opening Remarks"

1:10-1:45 **Dr. Ric Grummer**, University of Wisconsin-Madison: "Insulin Resistance in Transition Dairy Cows: Friend or Foe?"

1:45-2:45 **Dr. Heather White**, University of Wisconsin-Madison: "Hepatic methyl metabolism: Influencing success during the transition to lactation"

2:45-3:15 Break

3:15-4:15 **Dr. Richard Erdman**, University of Maryland: "Feeding choline and methionine to transition dairy cows"

4:15-5:00 **Dr. Ric Grummer**, University of Wisconsin-Madison: "What are your chances for success when feeding ReaShure precision release choline?"

5:00 Adjourn

5:15 Reception

February 3, 2015
Pre-Conference – "Improving Health and Production by Managing Transition Cows"

Sponsored by Elanco Animal Health

9:00 - 11:40 AM

9:00-9:10 **David Waagner**, Elanco Animal Health - Pre-conference Program Introduction

9:10-10:00 **Dr. David McClary**, Elanco Animal Health: "The Vital 90 Days and Why It's Important to a Successful Lactation"

10:00-10:50 **Dr. Marcus Kehrli**, National Animal Disease Center: "Immunological Dysfunction in Periparturient Cows: Evidence, Causes and Ramifications"

10:50-11:40 **Dr. Michael Overton**, Elanco Animal Health, "Economic Consequences in The Vital 90 Days"

11:45 - 12:55 PM Buffet Lunch

February 3, 2015
Ruminant Nutrition Symposium

1:00-1:20 **Dr. Charles Staples**, University of Florida: "25 Years of the Florida Ruminant Nutrition Symposium"

1:20 - 3:20 PM

Nutrition and Reproduction of Cattle

1:20-2:00 **Dr. Milo Wiltbank**, University of Wisconsin: "Nutrition and Reproductive Efficiency: Transition Period Management, Energy Status and Amino Acid Supplementation Alter Reproduction in Lactating Dairy Cows"

2:00-2:40 **Dr. Matthew Lucy**, University of Missouri: "Mechanisms Linking Postpartum Metabolism with Reproduction in Dairy Cows"

2:40-3:20 **Dr. Michael Day**, The Ohio State University: "Nutritional Effects on Beef Heifer Development, Puberty and Subsequent Reproduction"

3:20-3:50 Refreshment Break

3:50 - 5:10 PM

Calf Nutrition and Health

3:50-4:30 **Dr. Emily Miller-Cushon**, University of Florida: "Intensified Pre-Weaning Calf Feeding Programs: Impacts on Growth and Behavior"

4:30-5:10 **Dr. Michael Ballou**, Texas Tech University: "Dietary Strategies to Improve the Health of Dairy Calves"

5:10 PM Welcome Reception

February 4th, 2015

8:00 – 10:00 AM
Applied Beef Cattle Nutrition

8:00-8:40 **Dr. John Arthington**, University of Florida: "New Concepts in Trace Mineral Supplementation of Grazing Cattle Hydroxy Sources, Injectable Sources and Pasture Application"

8:40-9:20 **Dr. Terry Engle**, Colorado State University: "Copper and Selenium Metabolism and Supplemental Strategies for Grazing Beef Cattle"

9:20-10:00 **Dr. Alfredo DiCostanzo**, University of Minnesota: "Supplementation Strategies to Reduce Waste in Beef Cattle Systems"

10:00–10:30 Refreshment Break

10:30 – 11:50 AM
Applied Dairy Cattle Nutrition

10:30-11:10 **Dr. William Stone**, Diamond V: “Feeding Management and Methods to Reduce Feed Losses and Improve Dairy Cow Performance”

11:10-11:50 **Dr. Jonathan Goodson**, Evonik Industries: “Food Safety and Modernization Act: How Will It Affect the Feed Industry”

11:50 Symposium Adjourns

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

Michael Ballou, Texas Tech University
Michael Day, The Ohio State University
Alfredo DiCostanzo, University of Minnesota
Richard Erdman, University of Maryland
Terry Engle, Colorado State University
Jonathan Goodson, Evonik Corporation
Ric Grummer, University of Wisconsin-Madison
Marcus Kehrli, National Animal Disease Center, USDA
Matthew Lucy, University of Missouri
David McClary, Elanco Animal Health
Michael Overton, Elanco Animal Health
William Stone, Diamond V
Heather White, University of Wisconsin-Madison
Milo Wiltbank, University of Wisconsin-Madison

University of Florida
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John Arthington
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David M. Waagner, Elanco Animal Health, Valdosta, GA

25th Annual Florida Ruminant Nutrition Symposium

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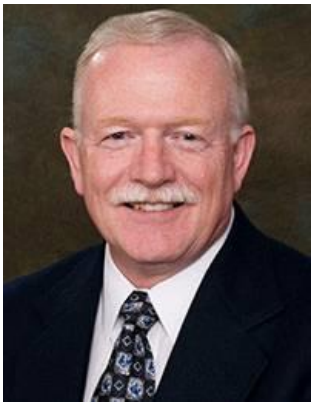
Dr. David McClary is a technical consultant for the Dairy Business Unit at Elanco. He received his BS from Western Kentucky University in 1970 and his DVM from Auburn University in 1974. Dr. McClary practiced for 4.5 years in Kentucky. In 1978 he returned to Auburn University CVM where he served as a resident and Assistant Professor in the Large Animal Clinic and completed his MS and certification as a Diplomat in the College of Theriogenology. In 1988, he joined Elanco Animal Health working on the development of bovine somatotropin. He has also been involved in a number of other research and development projects at Elanco including approval of monensin for use in dairy cattle and extensive post approval research with tilmicosin for treatment and control of bovine respiratory disease in beef and dairy cattle. He has served as a technical consultant for both the beef and dairy business units at Elanco supporting sales representatives. McClary was elected president of the American Association of Bovine Practitioners (AABP) 1990-1991. In 2008 he received recognition from that organization receiving the Pfizer-AABP Distinguished Service Award. In 2009 he received the W.S. Bailey Distinguished Alumnus Award, Auburn University, College of Veterinary Medicine.



Dr. Marcus Kehrli received the DVM degree in 1982 and the PhD in 1989 in immunobiology from Iowa State University. His research career at the National Animal Disease Center began in 1982 on bovine mastitis where he eventually became the Lead Scientist for the Immunology of Ruminant Perinatal Diseases Project until he joined Pfizer Animal Health in 1998. From 1998 to 2003, Dr. Kehrli was a Principal Research Investigator for Pfizer Global Research and Development, Veterinary Medicine Pharmaceutical Discovery, where his research focused on a pursuit of novel therapeutic solutions for livestock diseases. In 2003, Dr. Kehrli returned to National Animal Disease Center Research Leader of the Virus and Prion Research Unit, where he has implemented a broad, multidisciplinary program of applied and fundamental research to alleviate the economic impact of bacterial, viral and prion diseases on livestock and wildlife industries. His primary area of research expertise has been immunity to infectious diseases of cattle and swine. Currently, Dr. Kehrli is the Center Director for the National Animal Disease Center.

Dr. Michael W. Overton received his DVM from North Carolina State University and practiced veterinary medicine for 8 years in North Carolina. After a move to California to complete a Dairy Production Medicine Residency and his Masters of Preventive Veterinary Medicine degree, he worked as a Dairy Production Medicine Specialist at UC Davis-VMTRC in Tulare, CA for 6 years. Then, he joined the University of Georgia – College of Veterinary Medicine where he served as Professor of Dairy Production Medicine and chief of service for

the food animal program for about 7 years. In May 2012, Dr. Overton left UGA to assume a Dairy Analytics position with Elanco Knowledge Solutions. In this role, Dr. Overton is responsible for developing economic models and tools for both internal and external customers and for providing consultative services for dairies while also assisting in Global Marketing and Research and Development for Elanco. Dr. Overton and his wife, Carol, live in Athens, Georgia and their two children are pursuing post-graduate education in Georgia.

Dr. Milo C. Wiltbank is a Professor of endocrinology and reproductive physiology in the Department of Dairy Sciences at the University of Wisconsin in Madison. Dr. Wiltbank received his BS (1980) and MS (1982) degrees in from Brigham Young University in Utah. He then moved to the University of Michigan where he completed a PhD (1987). Dr. Wiltbank then went to Colorado State University for a postdoctoral fellowship. In 1991 he started his academic career in the Department of Dairy Sciences at the University of Wisconsin where he studies the regulation of ovarian function in dairy cattle. Basic studies focus on the regulation of hormonal receptors in the corpus luteum and developing ovarian follicle. Applied studies focus on development of methods that allow timed artificial insemination and improve pregnancy rates in dairy cattle.

Dr. Matthew Lucy is the Leader of the Animal Reproductive Biology Cluster and Professor of Animal Science at the University of Missouri-Columbia. He received a BS from Cornell University, MS from Kansas State University and the PhD from the University of Florida. He conducted postdoctoral research in the dairy research group at Monsanto (St. Louis, Missouri) before accepting a faculty position at the University of Missouri in 1994. He is known for his work on the reproductive physiology of high-producing dairy cows. Dr. Lucy's current research program examines the metabolic processes regulating the endocrinology and fertility of dairy cows and explores practical methods that evolve from this research. He is a recipient of the ADSA Hoyt Award (1990), the Midwestern Section ASAS Outstanding Young Researcher Award (2000), the ADSA Foundation Scholar Award (2000), the ADSA Pfizer Animal Health Physiology Award (2003) and the ASAS Animal Physiology and Endocrinology Award (2010). He is a past-President of the Dairy Cattle Reproduction Council (completing his term in 2011) and is a former Senior Physiology Section Editor for the Journal of Dairy Science. He currently serves as Editor-in-Chief of the Journal of Dairy Science.

Dr. Michael Day earned his BS in Animal Husbandry from the University of Missouri, Columbia, in 1980. He subsequently earned his MS and PhD degrees in reproductive endocrinology in the Department of Animal Sciences at the University of Nebraska- Lincoln, and joined the faculty at The Ohio State University in the Department of Animal Sciences in 1985. He is currently a Professor and Graduate Studies Chair in the Department. In 1996-97, Dr. Day took a sabbatical to work in at the Dairying Research Corporation in Hamilton, New Zealand. The focus of Dr. Day's research is the endocrine regulation of pubertal, follicular and uterine processes in cattle. Research targeting development and improvement of estrous cycle control in beef cattle has been a constant component of his program. He currently has ongoing and active research collaborations in Brazil in addition to his program in Ohio. Dr. Day has trained 21 graduate students and coauthored 90 journal articles, 147 abstracts and 40 papers in proceedings. His research has been primarily funded by USDA-NIFA and the AI industry. He contributes to many extension programs on a State, national, and international level.

Dr. Emily Miller-Cushon joined the Department of Animal Sciences at the University of Florida in 2014 as Assistant Professor of Animal Behavior and Welfare. She obtained her BSc degree from the University of Waterloo, Canada, in 2009 and PhD from the University of Guelph in 2014. The focus of her dissertation was the development of feeding behavior in dairy calves. She is generally interested in the relationship between management, behavior, and welfare of farm animals. Her research program focuses on how feeding patterns and social behavior of dairy calves respond to different early management factors and relate to performance and welfare.

Dr. Michael Ballou is an Associate Dean for Research and an Associate Professor of Nutritional Immunology in the College of Agricultural Sciences and Natural Resources at Texas Tech University. Dr. Ballou received his BS and PhD degrees in Animal Sciences and Nutritional Biology from the University of California Davis. His research program is focused on understanding now nutrition and management influence leukocyte responses and health of calves, heifers, and transition cows.

Dr. John Arthington is a graduate of the Animal Sciences Departments of Purdue University (BS) and Kansas State University (MS and PhD) and has been a member of the University of Florida since 1998. Dr. Arthington is a Professor Director of the UF-IFAS, Range Cattle Research and Education Center in Ona and his program focuses on productivity and environmental sustainability of range and grazing lands. His research focuses on the relationships among trace mineral nutrition and production stress, immune competence, well-being and productivity of beef cattle. He has mentored multiple MS and PhD students and post-doctoral associates, and contributed over 90 peer-reviewed manuscripts to the body of science supporting this field of study. Dr. Arthington' s academic program is also committed to the extension education mission of the Institute through participation and leadership in multidisciplinary and multi-state collaborations and services to our science and state, national, and international clientele groups.

Dr. Terry Engle is a Professor in the Department of Animal Sciences at Colorado State University. He received his BS and MS degrees in Animal Science from the Colorado State University, and the PhD degree in ruminant nutrition from North Carolina State University. Dr. Engle's teaching responsibilities include vitamin and mineral metabolism and animal metabolism. His research interests include trace mineral metabolism in ruminants with primary emphasis on the role of trace minerals and other nutrients on immune response, disease resistance, and lipid metabolism. Further research interests include molecular aspects of mineral absorption in ruminants.

Dr. Alfredo DiCostanzo is a Professor of beef cattle nutrition and management in the Department of Animal Science at the University of Minnesota. Dr. DiCostanzo received his BS degree from the Instituto Tecnológico y de Estudios Superiores de Monterrey Campus Queretaro in Mexico in 1981.He then moved to the University of Minnesota where he received his MS (1985) and PhD (1989) in animal nutrition. He then moved to the University of Missouri where he completed a postdoctorate in 1990. Hi research interests include nutrition and management factors affecting the biologic and economic efficiency of cow-calf and feedlot operations, evaluation of alternative feeds and feeding and management strategies to improve economic efficiency, and determination of nutrient requirements to enhance economic and environmentally sustainable beef production.

Dr. William Stone is a technical service director and field research for Diamond V. He grew up on a beef and hog farm in southeastern Wisconsin. After obtaining a DVM degree from the University of Wisconsin-Madison, he practiced veterinary medicine for 3 years in Monroe, Wisconsin. He moved to Cornell where he received his PhD on applied dairy cattle nutrition and management. He was a dairy nutritional and management consultant in central New York. From 1998 to 2007, Dr. Stone worked in a veterinary herd health/nutrition position with the PRO-DAIRY program at Cornell University. He then joined Diamond V where he directs the company's dairy research and technical support programs and works with agribusiness and dairy producers throughout the United States and internationally. His primary areas of interest are dairy cattle nutrition and feeding management, forage management, and identification of bottlenecks on dairies.

Dr. Jonathan Goodson received his BS, MS, and PhD degrees from the Department of Animal Sciences at The Ohio State University. Upon completion of his PhD, Dr. Goodson spent 2 years on a post-doctoral fellowship to study short-term metabolic control of liver fatty acid synthesis in the Department of Food Science and Nutrition at The Ohio University. Dr. Goodson then was employed as Dairy Program Director at Landmark, Inc. in Columbus, Ohio, and then Countrymark, in Delaware, Ohio. After 5 years, he left Countrymark and took a position of nutritionist and quality assurance manager at Gold Kist Inc. in Atlanta, Georgia, for 8 years. Then he moved to Southern States, Richmond, Virginia to work as a field nutritionist. He was then promoted to dairy product manager responsible for dairy feed sales in most of the east cost of the US. After 11 years at Southern States, he joined Degussa feed additives in 2003 as a technical support specialist, and became responsible for the technical support of the rumen-protected methionine source, Mepron. More recently, his responsibilities expanded to nutritional consulting to important integrated broiler customers. He provides formulation, quality assurance programs and on farm consultation.

The Vital 90™ Days and Why It's Important to a Successful Lactation

David McClary[1], Paul Rapnicki, and Michael Overton
Elanco Animal Health

Transition and the Vital 90 Days

The transition period for a dairy cow has traditionally been defined by the dairy industry as the period 3 weeks pre-calving to three weeks post-calving. An expanded period including the entire dry period, i.e., 60 days pre-calving to 30 days post-calving, more completely encompasses the actual period when physiological and nutritional adjustments determine if a successful subsequent lactation will be achieved. For the purposes of this paper, this expanded period will be described as The Vital 90™ Days.

Numerous physiological and metabolic changes (i.e., transitions) occur during the dry and early lactation periods in the dairy cow. These changes include:

- Cessation of milking at dry-off
- Changes in environment and ration composition
- Rapid fetal growth
- Decline in dry matter intake just prior to calving
- Initiation of colostrum production
- Hormonal changes, including declining progesterone and rising estrogen blood levels
- The process of giving birth
- Rapid increase in milk production

Along with physiological adjustments associated with transition, energy requirements essentially double overnight at the time of calving. Reynolds et al. (2003) showed liver glucose output doubling from 1,356 g/d at 11 days pre-calving to 2,760 g/day at 11 days post-calving. Those demands further increased to 3,283 gm/d by 22 days post-calving (Table 1). In addition to the demand for additional glucose for early lactation, energy balance is further compromised by a decline in feed intake in the peripartum cow (Grummer, 1995; Figure 1).

Along with a glucose deficit, the periparturient cow also commonly suffers a deficit in available protein. Much body protein is being used to support fetal growth in late gestation and the amino acid and glucose requirements for early lactation milk production (Overton, 2013). Bell et al. (2000) demonstrated a significant negative metabolizable protein (MP) balance in cows during early lactation. During the first 7 to

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10 days of lactation, high-producing dairy cows may mobilize as much as 1,000 g of tissue protein/d to satisfy the mammary gland's demand for amino acids and glucose. Bell concluded that a realistic estimate for MP requirements of a late-gestation cow was approximately 1,000 g/d. Because of the significant decline in feed intake just prior to calving, the suggested MP requirement for close-up dry cows is 1,200 g/d (Overton, 2013; Bell et al., 2000).

Table 1. Energy demand: measured glucose supply vs. estimated demands (Reynolds et al., 2003)

	Day relative to calving					
Variable	-19	-11	11	22	33	83
DMI, kg	9.7	9.8	14.1	16.9	19.4	21.8
Milk, kg	....	....	36.3	41.9	44.0	41.0
Liver net glucose output, g/d	1257	1356	2760	3283	3499	3650

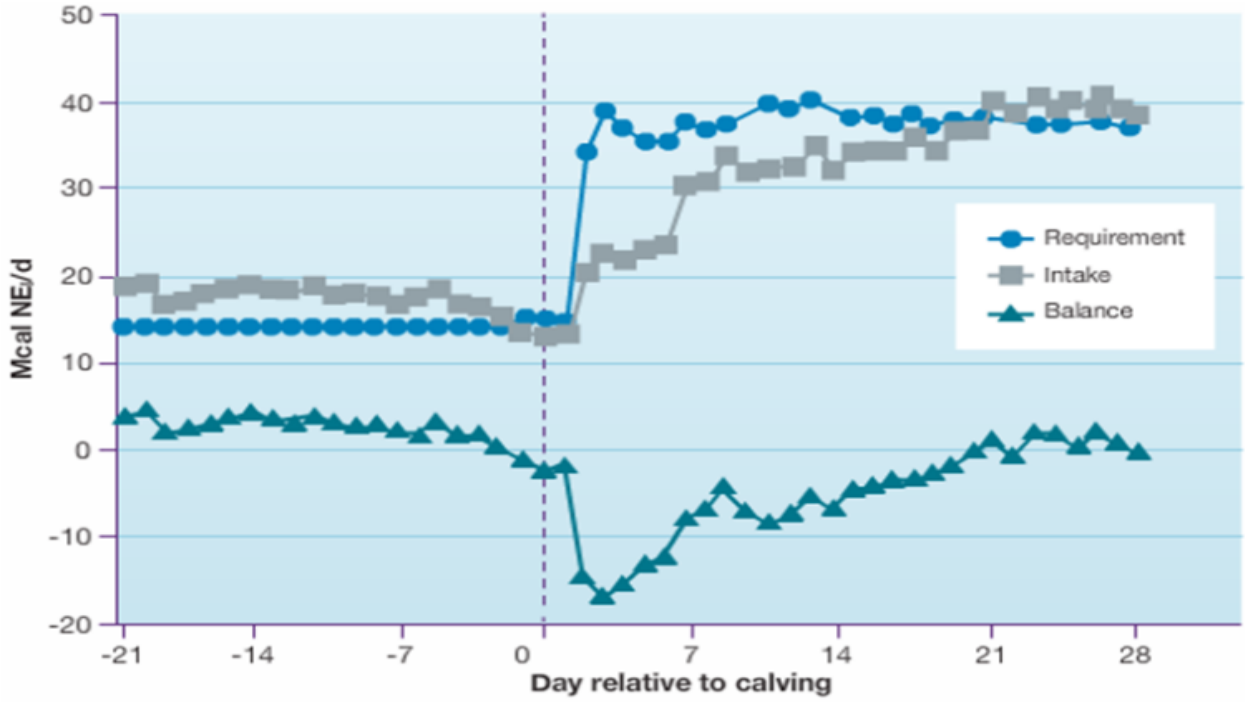

Figure 1. Energy requirement, energy intake, and energy balance of control cows during the transition period (Grummer, 1995).

The physiologic and metabolic changes occurring during transition also negatively affects immune function in the periparturient cow. Hoeben et al. (2000) demonstrated that immune response, as measured by neutrophil respiratory burst activity, was significantly reduced in the days just prior to and immediately post-calving (Figure 2). If immune function is impaired or suppressed, the cow becomes more susceptible to a number of periparturient disease conditions, such as retained fetal membranes, metritis, and mastitis.

Immunity encompasses a number of complex interactions that are designed to protect the animal from infection by a number of microbial organisms. The immune system is characterized by two primary branches: acquired immunity and innate immunity. Acquired immunity refers to the portion of the immune system that is commonly associated with antibody generation. Immunity is developed in response to first exposure to an antigen (foreign protein), such as a microbial agent or a vaccine antigen. In fact, the term "antigen" is a combination of the words **anti**body and **gen**erator. The immune response can be cell mediated or humoral and usually requires days to weeks to completely develop. Acquired immunity is generally specific to the microbial agent and has "memory" or tolerance such that it specifically responds to repeated exposure to that agent. The primary defense cell associated with acquired immunity is the lymphocyte, the white blood cell involved in antibody production.

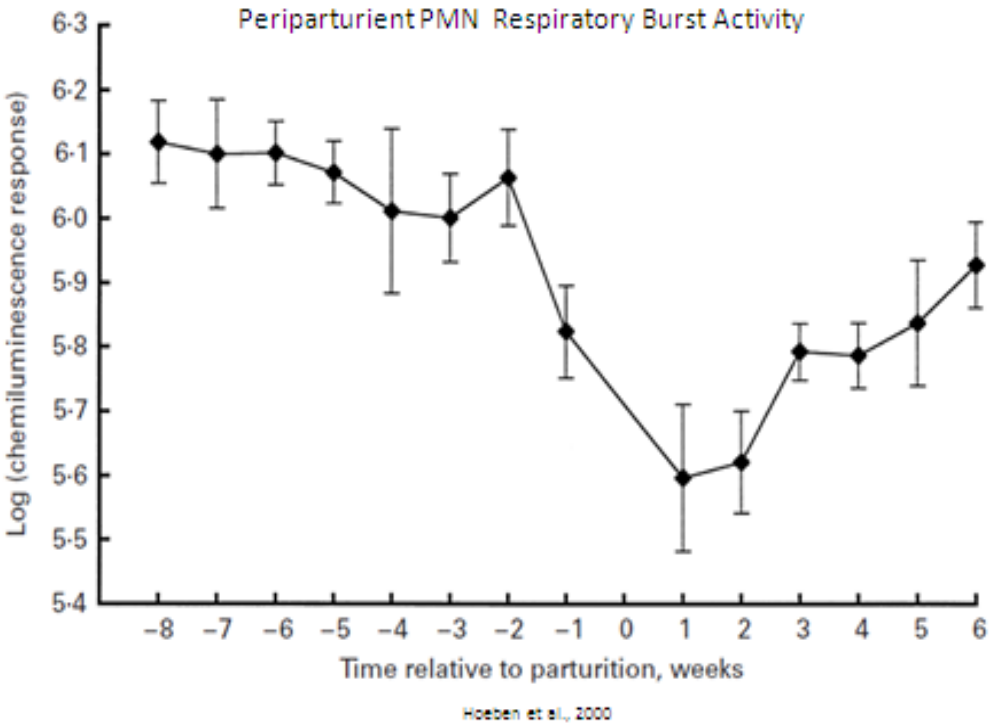

Figure 2. Impaired neutrophil function associated with reduced PMN respiratory burst activity in the periparturient cow (Hoeben et al., 2000).

In contrast to acquired immunity, innate immunity is nonspecific and has no memory of prior exposures. However, the innate responses to microbial exposure is

extremely rapid and very consistent. The primary defense cells in the innate system are neutrophils and macrophages. They commonly destroy bacteria by phagocytosis, which involves engulfing and digesting an invading microbe. Macrophages commonly reside in specific tissues such as the mammary gland or lungs and serve as sentinel cells that send out warning signals in the form of cytokines at the first indication of infection. Neutrophils respond to these signals by migrating, in large numbers, from the blood stream to the site of infection. When immune function is impaired, as described in the transition cow, neutrophils demonstrate a reduced ability to destroy bacteria.

Endocrine changes and physiologic stressors during transition contribute to impaired immune responses, but not all stress-related neuroendocrine responses are immune suppressive. The catecholamine response to stressors is, in fact, one of the early innate responses to stress, and is immune stimulatory. As a countermeasure, bacteria within the host, release their own neuroendocrine hormones, potentially initiating and enhancing pathogenic processes (Lyte, 2004).

Immune suppression during The Vital 90™ Days is multifactorial and can be related to hypocalcemia, elevated blood glucocorticoids levels, insufficient energy (hypoglycemia), and elevated ketones (ketosis) and elevated blood non-esterified fatty acids (NEFA) levels. Adequate nutrition, a clean environment, and strategic immunization are key components in restoring normal immune function and disease resistance in the periparturient period.

Negative energy balance is a normal physiological phenomenon in the early postpartum dairy cow and numerous other mammals. The primary concerns are the degree (depth) of negative balance and the cow's ability to adapt (duration), thus minimizing the length of time before returning to a positive balance. Successful lactations are dependent on how well energy balance is managed and immune function maintained during transition from pregnancy to lactation. Setting the cow up for a successful transition begins in the dry period, well before the initiation of the next lactation.

Periparturient Disease Conditions and The Vital 90 Days

Disease conditions that occur in the first 30 days of lactation often result from physiological changes and management decisions made during the prior 60 days. These diseases can generally be divided into those associated with negative energy balance or immune suppression. Common peripartum disease conditions associated with immune suppression include retained fetal membranes (RFM), metritis, and mastitis. Those associated with excessive negative energy balance include displaced abomasum, ketosis, and ovarian dysfunction (cystic ovarian disease or prolonged anestrus) (Figure 3).

Calcium can play a role in both categories of periparturient disease complexes. Cows experiencing clinical or subclinical hypocalcemia have impaired muscle function, gastrointestinal stasis, and reduced appetite increasing the risk of a metabolic disease

problem. Erb et al. (1988) showed an association between hypocalcemia and an increase in the chances for a metabolic disease condition, including displaced abomasum.

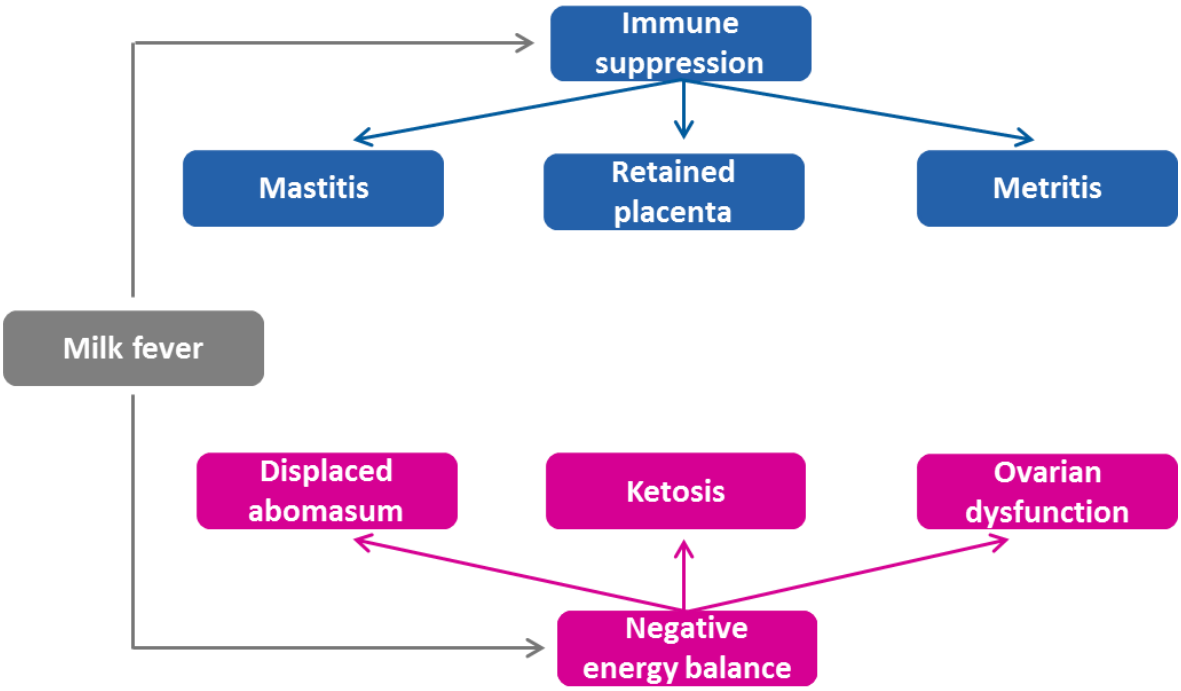

Figure 3. Diseases related to immune suppression and energy balance in the transition dairy cow (Duffield et al., 2009; Godden et al., 2006; Huzzey et al., 2007; Kimura et al., 2002; Loeffler et al., 1999).

Calcium also plays a critical role in immune function in the periparturient dairy cow. Intracellular Ca is an important component in early immune cell activation (Kimura et al., 2006). Activation of immune cells, such as circulating monocytes, is dependent on adequate concentrations of intracellular Ca. Reduced intracellular calcium stores negatively affect immune cells' response following an activating stimulus, thus contributing to the immune suppression seen in these animals. Periparturient hypocalcemia, with a corresponding decrease in monocyte intracellular Ca, results in immune suppression increasing the likelihood of periparturient disease such as mastitis. A New York study (Curtis et al., 1983) involving 2,190 cows demonstrated a very strong association between parturient hypocalcemia (milk fever) and mastitis. The odds ratio suggested that a milk fever cow was 8.1 times more likely to develop mastitis than a cow with normal blood Ca levels.

Lowered circulating Ca can itself contribute to a stress response in periparturient cows. Plasma cortisol typically increases three- to fourfold at the initiation of parturition. In cows with subclinical hypocalcemia, the increase in plasma cortisol may be five- to sevenfold. If a cow develops clinical hypocalcemia, plasma cortisol levels can increase ten- to fifteen fold compared to basal levels (Horst and Jorgensen, 1982).

Research has shown that the incidence of RFM may also be influenced by immune suppression. In a study comparing neutrophil function in cows with RP compared to those without RP, Kimura et al. (2002) demonstrated that neutrophils isolated from blood of cows with RP had a significant reduction in neutrophil function compared to those that did not retain. This impaired function continued for 1 to 2 weeks after parturition. Their work concluded that impaired neutrophil function played a major role in the likelihood of a cow suffering retained fetal membranes.

Periparturient diseases are often multiple entities. There are numerous interactions among the conditions associated with negative energy balance and immune suppression (Figure 4). The development of one disease condition often contributes to another such that conditions associated with negative energy balance contribute to an increased risk for conditions associated with immune suppression and vice versa.

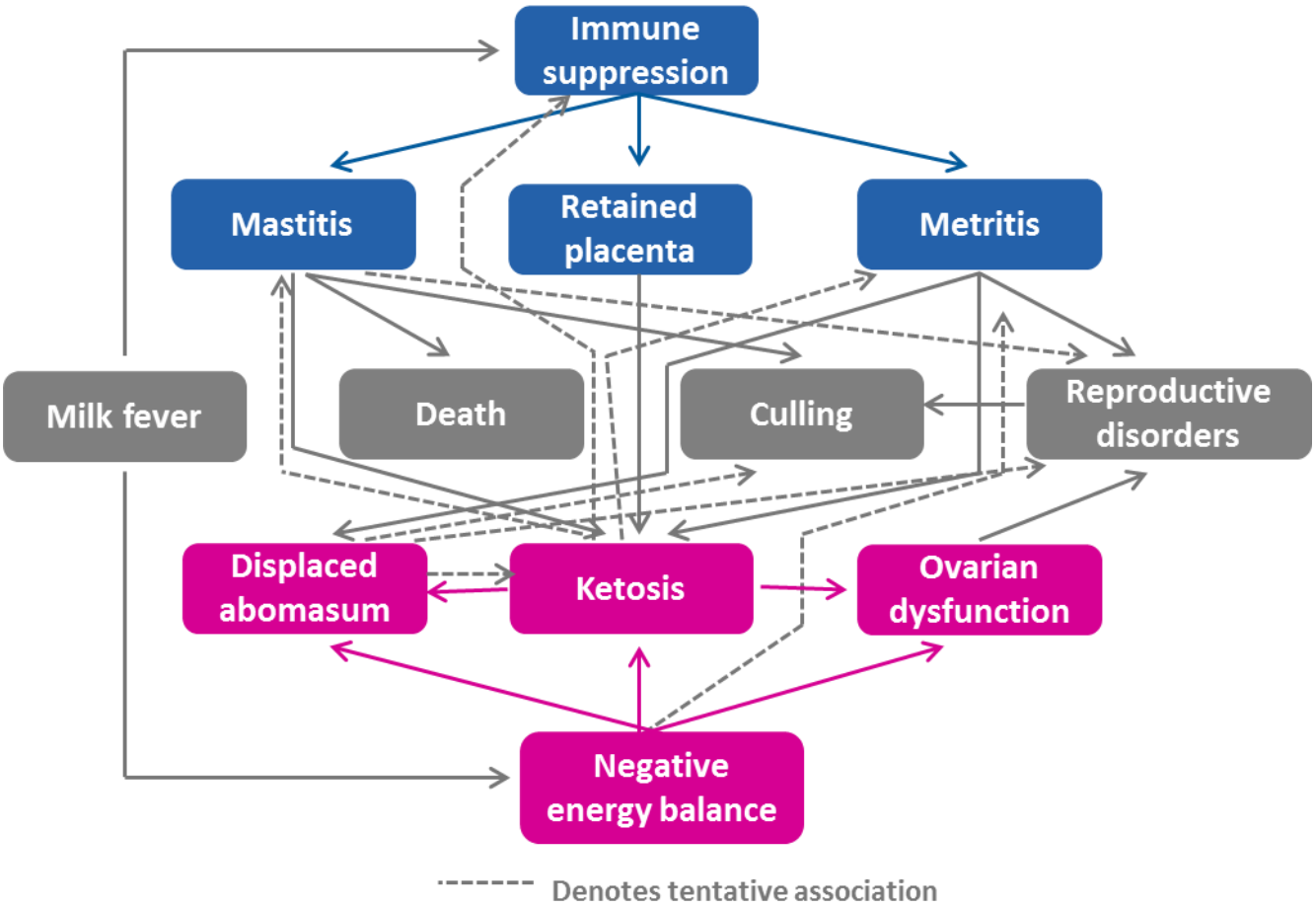

Figure 4. Complex interactions among diseases associated with immune suppression and energy balance in the transition dairy cow (Duffield et al., 2009; Godden et al., 2006; Huzzey et al., 2007; Kimura et al., 2002; Loeffler et al., 1999).

Periparturient Disease Prevention and Recognition during the Vital 90 Days

Dairy producers expend considerable time and financial resources in an attempt to assure the dairy cow has a successful dry period and transition into early lactation. The exact cost of these interventions are often not known, yet when asked to detail them dairy producers soon realize the investment during The Vital 90 Days can be substantial. Failure during this period leads to an increased incidence of disease and death loss. These failures place a significant negative burden on the operation.

Transition disease problems result in tangible and intangible consequences. The most obvious tangible consequence is financial loss. Beyond the tangible consequences there are also intangible or emotional consequences such as dealing with the stress and emotional frustration associated with higher morbidity and mortality in your client's herd. Furthermore, continually dealing with sick cows can negatively impact the morale of employees. Prevention of disease problems during The Vital 90 Days has the obvious tangible economic benefits but also intangible benefits of improved pride, confidence, and peace of mind.

The degree of success during transition has direct impact at both the cow and dairy level. Several key questions can be asked of dairy producers regarding recovery, future productivity, and/or disposition for cows experiencing any periparturient disease problems. These include:

- How many cows are in the sick pen, how long are they there, and how many make it out?
- How productive are the cows after a stay in the sick pen?
- Do all of your clients have an on-farm euthanasia protocol?
- What is the impact to the welfare of a cow if there is no euthanasia protocol?
- Do we have the medical interventions necessary to save every cow that develops a periparturient disease?

In addition, transition problems impact the dairy operation in general. When problems accumulate, frustration levels increase, and long term success of the operation suffer. Key questions that can be asked at the farm level include:

- What is a typical day like for a member of the hospital-pen treatment crew?
- Do farms experience protocol drift? Why is this?
- Are veterinarians frustrated by some of the treatments they see being used outside of their control?
- With the widespread use of fresh cow monitoring programs (e.g., daily temperatures for 10 to14 days), has a client ever treated their way out of a transition disease problem?

A key component often lacking or deficient when analyzing the incidence and associated cost of disease is inadequate disease records or no records at all. Kelton et al. (1998) published recommendations on recording and calculating the incidence for

eight clinically identifiable diseases of economic importance to the dairy industry. The diseases addressed were: milk fever, RFM, metritis, ketosis, left displaced abomasum, cystic ovarian disease, lameness, and clinical mastitis. This paper is commonly referenced when introducing guidelines and standards for the reporting of data related to the health of cattle. In addition to the eight conditions identified by Kelton et al. (1998), pneumonia should also be considered an economically important disease condition in some herds.

There continues to be a strong interest within the dairy industry in the recording and analysis of clinical disease data, with the goal of assisting dairy producers and their advisors in making impactful decisions.

Dairy producers and their advisors need to consider two broad categories of medical decisions:

1. Individual cow decisions
2. Herd health program decisions

Using on-farm records facilitates both categories of medical decisions and results in making a positive impact on both the individual cow and the dairy business operation. Having valuable information for making critical management decisions require accurate recording, and the ability to retrieve and analyze health records. Key components of this system are being able to:

- **Define** the conditions (diseases) to be tracked
- **Describe** the clinical signs of the disease
- **Detect and Monitor**
- **Decide (Individual Cow):** Record and Treat
    - Create standard protocols available for treatment options and recording
    - Utilize decision tools to choose specific protocol for a given case
- **Analyze** the available data

Accurate and complete dairy records start with consistent recording of health events. The basis for accurate recording is standardized definitions for common health conditions. The following list provides standardized disease definitions which, if used consistently, should improve the accuracy of disease detection, recording, and analysis. The following diseases commonly occur during The Vital 90 Days:

Metritis (METR)

- Metritis is recognized by an abnormal (smelly and watery) uterine discharge within 21 days of calving. On palpation per rectum, the uterus appears flaccid, not contracting normally, and fluid filled.
    - Mild clinical metritis is metritis without a fever or other clinical signs apart from the uterine changes.
    - Severe clinical metritis is metritis with the presence of clinical signs that may include fever, depression, and lack of strong appetite.

Ketosis (KETOSIS)

- Ketosis is recognized when animals are identified with elevated ketone bodies in the blood (> 1,200 µmol/L), milk (> 100 µmol/L), or urine in the absence of concurrent disease. The risk period for transition-related ketosis is usually the first 30 DIM, but testing is most commonly performed during weeks 1 and 2 after calving, when the risk is highest.
- Clinical ketosis is a more severe form of ketosis where the cow shows clinical signs of decreased appetite, decreased milk production, or abnormal behavior in the absence of another concurrent disease.
    - Primary clinical ketosis is clinical ketosis that occurs prior to or without any other concurrent disease.
    - Secondary clinical ketosis is clinical ketosis that occurs in conjunction with another disease process.

Displaced Abomasum (DA)

- Displaced abomasum (DA) is recognized when a ping is detected by thumping or tapping the cow's body wall while simultaneously listening with a stethoscope in the area between the 9^{th} and 12^{th} ribs above and below an imaginary line extending from the hip to the elbow on each side of the animal on the abdominal wall. DA can occur on either the right or left side.

Retained Fetal Membranes (RFM)

- Retained fetal membranes is recognized when the fetal membranes (placenta) are still visibly hanging from the cow's vulva 24 hours or more after calving.

Milk Fever (MF)

- Milk fever is identified if a cow of lactation 2 or more displays clinical signs that include muscle weakness, nervousness, muscle shaking, cold ears, and eventually the cow being unable to rise. This condition is caused by low blood calcium levels and usually occurs within 3 days of calving.

Mastitis (MAST)

- Clinical mastitis is recognized by visually observing abnormal milk from a quarter. Clinical mastitis can be classified as mild, moderate, or severe based on whether the cow shows any additional clinical signs beyond abnormal milk.
    - Severity score of 1, or Mild mastitis: Abnormal milk only
    - Severity score of 2, or Moderate mastitis: Abnormal milk + inflammation of udder (e.g., redness or swelling)
    - Severity score of 3, or Severe mastitis: Abnormal milk + inflammation of udder + sick cow (e.g., depression, poor appetite)
- Note that clinical mastitis can occur both within The Vital 90 Days and at other points in the lactation.

Ovarian Dysfunction (OVDYSF)

- Ovarian dysfunction is recognized when a cow is examined and determined to have ovarian problems that are causing abnormal patterns of heat expression (showing heat too often or not showing heat at all).
- While ovarian dysfunction can certainly impact the future reproductive performance, its definition is not a specific disease, and it typically is not diagnosed during The Vital 90 Days. In most cases it will not be tracked as an independent event.

Lameness (LAME)

- Lameness is recognized when a cow is observed walking or standing abnormally due to a problem in the foot, leg or hip.
- Note that lameness can occur both within The Vital 90 Days and at other points in the lactation.

Pneumonia (PNEU)

- Pneumonia is recognized when a cow is observed with altered breathing patterns and/or respiratory sounds due to a respiratory infection. Most cases of pneumonia have a fever but some do not.
- Note that pneumonia can occur both within The Vital 90 Days and at other points in the lactation.

When a disease condition is accurately detected, an appropriate treatment decision can be made. An important role of on-farm management software should be to facilitate guiding the delivery of treatments to the correct cows and compliance to prescribed therapies. Transition disease event entry should be promoted by veterinarians, consultants, and farm managers because it facilitates the delivery of the proper treatments to the correct cows. Farm management must define the approved treatment protocols to be used working with their farm's Veterinarian of Record. The Veterinarian of Record is the responsible party for providing appropriate oversight of drug use on the farm operation. Written protocols should include a protocol name, medications used, and specific directions for use (including duration of therapy and any milk and/or meat withdrawal periods). The primary purpose of data entry is to capture the data needed to guide the implementation of approved treatment protocols. With the supervision of the Veterinarian of Record, on-farm software can assist the animal care workers in delivering the highest quality medical and supportive care to the animals in their production unit.

Monitoring of disease consequences that occur during The Vital 90 Days can be utilized by the Veterinarian of Record, other consultants, and the farm management team to evaluate compliance to the approved herd strategy for the necessary medical treatment of clinical disease events when they occur. In addition, complementary reports related to transition disease incidence risk may be used to provide feedback to the management team about the herd health program strategy for managing negative energy balance and immune suppression during The Vital 90 Days.

Conclusion

The intent of this paper was the review of numerous concepts related to the modern dairy cow during the transition from dry to early lactation. Transition is not a single event or period but rather a progression through a multitude of events over approximately a 90-day period, thus the phrase “The Vital 90 Days.” Veterinarians and other dairy consultants should work with the producer in developing herd specific strategies for cows in this important period.

Most metabolic and infectious diseases occurring during early lactation are directly or indirectly attributable events during The Vital 90 Days. Dairy producers spend considerable time, effort, and resources during this period, yet rarely do they quantify their total economic investment or the financial consequences of failure during this period. Besides the direct costs, there are also intangible consequences impacting them and their herd. The last concept addressed identifies a practical process for identifying, treating, and recording disease problems. Management decisions during the high-risk period are key drivers for a cow’s health, well-being, and success in the subsequent lactation. Focusing on The Vital 90 Days can lead to a higher likelihood for reduced frustrations, higher profitability, and long-term success in a dairy operation.

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SESSION NOTES

Immunological Dysfunction in Periparturient Cows: Evidence, Causes and Ramifications

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Introduction

With a $40.5 billion Gross Domestic Value for milk produced in the U.S. during 2013, the dairy industry was the third largest sector of the 2013 U.S. animal agriculture economic engine. The value of milk produced in 2013 represented 24% of the total value of animal agriculture production; this figure had grown from $21 to 23 billion/year over a decade ago. The 2007 National Animal Health Monitoring System (**NAHMS**) Dairy Study reported that during 2006, 23.6% of cows were culled from operations, 26.3% and 23% were removed for reproductive and udder health problems respectively. In addition, 16.5% of cow mortalities were due to mastitis. Clearly, the economic value of controlling mastitis pathogens is immense. Most economic analyses of the cost of mastitis cite a 10% production loss as only one part of the overall cost of the disease. The majority (65 to 70%) of losses is associated with decreased milk yield resulting in lower production efficiency; the remaining costs are attributed to treatment. In addition to these direct losses, mastitis causes significant problems in milk quality control, dairy manufacturing practices, quality and yield of cheese, nutritional quality of milk, antibiotic residue problems in milk, meat and the environment, and genetic losses due to premature culling. These additional costs are very significant and are not always included in economic analyses of mastitis costs.

Because of the need for a safe, economical and stable supply of food, those of us serving the livestock health industry must be prepared to provide the best quality advice and care in managing our Nation's dairy herd. For dairy producers, the critical factor in providing a low somatic cell count milk supply is keeping cows free from mastitis. Mastitis is anything causing inflammation of the mammary gland, and infectious mastitis is caused by a plethora of microbial agents [1]. Nearly half of the Nation's herd of dairy cows will experience at least one episode of mastitis during each lactation. Research has already resulted in genetic selection for cows with lower somatic cell counts by the incorporation of this trait into the artificial insemination (**A.I.**) sire summary ranking indices. This approach mainly serves to reduce the normal increase in mastitis incidence that occurs as milk production goes up. Coliforms and environmental streptococci are the most common etiologic agents isolated from clinically severe mastitis cases on well-managed dairy farms [2, 3]. Clinical trials and experimental studies have demonstrated repeatedly *no benefits* of antibiotic therapy in

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cattle with clinical or subclinical coliform mastitis [4-6]. Hence, the advent of the *Escherichia coli* J-5 and other endotoxin core mutant vaccines in veterinary medicine many years ago provided us a tool to reduce the incidence and severity of clinical coliform mastitis [7-10]. However, there remains an unmet veterinary medical need of new ways to prevent or treat mastitis caused by environmental pathogens. For several years, research at the USDA's National Animal Disease Center in Ames, IA undertook a two-fold approach for improving the dairy cow's resistance to mastitis - immunomodulation and genetic selection for superior immune systems. In this paper, we will focus on the evidence for immune suppression in periparturient dairy cows, how this sets the cow up for infectious diseases such as mastitis, metritis and retained placental membranes, and some of the early research on immune modulation of the transition dairy cow and how that impacted resistance to mastitis.

Role of the Immune System in Mastitis

Immunity against infectious diseases of cattle is mediated by diverse, yet interdependent, cellular and humoral mechanisms. Many environmental and genetic factors influence the ability of livestock to mount effective defense strategies against the various pathogens and normal flora that they are exposed to throughout their lifetime. Innate resistance to infectious diseases reflects the inherent physiological attributes of an animal that make it more or less susceptible to disease development by a particular pathogen. There are several cell lineages that comprise the immune system (e.g., B-cells, T-cells, neutrophils, eosinophils, basophils, macrophages and mast cells). Each of these cell types has distinct responsibilities in providing host defense. Innate immunity represents the various immune components that are not intrinsically affected by prior contact with an infectious agent [11]. Lymphocytes provide the adaptive immune reactions that are antigen specific in nature and possess memory for future encounters with the same pathogen. In this paper we will present a novel approach of immune modulation of the innate immune system as a potential means to reduce antibiotic usage in veterinary medicine.

Our first understanding of cellular immunity is more than a century old and it actually involves research into the causes of bovine mastitis and the immune response. In his 1908 Nobel Lecture the Russian Zoologist, Élie Metchnikoff, described disease as consisting "of a battle between a morbid agent, the external microorganism, and the mobile cells of the organism itself. A cure would represent the victory of the cells, and immunity would be the sign of an activity on their part sufficiently great to prevent an invasion of microorganisms [12]." Metchnikoff cited the work of a Swiss veterinary expert, Zschokke, who found that plentiful phagocytosis of streptococci in the battle against infectious mastitis in cows, was a good sign. When phagocytosis was insignificant or not present, the cows were written off as no longer capable of producing good milk. This was later extended to include the idea that not only must the phagocytes engulf the microorganisms, but that these devouring cells must utterly destroy the microorganisms. In some cases, the streptococci of mastitis were found to "destroy the phagocytes after being engulfed by them thus liberating themselves to carry on their deadly work".

Today we have a far more detailed knowledge of the cow's immune response to pathogens in the mammary gland and elsewhere. Neutrophils are one of the most important cell types of native defense mechanisms because they respond quickly, within minutes, and do not require previous exposure to a pathogen to effectively eradicate the microbe. A major function of neutrophils is the phagocytosis and destruction of microorganisms that invade the body. Phagocytosis is probably the most widely distributed defense reaction, occurring in virtually all phyla of the animal world.

Neutrophils Are Critical Against Mastitis

Native defenses of cattle are continually challenged by exposure to pathogens (bacteria, fungi and viruses) and many factors affect the outcome of this interaction. Establishment of an infection in any organ or tissue is dependent upon a delicate balance between defense mechanisms of the body and the abilities of pathogens to resist unfavorable survival conditions. The neutrophil is one of the most important cells of the innate defense mechanisms because it can act quickly, within minutes, in large numbers and in most cases, does not require previous exposure to a pathogen to effectively eradicate the microbe. Studies have shown that it takes approximately 1 to 2 hours for neutrophils to accumulate in response to *E. coli* infection in tissues [13-16]. What this means is that microorganisms will have a 2-hour head start on the host immune response and any further delay in the inflammatory response will result in significantly more pathogens for the host to deal with. Unfortunately, delays in inflammatory responses in stressed animals are well documented [17-19], and some of the mechanisms responsible for delayed inflammation have been identified [20-22]. The importance of the neutrophil in protecting virtually all body tissues, especially against bacteria, has been repeatedly demonstrated experimentally and in nature [23-29]. Early and rapid accumulation of sufficient numbers of neutrophils is paramount in the ability of the host to effect a cure of invading pathogens [30]. Neutrophils can also release cytokines that in turn result in additional recruitment signals for more neutrophils [31-34]. Circulating ***neutrophils represent the major recruitable host defense against acute tissue infection***, such as mastitis [18, 19, 25, 35].

Immunosuppression in the Pathogenesis of Mastitis

A literal definition of immunosuppression is diminished immune responsiveness. This simplistic definition impacts a highly diverse system that affords protection against disease. Periparturient immunosuppression research was initiated by the observation that most clinical mastitis occurs in dairy cows in early lactation and the view that most bovine mastitis is caused by opportunistic pathogens and therefore these cows must be immunosuppressed. What evidence supported the hypothesis of periparturient immunosuppression? Practical experience teaches us that opportunistic infections are associated with severe compromises of host defense mechanisms. Over the past couple decades, an overwhelming amount of evidence of immunological dysfunction of lymphocytes and neutrophils in periparturient cattle and sows has been generated in research institutes around the world [17, 20, 36-76]. Periparturient immune dysregulation impacts the occurrence of infectious diseases of virtually any organ

system of livestock (e.g., gastrointestinal, respiratory and reproductive tracts all have increased disease incidence in postpartum animals).

First of all, there is an extremely high incidence of clinical disease in periparturient cows with nearly 25% of all clinical mastitis occurring during the first 2 weeks after calving. Clinical mastitis caused by virtually all pathogens, but especially coliform bacteria and streptococci other than *Streptococcus agalactiae*, has a very high incidence in early lactation. Cows must first become infected and then develop clinical mastitis. The rates of new intramammary infections (**IMI**) caused by environmental pathogens are highest during the first and last 2 weeks of a 60-day, nonlactating period of dairy cows [3, 77-79]. The rate of new IMI during these periods of peak susceptibility is 2 to 12 times higher than any other time in the production cycle of the cow. Most coliform and environmental streptococcal infections established in the nonlactating period and that are present at parturition result in clinical mastitis soon afterward [77, 80]. The proportion of all cases of clinical coliform mastitis that develop during the first 2, 4, and 8 weeks of lactation has been reported to be 25, 45 and 60%, respectively [81, 82].

The second piece of evidence supporting the notion of immunosuppression in the pathogenesis of mastitis was that we are traditionally taught that opportunistic infections are associated with severe compromises of host defense mechanisms. These two points led to experiments evaluating how functional a cow's immune system is around calving time. Over the past several years, an overwhelming amount of evidence of immunological dysfunction of lymphocytes and neutrophils in periparturient cattle has been generated in several research institutes around the world [17, 20, 36-75]. Today the data tells us the immune system becomes progressively more compromised at the end of gestation, cows become more readily infected in the mammary gland, then as the immune system "bottoms out" the first week or two after calving, these subclinical infections begin to win the battle with the cow's immune system and clinical mastitis results. This can also be extended to infectious diseases of virtually any system of the postpartum cow (gastrointestinal, respiratory and reproductive tracts all have increased disease incidence in postpartum cows).

What Causes Periparturient Immunosuppression?

Many neuroendocrine changes develop in cows during the periparturient period. Periparturient hormone fluxes may adversely affect immune cell function. Surprisingly, there is no effect of estrogen on bovine neutrophil function either during the follicular phase of the estrous cycle in cows or after administration of high doses of estradiol to steers [83, 84]. However, supraphysiologic concentrations of estradiol have been reported to suppress neutrophil function [85, 86]. These high concentrations of estrogens may be germane to immunosuppression and the high new IMI rates prior to calving. Before calving, total plasma estrogen concentrations increase in the cow, at least 10-fold greater than during estrus [87]. Moreover, during normal pregnancy, the progesterone binding capacity of human lymphocytes is increased (perhaps as a result of increasing estrogen levels) and the concentration of progesterone in serum during

pregnancy combine as sufficient to reduce lymphocyte functions [88, 89]. This raises the possibility that hormone sensitivities of immune cells during late gestation may be altered and result in functional changes in immune cells due to rising estrogen concentrations. Very high concentrations of both estrogens and progesterone are reached during the final days of gestation in cows [87]. This may be germane to the onset of impaired lymphocyte function in the prepartum cow whose lymphocyte hormone binding capacity may be higher than that in barren cows.

Many of the hormonal and metabolic changes that prepare the mammary gland for lactation take place during the 3 weeks preceding parturition. Lymphocyte and neutrophil function could be affected by prepartal increases in estrogen, prolactin, growth hormone, and/or insulin [87, 90-92]. During this critical period, the dairy cow's metabolism shifts from the demands of pregnancy to include those of lactation, with increased demands for nutrients. Negative energy and amino acid balances that exist during early lactation may also contribute to impaired neutrophil function and, thus, account for a portion of the periparturient immunosuppression observed.

The specific physiological factors contributing to periparturient immunosuppression and increased incidence of clinical disease have not been fully elucidated. We do know, however, that there is a very broad-based suppression of immune function in cows in the first week or two after calving. Wide variation in leukocyte functional activities has been documented between dairy cows and between different production stages (e.g., around calving time) [54, 56-58, 60, 93-99]. Most importantly, associations between neutrophil dysfunction and periparturient disorders in cows have been reported [45, 51, 59]. Periparturient immunosuppression is not limited to cattle. Investigations of immunosuppression and coliform mastitis in sows revealed depressed neutrophil function to be associated with the susceptibility to postpartum mastitis caused by *Escherichia coli* [76]. Defects in lymphocyte function also contribute to immune suppression during the periparturient period. In addition to reduced antibody production, other impacted roles of lymphocytes in periparturient cows include reduced production of cytokines that activate and direct both innate and adaptive immunity [44, 54, 56, 94, 100-102].

Today it is well recognized that the bovine immune system is less capable of battling pathogens during the periparturient period. The periparturient cow has suppressed immune competence, manifest as reduced capacity for nearly all types of immune cells that have been studied. Interestingly, there may be a teleological reason for immunosuppression in the Th1 branch of the immune system that may be essential in preventing unwanted immune reactions against self and fetal antigens exposed to the mother’s immune system as a result of normal tissue damage in the reproductive tract during parturition [103]. However, an inadvertent and perhaps unintended consequence of this suppression of the Th1 branch of the immune system is that many of the cytokines normally produced by these cells are critical to fully activate neutrophils that are absolutely critical to the defense of the mammary gland. Without a fully functional cellular immune system, both adaptive and innate branches of the cellular immune system operate at diminished capacity for immune surveillance and pathogen

clearance. This is the very circumstance that periparturient cows find themselves in and why it is so critical to manage transition cows to minimize their exposure to pathogens in the environment and to avoid metabolic disorders that might further stress their immune system.

The take-home message here is a multitude of factors of the immune system of a dairy cow become impaired as early as 2 to 3 weeks before she actually gives birth, and long before the elevation of endogenous cortisol which occurs from 36 hours before to 36 hours after calving. The cow's immune system then bottoms out and is seriously impaired for 1 to 2 weeks after calving. This effect is known as periparturient immunosuppression. Regardless of its causation, periparturient immunosuppression makes the dairy cow highly susceptible to the establishment of new infections, particularly in the mammary gland, and the subsequent progression of these new subclinical infections into clinical disease such as mastitis, metritis, and postpartum outbreaks of intestinal diseases such as salmonellosis, just to name a few.

What Are the Prospects for Immunomodulation to Prevent Disease?

Biotherapeutic immune modulators can be given to prevent or lessen disease symptoms caused by various viral and bacterial pathogens. A general goal of such a biotherapeutic compound is to provide the desired effect on host immunity for a sufficient period of time to sustain immunity through a period of immune dysfunction the host is experiencing. Cytokines are one class of compounds that have been investigated for potential biotherapeutic value. Administration of recombinant cytokines to modulate immunity in immunocompromised hosts is thought to prevent bacterial infections [104]. In an effort to study methods to ameliorate the effects of periparturient immunosuppression, several scientists have evaluated various cytokines that are part of the cow's normal immune system [41, 105-110]. Granulocyte-colony stimulatory factor (**G-CSF**) is a cytokine that triggers the bone marrow to produce leukocytes – neutrophils in particular, which in turn, fight infectious disease. Human G-CSF has been successfully used for many years as an adjunct therapy for cancer patients undergoing chemotherapy. In a series of studies, G-CSF has been evaluated for its effects on bovine immunity and as a prophylactic against mastitis [40, 111-115]. Our research findings indicate no adverse effects and that it can reduce the incidence and severity of clinical coliform mastitis by 50% during the first week of lactation following experimental challenge [116]. Granulocyte-colony stimulatory factor has also been shown beneficial against *Staphylococcus aureus* and *Klebsiella pneumoniae* mastitis [115, 117]. It is crucial to understand that immunomodulators work best in immunocompromised hosts; hence the periparturient period is an excellent time for such compounds to be given to cows as they will work to restore the immune system. Acceptable alternatives to the use of antibiotics in food animal practice need to be explored and the use of immunomodulators is a promising area for therapeutic, prophylactic, and metaphylactic approaches to prevent and combat infectious disease during periods of peak disease incidence. Research in the area of biotherapeutic immune modulation continues today.

What Does This All Mean for You?

Bovine mastitis is one of the most economically important diseases to dairy cattle industry. The pathogenesis is highly complex and involves many factors including various microbial etiologies, stress, management and environmental hygiene. Bovine mastitis has not been adequately controlled by vaccination or antibiotics. In many diseases, immunosuppression due to various stressors is responsible for increased susceptibility to bacterial colonization or growth. Over the past 50 years a considerable body of evidence of impaired neutrophil and lymphocyte function in periparturient dairy cows has emerged that coincides with the high incidence of new IMI 2 weeks prepartum and clinical mastitis in early lactation. To overcome this immunosuppression, immunomodulatory agents have been and are being evaluated for their ability to prevent economic losses associated with periparturient diseases such as mastitis. Researchers have investigated immunomodulation as an approach to provide dairy farmers with a new tool to prevent infectious disease in their herds although biotherapeutic products have not yet made it to the market place. The consequences of immune suppression are increases in infectious disease and premature loss from the herd both of which add significantly to the cost of production and decrease the profitability of dairy farming. Simple solutions will not likely be found for something as complex as immune suppression, however, without additional significant research into this topic we can be assured that no progress will be made.

Production of milk from mastitis-free cows is quite simple, right? Keep your cows in clean, dry and unstressful environments and feed them what they need, when they need it – far easier said than done! For years we have emphasized feeding cows optimal rations because the production and functional activities of leukocytes in combating microbial infection are complex and all involve expenditure of cellular energy, protein and other nutrients. The average cow has ~3,500 neutrophils per microliter of blood, this translates into ~1.4 x 10^{11} neutrophils in an 1,800 lb Holstein cow. The circulating half-life of neutrophils is about 6 hours, so the cow is replacing half of those cells every 6 hours from bone marrow stores. Clearly, a significant component of the dietary energy and protein consumption for maintenance is spent on replenishment of immune cells. The negative energy and protein balance of dairy cows during the periparturient period and up to peak lactation undoubtedly influence immune function. We know that cows without the stress of lactation recover from periparturient immunosuppression within 1 week after calving, whereas lactating cows remain immunosuppressed for 2 to 3 weeks postpartum [47, 48, 50]. The most we can do today is to give transition cows the best possible hygienic conditions and appropriate diets.

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SESSION NOTES

Economic Consequences in the Vital 90™ Days

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What is The Vital 90™ Days and What Is Important About it From an Economic Perspective?

The Vital 90™ Days begins approximately 60 days prior to calving and continues through the first 30 days of lactation. During this time, dairy cows experience a series of biological and physiological transitions that are usually accompanied by large changes in feed intake, dramatic shifts in hormonal profiles, and major fluxes in hepatic demands and function. The resulting negative energy and negative protein balance as well as immune suppression often lead to a multitude of metabolic and infectious problems including, but not limited to, retained fetal membranes, ketosis, metritis, displaced abomasum and mastitis among others.

Cows that experience one or more metabolic or infectious challenges during the Vital 90 Days usually experience higher culling and mortality risks. The reason for the higher risk being associated with this periparturient time is multi-fold. First, due to a large decline in feed intake and the hepatic challenge of increased demand for gluconeogenesis, cows are more likely to experience metabolic stress in addition to infectious challenge. Secondly, a significant proportion of cows that experience an adverse health issue actually suffer more than one such event increasing the negative impact on performance and increasing the culling risk. Finally, cows that experience negative health consequences in early lactation often have carryover effects that impact future milk production, reproductive performance, and even future disease risk (Duffield et al., 2009; Overton and Fetrow, 2008; Santos et al., 2013; Wilson et al., 2004). As a consequence, the short term culling and mortality risk is higher and there is carryover impact on future culling risk.

In a similar manner, diseases that occur during The Vital 90 Days is also associated with significant milk production losses. As in the case of culling, milk production losses can be measured in terms of immediate impact and in long term impact. Certain diseases such as metritis (with or without retained fetal membranes) and mastitis that occur during the immediate post parturient period are often associated with potentially large amounts of acute milk loss, but these disease events are also associated with longer term milk loss either via negative impacts to the lactation curve that are never fully recovered or via long term damage to milk secretory cells. Also, early lactation disease such as mastitis is expected to have a greater negative impact on milk production than a similar case that occurs later in lactation due to the lactational time at risk.

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One of the challenges to understanding/estimating the economic impact of disease consequences during The Vital 90 Days is the apparent association between diseases. As shown in Figure 1, the appearance of one disease challenge is often associated with another downstream disease issue (Duffield et al., 2009; Godden et al., 2006; Huzzey et al., 2007; Kimura et al., 2002; Loeffler et al., 1999). For example, cows with hyperketonemia (beta hydroxybutyrate, **BHBA** > 1.2 m*M*) had a greater risk of experiencing a left displaced abomasum (**LDA**), but cows with hyperketonemia in week one postpartum have a 6.1 times greater risk of experiencing an early lactation LDA as compared to cows developing hyperketonemia later (Duffield et al., 2009; McArt et al., 2012). This is not to say that one disease or issue causes another, but rather that there are often associations that exist such that when one condition is observed, there is a higher likelihood of seeing other related conditions. In general, cows that experience greater immune suppression during the periparturient period are more likely to experience retained fetal membranes, metritis, and mastitis; whereas cows that experience more severe negative energy balance are more likely to experience ketosis, displaced abomasum, and ovarian dysfunction.

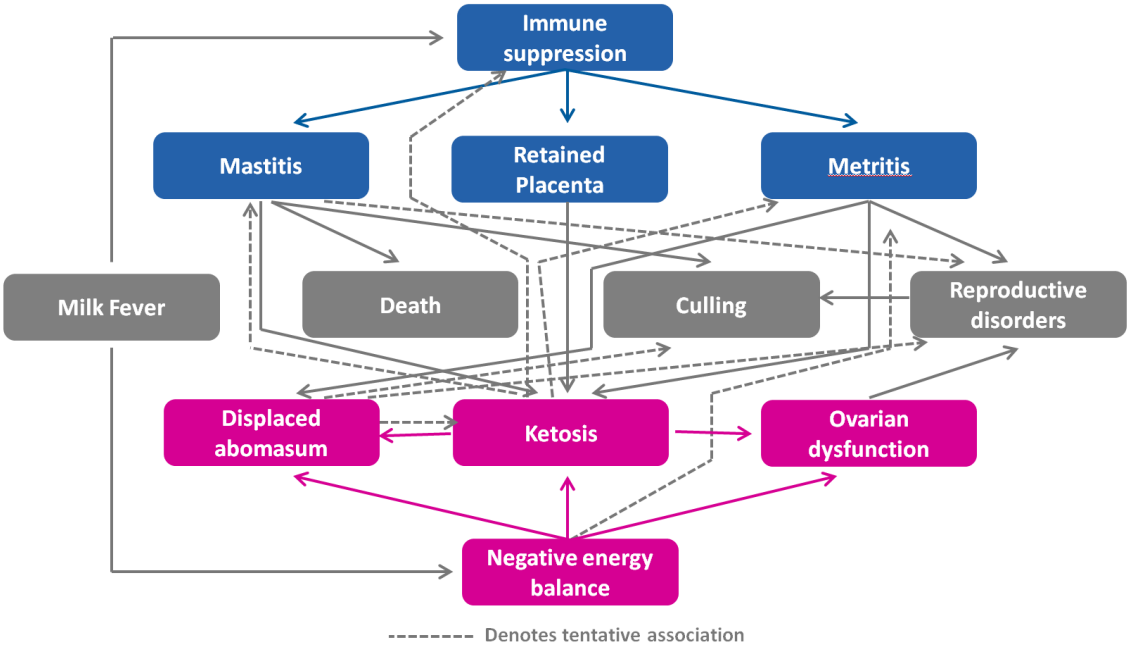

Figure 1. Demonstrated and tentative associations between various periparturient challenges in dairy cattle.

As a consequence of the multitude of negative impacts of metabolic and infectious challenges that occur during the Vital 90 Days, dairy producers and influencers typically spend considerable time, money and effort attempting to mitigate the negative impacts and consequences of these challenges. However, despite the high level of financial and management investment, few have carefully considered the full magnitude of cost incurred by each cow that calves. Elanco Knowledge Solutions, an analytics team within Elanco Animal Health, has developed a new tool to estimate and demonstrate the various costs of The Vital 90 Days at the farm level, and to present

a new industry metric for evaluating transition dairy cow performance, The Vital 90 Days Cost per Calving.

What Is the Economic Assessment Tool and What Does It Do?

The Economic Assessment Tool is part accounting tool and part economic model. It does not suggest or predict management needs but instead, tabulates all of the various costs currently being incurred during The Vital 90 Days other than routine feed costs or costs associated with milking during early lactation. The total cost estimate that is calculated by the Economic Assessment Tool is called The Vital 90 Days Cost per Calving.

This new metric, The Vital 90 Days Cost per Calving, is comprised of two major types of cost. The first cost for consideration is called the *Investment Cost* of the Vital 90 Days and is comprised of all of the vaccinations, therapeutics, nutraceuticals, feed additives, and management time and effort used by the dairy and its staff to mitigate health risks and to help increase the likelihood of a successful lactation. The tool allows each consultant to input the farm-specific protocols for each individual herd under consideration instead of relying on industry-wide estimates, and these inputs are stratified into first calving and second or greater calving to reflect potential management differences between animals calving for the first time and those returning to lactation. As mentioned previously, feed additives are considered in the Investment Costs but the base ration cost for each group is not considered.

The second source of costs in The Vital 90 Days is the *Consequence Cost.* Despite typical preventive efforts, 45 to 60% of cows typically experience one or more transition-related disorders (Ribeiro et al., 2013; Santos et al., 2010). *Consequence Cost* of disease refers to the total impact of disease occurring during this time and is subdivided into *Direct Disease Costs* and *Indirect Disease Costs.*

Direct Disease Costs include most of the commonly recognized costs associated with the impact of disease including diagnostics and therapeutics for clinical cases treated, the value of milk that must be discarded during treatment and any required withdrawal period, veterinary services, on-farm labor, and death losses that are directly associated with specific periparturient disease issues. Some people may refer to Direct Disease Costs as explicit costs since these often seem more tangible and typically occur at or very near to the time of disease diagnosis.

Indirect Disease Costs, however, are usually more implicit in nature and often represent the “lost opportunity cost” of disease. The contributors to *Indirect Disease Costs* include any predicted future milk production losses, future culling losses, on-going diagnostic or monitoring costs, future reproductive losses, and in many cases, other diseases that are attributable to the initial disease in question. By summing the Direct Disease Costs and the Indirect Disease Costs, the total Consequence Cost is determined.

When attempting to calculate the impact (and therefore, the cost) of a specific disease on the risk of other downstream diseases, the attributable risk and attributable cases must be estimated. Attributable risk is an epidemiological term that describes the difference in disease risk between an exposed population and an unexposed population. In order to calculate the attributable risk, the relative risk (**RR**) for the exposed population as compared to the non-exposed population should be adapted from the veterinary literature. For example, if a herd has a recorded incidence of 40% hyperketonemia in early lactation and cows with hyperketonemia have a RR of 8 for LDA, then cows with hyperketonemia are 8 times more likely to develop an LDA as compared to their non-affected herd mates. If the underlying risk of LDA for the herd as a whole is 3%, the risk of LDA in unaffected cows is ~ 0.8% and in hyperketonemic cows, the risk is ~ 6.3%. Consequently, the attributable risk of LDA given hyperketonemia is ~5.5%. Thus, ~88% of the total LDA cases in the herd can be attributed to hyperketonemia. The remaining 12% of the total cases (0.8% incidence in the "normal" cows) is found in cows that never experienced primary, early lactation hyperketonemia and these cases occurred due to unexplained reasons.

In the estimation of the *Indirect Disease Cost* of a specific disease, extreme care must be taken to avoid double or triple counting of costs. In the Economic Assessment Tool, two disease cost estimates are given for common transition issues that occur very early in lactation and that are linked to other costly outcomes further downstream such as clinical hypocalcemia, retained fetal membranes, hyperketonemia, and metritis. First, the ***Component Cost*** of a disease is reported. This cost estimate includes those *Direct* and *Indirect Disease Costs* that are directly attributable to the disease in question without consideration of further downstream impacts on other diseases. By summing all of the disease costs *not attributable to other diseases*, the Component Cost can be estimated with minimal risk of double counting disease costs.

Second, the ***Total Cost*** of a disease is also reported and this cost estimate includes the Component Cost and any additional *Direct* and *Indirect Disease Costs* incurred as the result of development of other disease issues that are predicted to result as a consequence of having the original issue in question. If the Total Cost of each disease reported were added together, a *greatly inflated disease cost estimate would be created*. Hence, the Total Cost of a disease is only applicable when discussing the potential impact of a product or management change on one very specific disease without regard to its carry-over impact on any other disease.

For example, in the case of hyperketonemia, there is a strong association between the presence of elevated ketones in the blood in the early post parturient period and a multitude of adverse health events including, but not limited to, an increased risk of culling, death, metritis, displaced abomasa, and future reproductive challenges. If the Total Cost of hyperketonemia was added to the Total Cost of metritis and the Total Cost of displaced abomasa, we would grossly overestimate the cost of hyperketonemia and the predicted number of cases of metritis and displaced abomasa would greatly exceed the herd's actual number of cases. However, if we add the

component costs of each disease, the grand total will more accurately reflect the total impact on the herd.

The Economic Assessment Tool adds the total investment cost of the far off, close up, and maternity/fresh cows to the total consequence cost (direct and indirect disease costs) and reports The Vital 90 Days Cost per Calving by parity. To correctly tabulate all of the Investment and Consequence Costs, a number of inputs must be made carefully and correctly. These inputs consist of the following sections:

1. General herd parameters - The first set of inputs involves whole farm inputs such as milk price, labor cost, replacement heifer cost, how waste milk is utilized, culling risk, etc. These inputs serve as the basis for the calculation of many other inputs and outputs such as the value of marginal milk and the cost of discarded milk during treatment.

2. Preventive protocols - In the Economic Assessment Tool, there is a detailed section for customizing each of the routine preventive protocols that occur during the far off, close up and calving/fresh periods. Expected costs, based on currently available market prices, are already embedded in the tool but can and should be customized for each herd.

3. Disease incidence - In order to correctly tabulate disease costs, the current incidence of the various common transition disorders must be input by parity group. Unfortunately, many dairies struggle with this area. Occasionally, herds may record disease issues in paper form but fail to enter the information in the computerized on-farm record system. Unfortunately, a far more common problem is simply a failure to utilize consistent disease definitions along with a failure to consistently record the occurrence of disease. Without proper disease information, the Economic Assessment Tool will vastly underestimate The Vital 90 Days Cost per Calving.

4. Treatment protocols - Each herd should have a standardized approach to treatment of common transition disorders such as milk fever, ketosis, metritis, mastitis, etc. The Economic Assessment Tool has a very detailed data input section that facilitates input and customization of the standard treatment protocols for each disease issue by parity group. As with the preventive protocol section, most of the commonly used pharmaceutical agents are already present in the tool but may be customized in terms of dose, duration, withdrawal and cost in order to more carefully and accurately reflect the farm's current protocols.

When attempting to understand any biological or economic system, the results and conclusions reached from analysis are greatly influenced by the accuracy and completeness of the inputs and other contributing factors. Similarly, the utility of the Economic Assessment Tool will not be optimized and its full value not realized if the inputs in the tool are incomplete or inaccurate. Hence, careful consideration of the herd's current general parameters, precise description of its preventive and treatment

protocols, and accurate calculation of true disease incidence is critical. During development and early field use of this tool, the single largest bottleneck to capitalization of the value of the Economic Assessment Tool is the inconsistencies or total failure to correctly and consistently define and record disease occurrence on dairies. When disease information is not captured, the tool will generate an estimate of cost that is lower than reality. For more information on the topic of disease recording, please see the companion paper in these proceedings titled "Disease Records for Impactful Decisions During The Vital 90™ Days."

If inputs have been carefully and accurately made into the Economic Assessment Tool, the output will represent the estimated cost of transitioning a cow through the Vital 90™ Days and will allow the user to now ask pertinent management questions such as 1) "What is the estimated economic impact of adding input X in the Vital 90 Day period?"; 2) "If the incidence of disease Y can be reduced by 30%, how does this impact my herd?"; 3) "Given the current level of inputs and disease incidence in my herd, what are the biggest opportunities to improve my profitability?"; 4) "How does a change in replacement heifer cost or market cow value impact the Cost per Calving?"

Dairy managers and consultants need better decision-making tools. Owners and managers are asked to consider using new or improved products on a routine basis. However, identifying the cost/benefit or return on investment for these opportunities can be difficult. One approach to these types of decisions is to assess the overall investment strategy and disease consequence costs of a proposed change. The Economic Assessment Tool is a new means to help answer these questions and to help better understand the total investment and consequence costs associated with freshening a dairy cow.

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SESSION NOTES

Nutrition and Reproductive Efficiency: Transition Period Management, Energy Status, and Amino Acid Supplementation Alter Reproduction in Lactating Dairy Cows

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Introduction

A number of reviews have highlighted the importance of nutrition in regulating bovine reproductive efficiency (Cardoso et al., 2013; Grummer et al., 2010; Santos et al., 2010; Wiltbank et al., 2006). The effects of nutrition in the embryo donor cow have been particularly emphasized (Santos et al., 2008; Sartori et al., 2010; Sartori et al., 2013; Velazquez, 2011; Wu et al., 2013). This review will specifically focus on some of our results related to how changes in nutrition can alter reproductive efficiency of dairy cattle.

Inadequate or excessive energy, protein, or specific amino acids can have effects at multiple stages of the reproductive process. First, effects during the early postpartum period have been postulated to alter the oocyte and subsequent embryo development after fertilization of this perturbed oocyte (Britt, 1992). Second, changes in circulating hormones and metabolites such as insulin, glucose, urea, or amino acids during the final stages of oocyte development, before ovulation, can profoundly impact fertilization or embryo development (Adamiak et al., 2005; Adamiak et al., 2006; Bender et al., 2014). A third obvious target of nutrition on the embryo is during the first week of development when changes in oviductal and uterine environment could alter development of the embryo to the blastocyst stage (Steeves and Gardner, 1999a; b; Steeves et al., 1999). Finally, changes in circulating energy metabolites such as glucose and propionate, and building blocks for cells such as amino acids, could alter the uterine lumen composition and subsequently influence hatching and embryo elongation. The elongating embryo secretes the protein interferon-tau that is essential to rescue the corpus luteum and can alter the concentrations of many substances in the uterine lumen (Groebner et al., 2011; Hugentobler et al., 2010). Alternatively, select nutrients in the uterine lumen can also alter interferon-tau expression (Kim et al., 2011). Thus, deficiencies or excesses of energy, protein, or specific amino acids could have targeted impact on a specific stage of oocyte/embryo development or may have multiple, potentially additive effects, on reproductive processes. Due to space limitations, many specific nutritional effects will not be approached in this particular review article, including effects of fatty acid supplementation, as well as vitamin and mineral

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supplementation or deficiencies; however, some of these aspects have been recently reviewed (Leroy et al., 2013; 2014; Santos et al., 2008; Velazquez, 2011).

Transition Period Nutritional Management: Effects of Dry Period Length

One extreme way alter energy balance during the transition period is to eliminate the dry period. Our studies, in collaboration with Ric Grummer's laboratory, have shown a positive effect of dry period elimination on energy balance, return to estrous cyclicity, and fertility (Grummer et al., 2010). When the dry period was eliminated, negative energy balance during the early postpartum period was effectively eliminated (Rastani et al., 2005). Time to first ovulation was reduced when comparing cows that had a 56 day dry period (31.9 + 4.4 d) with cows with a 28 d dry period (23.8 + 3.4 d) and cows with no dry period (13.2 + 1.2 d) (Gumen et al., 2005). Of particular interest to this review, the pregnancies per artificial insemination (**P/AI**) were increased in cows with no dry period (55%) compared with cows with a 56 day dry period (20%) (Gumen et al., 2005). Thus, changes in dry period management can reduce/eliminate negative energy balance during the early postpartum period and increase P/AI (Grummer et al., 2010; Gumen et al., 2005; Watters et al., 2009).

Transition Period Nutritional Management: Effects of Changes in Body Condition

The relationships between energy intake, energy output, and form of dietary energy (fiber vs. non-fiber carbohydrate, **NFC**) have been shown to produce profound effects on metabolic status of the cow and, in some cases, reproductive performance of both dairy and beef cattle. Part of this effect is due to a delayed return to cyclicity. Negative energy balance decreases dominant follicle growth and estradiol (**E2**) production probably related to the decrease in luteinizing hormone (**LH**) pulses as well as the decrease in circulating insulin and IGF-1 (Butler, 2003; 2005; Canfield and Butler, 1990). The magnitude of body condition score (**BCS**) loss after calving can increase in the percentage of cows that are not cycling at the end of the voluntary waiting period (Gumen et al., 2003; Lopez et al., 2005; Santos et al., 2004; Santos et al., 2009). An increase in percentage of anovular cows will lower reproductive efficiency in programs using detection of estrus or synchronized ovulation and timed artificial insemination (**TAI**) (Gumen et al., 2003; Santos et al., 2009). Cows with lower BCS near the time of AI have decreased fertility (Moreira et al., 2000; Souza et al., 2008) and this may be related to increased anovulation as BCS decreases (Santos et al., 2009).

In a recent retrospective study (Carvalho et al., 2014), we evaluated the effect of BCS near TAI on reproductive performance of lactating dairy cows treated with Double-Ovsynch protocol (Herlihy et al., 2012; Souza et al., 2008) to induce cyclicity and synchronize ovulation. Cows with low BCS (≤ 2.5) compared to cows with BCS ≥ 2.75 had greater incidence of anovulation (12.3% [21/171] vs. 4.9% [22/451]; $P = 0.0006$) and decreased P/AI (40.4% [105/260] vs. 49.2% [415/843]; $P = 0.03$). Thus, BCS near AI has a small but significant effect on fertility even when cows are induced into cyclicity using a GnRH-based protocol, such as Double-Ovsynch.

Potentially even more important to fertility than the absolute BCS at the time of AI is the amount of BCS loss between parturition and first AI (López-Gatius et al., 2003; Santos et al., 2009). Consistent with this idea, in experiment 2 of our study (Carvalho et al., 2014), we observed a much more dramatic effect on P/AI when we evaluated cows for BCS change between calving and 21 d after calving. The P/AI differed ($P < 0.001$) dramatically among BCS change categories and was greater for cows that gained BCS (83.5%; 353/423), intermediate for cows that maintained BCS (38.2%; 258/675), and least for cows that lost BCS (25.1%; 198/789). Thus, these results are consistent with the idea first introduced by Britt (1992), who postulated that energy status during the early post-partum period could alter follicular/oocyte quality resulting in negative effects on subsequent fertility in lactating dairy cows.

In experiment 3 (Carvalho et al., 2014), we decided to directly test this hypothesis by evaluating the effect of early postpartum body weight loss on embryo quality from superstimulated cows (Carvalho et al., 2014). The body weight of lactating dairy cows (n = 71) was measured weekly from first to ninth week postpartum and then all cows had superovulation induced using a modified Double-Ovsynch protocol. Cows were divided into quartiles by percentage of body weight change (Q1 = least change; Q4 = most change) from calving until third week postpartum. There was no effect of quartile on number of ovulations, total embryos collected, or percentage of oocytes that were fertilized; however, the percentage of fertilized oocytes that were transferable embryos was greater for cows in Q1, Q2 and Q3 than Q4 (83.8%, 75.2%, 82.6%, and 53.2%, respectively). In addition, percentage of degenerated embryos was least for cows in Q1, Q2, and Q3 and greatest for Q4 (9.6%, 14.5%, 12.6%, and 35.2% respectively). Thus the effect of changes in BCS during the early post-partum period on subsequent fertility at first AI could be partially explained by the reduction in embryo quality and increase in degenerate embryos by d 7 after AI in cows that lost more body weight from first to third week postpartum. This result is obviously consistent with the hypothesis introduced by Britt (1992). Thus, BCS and particularly BCS change can have dramatic effects on fertility and early embryo development in dairy cattle.

Effects of High Energy Diets on Fertility

Another somewhat opposite idea related to dietary energy intake and energy balance is an observed reduction in embryo quality when cows were fed excessive energy in the diet near the time of AI. Increases in feed intake or increased dietary NFC have been found to alter insulin (Adamiak et al., 2005; Adamiak et al., 2006) and progesterone (**P4**) concentrations (Sangsritavong et al., 2002; Vasconcelos et al., 2003), and superestimulatory success (Yaakub et al., 1999). Superestimulated beef heifers that were fed a high energy diet ad libitum (excessive energy) compared to 81% of ad libitum intake had reduced number of CL, reduced number of recovered structures, and dramatically reduced yield of transferrable embryos (Yaakub et al., 1999). Thus, excessive energy consumption can alter embryo development, although the mechanism(s) for these effects and whether the effects are on the oocyte or directly on the early embryo are not yet fully described.

We tested this idea by comparing the reproductive records of 49 free-stall Holstein-dairy herds in WI (herds using Dairy Comp 305 [n = 44] and PCDart [n = 5] software for management) with the composition of total mixed ration (**TMR**) diets. The nutritional information included all ingredients and nutrient composition of all mixes used. Size of herds enrolled in the data collection varied from 143 to 2,717 lactating cows (average 719.6 ± 77.2), were milked 2 (n = 6) or 3 (n = 43) times per day, with average production per cow of 39.0 ± 1.3 Kg/day, and average dry matter intake (**DMI**) of 25.1 ± 0.5 Kg/day. There was substantial variation in diet composition. For example, crude protein (**CP**) varied from 16.0 to 18.7%, rumen-degrable protein (**RDP**) from 9.1 to 12.3%, neutral detergent fiber (**NDF**) from 24.9 to 35.1%, NFC from 31.7 to 46.6%, starch from 20.1 to 30.8%, and fat from 3.1 to 6.7%. Milk production level was not associated with P/AI at first AI or other reproductive measures ($P > 0.10$). However, greater DMI tended to be associated with lower first service P/AI ($r = -0.25$, $P = 0.10$). Dietary content of CP, RDP, and fat was not associated with P/AI ($P > 0.10$). Percentage of dietary NDF was positively associated with first service P/AI ($r = 0.36$, $P = 0.01$). Most interestingly, greater energy content in the diet measured as NFC, NFC-intake, or starch were found to be detrimental to first service P/AI (NFC: $r = -0.54$, $P < 0.01$; NFC intake: $r = -0.42$, $P < 0.01$; starch: $r = -0.37$, $P = 0.03$), and all P/AI combined (NFC: $r = -0.51$, $P < 0.01$; NFC intake: $r = -0.44$, $P < 0.01$; starch: $r = -0.21$, $P = 0.20$). In conclusion from this study, diets containing more fiber and less rapidly digestible carbohydrates were associated with improved reproductive performance in high-producing dairy herds.

An important idea that needs to still be adequately tested is that excessive energy could lead to overstimulation of the follicle and oocyte leading to subsequent reductions in embryo development (Garnsworthy et al., 2008a; b; Rooke et al., 2009; Webb and Campbell, 2007). Some evidence for negative effects of overfeeding on embryo development is provided by a study using super-stimulated ewes in which overfeeding (2.2 times maintenance) dramatically reduced embryo quality compared to underfed (0.5 times maintenance) ewes (Lozano et al., 2003). This last study, as well as others in lactating cows (Sangsritavong et al., 2002; Vasconcelos et al., 2003), also observed that animals with greater feed intake had reduced circulating P4 concentrations. Previous studies have shown that increased circulating P4 concentrations during super-stimulatory treatments increased embryo quality and number of transferrable embryos (Nasser et al., 2011; Rivera et al., 2011). Lower circulating P4 may lead to increased LH pulses possibly leading to premature resumption of meiosis and ovulation of an oocyte of reduced fertility, as has been observed in persistent follicle models (Revah and Butler, 1996; Roberson et al., 1989). In addition to the effect of P4 during preovulatory follicle development, increasing circulating P4 concentrations after breeding, during early embryo development, can increase embryo development, particularly increasing length of the preimplantation embryo (Lonergan and Forde, 2014; Lonergan et al., 2013; Maillo et al., 2014; O'Hara et al., 2014a; O'Hara et al., 2014b; Wiltbank et al., 2014).

Excessive concentrations of insulin may decrease oocyte quality and subsequent embryo development. Adamiak et al. (2005) conducted an elaborate experiment

collecting oocytes via ultrasound-guided trans-vaginal follicular aspiration in beef x dairy crossbred heifers exposed to either maintenance or two times maintenance feeding levels over a period of three successive estrous cycles. The study found that the effect of feeding level on oocyte quality is dependent on body condition of the heifers; thus, the two times maintenance had a positive impact on oocytes recovered from heifers in a low BCS but had a negative impact on oocytes recovered from heifers of a moderately high BCS. In addition, many of the moderately fat heifers were hyperinsulinemic, which also had a negative impact on oocyte quality. In a similar study, heifers exposed to a high starch diet had a corresponding increase in circulating insulin concentrations and a subsequent decrease in blastocyst production rate (Adamiak et al., 2006). Thus, excessive energy intake may reduce embryo quality through elevations in LH pulses or through excessive insulin or other metabolic signal associated with consumption of a high carbohydrate diet or excess energy.

In later lactation Holstein dairy cows, energy intake generally exceeds energy output and therefore cows are in positive energy balance and circulating insulin is elevated. Acute restriction of feed intake reduced circulating insulin and increased circulating P4 in late lactation dairy cows (Ferraretto et al., 2014). We used this model to test specific hypotheses related to feed intake (ad-libitum intake vs. 25% feed restricted) and LH (± additional LH) in super-stimulated Holstein cows in late lactation using a 2 X 2 Latin square design (Bender et al., 2014). As expected, feed restriction had a substantial effect on circulating insulin concentrations without changing plasma glucose concentrations (Bender et al., 2014). Large changes were not observed in numbers of large follicles on the final day of super-stimulation, in the percentage of these follicles that ovulated, or in the number of CL on the day of flushing. Probably the most consistent and biologically-interesting result from this study was an interaction that was found between feed restriction and amount of LH during the superovulation protocol on the percentage of oocytes that were fertilized, and on the percentage of total structures that were graded as 1 and 2 embryos (best quality embryos) compared with degenerate embryos. It appears that combining ad libitum feeding and high LH reduced percentage of oocytes that were fertilized and subsequent embryo quality of fertilized oocytes. This is consistent with the idea that high LH combined with high insulin can reduce embryo quality. Conversely, feed-restricted cows with low LH in the super-stimulation protocol also had reduced fertilization of oocytes, reduced percentage of grades 1 and 2 embryos (of total structures), and increased degenerate embryos. However, increasing LH in feed-restricted cows increased embryo quality. Thus, there was an interaction between these two treatments on embryo quality that is consistent with the idea that optimizing super-stimulatory success requires consideration of both the hormonal and metabolic state of the treated cow with conditions that produce both high LH and high insulin (excess energy consumption) apparently being negative for fertilization and embryo quality (Bender et al., 2014).

In conclusion, it seems clear that negative energy balance during the first 3 weeks after calving can have a negative impact on fertility at the first AI, even though the AI occurred more than 5 weeks after the original negative energy balance. The harmful effect of negative energy balance during the transition period is manifest in

reduced embryo development during the first week after AI, suggesting a lingering effect of the transition problems on oocyte competence. In late lactation Holstein cows, feed restriction had a positive effect on embryo quality when supplemented with LH, but was negative in cows without additional LH. In dry Holstein cows on a maintenance diet, elevations in insulin reduced fertilization, suggesting a negative effect of insulin on oocyte quality, but did not alter subsequent embryo development or quality. Thus, breed, BCS, and current metabolic status of the cow need to be considered when deciding the optimal nutritional and hormonal programs to use during embryo production.

Effects of supplementation of specific amino acids on fertility

Some amino acids are limiting for optimal milk production as evidenced by an increase in milk and protein yields, and percentage of protein in milk after supplementation with specific, rumen-protected amino acids (Cho et al., 2007; Patton, 2010; Socha et al., 2005). Generally the first three rate-limiting amino acids for milk production are considered to be methionine (**Met**), lysine (**Lys**), and histidine (**His**) in most diets fed to lactating cows. In addition, many amino acids can have positive effects on physiological processes that are independent of their effects on synthesis of proteins. This has been termed “functional effects” of amino acids and methionine and arginine effects are the best studied “functional amino acids” that have been linked to reproduction (Bazer et al., 2011; Penagaricano et al., 2013).

Most amino acids are more concentrated in the oviduct and uterus than in the blood (Hugentobler et al., 2007). In addition to the mechanisms that concentrate amino acids in the uterus in non-pregnant ruminants, there are additional mechanisms that result in further increases in concentrations of amino acids in the uterine lumen in pregnant ruminants near the time of embryo elongation (Day 14 to 18 of development). Of particular interest for dairy cattle, the three amino acids that are considered rate-limiting for milk production, Met, His, and Lys, are the amino acids with the greatest increase in concentrations in the uterine lumen during embryo elongation (>10-fold increase on average (Gao et al., 2009c; Groebner et al., 2011)). Arginine is another amino acid that has been studied extensively in relation to reproduction (Wu et al., 2013) and it is also highly concentrated in the pregnant uterus.

The increase in specific amino acids in the uterus near the time of embryo elongation appears to be due to an induction of specific amino acid transporters in the uterine endometrial cells (Gao et al., 2009a; b; Groebner et al., 2011). The induction of these amino acid transporters is most likely induced by the protein interferon-tau that is secreted by the elongating conceptus (tissues that will generate the embryo and placenta). For example, interferon-tau treatment dramatically increased one specific amino acid transporter, SLC15A3, in both glandular epithelial (36-fold) and stromal epithelial (177-fold) uterine cells (Groebner et al., 2011). Thus, there is likely a positive feedback system occurring during this critical time of embryo elongation with uterine amino acids being essential for rapid embryo growth and embryonic interferon-tau production; whereas, interferon-tau stimulates active amino acid transport through the uterine epithelial cells to increase amino acid supply to the elongating embryo.

Disturbances in the temporal relationship between uterine blood flow, induction of uterine amino acid transport, uterine amino acid concentrations, embryonic growth, embryonic interferon-tau production, and rescue/regression of the corpus luteum may reduce fertility and increase pregnancy losses.

Numerous studies have evaluated the effects of rumen-protected amino acids, particularly methionine, on milk production. For example, a recent meta-analysis (Vyas and Erdman, 2009) evaluated the results from 35 experiments on production effects of postruminal supplementation with methionine. At low methionine intakes (25 g per cow per day) there were dramatic increases in milk protein (16 g of milk protein per gram of metabolizable methionine intake); whereas, the production response was more muted at high methionine intake (70 g per cow per day; increase of 4 g of milk protein per g of metabolizable methionine intake). Unfortunately, we have been unable to find studies in the scientific literature which were specifically designed and adequately powered to evaluate the effects of specific amino acids on reproductive efficiency of lactating dairy cows. The largest study (Polan et al., 1991) combined results from 259 cows at 6 Universities evaluating rumen-protected methionine and lysine supplementation. They detected no significant effect on days to first service, services per conception, or calving interval, although no details were provided on reproductive measures in each specific treatment group. It is obvious that large studies are needed to validly evaluate the effects of supplementing amino acids on measures of reproductive efficiency in lactating dairy cows.

One particularly interesting study (Coelho et al., 1989) used serum from lactating dairy cows in the media to grow head-fold stage rat embryos (Day 9.5 after breeding). Complete development of these embryos requires serum and development is normal in rat serum. When embryos were grown in serum from dairy cows embryonic development was abnormal when measured as total embryo protein, somite pairs, or percentage of the embryos that are abnormal (no neural tube closure, abnormal shape, no development of eyes and branchial arches). Supplementation of bovine serum with amino acids and vitamins produced normal development. Amino acid supplementation alone but not vitamin supplementation produced normal development. Supplementation of methionine alone was sufficient to produce normal development of the rat embryos in cow serum. In a separate experiment, use of serum from cows that were supplemented with rumen-protected methionine (110 g/d) also produced normal embryo development. Thus, bovine serum has such low methionine concentrations that normal development of rat embryos is retarded.

The requirements for complete development of bovine embryos have not yet been determined. Current culture conditions allow production of bovine embryos to the blastocyst stage (Day 7-8) and even allow hatching of a percentage of embryos (Day 9); however, conditions have not been developed that allow elongation of embryos in vitro, and definitely do not allow culture of bovine embryos to the head-fold stage that was analyzed in the rat embryo experiments. The methionine requirements for in vitro produced preimplantation bovine embryos (Day 7-8) was recently determined in studies from University of Florida (Bonilla et al., 2010). There was a surprisingly low methionine

requirement (7 µM) for development of embryos to the blastocyst stage by Day 7, however development to the advanced blastocyst stage by Day 7 appeared to be optimized at about 21 µM (Bonilla et al., 2010). Thus, the results of this study indicated that development of morphologically normal bovine embryos did not require elevated methionine concentrations (> 21 µM), at least during the first week after fertilization.

A recent study (Ikeda et al., 2012) evaluated whether methionine metabolism was required for normal development of bovine embryos. The researchers added ethionine or additional methionine to cultures of bovine embryos. Ethionine blocks metabolism of methionine into the one-carbon pathway (termed antimetabolite of methionine). Ethionine did not block development to the morula stage but blocked development to the blastocyst stage (Control = 38.5%; Ethionine = 1.5%). Development to the blastocyst stage in the presence of ethionine was partially restored by adding S-adenosylmethionine (**SAM**) which would restore the methylation pathway but not restore protein synthesis. Thus, methionine has an essential role in the development of the bovine embryo from morula to blastocyst, whichsuperstimulated is probably partially mediated by hypomethylation in the absence of sufficient methionine.

We recently evaluated the effect of supplementation with rumen-protected Met on early embryo development in superstimulated cows (Souza et al., 2012a; Souza et al., 2012b). We used superstimulated cows so that we would have sufficient statistical power by evaluating numerous embryos in order to validly test the in vivo effects of methionine supplementation on early embryo development in lactating dairy cows. In this experiment, cows were blocked by parity and calving date and randomly assigned to two treatments differing in level of dietary Met supplementation: 1) Met; diet composed of (% dry matter) corn silage (39.7), alfalfa silage (21.8), high-moisture corn (17.2), roasted soybeans (8.6), grass hay (4.6), canola meal (4.0), mineral-vitamin mix (2.7) and a blood meal-based product (ProVAAl Ultra; Perdue Agribusiness) with the rumen protected Met Smartamine (Adisseo), formulated to deliver 2,875 g of metabolizable protein (**MP**) with 6.8 Lys as % of MP and 2.43 Met as % of MP; 2) Control; cows fed the same basal diet but replacing ProVAAl Ultra by ProVAAl Advantage, which contains no added rumen protected Met, formulated to deliver 2,875 gr MP with 6.8 Lys as % of MP and 1.89 Met a % of MP. There was an increase in both kg of milk protein produced and percentage of protein in the milk (Souza et al., 2012b). Thus, from a protein production standpoint, Met appeared to be rate-limiting. We measured plasma Met concentrations in this study and found a large effect of feeding rumen-protected Met on circulating Met concentrations (Control = 16.8 µM vs. Met-supplemented = 22.9 µM).

Our primary interest was the effect of supplemental Met on embryo quality (Souza et al., 2012a). We evaluated a total of 570 embryos in this experiment and found no differences in fertilization or embryo quality. Thus, Met supplementation did not alter early embryo development, at least from a gross morphological standpoint.

Even though Met supplementation during the later stages of follicle development and early embryo development may not have produced morphological changes in the

early embryo, it is well known that Met during this time can have dramatic effects on the epigenome of the embryo (Sinclair et al., 2007). This means that the genes can be changed in such a way that they are not expressed in the same way due to addition of groups, generally methyl groups to the DNA of the cells. For example, a previous study in sheep restricted methyl donors by restricting Met, vitamin B12, and folate before and for the first 6 days after breeding (Sinclair et al., 2007). They then transferred normally-appearing embryos into control sheep and then evaluated the lambs after parturition. The embryos that were produced in low methionine produced lambs that had substantial differences in blood pressure and immune function. To test this idea in cattle, we evaluated whether the embryos that were recovered from cows that had been supplemented or not supplemented with Met had differences in gene expression (Penagaricano et al., 2013).

The objective of this part of the study was to evaluate the effect of maternal Met supplementation on the transcriptome of bovine preimplantation embryos (Penagaricano et al., 2013). Only high quality embryos from individual cows were pooled and then analyzed by a powerful technique that allows evaluation of all genes that are expressed in these embryos, called RNA sequencing (**RNAseq**). Remarkably, the small difference that we produced in circulating methionine produced a substantial difference in expression of genes in the embryo. A total of 10,662 genes were significantly expressed in the bovine embryos. A total of 276 genes were expressed differently, statistically, in embryos from cows supplemented or not supplemented with methionine. Most of these genes were turned off in embryos from cows that were supplemented with methionine. This would be expected since methionine supplementation leads to methylation of the DNA and this can inhibit expression of some specific genes until cells differentiate to the appropriate stage when gene expression should commence (Burdge et al., 2007; Wolff et al., 1998). Thus Met supplementation seemed to change gene expression in a way that may lead to improved pregnancy outcomes and improved physiology of the offspring. Many of the genes are involved in immune function and later stages of embryo development that may be critical for pregnancy progression and normal immune function after birth. Further studies are needed to determine if these changes in gene expression lead to changes in embryo development, reduced pregnancy loss, and altered physiology of the offspring.

Thus, supplementation of rate-limiting amino acids can have substantial effects on milk protein content and yield; however, effects on reproduction have not yet been adequately evaluated. The dramatic induction of the rate-limiting amino acids, Met, His, and Lys, in the uterine fluid of pregnant cows near the time of embryo elongation suggests that elevated amounts of these amino acids may be critical for this important stage of embryo development. Supplementation of cows with methionine during the final stages of follicular development and early embryo development, until Day 7 after breeding, did not lead to gross morphological changes in the embryos but did result in dramatic differences in gene expression in the embryo. Further studies are needed to evaluate whether supplementation with these essential amino acids to lactating cows

would have a beneficial impact on embryo survival and if these changes in the early embryo translate into changes in pregnancy outcomes or physiology of the resulting calf.

Conclusions

Fertility can be affected in a positive or negative way by deficiencies or excesses of energy/carbohydrates and protein/amino acids. Some of these effects may be occurring during the final stages of oocyte development within the preovulatory follicle but are only manifest by the blastocyst stage. For example, the effects discussed above using feed restriction and LH supplementation during follicle development can alter subsequent embryo development (Bender et al., 2014). In addition, some of the effects on embryo function may not be manifest in gross morphological appearance of the embryos but result in dramatic differences in gene expression as observed in the study that evaluated embryonic gene expression using RNASeq in embryos produced in dams that were supplemented or not supplemented with methionine (Penagaricano et al., 2013). There is still a great deal more fundamental biology that needs to be done to fully understand how embryo development can be most practically manipulated using nutritional strategies.

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SESSION NOTES

Mechanisms Linking Postpartum Metabolism with Reproduction in Dairy Cows

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Introduction

Milk production per cow is increasing in the United States. The period of peak milk production is in early lactation, usually within 30 to 60 days after calving when the cow's uterus is involuting and the cow's ovary is returning to estrous cyclicity. The competing processes of milk production, uterine involution, and the restoration of ovarian activity can be at odds, particularly if the unique homeorhetic processes that typify early lactation become imbalanced and the cow experiences negative energy balance and (or) metabolic disease during early lactation. A potential end result is that the cow does not become pregnant during the breeding period. Understanding the mechanisms that link the first 60 days of lactation with the subsequent reproductive success or failure is an important area of research for the dairy industry.

This paper will specifically focus on glucose because of its dual purpose as a major component of cow's milk and also a molecule that coordinates homeorhetic mechanisms that could possibly impinge upon postpartum uterine health and the subsequent establishment of pregnancy.

Why Glucose Is Involved in Both Lactation and Reproduction

Glucose metabolism presents an interesting challenge for the cow. The microorganisms in the rumen ferment carbohydrates to volatile fatty acids (**VFA**) that can be oxidized for energy. In addition to VFA, protein and fat passing into the lower digestive tract are absorbed and used for the synthesis of milk protein and fat. Seventy-two g of glucose are required for each kg of milk produced (Bell, 1995). Most of this glucose is converted directly into the milk sugar lactose. Although glucose is a major product of carbohydrate digestion in the rumen, it is rapidly fermented to VFA. Glucose, therefore, must be resynthesized in the liver of the postpartum cow via gluconeogenesis (Figure 1). An early lactation cow will produce 50 to 100 kg of milk per day. This equates to a glucose requirement for milk synthesis alone of 3.6 to 7.2 kg per day. The cow undergoes a series of homeorhetic adaptations that are aimed toward elevating glucose supply (Bauman and Currie, 1980). In addition to a large increase in hepatic gluconeogenesis shortly after calving, the cow assumes a state of insulin resistance that redirects glucose to the mammary gland (Giesy et al., 2012). In spite of these mechanisms, the postpartum cow has chronically low blood glucose concentrations because she fails to meet the glucose requirement.

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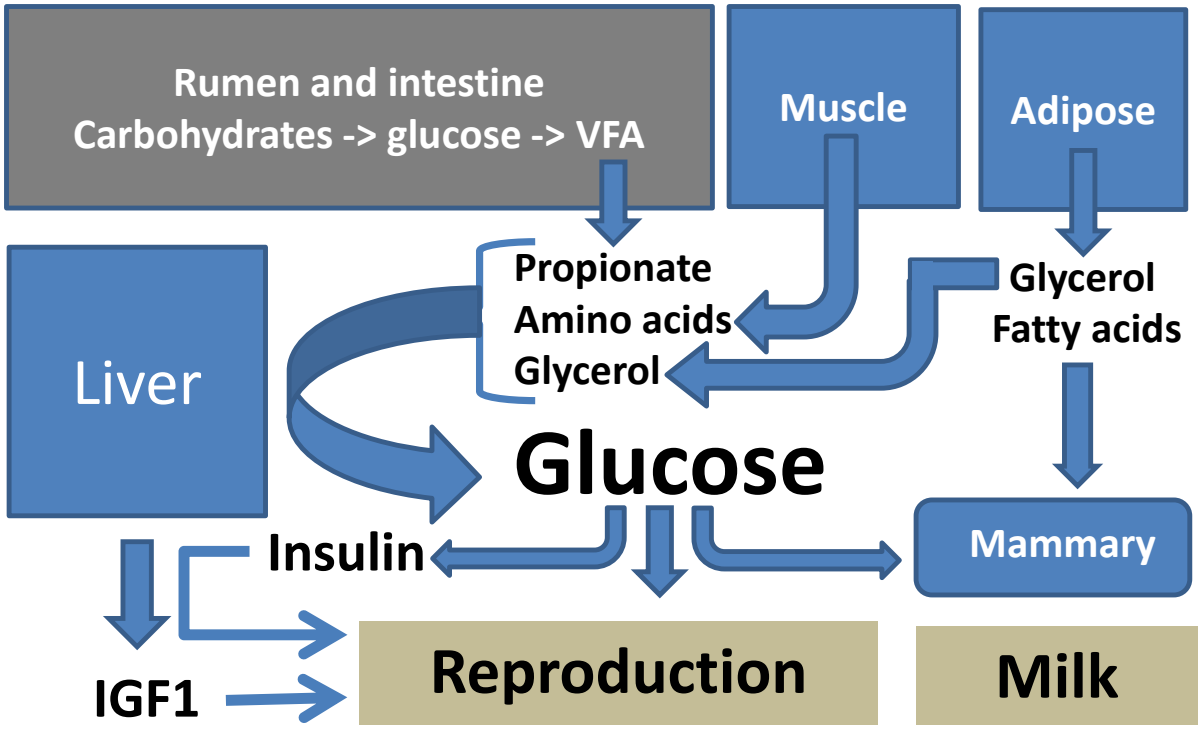

Figure 1. Metabolic processes in the early postpartum cow with potential to link glucose to the reproductive system. Glucose is synthesized in the liver via gluconeogenesis from substrates arising from rumen fermentation and the catabolism of muscle and adipose tissue. Glucose may ultimately control both circulating insulin (directly) and liver IGF1 production (via insulin-stimulated IGF1 synthesis and secretion). Glucose is also a required substrate for lactose synthesis during the production of milk. Low circulating glucose may impair reproductive processes that are needed to re-establish pregnancy during early lactation.

Glucose may coordinate whole animal metabolism through its capacity to orchestrate changes in endocrine hormones such as insulin and insulin-like growth factor 1 (**IGF1**) (Lucy, 2008). Glucose causes insulin release and insulin partitions nutrients toward adipose tissue and muscle. Insulin also stimulates the liver to release IGF1 into the circulation (Butler et al., 2003). As long as glucose remains low, insulin and IGF1 remain low, and the cow remains in a catabolic (tissue-losing) state during lactation. Glucose deficiency is only corrected when the mammary gland produces less milk in later lactation, blood insulin and IGF1 increase, and the cow partitions glucose toward adipose tissue and muscle (an anabolic state). The switch from the catabolic state to the anabolic state is a key regulator of the reproductive axis (Kawashima et al., 2012).

Associations Between Early Postpartum Blood Glucose and Reproduction

The blood concentrations of glucose decrease after calving. The decrease in blood glucose is theoretically caused by the glucose requirement for milk production. When we examined blood glucose concentrations in early postpartum cows we found that those that became pregnant after first artificial insemination (**AI**) had greater blood glucose concentrations on the day of calving and at 3 days postpartum

when compared with cows that did not become pregnant (Garverick et al., 2013). A relationship between serum nonesterified fatty acid (**NEFA**) concentrations and subsequent pregnancy also existed (cows that became pregnant at first AI had lesser postpartum NEFA concentrations when compared with cows that did not become pregnant at first AI).

Additional studies have established a link between early postpartum glucose and subsequent reproduction. For example, in the study Green et al. (2012), cows that did not get pregnant after first AI at approximately 60 days postpartum had lesser plasma glucose concentration during the first 30 days postpartum when compared with pregnant cows. Interestingly, the differences in blood glucose in cows that subsequently became pregnant or did not become pregnant were observed in both lactating and non-lactating cows (Green et al., 2012). Later postpartum (30 to 60 days postpartum) there was no relationship between plasma glucose concentration and pregnancy.

In separate studies performed in Ireland (Moore et al., 2014), blood glucose concentrations were examined in dairy cows that were known to have either high (**Fert+**) or low (**Fert-**) fertility. The Irish studies demonstrated greater glucose in Fert+ cows on the day of calving and one week later. Later postpartum, Fert+ and Fert- cows were similar for blood glucose concentrations. Finally, in their recent pooled analysis of studies of prepartum nutrition and subsequent postpartum reproduction, Cardoso et al. (2013) determined that greater blood glucose concentrations at weeks 3 and 4 postpartum were associated with shorter days to pregnancy.

The described relationships between blood glucose and pregnancy were for early postpartum blood glucose concentration, generally within the first month of lactation, and the establishment of pregnancy several months after the glucose measurements were made. The suggestion is that the early postpartum metabolic profile that includes blood glucose concentrations is predictive of subsequent postpartum fertility. A key question is how the metabolic profile of the early postpartum cow controls the reproductive processes leading to pregnancy that occur several months after the early postpartum period.

Blood Glucose Entry Rate Controls Other Metabolites in the Postpartum Cow

Glucose may be a mediator of postpartum reproduction because it acts as a substrate for the production of milk and is also essential for reproductive processes. It is impossible to say, however, whether any change in reproductive function is a consequence of a single hormone/metabolite or the collective action of several hormones or metabolites that change in a coordinated manner postpartum.

In attempt to address the possibility that glucose is the primary metabolic driver of the entire system we infused glucose into early postpartum cows in a physiologically relevant manner. Increasing daily doses from 500 to 1,500 g/d glucose were administered via jugular infusion by using a constant rate of glucose infusion (Lucy et al., 2013). Glucose infusion increased blood insulin concentrations. There was a marked decrease in both NEFA and beta-hydroxybutyric acid (**BHBA**) in response to glucose infusion. In addition to changes in insulin and circulating metabolites the glucose infusion increased circulating IGF1 concentrations. Insulin

may have mediated the stimulatory effects of glucose on IGF1 through its capacity to recouple the somatotropic axis. The infusion studies demonstrated that a single molecule such as glucose could rapidly reverse the metabolic profile that typifies early lactation (greater NEFA and BHBA with lesser insulin and IGF1). Based on these results, it is possible that glucose entry rate relative to demand in early lactation is coordinating the homeorhetic mechanisms. These same mechanisms may be impacting the reproductive systems that are undergoing restoration during the first 30 days postpartum.

How Can Early Postpartum Glucose Affect Reproduction Later Postpartum?

Inadequate blood glucose during early lactation theoretically compromises the function of tissues that depend on glucose. Metabolites such as NEFA and BHBA as well as insulin and IGF1 may also play a role in controlling tissue function. The first 30 days postpartum may be the most critical in terms of the impact that metabolites and metabolic hormones have on reproduction. Two essential processes occur during the first 30 days postpartum – the restoration of ovarian cyclicity and uterine involution. These two essential processes may be directly affected by glucose.

Restoration of ovarian cyclicity postpartum. The bulk of the research performed on metabolites and metabolic hormones has focused the re-initiation of ovarian cyclicity. Cows that are not cycling are infertile. Furthermore, fertility generally improves with each successive estrous cycle before the breeding period. There has been a traditional focus on understanding the mechanisms that control the timing of the restoration of ovarian activity before the breeding period. A common topic is the positive association between insulin, IGF1, and the day postpartum that the cow begins to cycle (Velazquez et al., 2008).

A variety of metabolites and metabolic signals can act at the level of the hypothalamus to increase gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) pulsatility (LeRoy et al., 2008). LeRoy et al. (2008) concluded that glucose and insulin were the most-likely molecules to exert an effect on hypothalamic GnRH secretion in the postpartum dairy cow. At the level of the ovary, both insulin and IGF1 promote the proliferation, differentiation, and survival of follicular cells (Lucy, 2008; Lucy, 2011). The most important actions of insulin and IGF1 are observed when either hormone acts synergistically with the gonadotropins [either follicle-stimulating hormone (FSH) or LH]. Glucose does control insulin secretion in the animal and ultimately controls hepatic IGF1 secretion via insulin release. Circulating glucose and the insulin/IGF1 systems, therefore, are functionally linked in the whole animal (Lucy 2011; Kawashima et al., 2012).

The associations between postpartum hormone and metabolites and subsequent reproduction are found early postpartum when the most-extreme homeorhetic states are known to occur. The early postpartum metabolic profile, therefore, may have the capacity to imprint ovarian tissue either through permanent effects on the genome (epigenetic mechanisms) or by changing the chemical composition of the cells themselves. Perhaps the best-studied example of this metabolic imprint is the relationship between early postpartum NEFA and its effect on the composition of the oocyte and function of follicular cells (Leroy et al., 2011). The possibility that there are permanent epigenetic modifications to the genome

during the early postpartum period that affect long-term developmental competence of follicular cells has not been demonstrated at this time

Uterine health and immune function. The re-initiation of ovarian activity postpartum is a traditional focus of studies of postpartum metabolism. Recently, however, greater emphasis has been placed on uterine health and the central place that uterine immune cell function occupies in determining the reproductive success of the postpartum cow (LeBlanc, 2012; Wathes, 2012). Under normal circumstances, uterine involution is completed during the first month postpartum. During involution, the uterus shrinks in size, reestablishes the luminal epithelium, and immune cells (primarily polymorphonuclear neutrophils or **PMN**) infiltrate the uterus to clear residual placental tissue as well as infectious microorganisms (LeBlanc et al., 2011). The postpartum cow has a depressed immune system particularly during the first month after calving. With respect to uterine involution and disease, the current theory is that the metabolic environment in postpartum cows suppresses the innate immune system through effects on PMN function (Graugnard et al., 2012; LeBlanc, 2012). In most cases, changes in circulating concentrations of nutrients and metabolites that occur in the postpartum cow are exactly opposite to those that would benefit the function of PMN. There is good agreement between in vitro analyses of PMN function and epidemiological evidence that indicates that an abnormal metabolic profile during the periparturient period predisposes the cow to uterine disease during the early postpartum period and infertility later postpartum (Chapinal et al., 2012).

Glucose is the primary metabolic fuel that PMN use to generate the oxidative burst that leads to killing activity. The glucose is stored as glycogen within the PMN. PMN undergo a brief period (approximately 14 days) of maturation and differentiation from progenitor cells within bone marrow prior to their release. It is during this time that glycogen is stored within the PMN. Glycogen concentrations in PMN within the postpartum cow decrease in a manner that is similar to the decrease in blood glucose postpartum (Galvão et al., 2010). Galvão et al. (2010) observed that cows developing uterine disease had lesser glycogen concentration in their PMN. Their conclusion was that the lesser glycogen reserve led to a reduced capacity for oxidative burst in PMN that predisposed the cow to uterine disease.

Most of the available data indicate that metabolic profile of the *prepartum* cow is equally important to that of the postpartum cow for subsequent uterine health and (or) the establishment of pregnancy (Castro et al., 2012). In their work in which an index for physiological imbalance was created, Moyes et al. (2013) concluded that an index that included NEFA, BHBA, and glucose was predictive of postpartum uterine disease especially when the *prepartum* index was used. In all likelihood the metabolic profile associated with uterine disease is initiated before or shortly before calving. This is not surprising given the relatively acute nature of the physiological events at the time of calving and the homeorhetic mechanisms at the initiation of lactation. A cow's homeorhetic capacity (i.e., capacity for gluconeogenesis, lipid mobilization, etc.) and her inherent resistance to disease are largely manifested after calving but the underlying biology is theoretically in place before she calves.

Implications of the Metabolic Profile Later Postpartum (During the Breeding Period)

Assuming that uterine involution is complete and the cow has began cycling then what are the implications of the metabolic profile of the cow during the breeding period? The metabolic profile of the later postpartum cow (greater than 30 days postpartum) still involves relatively low concentrations of glucose, insulin, and IGF1 although concentrations of NEFA and BHBA have typically normalized.

Estrous cyclicity during the breeding period. Patterns of estrous cyclicity for the lactating cows are less regular when compared with estrous cycle of nulliparous heifers. The same hormones that control when the cow begins to cycle (insulin, IGF1, and LH) also have an effect on cyclicity which relates to the functionality of the follicle and corpus luteum. The hormonal environment created by lactation (in this example low blood glucose, insulin and IGF1 concentrations) may potentially affect the capacity for ovarian cells to respond to gonadotropins. In the cycling cow, this could potentially affect estradiol production by the follicle as well as progesterone production by the corpus luteum. Low blood glucose could potentially compromise a variety of essential metabolic processes in ovarian cells including the oocyte that depends on glucose for energy (Berlinguer et al., 2012). In their recent study of bovine follicles, Walsh et al. (2012) concluded that steroidogenic acute regulatory protein (**STAR**) gene expression, the rate limiting enzyme in steroidogenesis, was specifically down-regulated by the metabolic profile found in early lactation, i.e. low glucose, insulin, and IGF1. There is also the potential for greater steroid metabolism in lactating compared with nonlactating cows that can be explained by greater dry matter intake in cows that are lactating (Wiltbank et al., 2011). Lesser circulating estradiol from the preovulatory follicle can lead to abnormal patterns of follicular growth, anovulatory conditions, multiple ovulation and also reduced estrous expression (Figure 2).

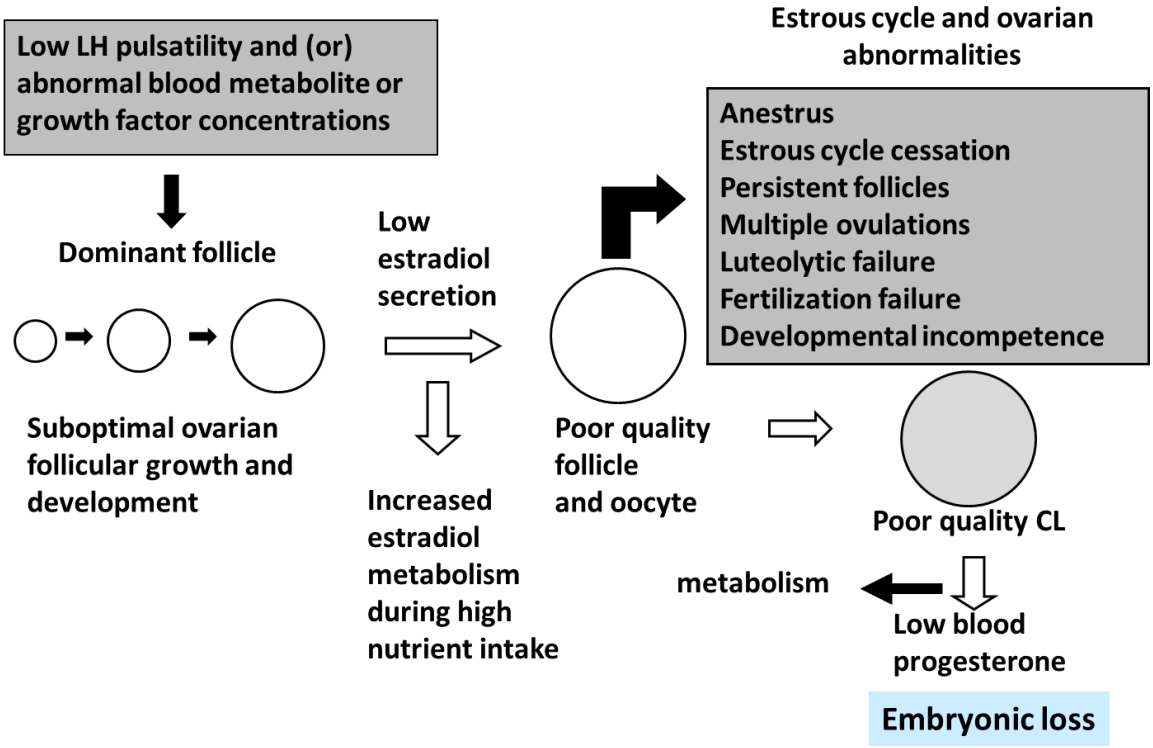

Figure 2. Mechanisms that link metabolism, metabolic hormones and metabolites to estrous cycle abnormalities, infertility, and embryonic loss in postpartum

cows. The cells of the follicle and the corpus luteum respond to changing concentrations of blood metabolites and growth factors. Steroids produced by the follicle and corpus luteum can have reduced circulating concentrations because they are metabolized by the highly metabolic liver of the lactating cow. An ovarian follicle may not be able to orchestrate the endocrine physiology of the estrous cycle because of reduced steroidogenic capacity and greater steroid metabolism. Endocrine failure by the follicle can lead to estrous cycle and ovarian abnormalities as well as a poor quality oocyte that may not develop after fertilization. The corpus luteum arises from the cells of the follicle. If the follicle is compromised then the corpus luteum may be compromised as well leading to low blood progesterone and embryonic loss.

Subnormal luteal function. Greater steroid metabolism has been implicated as a mechanism leading to low circulating progesterone in lactating cows. Low progesterone during the first weeks after insemination leads to slower embyronic development that predisposes the cow to embyronic loss (Lonergan, 2011). Progesterone stimulates uterine histotroph secretion and lesser uterine histotroph secretion, caused by low progresteorne concentrations, leads to slow embyronic development. The slowly developing embryos fail to reach adequate size to generate an adequate interferon-tau (**IFNT**) signal to the dam (Robinson et al., 2008). The pregnancy is lost because the mother fails to recognize the pregnancy and undergoes luteal regression as if she is not pregnant. Several authors have recently reviewed the mechanisms assoicated with subnormal luteal development and early embryonic loss (Pursley and Martins, 2011; Wiltbank et al., 2011; Bridges et al., 2013).

Glucose as a substrate for the developing embryo and fetus. Glucose is typically thought of as a key energy source for ATP production through mitochondrial oxidative phosphorylation. Glucose is not used primarily for metabolic fuel production, however, by either the mammary gland or the pregnancy. In the mammary gland, the bulk of the glucose is used to produce the milk sugar lactose. Likewise, in the uterus and placenta the bulk of the glucose is used to supply carbons for the synthesis of cellular components such as nucleotides, amino acids, lipids, etc. This latter phenomenon is known as the “Warburg effect” and typifies proliferating cells (Vander Heiden *et al.,* 2009).

In the study of Green et al. (2012) cows were either milked normally or dried off (not milked) immediately after calving. One major conclusion from the study was that for a given day of pregnancy, the fetus and placenta from a lactating cow were lighers and smaller than the fetus and placenta from a nonlactating cow. It was demonstrated that less glucose reached the fetus in a lactating compared with nonlactating cow, perhaps because maternal glucose concentrations were less during lactation (Lucy et al., 2012). The reduction in glucose reaching the pregnancy can potentially affect how the pregnancy develops because the pregnancy depends on glucose as a substrate for tissue synthesis and metabolic energy (Battaglia and Meschia, 1978).

The growth of the fetus and placenta, therefore, depends on the metabolic milieu of the cow (Figure 3). Low concentrations of glucose in postpartum cows may predispose the cow to pregnancy loss because the placenta may not have adequate

substrate for the creation of new cells. The incompetent and slowly developing placenta may eventually compromise the fetus. Once the fetus dies then the placenta dies and the corpus luteum regresses. The mammary gland has priority for glucose but neither the mammary gland nor the uterus/placenta has the capacity to concentrate glucose via a glucose transporter. Greater blood flow to the mammary gland dictates its greater capacity to extract glucose from the circulation.

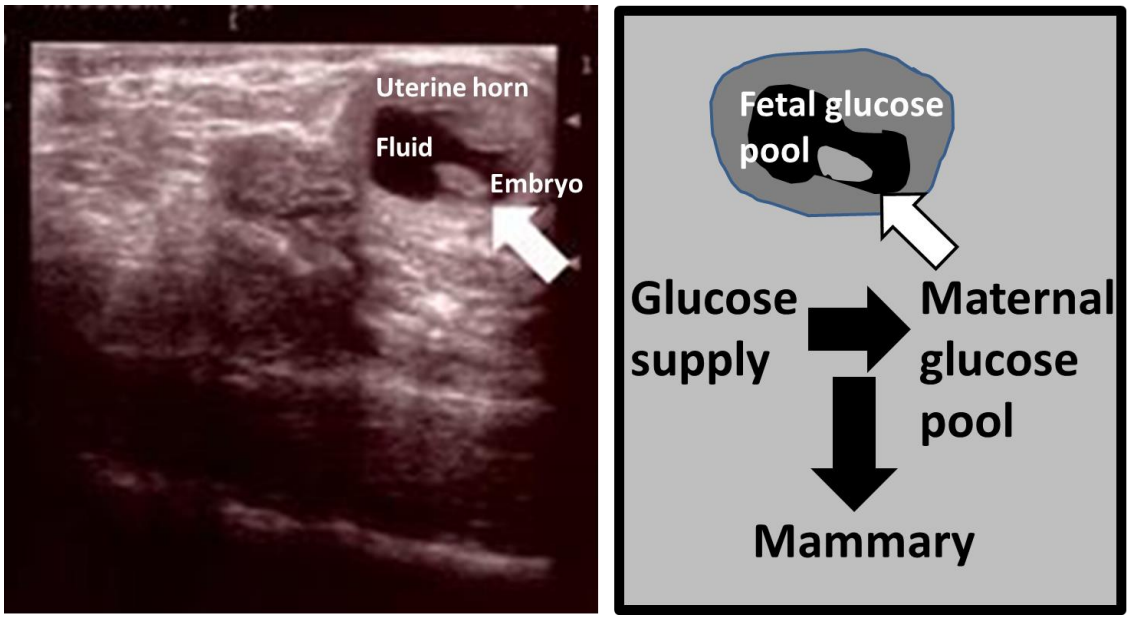

Figure 3. Ultrasound image of a bovine embryo in a lactating cow on day 27 (left) and model for glucose in the pregnant cow (right). The embryo is the small ovoid echogenic structure (arrow) floating within fluid inside the uterine horn. The embryo uses glucose as its primary carbon source for growth. Lactation causes a decrease in blood glucose concentrations because the mammary gland uses glucose for lactose synthesis. Glucose concentrations in placental fluids are less than glucose concentrations in the maternal circulation. The lesser blood glucose in lactating cows is associated with lower glucose concentrations in placental fluids compared with nonlactating cows (Lucy et al., 2012). Lesser glucose reaching the embryo may explain slower embryonic development in lactating cows.

Few published studies in cattle have asked the question “does slower growth of the fetal/placental unit lead to pregnancy loss?” In the horse, delayed development of the embryonic vesicle generally leads to embryonic loss (Carnevale et al., 2000). Several recent studies in the bovine have demonstrated that pregnant cows that undergo pregnancy loss have lesser blood concentrations of PAG leading up to the time that the pregnancy is aborted (Thompson et al., 2010; Pohler et al., 2013). The lesser blood PAG concentration may indicate that the cow is pregnant with a small embryo or fetus.

Conclusions

The endocrine and metabolic environment of the lactating cow affects the capacity of the cow to become pregnant postpartum. There is ample evidence that the hormones responsible for the homeorhetic mechanisms that support lactation

can also act on the uterus and ovary to affect their function prior to and during the breeding period. In addition to the hormonal environment, the metabolic environment created by lactation that includes low blood glucose and elevated NEFA and BHBA impinges upon the ovary as well as the immune system that plays a critical role in restoring uterine health in the postpartum cow. The specific mechanism through which the metabolic environment of early lactation deposits a lasting imprint on uterine and ovarian function is less clear. Also less clear are the mechanisms that link lactation to a predisposition for pregnancy loss in the lactating cow. The slow rate of embryonic or fetal growth in lactating cows with low blood progesterone and low blood glucose concentrations may be an important mechanism explaining pregnancy loss.

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SESSION NOTES

Nutritional Effects on Beef Heifer Development, Puberty and Subsequent Reproduction

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Introduction

Pregnancy success in the first breeding season of a heifer is primarily determined by the time at which puberty occurs relative to the start of her breeding season. Thereafter, the female's ability to rebreed in subsequent years and remain in the herd, and hence her lifetime productivity is influenced by timing of pregnancy in the first breeding season. The physiological endpoint of puberty is influenced by factors related to management of the annual cycle of beef production as well as the physiology and genetics of the female.

Beef production in almost all situations is seasonal in nature in order to coordinate feed resources with the nutrient requirements of the dam, and to some extent, the requirements of her calf. In the temperate regions of North America, most beef cows calve in late winter/early spring to match peak lactation (two to three months post-calving) with peak growth of cool-season grasses and other grazed forages. Some producers in these regions calve in the fall to take advantage of better weather for calving, fall pasture growth and/or traditionally greater calf prices at weaning in the spring. In the southern regions of the USA, fall and winter calving are much more prevalent as a result of the growth patterns of forages used in these regions. Regardless of the calving season employed, the time at which female cattle, including heifers, must become pregnant each year is constrained by this seasonal cycle, with typical breeding seasons being 2 to 4 months in duration.

A majority of *Bos taurus* heifers from breeds such as Angus, Hereford, Simmental, and Charolais are expected to calve for the first time at 22 to 24 months of age (at which time they are referred to here as primiparous cows) and at approximately 12-month intervals thereafter until 6 to 10+ years of age. To provide additional time for primiparous cows to recover from their first calving in preparation for their second breeding, they are often bred to calve 3 to 4 weeks before the multiparous cows in the same herd. This requires that heifers become pregnant for the first time at 12 to 15 months of age assuming a gestation period of slightly less than 9.5 months (280 to 285 days) and accounting for often more than two months variation in the birth date of heifers selected to serve as replacements. Thus, consideration of seasonal constraints implies that puberty should occur within this time frame. However, there are other

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factors that impact recommendations as to when puberty should occur relative to the first breeding season in heifers.

It has been demonstrated that conception rate of heifers increased by approximately 21% (Byerley et al., 1987; Perry et al., 1991) from their first ovulation to their third estrous cycle. It has also been shown that timing of conception in the first breeding season impacts lifetime productivity. Beef heifers that conceived early during their initial breeding season and calved as two-year-old females had a greater probability of becoming pregnant as primiparous cows (Burris and Priode, 1958), had greater lifetime production reflected in greater weaning weights, and tended to calve earlier in subsequent years (Lesmeister et al., 1973) compared with females that conceived later in their first breeding season. Hence, age at which puberty occurs and timing of this event relative to the breeding season will impact the time of conception in the first breeding season, lifetime productivity, and economic efficiency of beef production.

Economics of Age at First Calving

Considering the nature of seasonal beef production and the impact of time of puberty on conception in the first breeding season and lifetime productivity, a logical question is whether waiting to mate heifers at 18 or 24 months of age will circumvent many of these challenges. This question was a topic of discussion in the USA in the 1900s with regard to *Bos taurus* heifers in the temperate regions of the USA. The author of the first scientific paper found on this subject (McCampbell, 1921) concluded that the cow “never fully recovers from the shock of calving at this (2 years of age) age” and that “when a beef cow calves at 2 years of age, neither she nor her calves (in subsequent years) will be as large as they would have been had she dropped her first calf at 3 instead of 2 years of age.” However, after this report, most subsequent research demonstrated an advantage of calving at 2 versus 3 years of age relative to lifetime productivity. Heifers that calved at 2 years of age had a reduced calving rate of approximately 14% at 3 and 4 years of age as compared with heifers that calved first at 3 years of age but performed similarly thereafter (Withycombe et al., 1930). However, heifers that calved first at 2 years of age produced an average of 0.7 more calves than those calving first at 3 years of age by the time all cows were 6.5 years of age. The first calves from heifers were lighter at weaning than from older cows whether they calved first at 2 or 3 years of age, but no differences existed thereafter. A detailed economic analysis in this paper indicated that the difference in profit at the end of 4 years was $36.15/cow. This difference translates to approximately $500/cow in 2013!

A comprehensive international review of reports on age at first calving was provided by Morris (1980). Across experiments, lifetime production was either greater, or not significantly different, when heifers first calved at 2 versus 3 years of age, and overall, heifers calving at 2 years of age produced 0.7 more calves in their lifetime than if calving first at 3 years of age. The most comprehensive comparison of calving age in beef cattle was performed at the USMARC, Clay Center, NE, USA (Nunez-Dominquez et al., 1985, 1991). Cows were Angus, Hereford, or Shorthorn and F1 crosses of these

breeds. The authors evaluated a wide array of production characteristics in this experiment. Two key findings were that heifers bred to calve at 2 years of age produced 138 kg more of weaned calf weight in their lifetime and that economic efficiency (output - input) was 6 to 8% greater than in heifers bred to calve for the first time at 3 years of age.

More than 95% of heifers in the northern and central USA, comprised mainly of *Bos taurus* breeds, calve first at 2 years of age, whereas less than 50% of heifers in Florida and about 35% in Texas calve later than 2 years of age (Short et al., 1994). Breed is an important consideration as many cattle in these regions are crossbreeds of *Bos taurus* and *Bos indicus*. For comparision, in Brazil, the beef industry is dominated by straightbred females of the *Bos indicus* breed, Nelore, due to its high tolerance to heat and parasites. A limitation for female cattle efficiency in Brazil is the age at first calving (for review, see Nogueira, 2004), and essentially all Nelore females currently calve for the first time at 3 to 4 years of age (Malhado et al., 2013). At present, Brazil slaughters approximately 23% of its national cattle population each year, whereas in the USA, this figure is more than 35% of the national cattle population. As a result maintenance cost of breeding and slaughter animals per kilogram of beef produced is proportionally greater in Brazil than the USA. While several factors influence this discrepancy, a major contributing factor to this inefficiency is the delay of 1 to 2 years in breeding age of heifers. Hence, in the southern regions of the USA where delayed breeding of heifers is practiced in some herds, some of this inefficiency is inherent to production systems in these regions.

Nutritional Control of Age at Puberty

Most beef heifers are weaned from their dams at 6 to 8 months of age and it has been clearly demonstrated that plane of nutrition from weaning to the onset of the breeding season can impact age at puberty (for reviews see Patterson et al., 1992; Bagley, 1993). Traditionally, the recommendation has been that heifers be fed to attain 60 to 65% of their expected mature body weight by the onset of the breeding season. Various strategies have been employed to achieve this end point such as constant body weight gains from weaning to breeding or nutritional restriction followed by greater nutrient intake and compensatory gain. Taken together, findings from many experiments suggest that flexibility exists in how this target weight is attained to achieve acceptable pregnancy rates. Recent research has suggested that development of heifers to 50 to 57% of mature body weight may present an economic advantage over developing heifers to 60 to 65% of mature body weight (for review, see Endecott et al., 2013). However further research is necessary to assess the relative effects of these two strategies on cow longevity and economic efficiency. While some disagreement exists as to the ideal target weight for heifers at the start of their first breeding season, without question nutritional management during this phase is crucial to breeding success.

It has been reported that rate of growth pre-weaning and early post-weaning has a more profound effect on reproductive success in the first breeding season than that immediately preceding the breeding season (Roberts et al., 2009). This finding is

consistent with numerous earlier reports that indicated preweaning growth or weaning weight has a major impact on timing of puberty. Spontaneous precocious puberty (puberty before 300 days of age) occurs in up to 25% of *Bos taurus* beef heifers (Wehrman et al., 1996), and we have performed a series of experiments demonstrating that precocious puberty can be consistently induced by initiation of feeding a high-energy diet in beef heifers at approximately 3 months of age (Gasser et al., 2006 a,b,c,d; Table 1). Advantages of induction of precocious puberty in *Bos taurus* heifers in North America are limited given the seasonal schedule of beef production. Actually, precocious puberty in *Bos taurus* cowherds can be detrimental to efficiency of beef production due to precocious pregnancy and associated economic losses. However, in *Bos indicus* influenced cattle, opportunity exists to take advantage of this physiological response to nutritional intervention earlier in life to reduce age at calving in programs where heifers give birth after 2 years of age.

Table 1. The percentage of heifers that experienced precocious puberty and age at puberty[a]

		Early weaning, high concentrate diet (EWH)		Early weaning, control diet (EWC)	
Experiment	n	% Precocious puberty	Age at Puberty (d)	% Precocious puberty	Age at Puberty (d)
EXPT 1	18	89 (8/9)	262 ± 10	0 (0/9)	368 ± 10
EXPT 2	18	100 (9/9)	252 ± 9	56 (5/9)	308 ± 26
EXPT 3	10	80 (4/5)	275 ± 30	0 (0/5)	385 ± 14
EXPT 4	30	67 (10/15)	271 ± 17	20 (3/15)	331 ± 11

[a]Data from Gasser et al, 2006a, EXPT 1; 2006b, EXPT 2; 2006c, EXPT 3; and 2006d, EXPT 4.

Reproductive Technologies to Advance Age at Puberty

It is obvious that plane of nutrition, both postweaning and preweaning, can advance age of spontaneous puberty in heifers but variation in occurrence of this event is inherent in all groups of replacement females for a wide variety of reasons. The economic advantages of heifers becoming pregnant during the first part of their first breeding season have been clearly demonstrated. Thus, in order to optimize efficiency of each group of replacement females, it is important that all heifers reach puberty before or very early in their first breeding season. The challenge of attaining this endpoint is heightened since the identity of the heifers which have surpassed this threshold, and those that have not, is unknown. Even with excellent nutritional management, in most situations it is impossible, or not economically feasible, to provide a level of nutrition that ensures that all heifers reach this endpoint at the start of the season. Fortunately, hormonal technologies currently exist that can induce puberty in prepubertal heifers while at the same time synchronize estrus in postpubertal heifers

within a group. This standardization of reproductive status provides most heifers an excellent chance to become pregnant early in their first season.

Some *Bos taurus* in temperate regions of the US have not attained puberty at 12 to 15 months of age and the proportion varied from 6% to 81% between individual groups of heifers (Lucy et al., 2001). In Nelore cattle in Brazil, the proportion of heifers that were prepubertal at initiation of the breeding season, at approximately 24 months of age, exceeded 60% (Claro Jr. et al., 2010). If heifers fail to conceive in their first breeding season, whether at 1 or 2 years of age, then management options for them are limited. Either the non-pregnant heifer is fed an additional year with no return or she is removed from consideration as an animal to enter the breeding herd and instead is fed and marketed for slaughter. Most approaches, worldwide, to induce puberty in heifers use of an exogenous treatment with the hormone progesterone (normally produced by the ovary of females), either alone or in combination with other compounds such as gonadotropin-releasing hormone (**GnRH**), estradiol or equine chorionic gonadotropin (**eCG**) that aid in inducing ovulation. These induction protocols rely on the fact that progesterone treatment activates the reproductive system to result in puberty (Anderson et al., 1996; Day and Anderson, 1998). During and after the progesterone treatment, secretion of the hormone luteinizing hormone (**LH**) will increase, ovarian follicles are stimulated to grow, and the female ovulates spontaneously or in response to a second exogenous stimulus. The efficacy of these approaches has been well documented in *Bos taurus* heifers approximately 12 months of age (Rasby et al., 1998) and in Nelore heifers that were 24 months of age (Rodrigues et al., 2013). Typically, more than 80% of prepubertal heifers were induced to ovulate with the progestin treatment. In *Bos taurus* heifers, pregnancy to artificial insemination (**AI**) at the beginning of the breeding season did not differ between prepubertal and postpubertal heifers that were induced/synchronized with progesterone-based timed AI programs (Lamb et. al., 2006). Acceptable reproductive performance during the ensuing breeding season following induction of puberty with progesterone-based programs has been demonstrated in numerous reports with both *Bos taurus* and *Bos indicus* females. It is important to consider that hormonal induction of puberty is most effective in heifers that are approaching their spontaneous occurrence of puberty. In other words, there are age limits before which it is not possible to effectively induce the first ovulation with pharmacological manipulation (Hall et al., 1995), and these approaches are not a substitute for proper heifer development and nutritional management.

"Precocious" Breeding in *Bos indicus* Heifers

Reduction of the age at which heifers enter into production increases their lifetime productivity and improves economic efficiency. This increased efficiency could be realized in *Bos indicus* influenced heifers in the southern US, of which many still calve at 2.5 to 3 years of age. An even greater impact could be realized in countries such as Brazil, where essentially all heifers calve for the first time at 3 or 4 years of age. We (M.L. Day, M.P. Carvalho, R.A.C. Martins, A. D. P. Rodrigues, J. L. M. Vasconcelos, L. H. Cruppe unpublished) have been working in Brazil for the past 7 years to determine if an aggressive program of nutritional and hormonal intervention in Nelore (n = 2,345; n

= 433 in 2012–2013) and Nelore x Angus crossbred heifers (n = 414, 2012-2013; n = 738, 2013-2014) would result in acceptable pregnancy rates in heifers bred with timed AI at 12 to 15 months of age. Across these years, heifers were fed a variety of corn silage-based diets beginning at weaning (6 to 9 months of age) with approximate target weights at AI of 300 kg for Nelore heifers and 340 kg for Nelore x Angus heifers. In each year, heifers received a progestin based induction protocol that commenced 18 to 24 days before a timed AI protocol was initiated. In 2012–2013, ovulation was induced in approximately 80% of heifers before the breeding season and this proportion did not differ between the Nelore and crossbreed heifers. Two consecutive estrous synchronization protocols, separated by approximately 35 days, were then used in conjunction with timed AI (initial timed AI and resynchronization and AI of non-pregnant heifers); no natural service was used. Pregnancy rates after two rounds of AI were approximately 60% in Nelore and 80% in Nelore x Angus heifers. Pregnancy rate achieved in 2012–2013 for Nelore heifers was similar to that achieved with Nelore heifers in the previous two years. These results are similar to those that are often achieved with Nelore heifers that calve for the first time at three or four years of age under traditional management. The results in Nelore x Angus were confirmed in 2013 – 2014 with an approximate pregnancy rate after two AI of 77%. Pregnancy rates in the Nelore x Angus heifers in this program are similar to those we often attain with Angus crossbreed heifers in Ohio.

A second major question with this program was the ability Nelore-influenced cattle to rebreed as primiparous cows. Pregnancy rates after two AI for primiparous cows that calved at two years of age in 2013-2014 were 58% for Nelore and 88% for Nelore x Angus females. In each of these groups, nutritional supplementation was provided for 3 months after calving as this was during the dry season in Brazil. These preliminary findings indicate that a majority of Nelore and Nelore x Angus heifers can successfully calve at two years of age and rebreed as primiparous cows provided that they receive sufficient nutritional management and an aggressive hormonal approach in conjunction with timed AI. Evaluation of the economic benefit of using an approach of this nature in heifers that calve later than 2 years of age in southern regions of the USA is warranted.

Conclusions

Age at puberty in beef heifers influences economic efficiency of beef production through influences on both age at first calving (2 vs. 2.5+ years of age) and the time of conception of heifers in their initial breeding season. The seasonal nature of beef production and the advantages to production efficiency of a breeding season of restricted duration exacerbate the resultant loss in efficiency if puberty does not occur at the appropriate age. Current practices for the desired age at first conception vary by climate and the predominant breeds that best suit regional production practices. An overarching factor that influences age at puberty in heifers is the nutritional management that occurs during the development of the heifer. Highly effective hormonal technologies exist to aid in induction of puberty in well managed heifers of an appropriate age. Age at first ovulation and pregnancy in heifers can be substantially

influenced through implementation of nutritional and/or hormonal manipulation strategies. The appropriate level of intervention with these strategies, as well as age at first breeding, that yields optimal economic and reproductive efficiency is not consistent between regions, production systems, breed, etc. Application of knowledge and technologies and assessment of impacts on efficiency are necessary to determine the optimal approach in a given situation.

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SESSION NOTES

Intensified Pre-Weaning Calf Feeding Programs: Impacts on Growth and Behavior

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Introduction

There are a range of viewpoints on how best to feed and manage dairy calves early in life. Traditional approaches to rearing dairy calves have focused on stimulating early solid feed intake through restricting intake of milk or milk replacer. A conventional milk feeding rate is approximately 10% of a calf's birth weight, an amount that translates to between 4 and 5 L/day, supporting under 0.5 kg/d of weight gain (Appleby, 2001; Jasper and Weary, 2002). This conventional approach to feeding calves facilitates early weaning and has been viewed as economically appealing due to reduced feed costs. However, there is increasing on-farm adoption of alternative feeding programs which provide a higher plane of nutrition. Feeding programs which provide greater milk allowances support greater growth relative to outcomes of conventional restricted feeding, and thus are typically referred to as "intensified feeding," or "feeding for accelerated growth." These feeding programs provide quantities of milk that more closely resemble intake levels of a suckling calf, and allow "biologically appropriate" growth rates (Drackley, 2008), which fall between 0.75 and 1 kg/d (Tedeschi and Fox, 2009; Appleby, 2001).

In supporting increased intake, intensified feeding programs provide a number of immediate benefits, including greater growth prior to weaning, performance of natural feeding patterns, and improved welfare. Further, recent interest has turned to longer-term impacts of greater rates of weight gain early in life, such as improved performance in lactation. The increasing adoption of intensified feeding for dairy calves poses opportunities and challenges for other aspects of calf management, such as different approaches to housing, weaning methods, and provision of solid feed.

Early Impacts of Intensified Feeding

Plane of nutrition and growth

In contrast to the restricted amounts of milk provided in conventional feeding programs (10% of BW, or 4 to 5 L/d), calves provided more milk are able to double their nutrient intake (Khan et al., 2011a), consuming between 8 and 16 L/d when milk is provided *ad libitum* (Appleby, 2001; Jasper and Weary, 2002; Miller-Cushon et al., 2013a). In terms of milk replacer, conventional feeding programs typically provide 1 to

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1.5% of BW on a dry matter (**DM**) basis whereas intensified programs provide milk at 2 to 3% of BW on a DM basis. Some intensified feeding programs also alter the DM content of the milk replacer in addition to the feeding amounts; for example, providing milk replacer prepared with 18% compared to 12% DM (Terré et al., 2009).

Improved growth in intensified feeding programs can be accomplished by providing higher amounts of milk replacer (Diaz et al., 2001; Brown et al., 2005) as well as whole milk (Jasper and Weary, 2002). However, a calf's protein requirement increases with rate of body weight gain; thus, feeding a conventional milk replacer (containing 20 to 22% CP and 20 to 21% fat) at a greater rate will not supply sufficient protein for lean tissue growth and surplus energy will be converted to fat (Drackley, 2008; Brown et al., 2005). When energy is not limiting, calves have increased lean tissue growth when milk replacer contains 26 to 28% CP, and 15 to 20% fat (Diaz et al., 2001). In comparison, whole milk contains approximately 27% protein and 26 to 28% fat (Appleby, 2001; Shamay et al., 2005).

Intensified feeding programs have marked impacts on performance of the calf early in life, including improved rate of weight gain, structural growth, and efficiency of feed conversion (Diaz et al., 2001; Khan et al., 2007). Whereas conventional feeding programs typically support 0.3 to 0.6 kg/d in growth, intensified feeding programs allow weight gain ranging from 0.6 to over 1 kg/d. For calves provided milk *ad libitum*, average daily weight gain is typically between 0.8 and 1.2 kg/d (Appleby, 2001; Miller-Cushon et al., 2013a; Jasper and Weary, 2002). Advantages in structural growth (girth and height) in calves managed in an intensified feeding program have been noted both preweaning and postweaning (Khan et al., 2007).

Feeding behavior patterns and welfare

In addition to impacting growth, the milk feeding program greatly influences feeding behavior patterns of the calf. Intensified feeding systems, especially those that provide *ad libitum* access to milk or milk replacer, allow calves to exhibit a diurnal pattern of milk intake (Figure 1). Calves provided milk *ad libitum* have peaks of feeding activity at sunrise and sunset, and consume milk in 8 to 10 meals/d (Appleby, 2001; Miller-Cushon et al., 2013a). This pattern of milk intake and resembles the natural behavior of a calf nursing the dam (Lidfors et al., 1994; de Passillé, 2001). In contrast, calves fed according to conventional practice typically receive their milk allotment in two feedings per day (Figure 1), such that total time spent feeding during the day is greatly reduced. For example, calves provided milk at a rate of 5L/d spent about 10 min/d feeding, whereas calves provided milk ad libitum spent 45 to 60 min feeding (Appleby et al., 2001; Miller-Cushon et al., 2013a).

Calves fed restricted quantities of milk have frequent unrewarded visits to the feeder (De Paula Vieira et al., 2008; Borderas et al., 2009), suggesting that they are hungry (De Paula Vieira et al., 2008). Further, calves are highly motivated to suck and will spend considerable amounts of time engaged in non-nutritive sucking (Figure 1) when provided restricted amounts of milk. In addition to differences in feeding behavior,

calves provided restricted amounts of milk spent less time lying (Borderas et al., 2009; De Paula Vieira et al., 2008), vocalized more frequently (Thomas et al., 2001), and performed less play behavior (Krachun et al., 2010). Thus, intensified feeding systems have clear welfare implications for the calf, allowing performance of natural feeding behavior patterns and reducing hunger.

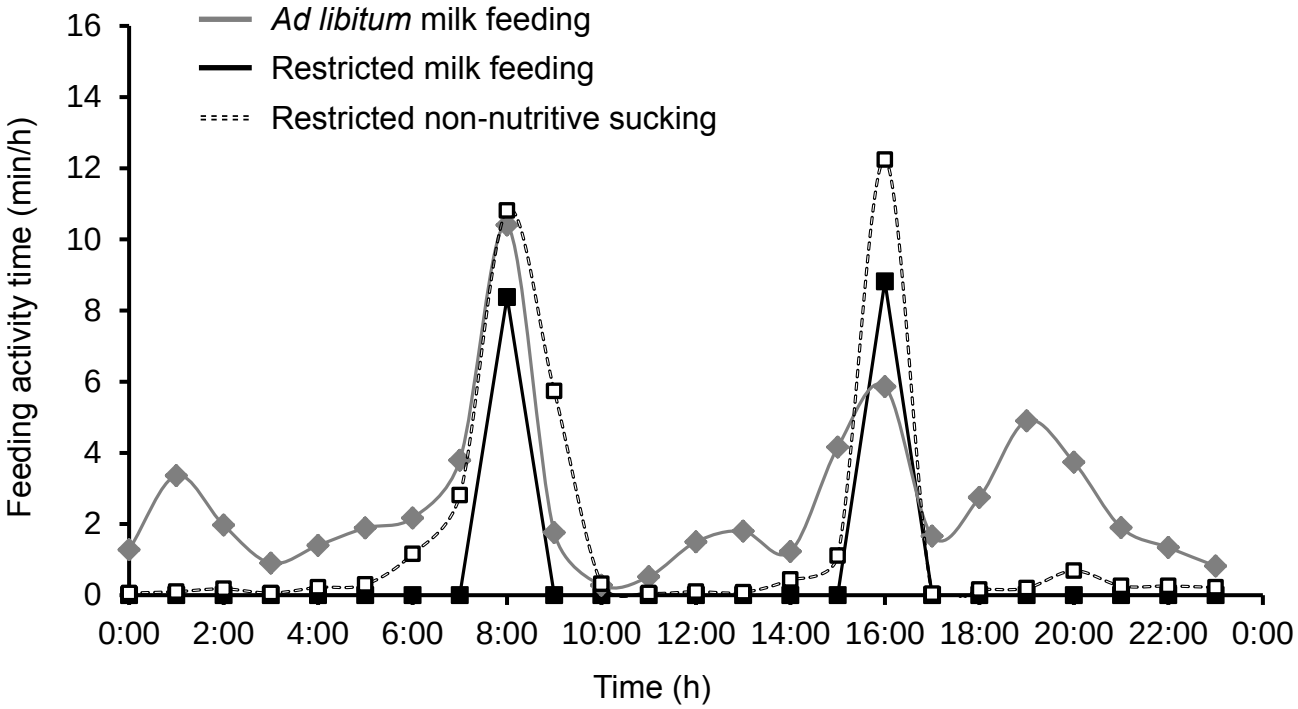

Figure 1. Diurnal feeding activity of calves provided milk *ad libitum*, and feeding and non-nutritive sucking activity for calves provided restricted amounts of milk (5 L/d). Adapted from Miller-Cushon et al. (2013b).

Longer-Term Effects of Intensified Feeding

From an economic perspective, motivation for feeding greater amounts of milk to calves depends in part on the potential long-term impacts of this feeding practice on performance of the calf. In controlled studies, early plane of nutrition has been found to have a number of impacts on longer-term production potential. In comparison to providing calves with restricted access to a low-energy milk replacer (23% crude protein, 15% fat), provision of whole milk to calves in *ad libitum* amounts was reported to have a range of long-term positive effects across different studies, including reduced age at conception and calving (Bar-Peled et al., 1997), increased BW at calving (Bar-Peled et al., 1997; Moallem et al., 2010), and improved milk production (Bar-Peled et al., 1997) or milk fat yield (Shamay et al., 2005; Moallem et al., 2010).

Similarly, results of studies comparing different amounts and qualities of milk replacer suggest that an intensified milk replacer feeding program reduces age at first calving (Raeth-Knight et al., 2009; Davis Rincker et al., 2011). Regression analysis of several published data sets suggests a positive impact of preweaning growth on later

milk production, with an improvement in milk production of 225 kg for an increase in pre-weaning average daily gain (**ADG**) of 100 g/d (Bach, 2011). Soberon et al. (2012) also reported a positive correlation between preweaning ADG with first lactation milk yield, suggesting an improvement in milk yield of 850 to 1,113 kg for every 1 kg of preweaning ADG. Davis Rincker et al. (2011) reported an economic analysis suggesting that, although cost of intensified feeding was greater than conventional, total costs by time of first lactation were not different.

Despite significant effects of intensified feeding programs on feeding behavior of the calf prior to weaning, there is little evidence to suggest that preweaning milk feeding level has a persistent effect on feeding patterns (Miller-Cushon, 2013a). However, Miller-Cushon (2013a) reported that, in the week after weaning, calves previously provided restricted amounts of milk consumed their solid feed more quickly and had larger meals, compared to calves provided milk *ad libitum*. Although differences in meal characteristics did not persist, differences in rates of intake after weaning suggest that previous experience with a restricted feeding scenario may have some impact on feeding motivation.

Challenges and Opportunities

Weaning strategies

Although intensified feeding programs hold much potential to improve short and long-term performance and welfare of dairy calves, there remain challenges with their implementation. The long-standing popularity of conventional restricted milk feeding programs was based on encouraging solid feed intake early in life and facilitating a smooth transition at weaning. Solid feed intake early in life is critical for rumen development, and consistent weight gain through weaning requires that the calf be consuming sufficient amounts of solid feed prior to removal of milk (Khan et al., 2011a). When provided greater quantities of milk, calves have less frequent and smaller meals of concentrate (Miller-Cushon et al., 2013a). Consequently, rumen development is delayed, such that post-weaning nutrient digestibility is lower in calves provided more milk (Terré et al., 2007; Hill et al., 2010). Thus, a challenge with an intensified feeding program is to support consistent growth through weaning.

Although greater weaning weights as a result of increased pre-weaning nutrition can be maintained into the post-weaning period (e.g. 8 kg weight advantage at 20 d post-weaning (Jasper and Weary, 2002) and 20 kg weight advantage at 56 d post-weaning; Figure 2), these results are not consistent. A number of studies indicate that weight gain of calves provided great quantities of milk may suffer at time of weaning if intake prior to weaning was low. For example, weight gain of calves provided milk replacer *ad libitum* may plateau during weaning whereas restricted-fed calves maintain consistent growth (ADG of -0.03 vs 0.6 kg/d; Figure 2). In some cases, differences in weight gain through weaning negated any body weight advantage arising from the pre-weaning feeding program (Borderas et al., 2009; DePassillé et al., 2011). This suggests that maintenance of greater body weights is extremely sensitive to weaning method.

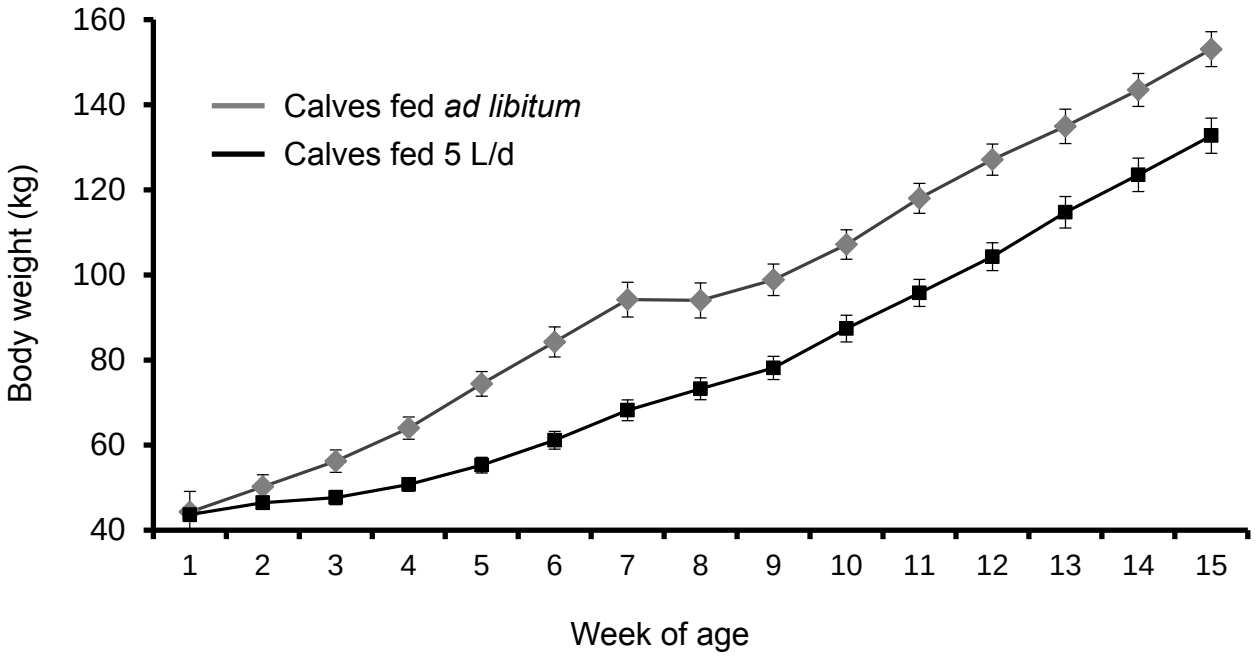

Figure 2. Growth of calves provided milk *ad libitum* or at a restricted level (5 L/d). Adapted from Miller-Cushon et al. (2013b). Weaning occurred during week 7.

The most important aspect of a weaning program is encouraging sufficient intake of solid feed intake prior to removal of milk. A gradual weaning process that encourages greater solid feed intake appears to maintain weight advantages for calves managed in intensified feeding systems. Khan et al. (2007) employed a step-down weaning method, reducing milk quantity 20 d prior to weaning at 7 weeks, and found that calves previously fed milk *ad libitum* maintained a weight advantage 40 d post-weaning. Age of weaning also influences post-weaning performance. de Passillé et al. (2011) reported that calves provided greater quantities of milk had no weight advantage over conventionally-fed calves after abrupt weaning at 7 weeks, but when weaned later (at 13 weeks), calves had begun consuming more solid feed and maintained a weight advantage over calves provided less milk. When considering potential for early growth to improve later production performance, maintaining improved growth through weaning is critical.

Solid feed intake and selection

In addition to the milk feeding program, solid feed provision is an important component of early management. When managed in conventional feeding systems, calves are typically provided *ad libitum* access to a high energy grain concentrate alongside restricted quantities of milk. Early intake of concentrate is critical for rumen development, as rumen papillae development occurs in response to butyrate produced through fermentation of carbohydrates (Warner et al., 1956; Sander et al., 1959). Provision of forage has long been discouraged, out of concern that it will displace

concentrate intake and, consequently, impair rumen development (Hill et al. 2008; Kertz et al. 1979). However, there is evidence to suggest that forage provision does not need to reduce concentrate intake (Khan et al. 2011b; Castells et al. 2012) and, further, may positively impact ruminal environment, reducing acidity of ruminal fluid (Suárez et al. 2007; Khan et al. 2011b) and improving feed efficiency (Coverdale et al. 2004). When offered a choice of hay and concentrate, calves selected a proportion of hay ranging between 5 and 30% of total DM intake (Castells et al., 2012; Miller-Cushon et al., 2013b; Khan et al., 2011b), depending on the type of hay provided and, potentially, other nutritional factors such as milk intake. Selection in favor of hay has been found to decrease after weaning, suggesting that calves may alter dietary selection patterns in response to energy requirements (Miller-Cushon et al., 2013b).

Housing and feeding management

Implementation of intensified feeding programs can also impact feeding management on a larger scale. Whereas conventionally-raised calves are typically housed individually, intensified feeding systems are often being adopted hand-in-hand with group-housing systems. Group housing of calves allows for the social facilitation of feeding behavior, resulting in calves beginning to consume solid feed earlier in life and consuming more solid feed prior to weaning (Hepola et al. 2006; De Paula Vieira et al. 2010). Group-housed calves also vocalized less during weaning (De Paula Vieira et al., 2010), suggesting that social contact is beneficial during this stressful transition. Calves housed with social contact gain weight more consistently through weaning (Chua et al., 2002), likely due in part to both greater intakes of solid feed prior to removal of milk and reduced stress. Thus, social contact may contribute to a successful weaning transition of calves managed in an intensified feeding program.

A major factor helping the implementation of intensified feeding programs is the growing adoption of computerized calf-feeding systems. These systems reduce the manual labor associated with increasing milk allotments, facilitate group-housing for calves while allowing for monitoring of individual intake, and provide control over feeding patterns and weaning programs. Calves fed by a computerized feeder are typically managed in larger groups, with 10 to 15 calves per feeder (Weber and Wechsler, 2001; Jensen and Holm, 2003). While social contact is valuable in supporting intake early in life, competition for access to artificial teats may be an issue. Even minimal competition for access to artificial teats (1:2 ratio of teat to calf) resulted in reduced milk intake in the early weeks of life for calves fed ad libitum (Figure 3). Further, calves chose to stand and feed at the same time, even when provided a single feeding space (Miller-Cushon et al., 2014), suggesting that calves may be motivated to feed in synchrony rather than adopting different feeding schedules.

Exposure to a competitive feeding environment also has potential to have longer-term impacts on feeding and social behavior. Compared to calves reared in a non-competitive feeding environment, calves reared with restricted teat access were found to persistently displace each other more frequently and consume their feed more quickly after weaning, despite having unrestricted access to feed buckets during the post-

weaning stage (Miller-Cushon et al., 2014). Persistent competitive behavior has potential to pose problems later in life, as competition for access to feed in adult cattle encourages large and infrequent meals (Hosseinkhani et al., 2008; DeVries and von Keyserlingk, 2009), which can negatively affect ruminal pH (Krause and Oetzel, 2006). Thus, as intensified feeding systems are increasingly adopted, further work is encouraged to assess longer-term effects of different management strategies on both performance and behavioral development of dairy calves.

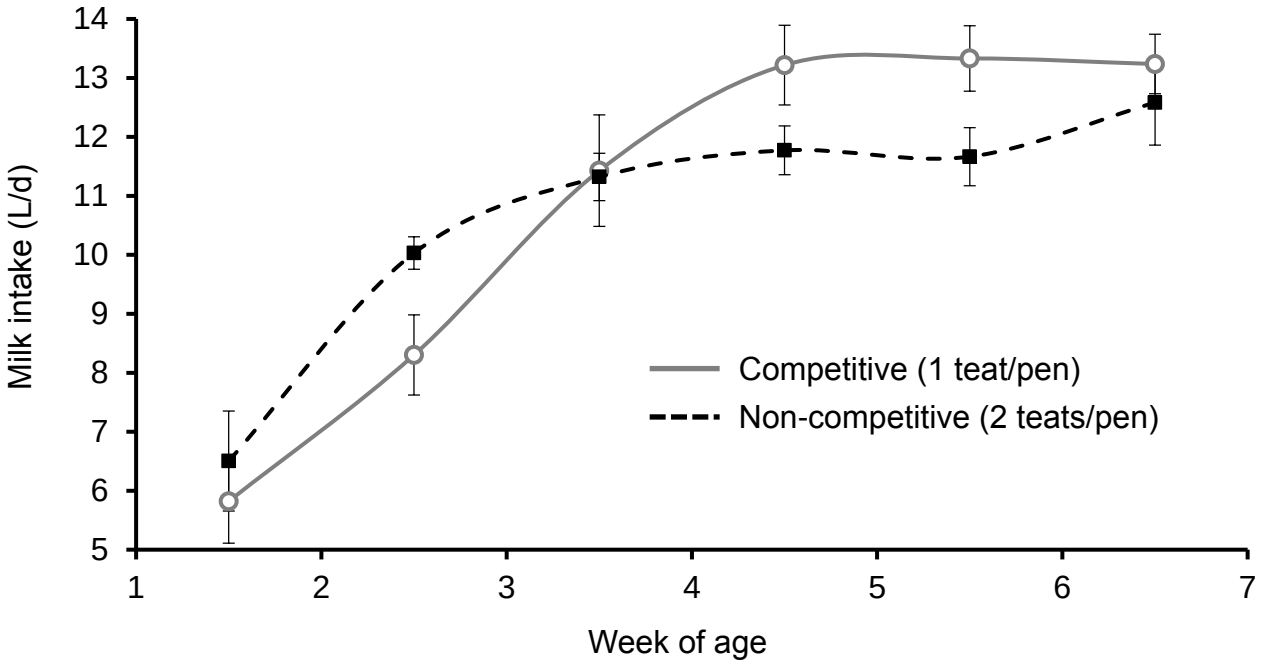

Figure 3. Milk intake of calves fed competitively (1 teat/pair of calves) or non-competitively (2 teats/pair of calves). Milk was provided *ad libitum*.

Conclusions

When managed in intensified feeding systems, calves will consume at least twice the amount of nutrients typically supplied according to conventional feeding strategies. Intensified feeding programs provide a higher plane of nutrition and support greater rates of growth. Feeding behavior is greatly influenced by feeding program, with access to greater quantities of milk allowing the expression of more natural feeding behavior patterns, such as those exhibited by a calf suckling the dam, and reducing behavioral indicators of hunger. Further, greater rates of gain prior to weaning are associated with earlier calving ages and improved milk production, suggesting that there may be a longer-term economic advantage to providing calves with more milk.

In successfully implementing intensified feeding programs, management and housing issues must also be considered. Successful weaning of calves providing greater quantities of milk requires a gradual process of reducing milk intake to encourage sufficient solid feed intake prior to removal of milk. There is also growing

evidence that provision of hay may be beneficial in encouraging greater total intake prior to weaning. Approaches to housing calves can impact outcomes of intensified feeding programs. Social housing for calves encourages greater solid feed intake and reduces stress through weaning. However, competition in group-housed calves may reduce milk intake when access to teats is restricted.

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SESSION NOTES

Dietary Strategies to Improve the Health of Dairy Calves

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Introduction

It is well documented that dairy calves are extremely susceptible to enteric diseases and mortality during the first few weeks of life. The latest reports from the USDA's National Animal Health and Monitoring System (NAHMS, 1993; 1996; 2007) report that the national mortality rate of heifer calves from 48 hours of life to weaning is approximately 7.8 to 10.8%. Producer perceived records indicate that scours account for 56.5 to 60.5% of all pre-weaned deaths. Approximately ¼ of all pre-weaned calves are therapeutically treated for scours, and the major causes of death from scours are due either to dehydration or to pathogen access to the blood causing septicemia. There is a high incidence of respiratory disease among dairy calves and it is the main contributor to high death losses, 1.8%, after weaning (NAHMS, 2007). The high incidences of disease indicate that we have much to learn about improving gastro-intestinal disease resistance among pre-weaned calves. Colostrum management, how much and the composition of fluid fed, the use of various additives such as prebiotics, probiotics, and proteins from hyper-immunized egg or plasma proteins, and housing can all influence the health of pre-weaned dairy calves. In addition, there are few data that indicate that early life nutrition can have long-term impacts on leukocyte responses and disease resistance (Ballou, 2012; Ballou et al., JDS *In Press*; Sharon and Ballou, unpublished). This is an exciting area of research that needs to be addressed further.

Why Are Calves Susceptible to Gastrointestinal Disease?

The calf is in a bit of a 'catch-22' situation early in life because it requires the passive absorption of many macromolecules from colostrum and milk, but this also increases the risk of translocation of pathogenic microorganisms. The gastrointestinal tract of many neonates undergoes a rapid maturation after parturition, and the timing of this depends largely on the species of interest. There are large gaps in our knowledge regarding how the gastrointestinal tract of a calf changes early in life; however, using gastrointestinal morbidity/mortality risk as an indirect measurement, the maturation occurs quite rapidly over the first few weeks of life. There are many components to the gastrointestinal immune system (Figure 1). Most of my discussion in this section was derived from animal models other than the calf, but the general principles can still be applied to the calf.

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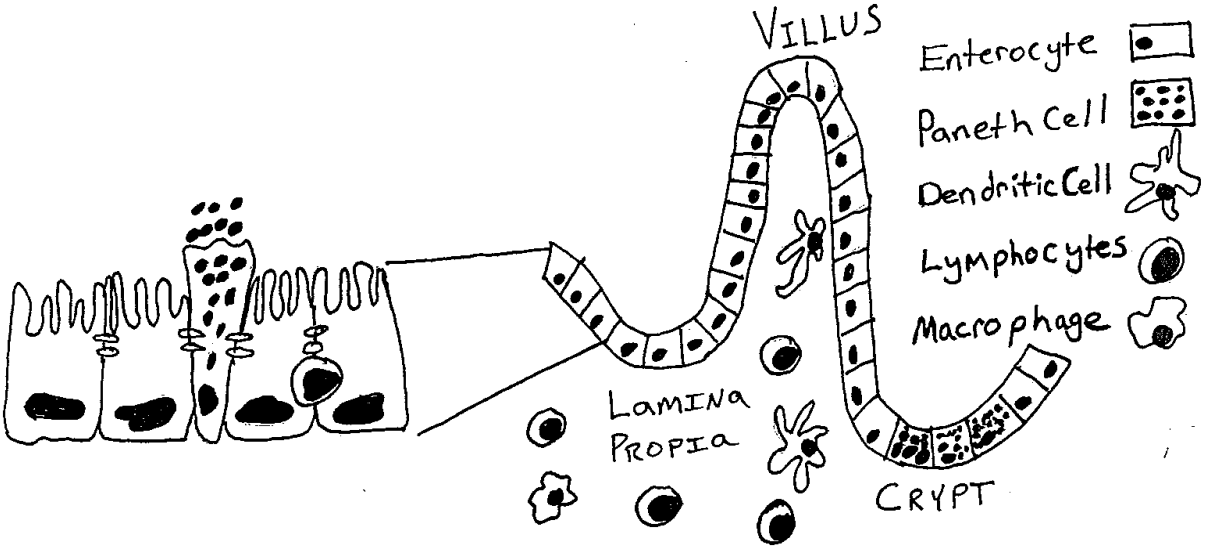

Figure 1. Schematic drawing of the small intestinal mucosa. The crypt-villus axis and common leukocytes found in the mucosa are shown on the right. The insert on the left is a magnification of the epithelial layer, depicting microvilli, tight junctions between epithelial cells, a Goblet cell secreting mucus, and an intraepithelial lymphocyte.

The epithelial cells that make up the mucosal surface and the tight junctions between those cells form a **physical barrier** that prevents luminal contents from flowing directly into systemic circulation. A breakdown in the tight junctions increases the likelihood of infectious disease because of increased bacterial translocation. Goblet cells are one of the types of epithelial cells found in the gastrointestinal tract, and they produce mucus that creates a layer that covers most of the intestinal epithelium. This mucus layer forms an additional physical barrier against potential enteric pathogens. Additionally, the mucus layer contains many antimicrobial factors that were secreted from immune cells in the intestinal mucosa. These antimicrobial factors include: defensins, lysozyme, and sIgA. Their function is to limit the interactions of live microorganisms with epithelial cells by creating a **chemical barrier**. Many leukocytes are found in the mucosa of the gastrointestinal tract as well as large lymphoid aggregates are localized in the submucosa of the distal region of the small intestines. These leukocytes contribute to the **immunological barrier** of the gastrointestinal tract. The majority of leukocytes found in the gastrointestinal (sub)mucosa contribute to adaptive immune responses and create memory that will help to prevent subsequent infections. Macrophages are found in the mucosa and could be involved in the clearance of some microorganisms, but neutrophils are rarely found in the mucosa and are only present in a pathologic state. Trillions of commensal microorganisms live in the gastrointestinal tract and they have a symbiotic relationship with the calf. These commensal microorganisms are part of a **microbial barrier** that limits the colonization of the gastrointestinal epithelium with more potentially pathogenic microorganisms.

These commensal microorganisms compete directly for substrates and space with the potentially pathogenic microorganisms and many of them produce antimicrobial factors and stimulate mucus production that further restrict potential pathogens from infecting the calf. These barriers work together to create a competent **Immune System** of the gastrointestinal tract. A defect in any of these components can increase the risk for infectious disease.

Many of the components of the gastrointestinal immune system begin to develop as early as the first trimester of gestation; however, further maturation of many of these barriers occurs only after birth (Guilloteau et al., 2009). This process of rapid intestinal maturation is known as “gut closure” and contributes to the **physical barrier**. The enterocytes, the nutrient absorptive cells that make up the majority of cells in the intestinal epithelium, are considered fetal-type at birth because they are largely vacuolated and can absorb intact macronutrients through pinocytosis. These fetal-type enterocytes are quickly replaced by more adult-like enterocytes. This process occurs from the proximal to distal intestines and from the crypt to the villus tip; therefore, even though the majority of the gastrointestinal tract may have undergone “gut closure” in the day and a half after birth there likely persist vacuolated, fetal-type enterocytes toward the villus tip of the lower regions of the intestines for a longer period of time. In addition to transcellular absorption of macromolecules, the gastrointestinal epithelium may also be more prone to paracellular absorption because of reduced tight junctions between the enterocytes. The mucus layer that covers the intestinal epithelium is dynamic and cannot be studied with traditional histological methods; therefore, very little is known regarding the postnatal changes in the mucus layer. Goblet cells respond to microbial exposure by increasing mucus secretion; therefore, it is conceivable that the mucus layer develops further during the post-natal period. Intestinal motility and the movement of digesta through the gastrointestinal tract can also reduce colonization of potentially pathogenic microorganisms, so a reduced intestinal motility can also contribute to the high incidence of enteric disease. Therefore, a compromised **physical barrier** of the intestines during the early post-natal period likely contributes to the high incidence of enteric disease and bacterial translocation.

The **chemical and immunological barriers** also can be compromised during the early post-natal period. Paneth cells begin to develop during gestation; however, the number of Paneth cells and the antimicrobial secretions increase throughout life. Additionally, the adaptive arm of the immune system is naïve at birth and develops over the life of the animal as the calf is exposed and re-exposed to antigens. Therefore, sIgA concentrations and diversity are low and will remain low until the calf begins to develop it’s own active immunity. Antibodies from colostrum are known to recirculate back to the mucosa of the intestines, and can offer some immediate protection from enteric pathogens; however, the half-life of many passively derived antibodies is 1 to 2 weeks. Therefore, the gastrointestinal tract will become more susceptible to those specific microorganisms again until they develop their own active immunity against them. This is probably why many calves start developing localized enteric disease and scours during the 2nd or 3rd week of life. The fact is young animals will always be at an increased risk for infectious diseases until they develop their own active immunity. It’s one of the

benefits of getting older, the adaptive arm of the immune system becomes ‘wiser’ because of what it has been exposed to and experienced.

The calf *in utero* is developing in a relatively sterile environment and upon parturition and during the post-natal life they are exposed to a greater number and diversity of microorganisms. There is a progression in the microbial colonization of the gastrointestinal tract, with facultative anaerobes from the environment (ie: *Enterobacteriaceae*, *Streptococcus*, and *Staphylococcus)* dominating during the early post-natal period. There will be a switch to where strict anaerobes (ie: *Bifidobacterium*, *Bacteroides*, *Lactobacilli*, and *Clostridia*) will dominate and account for greater than 99% of the bacteria in the intestines for the rest of the animal’s life. Therefore, the **microbial barrier** of the gastrointestinal tract is also compromised during early life and likely contributes to the greater incidence of enteric disease.

Therefore, from a systematic perspective, there are many holes in the gastrointestinal immune system defense during early post-natal life. This greatly increases the relative risk for enteric disease. It is well known that what an animal is fed during the neonatal period will influence the development of the gastrointestinal immune system and enteric disease resistance. It should be noted that a lot more basic research on the development of the post-natal gastrointestinal immune system in calves is needed and should be a research priority.

Maturation of the Gastrointestinal Immune System and Preventing Pathogen-Host Interactions

A common management strategy in the dairy industry is to feed approximately 4 L of colostrum within the first 6 to 12 hours of birth. Then calves are switched to either milk or milk replacer. It is well known that bioactive compounds in colostrum and transition milk directly influence the maturation of the gastrointestinal immune system. Our current colostrum management protocols are designed to ensure as many calves as possible get adequate passively derived immunoglobulins as possible. I don’t want to down play the importance of passive transfer of immunoglobulins because it is essential in preventing systemic and local enteric diseases while the gastrointestinal tract matures; however, current colostrum management programs completely ignore the role that colostrum and transition milk play in the maturation of the intestinal immune system. Enteric disease would likely be reduced if we fed calves to hasten the maturation of the gastrointestinal immune system. Most of our management decisions after feeding colostrum are aimed at reducing the interaction of potentially pathogenic microorganisms with the intestinal epithelial cells.

Prebiotics, probiotics, and proteins from hyper-immunized egg or spray-dried plasma all have shown some merit in improving the resistance to enteric disease. Prebiotics are dietary components that are not easily digested by the calf, but are used by bacteria in the lower intestines to improve their growth. Probiotics are a vague term, but generally are live microorganisms that provide ‘some’ health benefit. At first glance

this may seem bad, why would one want to improve the growth of bacteria in the lower intestines? As mentioned before, the intestinal tract is not sterile. Soon after birth, a wide range of bacterial species colonizes the gastro-intestinal tract of calves. Most of these bacterial species do not pose any immediate threat to the survival of the calf and in the past were called “good bacteria” and, of which, many of the common probiotic species are routinely classified as, including: *lactobaccilus* species, *bifidobacteria*, *Enterocooccus faecium*, and *Bacillus* species. Remember that the microbial intestinal barrier soon after birth is colonized primarily by facultative anaerobes and subsequently becomes inhabited largely by strict anaerobes. Most of the probiotic microorganisms are strict anaerobes. Many of the probiotic species also have a direct bactericidal activity or compete with the more pathogenic microorganisms for limited resources. In addition, probiotics are themselves bacteria and they may “prime” the immune system of the calf by staying alert, as even the immune system recognizes the “good” bacteria as foreign. The common, commercially-available prebiotics available are the fructooligosaccharides (FOS), mannanoligosaccharides (MOS), lactulose, and inulin.

Data on the influence of prebiotics and probiotics alone on the health of dairy calves is equivocal. There are data that show improvements in reducing scouring and improving growth (Abe et al., 1995), whereas equally as many studies show no benefits to including either prebiotics or probiotics in milk (Morrill et al., 1995). The lack of a clear effect in calves is likely due to many environmental factors. Research does however support that many prebiotics and probiotics are generally safe and do not have any adverse effects on calf health or performance. In fact, most regulatory agencies around the world classify most prebiotics and probiotics as Generally Regarded As Safe (GRAS). Lastly, it is important to note that not all probiotic species and further, not all strains of a specific species, i.e. not all *Lactobaccilus acidophilus* strains behave similarly. Therefore, I would recommend only using probiotic species and strains that have been reported, through 3rd party research, to improve health and performance of calves. Additionally, viability/stability of the product should be confirmed as many of the probiotic species can become nonviable during processing and storage.

Another strategy to reduce the interaction of pathogenic microorganisms is to feed egg protein from laying hens that were vaccinated against the very microorganisms that cause gastro-intestinal diseases in calves. The laying hens will produce immunoglobulins (IgY) and concentrate those proteins in their eggs, which can recognize the pathogen, bind to it, and prevent its interaction with a calf’s gastro-intestinal tract. Inclusion of whole dried egg from these hens decreased the morbidity due to various bacteria and viruses. In addition to the use of hyper-immunized egg protein, spray-dried plasma proteins can improve gastro-intestinal health of calves. Spray-dried plasma is exactly like it sounds, plasma that is spray-dried to preserve the functional characteristics of the diverse group of proteins in plasma. The use of spray-dried plasma has been used for many years in the swine industry to improve performance and health during the post-weaned period. The addition of spray-dried plasma proteins in milk replacer reduced enteric disease in calves (Quigley et al., 2002).

In 2010, our group evaluated the effects of supplementing a blend of prebiotics, probiotics, and hyper-immunized egg proteins to Holstein calves from immediately after birth through the first 3 weeks of life (Ballou, 2011). Calves given the prophylactic treatment (n = 45) were administered directly into the milk 5 x 10^9 colony forming units per day (from a combination of *Lactobacillus acidophilus, Bacillus subtilis, Bifidobacterium thermophilum, Enterococcus faecium,* and *Bifidobacterium longum*), 2 grams per day of a blend of MOS, FOS and charcoal, and 3.2 grams per day of dried egg protein from laying hens vaccinated against K99+ *Escherichia coli* antigen, *Salmonella typhimurium*, *Salmonella Dublin*, coronavirus, and rotavirus. Control calves (n = 44) were not given any prebiotics, probiotics, or dried egg protein. All calves were fed 2 L of a 20% protein / 20% fat, non-medicated milk replacer twice daily. Prior to each feeding fecal scores were determined by 2 independent, trained observers. Briefly 1 = firm, well-formed; 2 = soft, pudding-like; 3 = runny, pancake batter; and 4 = liquid splatters, pulpy orange juice. The prophylactic calves refused less milk ($P < 0.01$) during the first 4 days of life (57 vs 149 grams of milk powder). There were no differences in starter intake or average daily gain due to treatments. However, calves that received the prophylactic treatment had decreased incidence of scours ($P < 0.01$) during the first 21 days of life (25.0 vs 51.1%). Scours were classified as a calf having consecutive fecal scores ≥ 3. The intensity of disease in this study was low and only 1 out of 90 calves died during the experiment. These data support that a combination of prebiotics, probiotics, and hyper-immunized egg protein can improve gastro-intestinal health and could be an alternative to metaphylactic antibiotic use. Future research should determine the efficacy of that prophylactic treatment in calves that are at a higher risk of developing severe gastrointestinal disease and subsequently death as well as investigate the mechanism(s) of action within the gastrointestinal immune system.

Plane of Nutrition

The interest in the plane of nutrition that calves are fed during the pre-weaned period has increased primarily because data indicate that calves fed a greater plane of nutrition are younger at first calving and they may have improved future lactation performance (Soberon et al., 2012). More large prospective studies in various commercial settings should confirm that calves fed greater planes of nutrition during the pre-weaned period have improved future lactation performance. Most data on how plane of nutrition influences the health of calves during the first few weeks of life is limited to small, controlled experiments with fecal scores as the primary outcome variable (Nonnecke et al., 2003; Ballou, 2012). Many studies observed that the calves fed the greater plane of nutrition had more loose feces or greater fecal scores (Nonecke et al., 2003; Bartlett et al., 2006; Ballou et al., In Press JDS), while others reported no differences in fecal scores (Ballou, 2012; Obeidat et al., 2013). It is important to note, that no study has reported greater fecal scores among calves fed a lower plane of nutrition when compared to calves fed a greater plane of nutrition. It has been suggested that the greater fecal scores were not due to a higher incidence of infection or disease, but may be associated with the additional nutrients consumed. A couple of recent studies from my lab are confirming that calves fed greater quantities of milk solids early in life have greater fecal scores; however, when the dry matter percentage

of the calves feces were determined there were no differences between calves fed differing quantities of milk solids (Liang and Ballou, unpublished).

It was unknown whether the digestibilities of nutrients of calves fed varying planes of nutrition were different during the first week of life. Decreased nutrient digestibilities would likely increase the risk of enteric disease because the increased supply of nutrients to the lower gastrointestinal tract could provide a more favorable environment for pathogenic microorganisms to thrive. My lab recently tested the hypothesis that feeding a higher plane of nutrition during the first week of life would decrease the percentages of dietary nutrients that were digested and absorbed (Liang and Ballou, unpublished). Our justification for this hypothesis was that the reduced plane of nutrition during the first week of life would allow the gastrointestinal tract time to adapt to enteric nutrition, without overwhelming the system. However, after conducting a digestibility trial with Jersey calves during the first week of life we had to reject that hypothesis. In fact, there was no difference in the percentage of intake energy that was captured as metabolizable energy, averaging 88% across treatments for the first week of life. We separated the first week of life up into 2 three-day periods and observed a tendency (P = 0.058) for more of the intake energy to be captured as metabolizable energy during the 2nd period (85.9 versus 91.2 ± 2.0; 1st and 2nd period, respectively); however, the first period was likely underestimated because residual meconium feces would decrease the apparent digestibility. There was a treatment x period interaction (P = 0.038) for the percentage of dietary nitrogen retained. The calves fed the greater plane of nutrition had improved nitrogen retention during the first period (88.0 versus 78.7 ± 1.20; P = 0.004), but was not different from calves fed the reduced plane of nutrition during the second period (85.3 versus 85.0 ± 1.20; P = 0.904). Most of the difference in nitrogen retention during the first period could be explained by differences in apparent nitrogen digestibility. It should be noted that apparent digestibility was likely more underestimated among the calves fed the restricted milk replacer during the first period because an equal quantity of meconium feces collected across the treatments during period 1 would underestimate the calves fed the restricted quantity of milk replacer more. The data from the digestibility study indicate that calves not only tolerate greater quantities of milk during the first week of life, but they incorporate those nutrients into lean tissue growth. The gastrointestinal immune system and implications to enteric health should be further investigated.

Over the past 7 years, our group has conducted research to better understand how plane of nutrition during the pre-weaned period influences leukocyte responses and resistance to infectious disease during the pre- and immediate post-weaned periods (Ballou, 2012; Obeidat et al., 2013; Ballou et al., In Press, JDS; Liang and Ballou, unpublished; Sharon and Ballou, unpublished). The results indicate that plane of nutrition influences leukocyte responses of calves (Ballou, 2012; Obeidat et al., 2013; Ballou et al., In Press, JDS). In 2 studies, we reported that when calves were fed a lower plane of nutrition their neutrophils were more active during the pre-weaned period, as evident by increased surface concentrations of the adhesion molecule L-selectin and a greater neutrophil oxidative burst (Obeidat et al., 2013; Ballou et al., In Press, JDS). After weaning the elevated neutrophil responses were no longer apparent in either of

those studies. The exact mechanisms for the more active neutrophils among the low plane of nutrition calves are not known, but could be due to increased microbial exposure because of increased non-nutritive suckling, altered microbial ecology of the gastrointestinal tract, or reduced stress among the calves fed the low plane of nutrition. If the neutrophils are more active because of increased microbial exposure, calves fed a lower plane of nutrition could be at an increased risk for disease during the pre-weaned period if exposed to more virulent pathogens. Ongoing research in my laboratory is trying to understand the behavior and potential microbial exposure when calves are fed varying planes of nutrition and its influence on risk for enteric disease and immunological development. In fact, a few studies have shown that plane of nutrition during the pre-weaned period influence adaptive leukocyte responses. Pollock et al. (1994) reported that antigen-specific IgA and IgG_2 were reduced when calves were fed more milk. In agreement, Nonnecke et al. (2003) reported that less interferon-γ was secreted when peripheral blood mononuclear cells were stimulated with T-lymphocyte mitogens. However, not all data indicate that adaptive leukocyte responses are reduced when greater quantities of milk are fed; Foote et al. (2007) did not observe any difference in either the percentage of memory CD4+ or CD8+ T lymphocytes or antigen-induced interferon-γ secretion. All the leukocyte response data taken together suggest that calves fed lower planes of nutrition may have more active innate leukocyte responses driven by increased microbial exposure, which may explain the greater adaptive leukocyte responses. In a relatively sanitary environment this increased microbial exposure may improve adaptive immune development in the absence of clinical disease, but in a dirty environment it would likely increase the risk of enteric disease.

How plane of nutrition influences resistance to enteric disease is even less clear than how the leukocyte responses are affected. Quigley et al. (2006) reported that feeding a variable, greater plane of nutrition to high-risk Holstein bull calves, purchased from a sale barn and raised on bedding contaminated with coronavirus, increased the number of days calves had scours by 53% and also increased the number of days calves received antibiotics, 3.1 versus 1.9 days. In contrast, a more recent study reported that calves fed a greater plane of nutrition had improved hydration and fecal scores improved faster when they were challenged with *Cryptosporidium parvum* at 3 days of age (Ollivett et al., 2012). In a recent study from my lab, we orally challenged calves fed either a restricted plane or a greater plane of milk replacer at 10 days of age with an opportunistic pathogen, *Citrobacter freundii* (Liang and Ballou, unpublished). The calves fed the greater plane of nutrition had a greater clinical response to the challenge as evident by increased rectal temperatures ($P = 0.021$) and numerically greater peak plasma haptoglobin concentrations (511 versus 266 ± 108 μg/mL; $P = 0.118$). There also was a tendency for total mucosal height of the ileum to be increased among calves fed the greater plane of nutrition (921 versus 752 ± 59.1 μm; $P = 0.059$). The increased surface area of the lower gastrointestinal tract could partially explain the increased clinical response among the calves fed the greater planes of nutrition. Current data indicate that their likely is a pathogen:host interaction on the effects that plane of nutrition influence enteric disease resistance. Larger data sets with naturally occurring disease incidence and more experimentally controlled relevant disease challenges that

are focused on the gastrointestinal immune system are needed before definitive conclusions on the role that plane of nutrition plays on enteric health of calves during the first few weeks of life. However, current data do not support that feeding greater planes of nutrition during the first few weeks of life are going to dramatically reduce enteric disease, so if you hear, “We have high incidences of disease and death in dairy calves because we restrict the quantity of milk they are fed” this is likely not true.

In contrast to health during the first few weeks of life, the plane of nutrition during the pre-weaned period seems to influence leukocyte responses and disease resistance among calves after they are weaned (Ballou, 2012; Ballou et al., In Press, JDS; Sharon and Ballou, unpublished). Jersey bull calves that were fed a greater plane of fluid nutrition had improved neutrophil and whole blood *E. coli* killing capacities after they were weaned when compared to Jersey calves fed a more conventional, low plane of nutrition (Ballou, 2012). These effects were only observed among the Jersey calves in this study and not the Holstein calves. In a follow-up study, Jersey calves that were previously fed a greater plane of milk replacer had a more rapid up-regulation of many leukocyte responses, including neutrophil oxidative burst and the secretion of the pro-inflammatory cytokine tumor necrosis factor-α, after they were challenged with an oral bolus of 1.5×10^7 colony-forming units of a *Salmonella enterica* serotype *Typhimurium* (Ballou et al., In Press, JDS). The increased activation of innate leukocyte responses among the calves previously fed the greater plane of nutrition reduced ($P = 0.041$) the increase in plasma haptoglobin and those calves also had greater concentrations of plasma zinc. The calves fed the greater plane of nutrition also had improved intake of calf starter beginning 3 days after the challenge ($P = 0.039$). These data indicate that the Jersey calves previously fed a greater plane of nutrition had improved disease resistance to an oral *Salmonella typhimurium* challenge approximately a month after weaning.

Recently, we completed a viral-bacterial respiratory challenge on calves a month after weaning that were previously fed either a restricted quantity or a greater plane of milk replacer (Sharon and Ballou, unpublished). Each calf was challenged intranasally with 1.5×10^8 plaque forming units of bovine herpes virus-1 per nostril and 3 days later were given either 10^6, 10^7, or 10^8 colony forming units of *Mannheimia haemolytica* intratracheally in 50 mL of sterile saline (n = 5 per plane of nutrition and bacteria dose combination; N = 30). Calves were observed for 10 days after the *Mannheimia haemolytica* challenge. The bovine herpes virus-1 challenge decreased calf starter intake by 21.2% in both plane of nutrition treatments. The *Mannheimia haemolytica* challenge further decreased calf starter intake, but again was not different between planes of nutrition (7.6%). All calves survived the entire observation period, but 2 calves were euthanized (were completely anorexic and did not respond to antimicrobial / anti-inflammatory treatments) 2 days after the end of the observation period and 2 calves died within a week of completing the observation period. All calves that died or were euthanized were previously fed the restricted plane of nutrition (1, 2, and 1 calves challenged with 10^6, 10^7, or 10^8 *Mannheimia haemolytica,* respectively). Necropsies of all 4 calves were consistent with severe pneumonia. Hematology and plasma data during both challenges indicated that calves previously fed the restricted quantity had a

greater clinical response as evident by greater percentages of neutrophils in peripheral circulation ($P = 0.041$) and plasma haptoglobin concentrations ($P \leq 0.097$). Therefore, the calves previously fed the restricted quantities of milk replacer had a more severe response to the combined viral-bacterial respiratory challenge, and the response was relatively independent of the *Mannheimia haemolytica* dose.

Therefore, the 3 studies from our group are promising that early plane of milk replacer nutrition can influence the health of dairy calves within a month of weaning. Further, it appears that both enteric and respiratory health is improved with feeding greater planes of nutrition during the pre-weaned period. As was noted for enteric health during the pre-weaned period, larger data sets with naturally-occurring disease and additional experimentally-controlled challenges with leukocyte responses are needed before definitive conclusions can be draw. Further, it is of interest whether or not the improved health observed within the first month of weaning would persist later into life and improve resistance to other diseases that are common during the life cycle of dairy cattle, including: gastrointestinal, respiratory, metritis, and mastitis.

Implications

Dairy calves are extremely susceptible to disease in the first few weeks of life, which may be related to the naïve gastrointestinal immune system of calves. Increasing the plane of nutrition in the first week or 2 appears to increase fecal scores, although the dry matter percentages of the feces were not different. Additionally, the digestibility of nutrients during the first week of life is high and does not appear to be impaired by feeding a greater quantity of milk replacer solids. However, resistance to enteric disease during the first few weeks of life does appear to be influenced by plane of nutrition, but more data are needed before more definitive conclusions can be made. Some early data are suggesting that feeding a greater plane of nutrition during the pre-weaned period may improve leukocyte responses and disease resistance of calves that extends beyond the pre-weaned period, but as for the effects of plane of nutrition on risk for enteric disease, more data are needed before we fully understand how early life plane of nutrition influences disease resistance later in life.

In addition to plane of nutrition, the uses of prebiotics, probiotics, and proteins from hyper-immunized egg or spray-dried plasma were all shown to reduce the incidence of gastrointestinal disease. If your calves have a high early mortality rate, I would recommend you look into using a research-backed product with prebiotics, probiotics, or proteins from hyper-immunized egg or spray-dried plasma.

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SESSION NOTES

New Concepts in Trace Mineral Supplementation of Grazing Cattle
Hydroxy Sources, Injectable Sources and Pasture Application

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Introduction

The trace mineral nutrition of grazing cattle is complicated by several factors among which are the impacts of trace mineral antagonists in grazed forage and the reliance on predictable, uniform intake of free-choice mineral supplements. Numerous options are available to assist in the management of trace mineral nutrition of grazing cattle. In recent years, significant research efforts have been focused on new technologies, which have revealed insight toward their utilization in trace mineral supplementation programs. This article will focus on three of these technologies, (1) hydroxy trace minerals, (2) injectable trace minerals, and (3) pasture application of Se.

Hydroxy Trace Minerals

One of the newest technologies to impact the trace mineral nutrition of livestock is the creation of hydroxy trace mineral sources of Cu, Zn, and Mn. These specific crystalline inorganic mineral sources are formed by covalent bonds within a crystalline matrix. This covalent bond structure differs from the ionic bonds present in common sulfate-based minerals and is more similar to the covalent bonds present in organic trace mineral sources. Whereas organic trace minerals are covalently bound to a carbon-containing ligand, hydroxy trace minerals are covalently bound to an OH group. One of the most functional characteristics of hydroxy trace minerals is their lack of solubility at neutral pH ranges, such as the rumen of healthy cattle. Dissolution of the metal occurs at lower pH values, which are common in the lower gastrointestinal tract. In addition to these nutritional characteristics, the crystalline matrix of the hydroxy trace minerals allows for exceptional handling characteristics. They are non-hydroscopic and free of dust leading to handling and mixing advantages absent in most other inorganic and organic trace mineral sources. Within the blended formulation, hydroxy trace minerals are highly stable, particularly when compared to sulfate counterparts. This stability aids in the reduction of oxidative loss of fat-soluble vitamins. Lu et al. (2010) reported a 52% reduction in vitamin E loss when broiler feeds were supplemented with 200 mg/kg of hydroxy Cu vs. Cu sulfate (Figure 1). Hydroxy trace minerals have also been suggested to have greater bioavailability compared to sulfate counterparts (Spears et al., 2004) and due to their lower solubility, they may avoid certain trace mineral antagonisms in the rumen (i.e. Cu x S x Mo; Arthington and Spears, 2007). Additional to these functional characteristics, hydroxy trace minerals are also highly concentrated allowing for greater flexibility with formulation space. For example, a

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mineral supplement containing 4,000 mg/kg Zn would require only 0.73% of the formulation space for hydroxy Zn inclusion (IntelliBond [Micronutrients]; 55% Zn), but would require 2.7% of the formulation space for organic Zn inclusion (i.e. Bioplex [Alltech] Zn; 15% Zn).

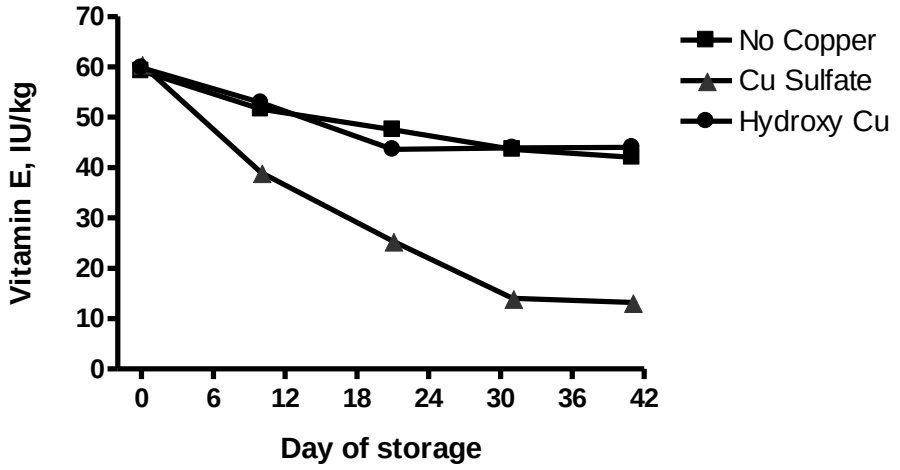

Figure 1. Vitamin E stability of broiler feeds supplemented with no Cu or 250 mg/kg of Cu from sulfate and hydroxy sources. Adapted from Lu et al. (2010). Vitamin E concentrations are less ($P < 0.05$) in Cu sulfate-supplemented feeds on each sampling day compared to the other two treatments. Average standard deviation of the mean = 3.30, 3.60, and 3.85 for No Cu, Cu sulfate, and hydroxy Cu, respectively.

Limit-Creep Feeding and the Effects of Trace Mineral Source on Voluntary Intake by Cattle

We have had a long-term interest in nutritional management applications that will optimize the trace mineral status in beef calves prior to weaning. Weaning is one of the most stressful events that a calf will encounter throughout its lifetime and trace mineral loss is a consequence of that stress. Normal calf management practices such as castration and vaccination also contribute to stress and trace mineral loss. Therefore, optimizing the trace mineral nutrition of calves, prior to weaning will help to ensure adequate trace mineral status following recovery from the stress of weaning. One area of investigation is the use of “limit-fed” creep supplements. The concept of “limit-fed” is essential in this application. Many studies have confirmed that the efficiency of added gain among creep-fed calves is poor, in fact, the poorest of all phases of the beef production system. Therefore, we sought to use limited creep feeding as a system for delivering trace minerals to pre-weaned calves. In our first study, we discovered that calves had a strong aversion to consumption of mineral-fortified creep feed, which did not exist in calves consuming the same supplement without mineral fortification (Figure 2; Moriel and Arthington, 2013). We hypothesized that the sulfate sources of minerals, particularly Cu and Zn, were disassociating in the calves’ mouths, causing a taste aversion, such as a person might experience with a metallic taste experience. This

hypothesis is supported by the highly soluble nature of Cu- and Zn sulfate. Visual observation of the calves' reactions as they attempted to consume the supplements also supported our hypothesis.

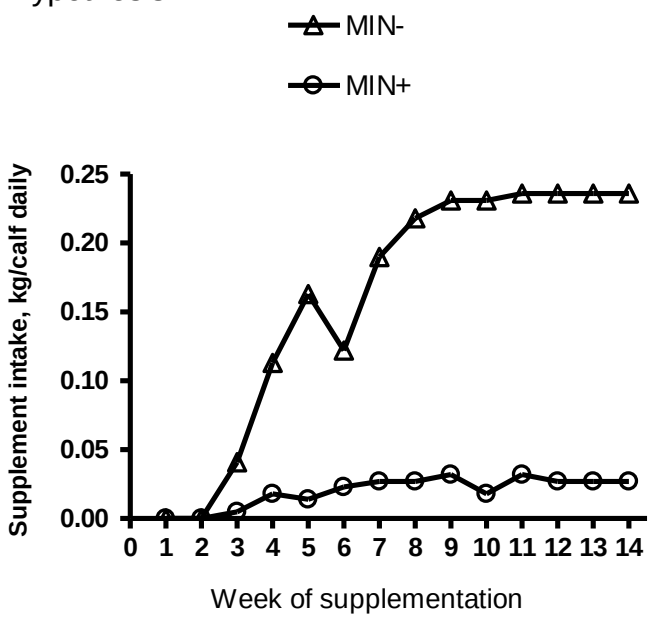

Figure 2. Voluntary intake (as-fed) of limit creep feed supplements with (MIN+; sulfate sources of Cu, Zn, and Mn) or without (MIN-) mineral fortification (pooled SEM = 0.008). Calves limited to a maximum of 230 g/calf daily. Average daily intake over the entire supplementation period was greater ($P < 0.001$) for MIN- vs. MIN+ (0.16 vs. 0.02 kg/calf daily; SEM = 0.006). Figure adapted from Moriel and Arthington (2013).

To test our hypothesis, we designed a study to evaluate the preference for intake of three experimental supplements, each containing the same base ingredient formulation, but differing by source of Cu, Zn, and Mn. This was achieved in 4 individual studies. These studies involved 8 pens of early-weaned calves (2 calves/pen) with an average age of 120 days and an average body weight of 115 kg. Each pen was provided free-choice access to concentrate and grass hay. On each study day at 1000 h, all feed was withdrawn from the pens and calves were offered three different mineral fortified supplements, for a 4-hour period. The supplements were provided in three separate feeding containers. The supplements differed by the source of Cu, Zn, and Mn, which were hydroxy- (IntelliBond; Micronutrients, Inc.), organic- (Bioplex; Alltech, Inc.), and sulfate-sources. The supplements were created using a base mixture containing 52, 46, and 2% cottonseed meal, ground corn, and salt fortified with 2,000, 750, and 3,000 mg/kg of only Zn (Experiment 1), only Cu (Experiment 2), and only Mn (Experiment 3), respectively. The last evaluation (Experiment 4) contained the same base supplement mixture fortified with a mixture of Zn, Cu, and Mn. Preferential intake was measured over 7- (Experiments 1, 2, and 3) and 14-d (Experiment 4) evaluation periods. Results are expressed as preferential intake as a % of the amount of supplement offered. These results reveal a lesser preferential intake of supplements fortified with organic sources of Cu and Zn compared to supplements fortified with hydroxy and sulfate sources of these elements (Figure 3).

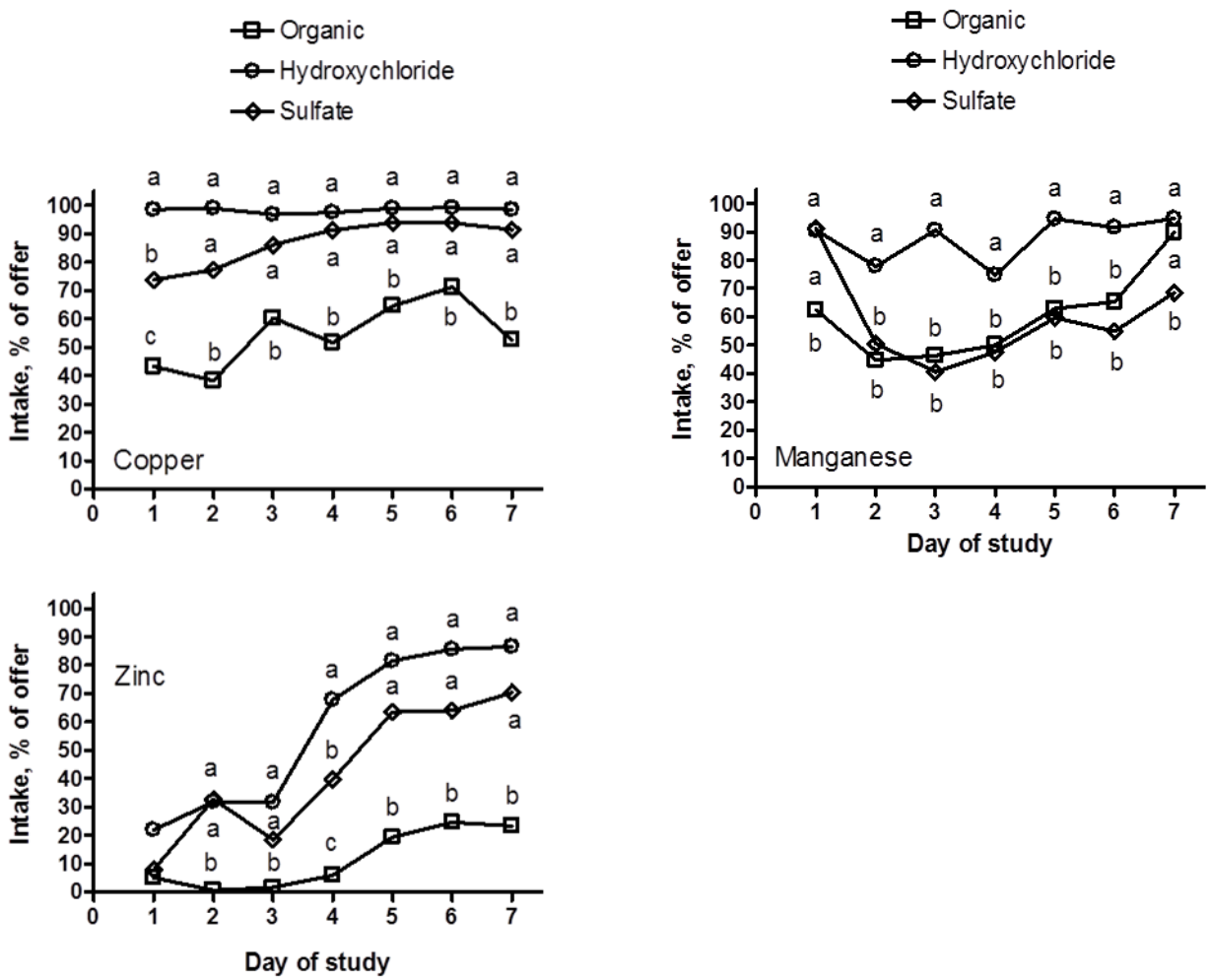

Figure 3. Preferential intake of supplements individually fortified with Cu (750 mg/kg), Zn (2,000 mg/kg), and Mn (3,000 mg/kg) from organic (Bioplex; Alltech, Inc.), hydroxy chloride (IntelliBond; Micronutrients, Inc.), or sulfate sources. Pooled SEM = 7.71, 7.90, and 7.34 for Cu, Zn, and Mn, respectively. Means with unlike superscripts within day differ ($P < 0.05$).

In the case of Mn, the preferential intake of the hydroxy source was greater than both organic and sulfate sources for all days, except 1 and 7. When all three trace minerals were combined together, preferential supplement intake differences were dramatically different with calves almost exclusively selecting the supplement fortified with the mixture of hydroxy Cu, Zn, and Mn (Figure 4).

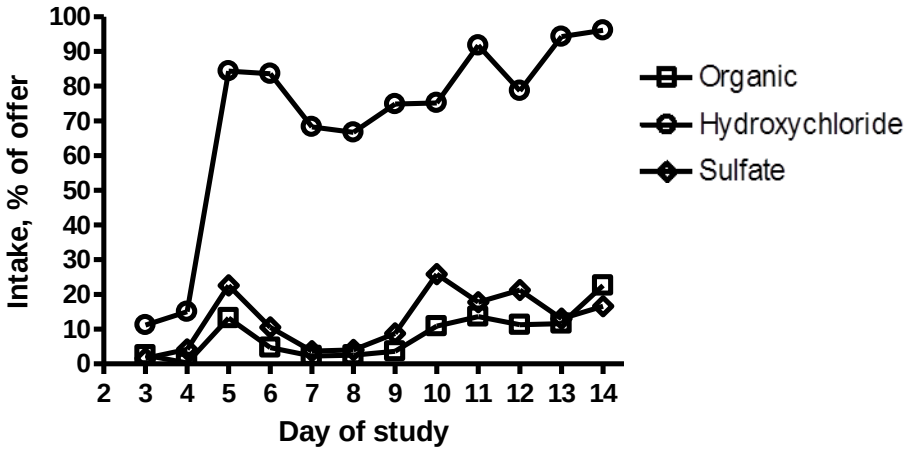

Figure 4. Preferential intake of supplements fortified with Cu (750 mg/kg), Zn (2,000 mg/kg), and Mn (3,000 mg/kg) from organic (Bioplex; Alltech, Inc.), hydroxy chloride (IntelliBond; Micronutrients, Inc.), or sulfate sources. Pooled SEM = 7.72.

With this knowledge, we designed the next limit-fed creep feeding study with three treatment formulations; (1) mineral fortification with hydroxy sources of Cu, Zn, and Mn, (2) mineral fortification with sulfate sources of Cu, Zn, and Mn, and (3) no mineral fortification (Table 1). In this study, voluntary intake increased over the 13-week supplementation period ($P < 0.001$), but there was no treatment x time interaction for voluntary creep feed intake ($P = 0.33$). Nonetheless, over the entire supplementation period, calves provided mineral-fortified creep with hydroxy sources of Cu, Zn, and Mn tended to consume more ($P = 0.10$) of the limit-creep feed offered than calves provided sulfate sources of these elements (7.4 vs. 4.9 kg; SEM = 0.97; Figure 5).

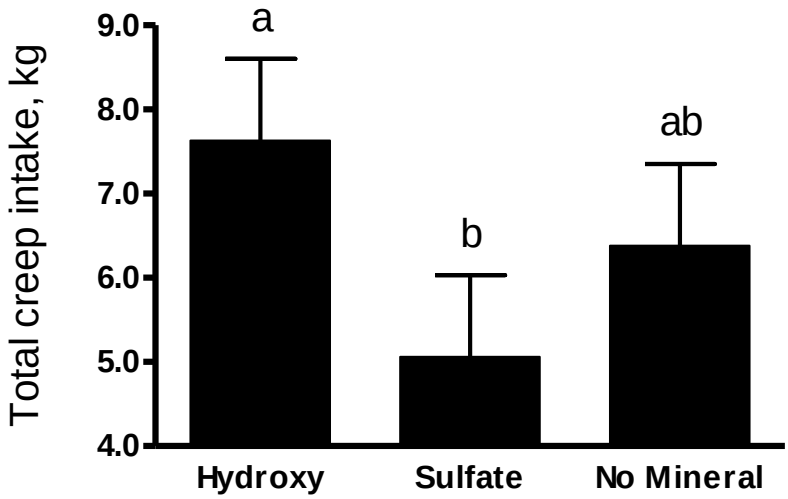

Figure 5. Total creep intake/calf over an 89-d study. Total creep intake tended ($P = 0.10$) to be greater over the entire 89-day supplementation period for calves provided mineral-fortified creep with hydroxy sources of Cu, Zn, and Mn compared to calves provided sulfate sources of these elements (7.4 vs. 4.9 kg; SEM = 0.97). Means with unlike superscripts differ ($P < 0.05$).

Table 1. Ingredient composition of limit-fed creep supplements[1]

Item	Hydroxy	Sulfate	No mineral
	---------- % ----------		
Soybean meal	73.75	73.75	73.75
Alfalfa meal	10.00	10.00	10.00
Wheat middlings	5.08	4.87	6.40
Molasses, dried	5.00	5.00	5.00
Ca carbonate	2.50	2.50	2.50
Salt	1.25	1.25	1.25
Fat, liquid	1.00	1.00	1.00
Ca propionate	0.10	0.10	0.10
Zn sulfate	0	0.63	0
Mn oxide	0	0.50	0
Cu sulfate	0	0.31	0
Na selenite (1% suppl.)	0.08	0.08	0
Ethyleneiamine dihydroiodide (EDDI)	0.01	0.01	0
Co carbonate	0.002	0.002	0
IntelliBond[2] M	0.68	0	0
IntelliBond[2] Z	0.41	0	0
IntelliBond[2] C	0.13	0	0

[1] Diets formulated to provide 750, 2,000 and 3,000 mg/kg of Cu, Zn, and Mn, respectively. Creep supplements provided in cow exclusion areas in amounts not to exceed 230 g/calf daily. [2] Micronutrients Inc.

Liver tissue collected for biopsy from calves at the time of weaning revealed greater concentrations of Co, Cu, and Se among calves consuming mineral-fortified creep feed, irrespective of source, compared to calves consuming creep feed without mineral fortification ($P \leq 0.004$; Table 2). Although differences in liver concentrations of Cu and Co were detected among treatments, all were within normal ranges for cattle. However, calves not provided mineral-fortified creep feed were highly deficient in Se (average = 0.16 mg/kg DM), whereas calves provided mineral-fortified creep feeds were only marginally deficient (0.52 mg/kg DM).

These results provide meaningful insights to the management of trace mineral nutrition of pre-weaned calves. Research efforts in 2015 will further focus on the applications of hydroxy Cu, Zn, and Mn in both limit-fed creep feeding applications and free-choice, salt-based trace mineral supplementation systems.

Table 2. Effect of mineral fortification of limit creep feed using sulfate or hydroxy sources of Cu, Zn, and Mn on liver trace mineral concentrations of weaned calves[1]

Item	Hydroxy[2]	Sulfate[2]	No mineral	No creep	SEM	Hydroxy vs. sulfate	Mineral vs. no mineral	Creep vs. no creep
	---------- mg/kg (DM basis) ----------							
Co	0.36	0.13	0.08	0.04	0.037	0.001	< 0.001	0.001
Cu	241	179	114	98	26.9	0.13	0.001	0.007
Fe	204	223	259	267	80.2	0.86	0.46	0.56
Mn	8.8	9.0	6.1	7.7	1.43	0.92	0.15	0.84
Mo	3.5	2.8	2.9	2.5	0.64	0.45	0.45	0.32
Se	0.60	0.43	0.18	0.14	0.120	0.31	0.004	0.02
Zn	172	171	172	153	17.5	0.99	0.54	0.21

[1]Calves were provided limit-creep feed, 3 times weekly, in amounts not exceeding 230 g/calf daily, except for the No creep treatment. Liver biopsy samples were collected at weaning following an 89 day period of limit-creep supplementation.
[2]Creep feed contained 750, 2,000 and 3,000 mg/kg of Cu, Zn, and Mn, respectively, from IntelliBond (hydroxy-sources), and Cu- and Zn-sulfate and manganous oxide (sulfate).

Injectable Trace Minerals

Injectable trace minerals (**ITM**) have been available for many years, but the technology, targeted application, and scientific assessment of efficacy has more recently been a subject of attention. An advantage of ITM, compared with traditional oral supplementation methods is the targeted delivery of a known amount of trace minerals to individual animals. This removes the variability associated with annual fluctuations in voluntary intake, which is common among cattle provided free-choice mineral formulations (Arthington and Swenson, 2004). In addition, ITM can be used within production environments that might experience difficulty managing the routine delivery of free-choice mineral mixes, such as extensive rangeland systems, seasonal grazing of mountain meadows, and seasonally flooded pastures. Further, the contribution of wildlife to the overall consumption and disappearance of free-choice mineral mixes also can cause complications in these production environments and add further value to the use of ITM. Our interest in ITM investigation originated from research findings from colleagues at other Universities which included increased mineral status (Pogge et al., 2012),increased feed efficiency (Clark et al., 2006), reduced treatments for illness (Berry et al., 2000), and reduced morbidity treatment costs (Richeson and Kegley, 2011) in stressed feeder calves. Our specific aim was to assess measures of mineral status, performance, and immune competence in beef calves receiving ITM or a control injection of sterile saline.

In the first experiment (Arthington and Havenga, 2012), we evaluated a single 7 mL subcutaneous injection of ITM (MultiMin®) containing 15, 40, and 10 mg/mL of Cu, Zn, and Mn, respectively as disodium EDTA chelates, and 5 mg/mL of Se as Na selenite or 7 mL sterile saline (Control). These treatments were administered to weaned steer calves concurrently with a single dose of a commercially-available modified live vaccine (Arthington and Havenga, 2012). All calves enrolled in the study were determined to be seronegative for the key viral pathogens targeted by the vaccine (BHV-1, BVDV-1, and BVDV-2). As a response variable, we measured serum neutralizing antibody titers following vaccination. On the day of vaccination and treatment administration, serum concentrations of Cu, Zn, Mn, and Se were similar among all steers and all values were within the sufficient range for cattle, suggesting that there were no pre-existing mineral deficiencies among the group of steers utilized in this study. By d 14 after treatment administration, steers receiving the saline control treatment experienced a decrease in serum Zn and Se concentrations and on that sampling day were less than steers receiving ITM. Neutralizing antibody concentrations to BVD-1 and 2 and BHV-1 (the primary causative pathogen for infectious bovine rhinotracheitis - IBR) increased in all steers following vaccination. Antibody titers against BHV-1 were greatest for steers receiving ITM vs. Control on day 14, 30, and 60 post-vaccination (Figure 6). Additionally, there were no visible signs of injection site inflammation which were sometimes common in earlier ITM preparations, particularly Cu-containing injectable supplements (Boila et al., 1984; Chirase et al., 1994).

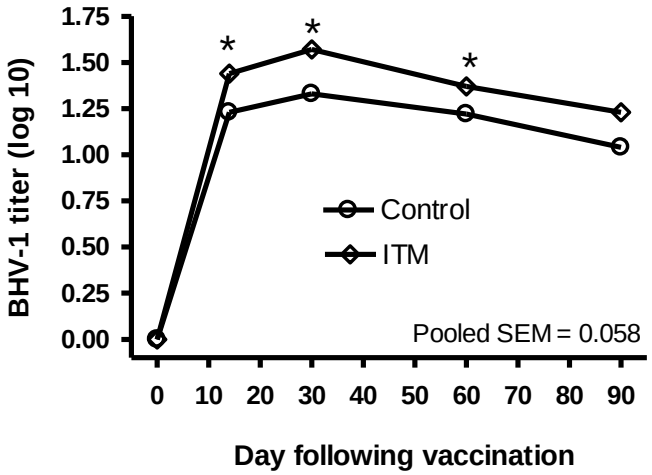

Figure 6. Bovine herpesvirus-1 (BHV-1) serum titers of calves provided a 7-mL injection of trace minerals (ITM) or 7 mL of sterile saline (Control). Seronegative calves were vaccinated on d 0. * = Values within the day and between treatments differ ($P < 0.05$). Data adapted from Arthington and Havenga (2012).

In the next experiment, 34 yearling heifers were randomly assigned to receive 4, 2.5 mL injections of ITM or sterile saline (Control) on d 0, 51, 83, and 127 of the study (Arthington et al., 2014). The ITM product used in Experiment 2 contained 15, 60, and 10 mg/mL of Cu, Zn, and Mn, respectively as disodium EDTA chelates, and 5 mg/mL of

Se, as Na selenite (MultiMin 90®; Multimin USA). The heifers grazed winter, stockpiled limpograss pastures and were provided free-choice, stock salt with no added trace minerals. On day 51, at the time of the second injection, all heifers were challenged with a 10-mL injection of a 25% porcine red blood cell solution to represent a novel exposure to a pathogen. The production of antibodies against the porcine red blood cells (via hemagglutination procedures) was found greater for heifers receiving ITM, compared to Control (Figure 7). Heifers receiving ITM had a 21% greater ADG compared to Control heifers (0.69 vs. 0.57 lb/d). In addition, by the end of the evaluation, heifers receiving ITM had greater liver concentrations of Se compared to control heifers (0.88 vs. 0.48 mg/kg; DM basis).

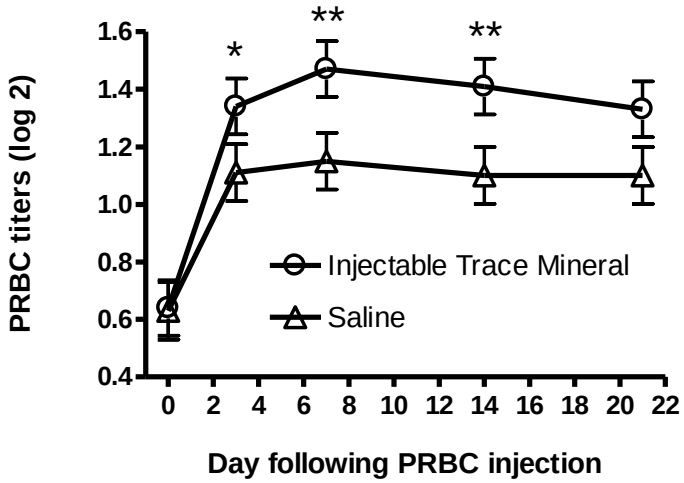

Figure 7. Effect of injectable trace minerals (ITM) on humoral immune response to porcine red blood cell (PRBC) injection. Heifers received ITM or sterile saline (2.5 mL) on d 0 and 51 and humoral immune response to PRBC was evaluated on d 51 following the second treatment administration. Treatment means differ (**P < 0.03) on d 7 and 14 and tend to differ (* P > 0.10) on d 3. Data adapted from Arthington et al. (2014).

Collectively, these findings suggest that the trace mineral status of cattle can be increased by administration of ITM. Additionally, antibody production to vaccine appears to be heightened in calves receiving ITM. These responses appear to be evident even in calves exhibiting adequate trace mineral status. It is unclear; therefore, if these observed increases in antibody titers are responses to increased trace mineral status or a priming response to the immune system. Nonetheless, this heightened immune response may be an important contributing factor to the improved measures of health and performance reported by other investigators in previous studies.

Pasture Application of Selenium

Selenium is an essential trace element for all categories of livestock. In grazing cattle, Se nutrition is complicated by the regional differences in soil Se abundance causing variation in plant Se content. Of the trace elements commonly found to be deficient in forage (i.e. Se, Cu, Co, Zn, and sometimes Mn), Se is the only trace mineral

that is sometimes found in toxic concentrations in forages grown in specific regions of the US. The range between adequate and toxic concentrations is narrower for Se compared to other essential trace minerals; however, in most regions of the country, Se-deficient forage is much more common than cases of Se excess. In a survey of 253 cow/calf operations in 18 US states, over 18% were classified as marginally or severely Se deficient by blood Se parameters (Dargatz and Ross, 1996). Among the states analyzed, those located in the southeast region had the greatest percentage of operations classified as marginally or severely Se deficient (35.8%). A complicating factor impacting Se supplementation is the FDA control over maximum Se fortification of free-choice cattle mineral supplements, which limits Se supplementation to a level not exceeding 3 mg/head daily (21CFR573.920 rev. April 1, 2014). In almost all situations, this upper limit is sufficient to supply adequate Se nutrition to grazing cattle; however, this assumes a consistent intake at the target level for which the free-choice supplement was formulated. Unfortunately, we know that there are significant and often dramatic fluctuations in free-choice intake of salt-based mineral supplements. During periods of reduced voluntary intake, the potential occurrence of Se deficiency becomes a concern. This is further accentuated in scenarios involving high-S diets (i.e. > 0.30 % S; DM basis), which is a major antagonist impacting Se metabolism.

One potential method for addressing Se nutrition in grazing cattle is the implementation of pasture Se applications with the intent of increasing plant Se content and thus the Se status of cattle grazing these forages. In Florida, spraying bermudagrass with Na selenate at Se application ranges of 120 to 480 g/ha resulted in substantial increases in forage Se content by 2 wk after application, decreasing rapidly by 12 wk post-application (Table 3; Valle et al., 1993).

Table 3. Average forage Se concentrations (mg/kg; DM basis) at different weeks after spraying with Na selenate[1]

	Weeks after spraying Na selenate				
Se application rate, g/ha	2	4	6	12	18
0	1.4[a]	0.9[a]	1.5[a]	0.5[a]	0.4[a]
24	2.9[a]	2.7[a]	2.3[a]	0.7[b]	0.8[b]
120	12.8[a]	6.3[b]	4.8[bc]	0.5[c]	0.6[b]
240	26.1[a]	15.5[b]	11.9[b]	0.8[c]	1.0[b]
480	51.5[a]	28.2[b]	25.7[b]	0.7[c]	0.7[b]

[1]Data adapted from Valle et al. (1993). Means are based on 4 replicates per treatment.
[2]Means with unlike superscripts within each row differ ($P < 0.05$).

Selenium from selenate sources appears to be much more available for plant uptake compared to selenite sources (Archer, 1983). Feeding forages grown on Se-fertilized hay fields impacts both Se status and performance of grazing cattle. In one study (Hall et al., 2013), weaned Angus-type calves were fed Se-fertilized alfalfa hay over a 7-week period. Alfalfa hay was grown on fields receiving applications of Na

selenate in amounts providing 0, 23, 45, or 90 g Se/ha. These application rates resulted in a linear (R^2 = 0.997) response for Se application rate and subsequent Se content of alfalfa hay harvested 40 d after Se application (Figure 8; Inset A). In addition, calves consuming these hay treatments (approximately 2.5% BW daily) experienced a linear (R^2 = 0.979) increase in whole blood Se concentrations as Se application rate (and Se content of hay) increased (Figure 8; Inset B).

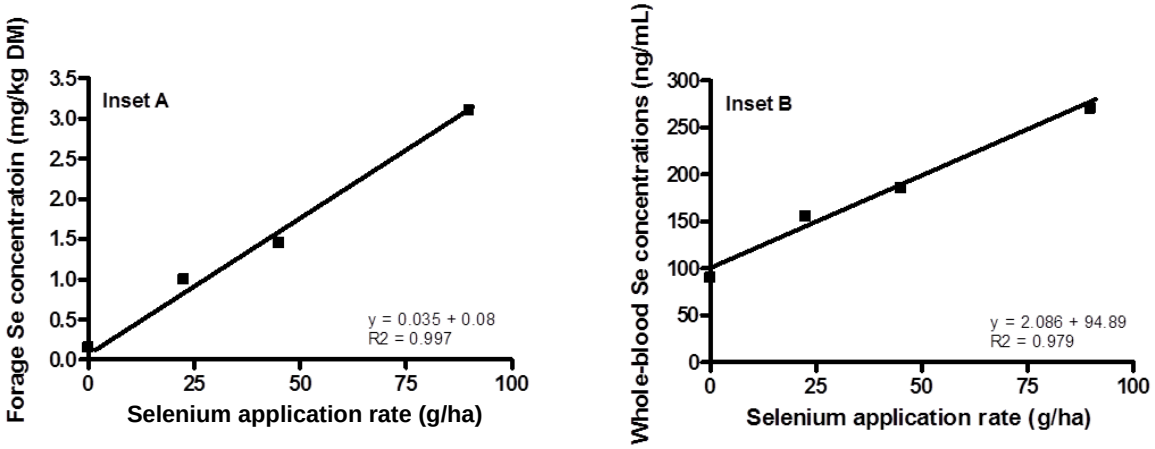

Figure 8. Effects of Na selenate application to alfalfa hay fields on subsequent forage Se content (A) and Se status (B) of calves consuming the hay. Data adapted from Hall et al. (2013).

In a recent study at the UF/IFAS, Range Cattle REC, we produced a high-Se hay crop by spraying a Jiggs bermudagrass hayfield with Na selenate at a rate of 257 g Se/ha. Selenium content of hay, harvested 8 wk after Na selenate application, was greater for Se-treated vs. control pastures (7.73 ± 1.81 vs. 0.07 ± 0.04 mg/kg DM; $P < 0.001$). In a subsequent study, this hay crop was fed to weaned calves and Se status was evaluated over a 42-d study. Calves were stratified by initial BW and randomly assigned to treatments including high-Se hay, low-Se hay + supplemental Na selenite, or No supplemental Se (n = 14, 14, and 4 calves, respectively). Calves were housed in drylot pens (2 calves/pen; 7, 7, and 2 pens per treatment). A pair-feeding design was utilized, whereas each pen of high-Se hay calves was paired to a pen of Na selenite - supplemented calves. Calves assigned to the high-Se hay treatment were provided ground, high-Se hay for a 4 h period each morning. Pen DMI was calculated and total daily Se intake/pen was estimated. Each Na selenite paired pen was then provided the same daily amount of Se via Na selenite hand-mixed into a limit-fed grain supplement. Therefore, each pen of calves receiving high-Se hay had a paired partner pen of calves receiving the same amount of Se via Na selenite. Liver Se concentrations remained unchanged for the negative control calves receiving no supplemental Se over the 42-d feeding period, but they were increased ($P < 0.001$) in calves receiving both high-Se hay and Na selenite treatments. Calves receiving high-Se hay had greater ($P < 0.05$) liver Se concentrations on d 21 and 42 than calves receiving Na selenite (Figure 9).

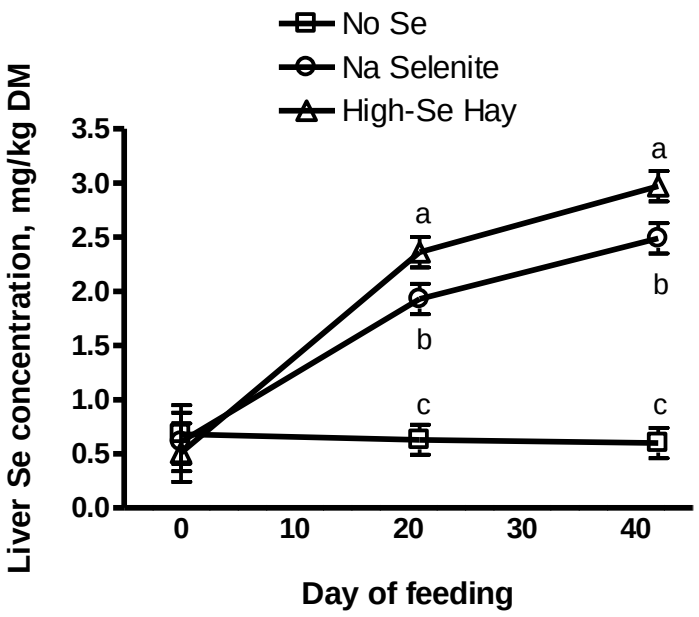

Figure 9. Liver Se concentrations among calves offered high-Se hay or a Na selenite supplement. Basal diet contained 0.6 mg Se daily (No Se treatment). Calves fed high-Se hay and the Na selenite supplement were pair fed to control overall daily Se intake (average 2.8 mg Se/d). [a,b,c] Means differ within day; $P < 0.05$.

Interestingly, this difference was attributed only to the paired pens consuming < 3 mg Se daily (Figure 10). From these initial data, we hypothesize that there is a differential availability of Se in forage vs. inorganic sources dependent upon the total daily intake with a critical point of approximately 3 mg/d in beef calves. We are currently examining these data further in both periparturient cows and calves.

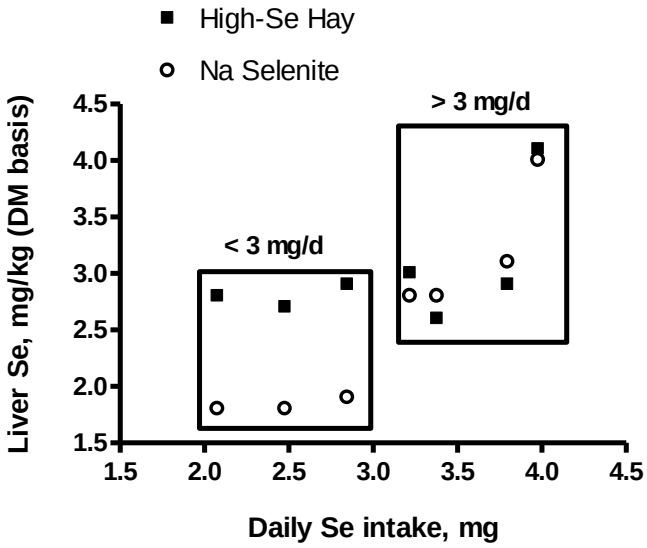

Figure 10. Liver Se concentrations (d 42) among pair-fed calves calves. X-axis denotes average daily Se intake (mg/d) among each pair-fed calf group.

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SESSION NOTES

Copper and Selenium Metabolism and Supplemental Strategies for Grazing Beef Cattle

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Introduction

Trace minerals have long been identified as essential components in the diets of domestic livestock species. Chromium, cobalt, copper, iodine, iron, manganese, molybdenum, nickel, selenium, and zinc are included in the category of essential trace minerals (or microminerals). Trace minerals exist in cells and tissues of the animal body in a variety of chemical combinations, and in characteristic concentrations, depending on the trace mineral consumed and the tissue in which the trace mineral is metabolized (McDowell, 1992; Underwood and Suttle, 1999). Concentrations of trace minerals must be maintained within narrow limits in a cell (McDowell, 1989, 1992; Underwood and Suttle, 1999). Trace mineral deficiencies, toxicities, and imbalances require the animal to metabolically compensate for the nutrient deviation (McDowell, 1989, 1992; Underwood and Suttle, 1999). In doing so, certain metabolic diseases can manifest and overall animal production can be depressed, thus decreasing overall animal performance and health.

Supplementation of minerals to beef cattle has been shown to have positive effects on reproduction, immune status, disease resistance, and feed intake when specific trace minerals are deficient or imbalanced in the diet. Trace minerals have been identified as essential components for carbohydrate, lipid, protein, and vitamin metabolism, and have been shown to be involved in hormone production, immunity, and cellular homeostasis. In general, trace minerals function primarily as catalysts in enzyme systems within cells. Enzymes requiring trace minerals for proper function can be classified into two categories: 1) metal activated enzymes and 2) metalloenzymes. The requirement for a metal in metal-activated enzymes may or may not be absolute; however, the presence of a metal is typically required for optimizing enzyme activity. Metalloenzymes are enzymes that contain a tightly bound metal ion at or near the active site. The metal ions bound to metalloenzymes are actively involved in enzyme function and removal of the metal ion renders the enzyme non-functional. Enzymes associated with the electron transport, bone metabolism, immune function, oxidative stress protection, and gene expression require trace minerals for proper function (Underwood and Suttle, 1999). The specific functions of copper and selenium fall mainly into the catalytic and regulatory categories as described above. The intent of this review is to briefly discuss: 1) the functions of copper and selenium; 2) troubleshooting a potential

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trace mineral deficiency; 3) supplementation strategies, and 4) possible factors that may impact trace mineral requirements in ruminants.

Functions of Copper and Selenium

***Copper**:* Copper is second only to zinc in the number of enzymes that require this metal for appropriate function (Underwood and Suttle, 1999). Copper is therefore essential to proper physiological function and is involved in an array of metabolic systems. These include iron metabolism, cellular respiration, cross-linking of connective tissue, central nervous system formation, reproduction, and immunity (McDowell, 1992).

In order for hemoglobin synthesis to occur, iron must be converted to the ferric form before being incorporated into the hemoglobin molecule. This process is accomplished by ceruloplasmin, which is a copper containing enzyme synthesized in the liver (Saenko et al., 1994). Copper is also an essential component in the enzyme cytochrome oxidase. This enzyme acts as the terminal oxidase in the electron transport chain and is essential to cellular respiration by converting oxygen to water (Spears, 1999). Cytochrome oxidase is also necessary for proper central nervous system function. Cross-linking of connective tissue is also facilitated by a copper-containing enzyme, lysyl oxidase (Harris and O'Dell, 1974).

The requirement for copper for optimal reproductive performance has also been widely documented, although a specific copper-linked enzyme that is responsible has not been identified. It is likely that an array of copper-containing or copper-activated compounds is involved in the reproductive process making this identification even more difficult. Corah and Ives (1991) noted that clinical signs of copper deficiency associated with reproduction include decreased conception rate, overall infertility, anestrus and pregnancy loss. Some of these problems may be associated with the function of a major enzyme: copper-zinc superoxide dismutase. This copper-containing enzyme functions as an antioxidant to protect cells involved in reproduction from oxidative stress. The same copper-zinc superoxide dismutase has also been implicated in contributing to proper function of the immune system as well (Miller et al., 1979).

Signs of copper deficiency in ruminants include anemia, bone and connective tissue disorders, neonatal ataxia, cardiovascular disorders, depigmentation of hair or wool, impaired immunity, and infertility. Copper deficiency can be produced by removal of copper from the diet but more often, under practical conditions, copper deficiency is produced by antagonists present in the diet or water. High concentrations of sulfur, molybdenum, iron, and zinc have been shown to inhibit the absorption of copper (Miltimore and Mason, 1971; Huisingh et al. 1973; Ward, 1978; Suttle 1974, 1975, 1991; Phillippo et al., 1987).

Selenium: Selenium was first identified in the 1930's as a toxic element to some plants and animals. However, selenium is now known to be required by laboratory animals, food animals, and humans (McDowell, 1992; Underwood and Suttle, 1999). Selenium is necessary for growth and fertility in animals and for the prevention of a

variety of disease conditions. Rotruck et al. (1973) reported that selenium functions as a component of glutathione peroxidase, an enzyme that inactivates oxygen radicals such as hydrogen peroxide and prevents oxygen radicals from causing cellular damage.

Since the discovery by Rotruck et al. (1973), selenium has been shown to affect specific components of the immune system (Mulhern et al., 1985). Earlier research by Reffett et al. (1988) reported lower serum immunoglobulin (**Ig**) M (an antibody produced by B cells) concentrations and anti-infectious bovine rhinotracheitis virus (**IBRV**) titers in selenium deficient calves challenged with IBRV than when compared to selenium adequate calves. Polymorphonuclear leukocyte function was reduced in goats (Azizi et al., 1984) and cattle (Gyang et al., 1984) fed selenium deficient diets compared with controls receiving selenium-adequate diets. Some studies have shown increased T-lymphocyte proliferation following *in vitro* stimulation with mitogen while others have not (Spears, 2000). Bovine mammary endothelial cells growing in selenium deficient cell culture media were found to exhibit enhanced neutrophil adherence when stimulated with cytokines (Maddox et al., 1999; Spears, 2000). These findings indicate that selenium may impact neutrophil migration into tissues and subsequent inflammation.

Several other selenium containing proteins (selenoproteins) have been purified since Rotruck et al. (1973) reported selenium's involvement in glutathione peroxidase. These include several glutathione peroxidase enzymes (1-4), iodothyronine 5´-deiodinase Type I, II, and III which are involved in thyroid hormone metabolism (conversion of T_4 to T_3), thioredoxin reductase, selenoprotein P (selenium transporter), and selenoprotein W (may serves as an antioxidant; Arthur and Beckett, 1994; Sunde, R. A., 1994; Underwood and Suttle, 1999).

Signs of selenium deficiency include white muscle disease, Heinz-body anemias, reproductive disorders (embryonic mortality, infertility, and retained placenta), impaired immune function, and growth impairment (Underwood and Suttle, 1999). The majority of these disorders are caused by a reduction in the antioxidant capacity of cells due to a reduction in selenium. Under practical conditions selenium deficiency can be induced by intake of low selenium diets, consumption of diets high in sulfur and possibly calcium (Harrison and Conrad, 1984; Miller, et al., 1988, NRC, 2000). Furthermore, since vitamin E is involved in oxidant protection within a cell, a vitamin E deficiency may increase the amount of selenium needed to prevent oxidative stress within a cell.

Troubleshooting a Potential Copper and Selenium Deficiency

As discussed by Arthington (2002), the first step in identifying trace mineral deficiencies is to attempt to rule out other more directly contributing factors that can be the cause of decreased animal performance (i.e. infectious diseases, other nutrient deficiency, etc.). For example, if average cow body condition score is below 5 (moderate), chances are far greater that decreases in reproduction and/or immune competence are a result of energy/protein deficiency rather than a trace mineral deficiency. Also be sure that appropriate trace mineral supplementation is being offered.

If other contributing factors such as disease or energy/protein deficiencies or imbalances are ruled out, it is then important to understand the trace mineral contribution from the available feedstuffs and water. Collect forage samples, being careful to select forage that the animals are actually grazing or consuming. Perform a standard trace mineral evaluation of the forage. Also, do not forget to analyze the drinking water, especially in drought-type conditions (Arthington, 2002). As mentioned previously, sulfur can decrease copper and selenium availability. Excessive levels of iron may also depress the utilization of copper. In some instances it may be important to confirm or disprove a potential copper and/or selenium deficiency by examining tissue status through blood and/or liver collection. Consider this option carefully before proceeding.

Blood is commonly used to determine an animal's trace mineral status or to diagnose deficiency or toxicity because of ease of collection (Bull, 1980). Trace mineral-dependent enzymes have also been analyzed to determine trace mineral status since collection is easy and possible trace mineral contamination can be avoided (Bull, 1980). However, the most reliable method of diagnosing a mineral deficiency is to monitor an animal's response to the supplementation of a particular trace mineral (McDowell, 1992) by monitoring health and (or) production after supplementation, since conventional indices of trace mineral status (blood or liver concentrations) are only approximate measurements (Suttle, 1994). Because of the significant cost and time constraints of such experiments, the analysis of animal tissue (s) for trace mineral concentration is the most commonly used indicator of trace mineral status (McDowell, 1992).

Copper: Substantial storage of copper in the liver is possible (NRC, 2000), and therefore analysis of liver copper concentration is considered the best method of classifying copper status and to document changes in copper status (Hemken et al., 1993). However, determination of copper status via the analysis of copper dependent enzymes including ceruloplasmin and copper-zinc superoxide dismutase is also common. Analysis of serum copper concentrations to estimate mineral status is done, but the minimum liver copper concentration necessary to maintain normal plasma copper concentrations in ruminants is approximately 40 mg Cu/kg DM (Underwood, 1977), making serum evaluation a less valuable method to classify copper status, particularly if cattle are subclinically deficient. Analysis of blood samples alone for diagnosis of copper status can be misleading, and therefore should be accompanied by liver and forage analyses for copper concentration (Corah and Arthington, 1993).

***Selenium*:** For several animal species, selenium concentrations in liver adequately portray selenium status (McDowell, 1992). Furthermore, tissue activity of glutathione peroxidase (a Se dependent enzyme) is a relatively good status indicator of selenium because tissue (i.e. liver tissue) and plasma glutathione peroxidase activity increase or decrease rapidly during selenium depletion or repletion (McDowell, 1992). However, glutathione peroxidase activity does not reveal the overall status within the tissue. Blood selenium concentrations indicate current selenium status but are difficult to use to determine selenium storage in the body (NRC, 1996).

Trace Mineral Supplementation Strategies for Grazing Beef Cattle

Prior to selecting a supplement strategy for trace minerals, it is important to try and estimate intake of the dietary essential trace elements from the pasture, water, and other protein/energy supplements being offered. This will require mineral analysis of all feed ingredients. It is also helpful to try and understand seasonal variations of minerals in forages and water that cattle are consuming. This requires feed and water sampling several times over the course of a year. Once it has been determined that a trace mineral (or trace minerals) are inadequate, a supplementation strategy should be developed. There are many different supplementation strategies for trace minerals which can include: 1) direct supplementation of the minerals needed. This type of mineral supplement would include free-choice lose dry mineral or compressed mineral blocks; 2) Energy and/or protein supplements fortified with minerals. These include protein blocks, lick tanks, range cake, etc.; and 3) injectable trace minerals.

Factors That Can Alter Trace Mineral Metabolism

Despite the involvement of certain trace minerals in animal production and disease resistance, deficiencies of trace minerals have not always reduced performance or increased the susceptibility of domesticated livestock species to natural or experimentally-induced infections (Spears, 2000). There are many factors that can affect an animal's response to trace mineral supplementation such as the duration and concentration of trace mineral supplementation, physiological status of an animal (i.e. pregnant vs. non pregnant), the absence or presence of dietary antagonists, environmental factors and the influence of stress on trace mineral metabolism (Baker et al., 2003). For the purpose of this portion of the review, five areas deserve attention when discussing potential factors that may affect the trace mineral requirements of ruminants: breed, gestational status, stress, trace mineral antagonists, and age.

***Breed**:* Although species differences in trace mineral metabolism have long been recognized, differences been between breeds within a species have only recently been noted. Differences in trace mineral metabolism between breeds of dairy cattle have been reported. In an experiment by Du et al. (1996), Holstein (n = 8) and Jersey (n = 8) primiparous cows and Holstein (n = 8) and Jersey (n = 8) growing heifers were supplemented with either 5 or 80 mg of copper/kg DM for 60 days. At the end of the 60 day experiment, Jerseys had higher liver copper concentrations relative to Holsteins across both treatments. Furthermore, liver copper concentrations increased more rapidly and were higher in the Jerseys supplemented with 80 mg of copper/kg DM compared to Holsteins supplemented with 80 mg of copper/kg DM by day 60 of the experiment. Overall serum ceruloplasmin oxidase activity was higher in Jerseys than Holsteins. Additionally, Jersey cows and heifers had higher liver iron and lower liver zinc concentrations than did Holstein cows and heifers at day 60 of the experiment. These data indicate that Jerseys and Holsteins metabolize copper, zinc, and iron differently.

Ward et al. (1995) conducted a metabolism study in which Angus (n = 8) and Simmental (n = 8) steers were placed in metabolism crates to monitor apparent absorption and retention of copper. At the end of the 6-day metabolism experiment, plasma copper concentrations and apparent absorption and retention of copper were higher in Angus relative to Simmental steers. The authors indicate, from their data as well as from others, that Simmental cattle may have a higher copper requirement than Angus cattle and that these different requirements may be related to differences in copper absorption in the gastrointestinal tract between breeds. Furthermore, it has also been suggested that these breed differences in copper metabolism may not be due solely to differences in absorption, but also to the manner in which copper is utilized or metabolized post-absorption. Gooneratne et al. (1994) reported that biliary copper concentrations are considerably higher in Simmental cattle than in Angus cattle. It is apparent that differences in copper metabolism exist between Simmental and Angus cattle both at the absorptive and post-absorptive levels.

An extensive study comparing the mineral status of Angus, Braunvieh, Charolais, Gelbvieh, Hereford, Limousin, Red Poll, Pinzgauer, and Simmental breeds consuming similar diets has also been conducted (Littledike et al., 1995). This work compared not only copper, but also zinc and iron status between all previously mentioned breeds of cattle. In adult cattle, it was shown that Limousin liver copper concentrations were higher than all other breeds, except for Angus. This same trend was not seen for zinc or iron; with very little breed differences observed except for lower liver zinc concentrations in Pinzgauer when compared to Limousin. Serum zinc and copper concentrations did not differ by breed.

Gestational Status: Although little data have been published examining the effects of gestational status on trace mineral metabolism in cattle, several experiments have been conducted using laboratory animals and humans that indicate trace mineral metabolism is altered during pregnancy. Studies using rats have shown that the overall maternal body stores of copper increase during pregnancy and then decrease during lactation (Williams et al., 1977). Vierboom et al. (2002) reported that pregnant cows tended to absorbed and retained more copper than non-pregnant cows and sheep. These data indicate that certain physiological and/or metabolic parameters are altered in pregnant cows that enhance the apparent absorption and retention of certain trace minerals.

The above data indicate that copper metabolism is altered in pregnant vs. non-pregnant animals. Further research is required to determine the metabolic mechanisms that enable pregnant animals to alter copper metabolism as well as an animal's specific metabolic requirement for both maintenance and fetal development. Additional research to determine the effects of gestational status on the metabolism of other trace minerals as well as if breed differences exist relative to trace mineral metabolism and gestational status is needed.

Stress: As mentioned earlier, minerals such as copper and selenium are involved in immune responses. Deficiencies and/or imbalances of these elements can

alter the activity of certain enzymes and function of specific organs thus impairing specific metabolic pathways as well as overall immune function.

Stress and its relationship to the occurrence of disease have long been recognized. Stress is the nonspecific response of the body to any demand made upon it (Selye, 1973). Stressors relative to animal production include a variety of circumstances such as infection, environmental factors, parturition, lactation, weaning, transport, and handling. Stress induced by parturition, lactation, weaning and transport has been shown to decrease the ability of the animal to respond immunologically to antigens that they encounter. Furthermore, research has indicated that stress can alter the metabolism of trace minerals. Stress in the form of mastitis and ketosis has been shown to alter zinc metabolism in dairy cattle. Orr et al. (1990) reported an increase in urinary copper and zinc excretion in cattle inoculated with IBRV. Furthermore, Nockels et al. (1993) reported that copper and zinc retention was decreased in steers injected with adrenocorticotropic hormone (**ACTH**, a stressor), in conjunction with feed and water restriction.

Trace Mineral Antagonists*:* Many element-element interactions have been documented (for an in depth review see Puls, 1994). These include zinc-iron, copper-iron, copper-sulfur, copper-molybdenum, and copper-molybdenum-sulfur interactions and interactions between elements and other dietary components. Peres et al. (2001) used perfused jejunal loops of normal rats to characterize the effects of the iron:zinc ratio in the diet on mineral absorption. When the iron:zinc ratio in the diet was held below 2:1, no detrimental effects on absorption were observed. However, once concentrations were increased to yield a ratio between 2:1 and 5:1, zinc absorption was decreased. Similar effects have also been seen for copper absorption, with depressed copper uptake in the presence of excess iron (Phillippo et al., 1987).

The best known of mineral interactions that can cause a reduction in copper absorption and utilization is the copper-molybdenum-sulfur interaction. However, even molybdenum or sulfur alone can have antagonistic effects on copper absorption. Suttle (1974) reported that plasma copper concentrations were reduced in sheep with increasing concentrations of dietary sulfur from either an organic (methionine) or inorganic (Na_2SO_4) form of sulfur. In another experiment, Suttle (1975) demonstrated that hypocupraemic ewes fed copper at a rate of 6 mg copper/kg of diet DM, with additional sulfur or molybdenum, exhibited slower repletion rates than sheep fed no molybdenum or sulfur. However, when both molybdenum and sulfur were fed together, copper absorption and retention was drastically reduced. Current research would support these findings and suggest that in addition to independent copper-sulfur and copper-molybdenum interactions, there is a three way copper-molybdenum-sulfur interaction that renders these elements unavailable for absorption and/or metabolism due to the formation of thiomolybdates (Suttle, 1991).

Ward (1978) investigated the independent effect of molybdenum on copper absorption and concluded that elevated molybdenum intake reduces copper availability and can lead to a physiological copper deficiency. Based on this and previous

experiments, it appears that the ratio of the antagonistic elements seems to be more important than the actual amounts. Miltimore and Mason (1971) reported that if copper:molybdenum ratios fall below 2:1, copper deficiency can be produced. Huisingh et al. (1973) further concluded, in their attempt to produce a working model of the effects of sulfur and molybdenum on copper absorption, that both sulfur (in the form of sulfate or sulfur-containing amino acids) and molybdenum reduce copper absorption due to the formation of insoluble complexes. They also noted that sulfur and molybdenum interact independently and suggested that they may share a common transport mechanism.

Mineral to mineral interactions are not the only possible inhibitors of mineral absorption. Other dietary components can also inhibit or enhance the amount of mineral that is absorbed. Protein containing sulfur-containing amino acids is an example of a dietary component that can affect mineral metabolism. Snedeker and Greger (1983) reported that high protein diets significantly increase apparent zinc retention. In contrast, diets high in sulfur-containing amino acids have been shown to decrease copper absorption, most likely due to the formation of insoluble copper-sulfur and potentially copper-molybdenum-sulfur complexes (Robbins and Baker, 1980).

In his review, O'Dell (1984) also noted the potential for carbohydrate source to affect copper absorption. This is attributed to phytate as well as oxalate concentrations in the diet. Fiber can also act as a mineral trap due to its relatively large negative charge that serves to bind the positively charged divalent metal cations rendering them unavailable for absorption (van der Aar et al., 1983).

***Age**:* Age has also been shown to affect the mineral of cattle. Trace mineral requirements have been reported to vary with age of dairy cattle (NRC, 2001). Wegner et al. (1972) reported that dairy cattle in their second to fifth lactations had higher serum zinc concentrations than either first lactation or bred heifers. This change in mineral needs over time is most obvious in young growing animals.

Summary

The interactions between trace minerals, animal production and stress are extremely complex. Many factors can affect an animal's response to trace mineral supplementation such as the duration and concentration of trace mineral supplementation, physiological status of an animal (pregnant vs. nonpregnant), the absence or presence of dietary antagonists, environmental factors, and the influence of stress on trace mineral metabolism. Prior to formulating a trace mineral supplementation strategy, it is important to understand (to the best of your ability) mineral intake from grazed forages and water. Future research is needed to better understand the mechanisms by which trace minerals are absorbed and metabolized in beef cattle.

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SESSION NOTES

Supplementation Strategies to Reduce Waste in Beef Cattle Systems

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Introduction

Production conditions are rapidly changing for operators in all sectors of the beef industry. The decade beginning in 2000 brought about increases and volatility in input costs, which fortunately have been followed by increases in feeder and fed cattle prices.

Some often cited reasons why both grain and forage prices are and will continue to be high include utilizing corn for ethanol production and the pressure corn grain prices placed on shifting other crop and forage land to corn grain production. Yet, current times of increased income present an ideal opportunity to improve resource management. This will be necessary to permit continued profit under increased input costs and high volatility — the new norm in livestock production conditions. Since 2006, yearly variability (coefficient of variation) in corn grain prices recorded for NW Iowa (starting in October of the year) ranged from 8.4% to 20%. In the same period, variability in prices of dry distillers' grains and for the same region ranged from 8.2% to 24%. Forage price and price variation were not immune to these changes. Prices and volatility of good and fair quality bermudagrass hay in the Southeast have been markedly affected since 2005 (Figure 1; https://www.marketnews.usda.gov/mnp/ls-home).

As hay prices increased, their impact on overall feed costs also increased. From 2008 to 2013, purchased and homegrown feed costs (including mostly hay and some concentrate and mineral supplements) for operations in the Fruitful Rim region (representing Florida and other coastal states) increased from $0.56 to $0.64 for every dollar spent on feeding beef cows (http://www.ers.usda.gov/data-products/commodity-costs-and-returns.aspx). Due to recent price increases for feeder cattle, the relative contribution of feed costs to feeder breakeven price decreased. Although this may make some beef cattle system operators disregard the impact of feeding costs on profit, we argue that the differential between feed costs and gross feeder calf income must be used to enhance profits while feeder cattle prices are high. The differential should also be used to prepare the enterprise for the inevitable drop in feeder cattle prices already forecast for 2016 by many economists.

This contribution to the 26th Florida Ruminant Nutrition Symposium will focus on managing hay supplies and intake in beef cow-calf operations as a tool to retain biological and economic efficiency. Where appropriate, references to effects of intake

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management on rumen function and ruminant intake control will be made to aid in advancing our knowledge in these areas.

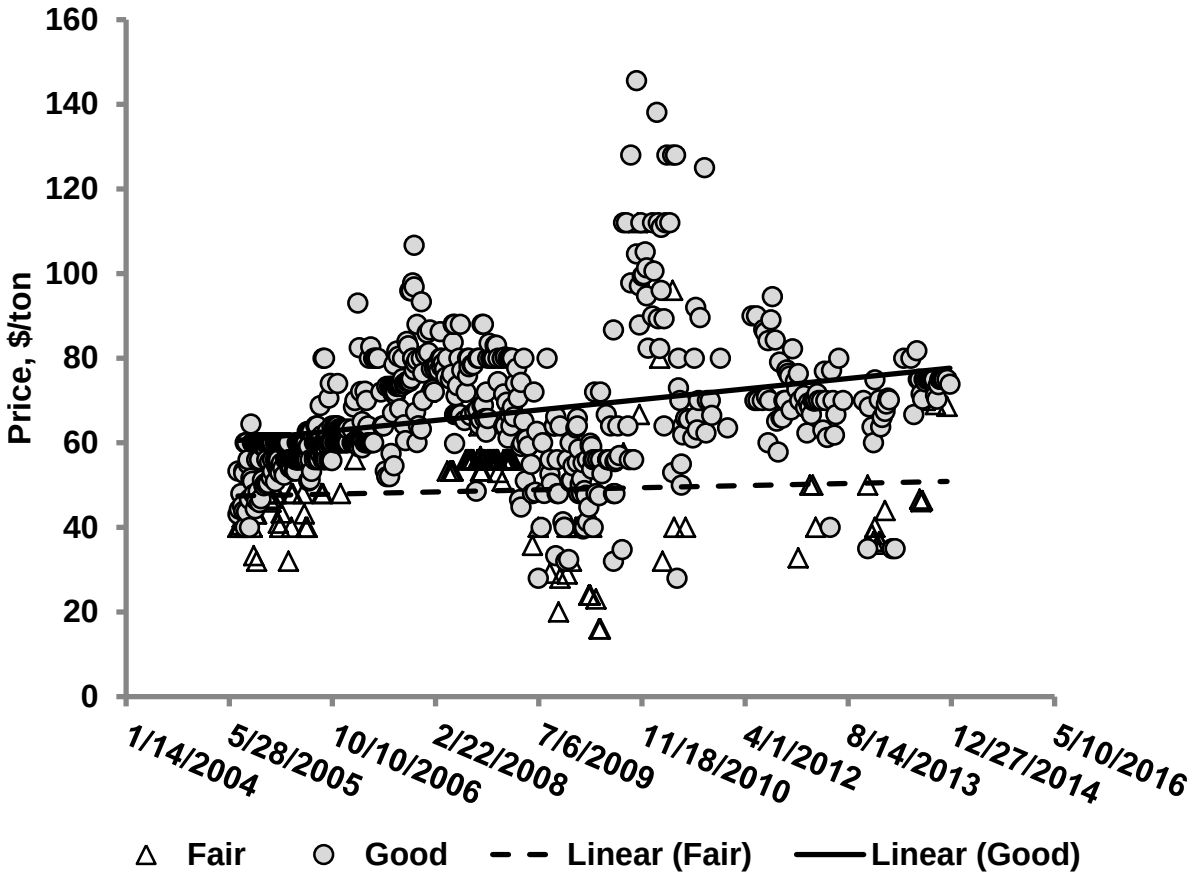

Figure 1. Hay prices ($/ton) recorded by USDA Agricultural Marketing Service for Bermuda grass hay of fair or good quality in the Southeast from September, 2005 to December, 2014.

Hay Procurement and Storage

Under most operating conditions in cow-calf enterprises, hay is purchased as a supplement to homegrown forage supplies due to a shortage of forage of the appropriate quality or reduced supply owing to insufficiency of hay acres, drought or both. A mature, 1,200-lb cow of British × Brahman breeding requires procurement or supply of hay with 52% or 60% TDN during late and early lactation, respectively. Using dry matter intake (**DMI**) guidelines (NRC, 2000), such a cow will consume 2% of her body weight (**BW**) as DMI. This means that cow-calf operators must provide a minimum of 24 lb of DM/cow daily or the equivalent of 740 lb DM/cow monthly. Estimates made from data derived from USDA on feeding hay (http://www.ers.usda.gov/data-products/commodity-costs-and-returns.aspx) indicate that a supplemental hay feeding period of 120 days is needed. This value translates to a total DM need of 2,880 lb DM/cow, 3,400 lb as-is/cow, or about 3 medium-sized bales per cow. These values assume no losses during storage or feeding.

Hay was stored under protective structures in only 1 out of 3 feedlots regardless of the size or region of the country surveyed (USDA, 2013). Although similar national data for cow-calf operations does not exist, we expect that a similar or smaller proportion of cow-calf operations store their hay under protective structures.

A 100-cow herd will need to store up to 300 hay bales (84% DM). This would require a hay storage building measuring 65' × 60' with 16' height at the eaves. Estimates of building costs for this building range from $35,000 to $60,000 depending on materials, concrete costs and nearness to building suppliers. The reader is referred to specific commercial and university contacts and websites to address questions regarding building size and structure for their individual situation. For a 20-year depreciation schedule, and a building cost of $45,000, the share of structure costs per cow per year would be $22.50/cow or $13.25/ton. At hay feeding costs of $120/cow/year, composite hay (as-is) losses of 19% from procurement to feeding would break even with the costs of building this structure. This hay loss value will be significantly greater if we include measured hay waste losses during storage and feeding under experimental conditions.

Although many extension-based publications report hay losses during storage, it is generally difficult to reference the original research articles from which these publications are sourced. Yet, DM losses from 1,000-lb large round bales (fixed-chamber baler) of bermudagrass hay stored on the ground in a single row for 8 months ranged from 3.4% for bales stored in a barn to 9.7% or 14.1% for bales stored outside with the axis-oriented North-to-South or East-to-West, respectively (Huhnke, 1990a). Thus, although 100% reduction in storage losses is impossible, a reduction in hay DM loss from 14% or 10% to 3% through storage in a barn is feasible. More importantly, hay in vitro DM digestibility (**IVDMD**) decreased significantly ($P < 0.01$) from 56.1% or 52.3% at the beginning to 48.9% or 45.2% at the end of the storage period for bales stored outside in a single row with the axis-oriented from North-to-South or East-to-West, respectively. This decrease in IVDMD reduced the energy concentration of hay beyond the minimum required to feed late-gestating beef cows. Although IVDMD from hay in bales stored in a barn also decreased significantly ($P < 0.01$; 57.9% vs. 54.5% at the beginning and end of storage period, respectively), the magnitude of the decrease was 50% less (Huhnke, 1990a). The author of the study reported similar effects of weathering on DM and IVDMD losses in large round wheat hay bales during storage in a separate study (Huhnke, 1990b).

Hay Feeding

Current State of Knowledge

Most cattle producers in the U.S. feed hay to cattle in the form of a round bale for the simplicity of handling and management. Depending on herd size, facilities and equipment, producers deliver hay to last for at least one day. Options for managing feed intake by producers with small herds and no access to equipment are few. When

relying on bale feeders, the minimum feeding unit is a bale. Because a single mature cow accounts for disappearance (intake and waste) of up to 40 lb DM, when single bales (weighing 1,000 lb) are placed on feeding sites, producers need approximately 25 cows to use an entire bale in a single day. Alternatives to bale feeder feeding are rolling the bale out on the pen surface, or investing in feeding delivery equipment and concrete or wooden bunks to manage intake at increments on par with the number of cows in the group.

As expected, feeding forage on the pen surface and delivering forage at amounts greater than needed for a single day leads to greater forage wastage. Relative to hay waste of 5% in a hay ring feeder, cows fed loose hay on the pen surface wasted from 11% when offered a 1-day supply to 31% when offered a 4-day supply (Smith et al., 1974). Similarly, forage DM waste was 24% (Year 1) to 34% (Year 2) when calves were permitted to graze windrowed forage, whereas offering forage from the same source as the dried hay in a ring feeder led to forage DM waste of 12% to 13% DM for years 1 and 2, respectively (Volesky et al. 2002). Results from this study were confounded by moisture concentration of forage and forage placement.

The extent of waste management by using feeding structures varies widely. Cows given free-choice access to hay delivered in a manner to prevent the feeder from being empty in a 24-h period wasted more hay from trailer- and cradle-type feeders than cows given access to hay delivered in a ring or cone feeder (cone over a ring) (Buskirk et al., 2003). Measures of cow behavior, particularly, negative interactive behaviors, apparently arising from hay feeder design were correlated with hay waste. Cradle design feeders led to more aggressive behavior at the feeder, caused cows to access the feeder in a manner inconsistent with the manufacturer's projections, and caused greater feeder occupancy (Buskirk et al., 2003).

Alternatively, limiting access to the hay feeder is an option to reduce waste that is particularly appropriate for small herds owned by operators who have off-farm jobs. In a recent study, lactating beef cows (with calves) were permitted limited access to hay ring feeders for 4 or 8 hours or given 24-hour access (Cunningham et al., 2005). Cows given access for 4, 8 or 24 hours consumed 20.1, 28.2 or 29.3 lb of DM daily and wasted 2.4, 4.0 or 6.4 lb of DM daily, respectively. In this study, total disappearance (intake + waste) but not waste alone increased linearly with access time. In a similar study, cows in their last trimester of gestation were given access to hay in ring-type feeders for 6, 9 or 24 hours (Miller et al., 2007). There was a linear trend for cows given longer access time to consume more hay, but a quadratic trend for cows to waste more hay. The latter was because less hay was wasted when cows were given access to hay for 9 hours, than for 6 or 24 hours. Estimates of hay waste for cows offered access for 9, 6, and 24 hours were 8.5% vs 16.1% and 16.4% of DM offered, respectively.

Interactive Hay and Supplement Feeding Factors Affecting Waste

It is clear that a variety of factors including amount of forage offered, feeder type (as illustrated above or lack thereof), intrinsic forage or supplement characteristics (processing or supplement type) and access time to forage all interact to influence the

amount of forage or supplement waste. For a series of experiments, we hypothesized that hay placement, hay processing and energy supplement type and placement (in a feeder or on the pen surface) each affect DMI and hay or energy supplement waste. We further hypothesized that greater access time to round bale feeders would result in greater hay intake and waste.

Materials and methods

In a series of 3 short-term (10-day), experiments with Latin Square designs, we examined whether hay processing (whole or ground) and placement (hay ring feeder or bunk vs. pen surface; Experiment 1), energy supplement placement (bunk, tire or on the pen surface) and type (wet beet pulp or dry corn grain screenings; Experiment 2; hay was fed in hay ring feeder) and access time to hay (6, 14 or 24 hours; Experiment 3; hay was fed in hay ring feeder) would affect hay, energy supplement or mineral supplement DMI or waste by late-gestating beef cows. Cow BW was measured at the start and end of each Latin Square period after withdrawing feed and water for 16 hours to eliminate effects of gut fill on weight.

In all experiments energy, protein, vitamins and minerals required for maintenance and gestation were determined based on breed and weight (NRC, 2000), and feed was offered accordingly. A 5-yr-old, 1,350-lb Angus cow at a body condition score of 5 (250 d in gestation) was used as a model to calculate nutrients required for maintenance and gestation. The original calculation for DMI was based on brome hay containing 56% total digestible nutrients (**TDN**) and 10.5% crude protein (**CP**). Estimating a DMI of 1.9% of BW yielded an expected intake of 26 lb of brome hay/cow daily with a 0.48 Mcal NE_m energy deficit. Wet beet pulp (**pulp**) or dry corn grain screenings (**screenings**) containing 65 or 87% TDN, respectively (Table 1) were used to supplement energy in Experiment 2 resulting in the need to feed 10 lb of pulp DM or 2.7 lb of screenings DM to eliminate the energy deficit. The projected daily NE_m deficit was ignored in Exp. 1 due to the short term of this experiment and the objective of focusing on effects of hay processing and placement in this experiment.

In experiments 1 (1,343 lb; 12 cows/group and 3,600 ft^2/cow) and 2 (1,418 lb; 10 cows/group and 4,800 ft^2/cow), cows had access to a 225-lb vitamin and mineral supplement tub rated by the manufacturer to supply sufficient nutrients for 25 to 30 head (Table 1). Consumption of 0.25 to 0.50 lb daily was expected. Each treatment group had free choice access to a 50-lb white-salt block. In Experiment 3 (1,327 lb; 8 cows/group and 546 ft^2/cow), cows had access to a free choice, loose complete vitamin and mineral mixture to meet their mineral needs (Table 1). Loose complete vitamins and minerals were mixed at a 50:50 ratio with granulated white-salt. Water was accessible at all times.

Feed offered in the form of hay or supplement was weighed immediately before delivery. Individual round bales were sampled by taking 15 cores per bale from the twine or round side of the bale before delivery for nutrient analyses and the twine was removed. Supplement samples were collected for analyses at the start of every period

by collecting 5 random grab samples. All feed samples were then frozen for further analyses.

Table 1. Nutrient concentration means of grass hay, wet beet pulp and dry corn screenings (dry matter basis) and guaranteed analyses (as-is) of mineral supplement for each experiment

	Hay [1]			Mineral supplement		Beet pulp	Corn screenings
Experiment:	1	2	3	1 and 2	3	2	2
Nutrient							
DM, %	89	89.6	90	96.9	-	26.6	89.8
CP, %	10.4	10	8.8	9.7	-	7.4	6.8
ADF, %	36.8	37	46.4	0.01	-	34.7	3.4
NDF, %	58.3	59.1	68.1	0.7	-	53.5	10.9
ASH, %	6.3	6.3	7.5	29.4	-	17.3	2.2
TDN, %	63.8	63.7	52.1	81.4	-	64.7	86.9
Ca, %	-	-	-	5	13	-	-
P, %	-	-	-	3.5	6	-	-
Mg, % max	-	-	-	1.5	1.5	-	-
K, % min	-	-	-	4	1.5	-	-
Zn, ppm	-	-	-	3,750	3,600	-	-
Mn, ppm	-	-	-	1,250	3,600	-	-
Cu, ppm	-	-	-	1,250	1,200	-	-
Co, ppm	-	-	-	30	12	-	-
I, ppm	-	-	-	68	60	-	-
Se, ppm	-	-	-	13	27	-	-
Vit A, IU/lb	-	-	-	80,000	300,000	-	-
Vit D3, IU/lb	-	-	-	20,000	30,000	-	-
Vit E, IU/lb	-	-	-	100	300	-	-
NaCl, %	-	-	-	-	25	-	-

[1] Average nutrient concentration across experimental periods.

Deliveries occurred daily for cows fed hay in bunks or those supplemented with screenings or pulp. Hay deliveries to ring feeders as whole round bales were made after visual observations of the amount of hay left in the ring. Additional bales were not delivered if the hay left in the feeder was expected to last over 12 hours. Hay deliveries on the pen surface were based either on projected intake (processed hay piled on the pen surface) or by rolling a whole bale out on the pen surface. Daily hay deliveries to feed bunks were based on estimates of intake and waste (29 lb DM/cow) for that group. Delivery time and amount were recorded at the time of delivery.

Hay or supplement waste (left on the pen surface or in the structure where it was delivered) was collected when additional hay or supplement was delivered by measuring the overall waste area and randomly sampling the hay within a 1-ft^2 metal quadrat placed on the hay to obtain representative sub-samples. Subsamples were collected from an area approximately 2% of the size of the total area occupied by the waste and waste samples were frozen for further analysis. Waste was expressed as a percentage of measured DMI. This was done to present results in terms of required feed inventory (feed intake + waste) rather than as percentage of feed offered (feed offered is based on estimated intake and waste).

Experiment 1 results: Effects of hay processing and hay placement

Feeding long or ground hay to beef cows in a feeder (hay ring or feed bunk) or on the pen surface did not affect ($P > 0.33$) hay intake expressed as lb/d or proportion of cow BW (Table 2). Hay waste was greater ($P < 0.01$) when hay was fed on the pen surface rather than in a feed bunk or hay ring. Intake of mineral supplement was affected by hay processing. Cows fed processed hay (on the pen surface or in a bunk) consumed more ($P < 0.01$) mineral supplement than those fed long or whole hay (on the pen surface or in a hay ring feeder). A trend for greater ($P = 0.08$) mineral supplement intake by cows fed on the pen surface was observed. Similarly, a trend ($P = 0.058$) for an interaction between feeder type and processing was observed for mineral supplement intake because cows fed long hay in a ring feeder consumed the least amount of mineral supplement. Yet, cows fed ground hay in a bunk or on the pen surface consumed the greatest amount of mineral supplement; consumption of mineral supplement by cows fed long hay on the pen surface was intermediate. Total DMI averaged 2% of the cow's BW and was not affected by hay feeding method or processing.

Estimates of waste resulting from placing hay in a feed bunk or hay ring feeder were similar to those reported previously. Estimates of waste from placing processed or unprocessed hay on the pen surface were also similar to those reported previously. Thus, feed inventory required when using a hay ring feeder or a feed bunk would need to be nearly 5% greater than the expected intake or it would need to provide an additional 1.35 lb DM/cow daily. The feed inventory required if a feeder is not used would need to include an extra 5 lb DM/cow daily over the expected DM intake or be about 19% greater than the expected daily DM intake of the cow.

We did not expect hay feeder or hay processing to impact mineral supplement intake. Cow eating behavior and eating rate may have been affected by hay processing, which may have resulting in greater mineral supplement intake by increasing the hay intake rate. Dairy cows fed alfalfa hay chopped to a theoretical length of 15 mm ate at a faster rate (11% more lb/min) than those fed the same hay chopped to a theoretical length of 30 mm (Nasrollahi et al., 2014). Absence of a feeder likely resulted in increased trampling and led to the greater hay waste measured when hay was placed on the pen surface. This likely prompted cows to spend more time at

the mineral supplement feeder to compensate for the perceived lack of “good feed” at the site where the hay was fed.

Table 2. Hay, mineral supplement and total DM intake and waste by cows fed whole or processed hay in structures (ring feeder or feed bunk) or on the pen surface (Experiment 1)

	Placement		Processing			*P*-values		
Item	Pen surface	Structure	Whole	Processed	SE[1]	Placement	Processing	Placement x Processing
Hay								
Intake, lb/day	24.9	26.2	25.8	25.4	1.1	0.33	0.70	0.50
Intake, % BW	1.9	2	1.9	1.9	0.1	0.33	0.70	0.40
Waste, % [2]	19.1	4.6	13.6	10.1	2.2	<0.01	0.26	0.60
Mineral supplement								
Intake, lb/day	1.5	1.3	1.1	1.7	0.1	0.08	<0.01	0.06
Total								
Intake, lb/day	26.5	27.6	26.9	26.9	1.1	0.42	0.97	0.40
Intake, % BW	2	2.1	2	2	0.1	0.42	0.98	0.40
Waste, % [2]	18.1	4.4	13	9.5	2	<0.01	0.22	0.6

[1] Standard error.

[2] Waste expressed as a proportion of intake

Experiment 2 results: Effects of supplement type and placement

Feeding cows hay and no energy supplement or hay and screenings in a feed bunk led to greater ($P < 0.05$) hay consumption than other approaches (Table 3). Feeding cows hay and screenings in a tire led to intermediate consumption of hay, which was greater ($P < 0.05$) than hay consumption by cows fed hay and pulp delivered in a bunk or tire.

Table 3. Hay, supplement and total DM intake and waste by cows fed wet or dry energy supplements (suppl.) placed in structures (ring feeder or feed bunk) or on the pen surface (Experiment 2)

	Control	Wet beet pulp			Dry corn screenings		
Item	No suppl.	Bunk	Tire	Pen surface	Bunk	Tire	SE[1]
Hay							
Intake, lb/d	29.1[a]	22.7[c]	24.3 [c]	25.6[bc]	28.7[a]	26.5[b]	1.1
Intake, %BW	2.1[a]	1.6[c]	1.7[c]	1.8[bc]	2.0[a]	1.9[ab]	0.1
Waste, % [2]	9.8[a]	18.1[c]	10.4 [ab]	11.7[ab]	11.2[ab]	12.1[b]	1.2
Energy suppl.							
Intake, lb/day	0.0[a]	7.7[b]	7.7 [b]	6.6[c]	2.9[d]	2.9[d]	0.2
Waste, % [2]	0.0[a]	2.1[a]	2.4 [b]	21.9[c]	0.00[a]	0.00[a]	1.1
Mineral suppl.							
Intake, lb/day	1.0[a]	0.7[d]	0.9[abcd]	0.9[abc]	0.7[cd]	0.8[bcd]	0.1
Total							
Intake, lb/day	30.2	30.9	32.4	33.5	32.4	30.2	1.3
Intake, % BW	2.1	2.2	2.3	2.4	2.3	2.1	0.1
Waste, % [2]	9.5[bc]	13.5[a]	8.1[c]	12.8[a]	9.9[bc]	10.6[b]	1.1

[a,b,c,d] Within a row, least square means without common superscript letters differ ($P < 0.05$).

[1] Standard error.

[2] Waste expressed as a proportion of intake.

Mineral supplement intake was lower ($P < 0.05$) for cows fed pulp in a bunk compared to those fed no supplement or pulp on the pen surface. Mineral supplement intake was similar ($P > 0.10$) among cows fed pulp in a bunk or tire or those fed screenings in a bunk or tire. Mineral supplement intake was greater ($P < 0.05$) for cows fed no energy supplement than those beet pulp in a bunk or screenings in a bunk or tire.

Hay or supplement waste differed based on supplement type and placement. None of the screenings was wasted when it was fed; resulting in a similar ($P > 0.10$) energy supplement waste value to that in cows fed no energy supplement. Hay waste was greatest ($P < 0.05$) when pulp was placed in a bunk; yet, energy supplement waste was greatest ($P < 0.05$) when wet beet pulp was placed on the pen surface. Both of these treatments resulted in the greatest ($P < 0.05$) total feed waste in spite of giving lower supplement and hay waste, respectively. Feeding pulp in a tire resulted in relatively low hay and supplement waste, which resulted in among the lowest total feed waste values.

When feeding pulp, delivering the supplement in a tire feeder led to less total feed waste comparable to other placements for the supplement or to feeding screenings in the tire. Nevertheless, feed wastes were substantially lower when cows were fed screenings in the tire or bunk versus feeding pulp in the bunk or on the pen surface. Cows fed wet beet pulp in the bunk may have had among the lowest mineral intakes because they spent more time at the bunk than at the mineral feeder. The greater time spent at the bunk on this treatment may explain why more hay was wasted on this treatment than others.

Experiment 3 results: Effects of access time

Cows given access to hay feeder rings for 24 hours consumed and wasted more ($P < 0.05$) hay than those given 6 or 14-hour access (Table 4). Cows given access to hay for 6 hours consumed and wasted less ($P < 0.05$) hay than those given access for 14 hours.

Table 4. Hay DM intake and waste by cows given access to hay in feeder rings for 6, 14, or 24 hours (Experiment 3)

	Access to hay rings, hours				Contrast *P*-values	
Item	6	14	24	SE[1]	6- or 14-hour access vs. 24-hour access	6 vs. 14 hours
Hay						
Intake, lb/day	21.2	24.5	27.3	0.2	< 0.01	< 0.01
Intake, %BW	1.6	1.8	2.1	0.0	< 0.01	< 0.01
Waste, % [2]	0.1	4.3	7.7	0.5	< 0.01	< 0.01

[1] Standard error.
[2] Waste expressed as a proportion of intake.

Average BW was not affected by access time to hay feeders; therefore, on all treatments, the energy consumed from hay was sufficient to maintain BW and fetal growth. Assuming that 11.43 Mcal NE_m/d were required for these functions for cows weighing 1,327 lb (94 kcal NE_m/kg $BW^{0.75}$), then cows in each of these treatments consumed 102, 91 and 79 g DM/kg $BW^{0.75}$ for maintenance and fetal growth. These

values reflect NE_m concentrations achieved when cows consumed feed for 24 hours (ad libitum) or for 14 or 6 hours of 0.92, 1.03 or 1.19 Mcal/kg DM, respectively (g DM/kg $BW^{0.75}$ divided by NE_m expressed as kcal/kg BW $^{0.75}$). Corresponding ME concentrations were 1.76, 1.87 and 2.04 Mcal/kg DM. The expected ME concentration based on chemical analyses of hay fed to these cows was 1.88 Mcal/kg DM. Therefore, cows given 14-hour access to hay feeders achieved the expected diet metabolizability of hay.

Cattle limit-fed a high-energy diet had greater diet dry matter digestibility (Klinger et al., 2007) than those fed a high-forage diet ad libitum. In the present experiment, DE concentration derived from ME reflected the finding that cows given access for 14 hours digested hay at expected values while those fed for ad libitum access had 6.1% less energy digestibility. Cows given access to hay for 6 hours had 9.4% greater energy digestibility.

Conclusion

When forage and grain prices are high, cow-calf operators should focus management efforts to preserve feed resources. The value of hay DM lost during storage nearly pays for construction costs of a new hay barn. Hay DM waste during feeding can range from a minimum of 5% when hay ring feeders are used to as much as 10 to 18% when wet energy supplements are fed. Therefore, when hay losses during storage and feeding are considered, the total hay waste could be as much as 30% of the harvested or purchased hay.

Zero waste is impossible, but literature values and those from the current experiment place hay waste at feeders at 5% and hay losses during storage at 3%. At current hay prices ($70/ton) and projected needs for a cow fed hay for 120 days (1.7 ton as-is), the value of differential loss between cumulative 30% or 8% losses is $26/cow or $2,600 in a 100-cow herd. As indicated above, construction costs for a hay barn of $45,000 depreciated over 20 years in a 100-cow herd were determined to be $22.50/cow. Thus, it may be more cost-effective to invest in a hay barn than continuing to store hay outside in situations where hay waste is high during either storage or feeding or if wastage of hay is increased because of poor choice and placement of an energy supplement.

When no energy supplement was used in Experiment 1, mineral supplement intake was at least 75 to 100% greater than that recommended by the manufacturer. The site selection for mineral feeders was far from water or feed sites and surface area allocation per cow in these experiments was nearly 1 tenth of an acre. Effects of cold weather could not be discounted. Under these conditions, consumption of mineral at intakes recommended by the manufacturer were only achieved when cows were fed long hay. Further evidence that energy supplementation reduces excessive mineral supplement consumption was provided by the observation that energy supplementation with either dry supplements or a wet supplement (placed in a bunk), prevented over consumption of the mineral supplement (Experiment 2). In this experiment, cows fed no energy supplement consumed the mineral at nearly the same rate as cows in

Experiment 1 (1 vs 0.9 lb/d, respectively). Energy supplementation to prevent over consumption of mineral supplements is not recommended, but cow-calf operators are encouraged to manage mineral supplementation by limiting the rate at which they replace minerals in feeders after cows empty them.

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SESSION NOTES

Feeding Management and Methods to Reduce Feed Losses and Improve Dairy Cow Performance

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Introduction

Feed costs constitute the greatest percentage of total production costs. Much effort is often placed on formulating optimized rations for cost and production, yet scant attention is given to the implementation of the feeding program. Feedstuff shrink can be huge or minimal, whereas totally mixed rations (**TMR**) can be extremely consistent throughout the bunk or extremely variable. We will discuss shrink and ways to reduce it, and protocols that can be implemented to assist in making a consistent TMR.

Shrink the Shrink

Feedstuff shrink has different definitions. In this discussion, shrink is defined as the amount of feedstuff dry matter that was purchased but not consumed by the cow. Shrink can also occur when loads are being prepared and an ingredient is added beyond the ability of the animal to make use of the excess nutrients.

Shrink represents money down the drain, and it can really add up. For a 1,000 cow dairy averaging 52 pounds of dry matter intake (**DMI**) at $0.13 per pound of dry matter (**DM**), each percentage point of TMR shrink is worth about $25,000. Sometimes simple, relatively inexpensive changes made in the forage and feeding systems result in substantial cost savings.

Greene (2014) discussed various approaches dairies can take to reduce feedstuff losses. Silage losses need to be considered from harvest to the TMR mixer. Ruppel (1995) found in bunker silos filled with haylage that DM loss decreased by about one percentage point for every additional pound of dry matter density achieved in the silo. High losses frequently occur in drive-over piles where the sides are steep and inadequately or not packed at all. Plastic with reduced oxygen permeability, or two layers of quality plastic, will also reduce top spoilage. An important key to minimizing spoilage beneath the plastic is to make sure that air does not infiltrate and traverse beneath the plastic cover. This occurs when the edges of the plastic are not properly weighted down, or when the plastic becomes damaged, and there isn't sufficient weight placed on top of the plastic to keep it tightly adhered to the silage. Gravel-filled silage tube bags help to hold plastic tightly to the silage surface and they will not blow back in the wind.

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Additional losses occur on many dairies at feed-out. Defacers or silage rakes help to keep the silage face straight without disrupting deeper silage layers. Ideally silage would either be loaded directly into the TMR mixer very close to the silage face, or moved to a commodity bay or building for load preparation. Depending on the travel distance and the surface smoothness, considerable waste can occur when loading the mixer.

Dry matter losses were carefully measured for silages and a variety of ingredients in different storage structures (Table 1, adapted from Greene, 2014). On-farm scales and feed management software are necessary to properly measure shrink. Dry matter was measured on silage throughout the filling process, sometimes as frequently as every third load of silage delivered to the bunker. The same equipment was used to measure silage dry matter at feed-out. Amazingly, corn silage losses of only 4.8%, and haylage losses of only 5.6%, were measured on two dairies (Table 1). These dairies weren't doing anything "special", other than doing everything right.

Table 1. Measured shrink values on dairy herds

Ingredient	Herds	Range, %	Weighted mean, %
Corn silage (pile, pit)	15	4.8 – 16.0	9.1
Corn silage (bag)	8	6.5 – 14.0	9.9
Haylage (pile, pit)	12	5.6 – 16.0	10.2
Haylage (bag)	11	8.5 – 17.0	10.7
Feed center (3 sided, open front)	16	2.5 – 11.0	6.7
Feed center (under roof, enclosed)	5	2.0 – 7.0	4.0
Bulky Ingredients (cottonseed hulls, whole cottonseed)	14	3.5 – 14.0	11.3
Upright/overhead storage	7	2.0 – 7.0	4.0
Wet byproducts	13	12.0 – 40.0	23.0
Bagged ingredients	16	2.0 – 19.0	8.1

Large losses in grains can occur from sloppy handling of the ingredients during loading, and from the wind. Shrink is increased every time an ingredient is added to the mixer. It is more efficient if smaller inclusion ingredients are included in a premix. The feeder will only have one ingredient, the premix, to add to the mixer when mixing the TMR. Adding wet ingredients (molasses or whey, for example) to the premix can also help to reduce losses from wind, but be careful that the mix density does not become high enough to impede proper mixing of the premix.

Upright bins reduce shrink by minimizing losses to wind, and eliminating losses that occur when moving with the loader bucket. Shrink is also reduced by the ability to

accurately add the ingredient to the mixer. Downspouts, ideally extending below the top of the mixer, further reduce losses from wind.

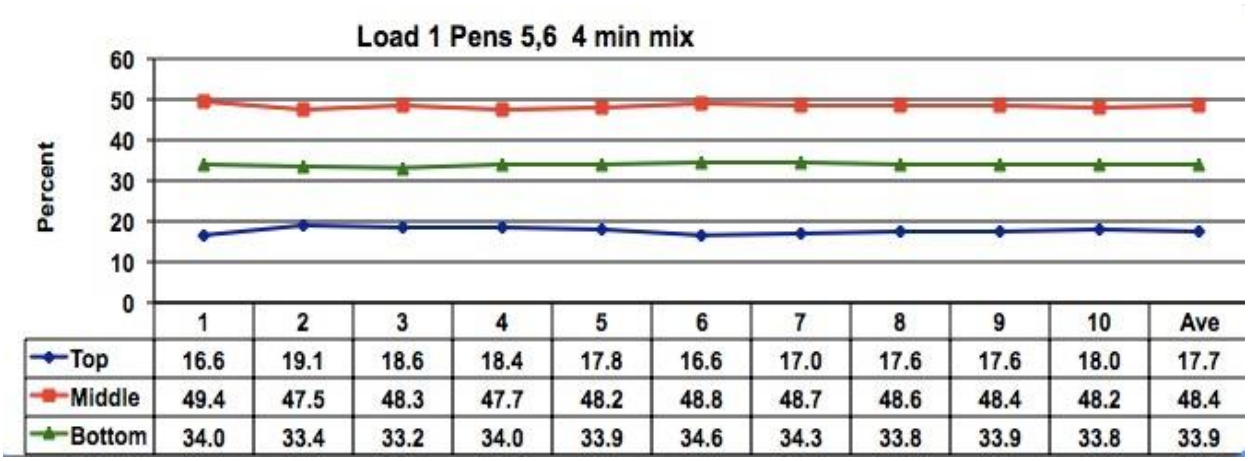
More dairy producers are constructing enclosed feed centers to reduce shrink from wind, wildlife, and weather damage. Feed centers also help to enhance TMR uniformity and accuracy by having the forages that will be fed that day out of the weather, and being able to load out of the wind.

TMR Audits

A TMR Audit consists of an intensive evaluation of the feeding system (Oelberg and Stone, 2014). One of its primary objectives is to reduce the amount of variation between the formulated and consumed ration. The Diamond V Technical Services team has conducted several thousand TMR Audits on dairies across the United States. Anecdotally, we have observed an improvement in performance as feeding routines were changed and TMRs became more consistent.

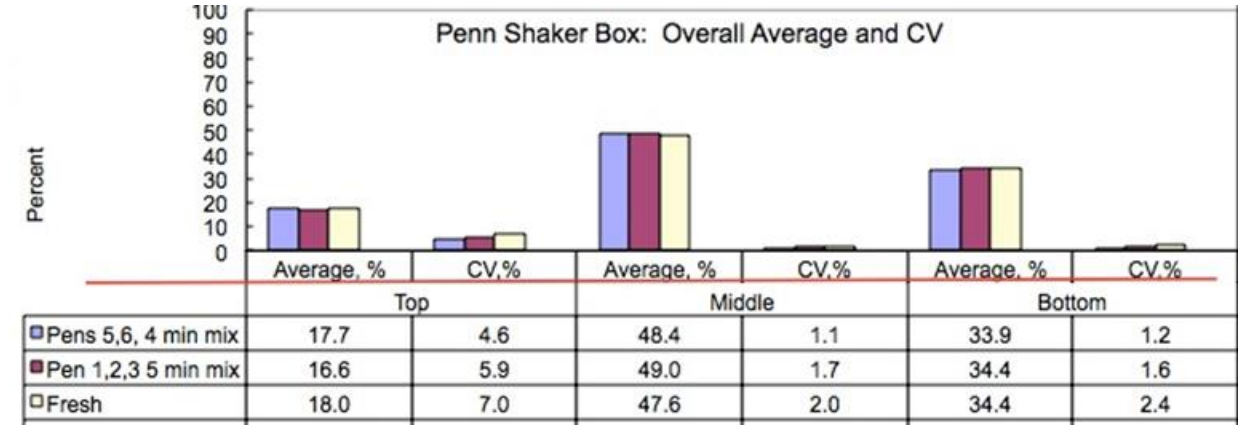
Forage within a bunker silo varies in DM and nutrients primarily across the vertical, but also somewhat across the horizontal, aspect of the silo. To minimize this variation, forages should first be defaced (starting from the bottom and working up), and then pushed into a central pile with the loader bucket and further mixed with the loader bucket. The feeder should be careful to include any forage at the bottom of the silo that was not removed with the defacer. This basic procedure, which should be taught to all feeders, helps to make the TMR consistent throughout all loads of feed.

One of the objective measurements in a TMR audit is an evaluation of the TMR particle size distribution along the length of the feedbunk. Ten TMR samples, approximately 1.4 L in volume and moderately packed, are collected along the feedbunk in a proportional distance to the unloaded TMR. TMR samples are then run through the Penn State Particle Separator (two sieve and pan) according to the recommendations of Lammers et al. (1996) and Kononoff et al. (2003). The particle size distributions are graphed and the coefficient of variation (**CV**) for each sieve and the pan determined. Our goals are to have CVs less than approximately 2.5% for the middle sieve and pan. The top sieve often has much less material on it, and hence it can be more difficult to have a small CV for the material retained on this sieve. However, the top sieve CV can be kept to less than 10% even with relatively small amounts of TMR retained on it. TMRs can be highly consistent (Figures 1a and 1b) or variable (Figures 2a and 2b).



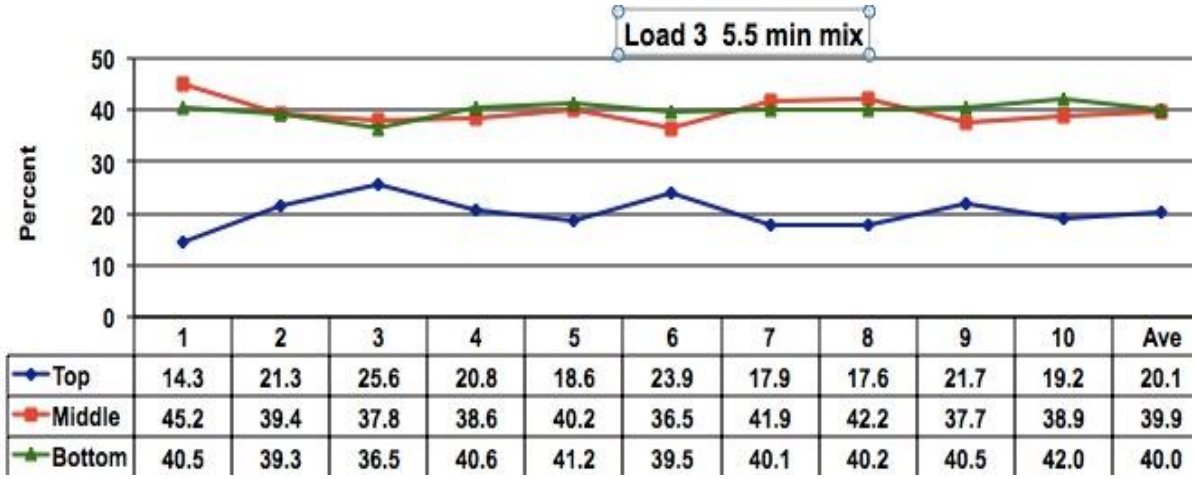
	1	2	3	4	5	6	7	8	9	10	Ave
Top	16.6	19.1	18.6	18.4	17.8	16.6	17.0	17.6	17.6	18.0	17.7
Middle	49.4	47.5	48.3	47.7	48.2	48.8	48.7	48.6	48.4	48.2	48.4
Bottom	34.0	33.4	33.2	34.0	33.9	34.6	34.3	33.8	33.9	33.8	33.9

Figure 1a. An example of an extremely consistent TMR that was prepared by a twin-screw vertical mixer wagon. The figure contains the percentages of the TMR retained on different sieves of a Penn State particle separator from ten sequential samples taken from a load of TMR.



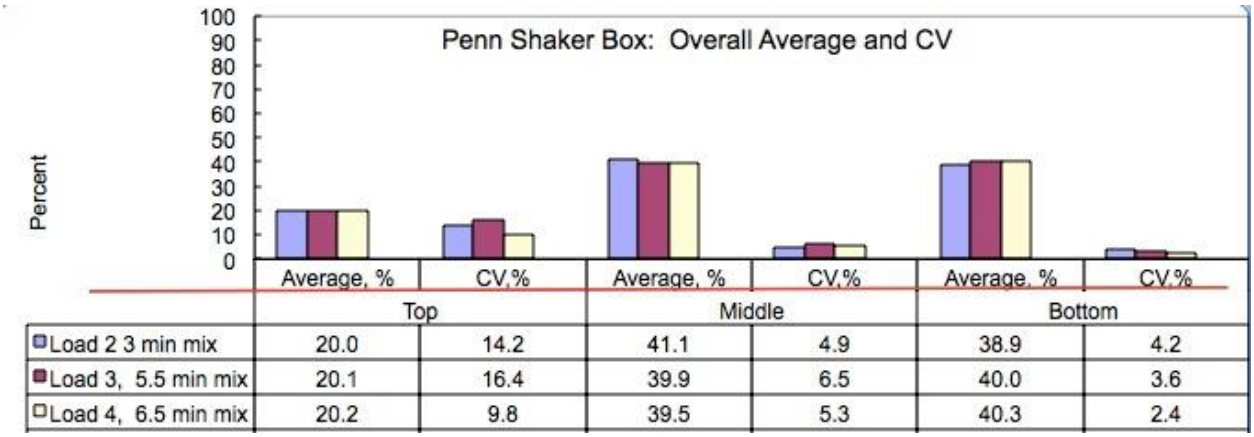
	Top		Middle		Bottom	
	Average, %	CV,%	Average, %	CV,%	Average, %	CV,%
Pens 5,6, 4 min mix	17.7	4.6	48.4	1.1	33.9	1.2
Pen 1,2,3 5 min mix	16.6	5.9	49.0	1.7	34.4	1.6
Fresh	18.0	7.0	47.6	2.0	34.4	2.4

Figure 1b. The average percent of TMR retained and CV from three loads of TMR (including the results from Figure 1a) where ten samples of TMR were collected sequentially along the feedbunk and then shaken through the Penn State particle separator. All loads and screens were within our goal ranges for CV of less than less than 2.5% for the middle sieve and pan, and ideally less than 10% for the top sieve.



	1	2	3	4	5	6	7	8	9	10	Ave
Top	14.3	21.3	25.6	20.8	18.6	23.9	17.9	17.6	21.7	19.2	20.1
Middle	45.2	39.4	37.8	38.6	40.2	36.5	41.9	42.2	37.7	38.9	39.9
Bottom	40.5	39.3	36.5	40.6	41.2	39.5	40.1	40.2	40.5	42.0	40.0

Figure 2a. An example of an inconsistent TMR that was prepared by the same type of twin-screw vertical mixer wagon used to prepare the TMR in Figure 1a. The figure contains the percentages of the TMR retained on different sieves of the Penn State particle separator from ten sequential samples taken from a load of TMR.



	Top		Middle		Bottom	
	Average, %	CV,%	Average, %	CV,%	Average, %	CV,%
Load 2 3 min mix	20.0	14.2	41.1	4.9	38.9	4.2
Load 3, 5.5 min mix	20.1	16.4	39.9	6.5	40.0	3.6
Load 4, 6.5 min mix	20.2	9.8	39.5	5.3	40.3	2.4

Figure 2b. The average percent of TMR retained and CV from three loads of TMR (including the results from Figure 2a) where ten samples of TMR were collected sequentially along the feedbunk and then shaken through the Penn State particle separator. None of the loads met the goals of CV of less than 2.5% for the middle sieve and pan, and ideally less than 10% for the top sieve. To address these problems and improve consistency, a defacer was purchased, mix times were made more uniform via a timer, and the order of adding ingredients was changed.

The primary factors contributing to TMR variability within and between loads include the following:

1. Equipment wear (augers, kicker plates, knives, etc.)
2. Mix time after the last ingredient
3. Load size
4. Levelness of mixer during mixing
5. Loading position on the mixer box
6. Hay/straw quality and processing
7. Loading sequence
8. Liquid distribution
9. Vertical mixer auger speeds
10. Hay restrictor plate setting in vertical mixers

Equipment wear. Feed mixing equipment is not routinely evaluated like most milking equipment. Worn equipment doesn't work properly. The kicker plate is mounted on the lateral aspect of the leading edge of the auger. Most, but not all, vertical mixers utilize a kicker plate to remove feed from along the bottom wall of the mixer. This allows feed from the upper aspect of the mixer to move down the wall. The mixing process occurs as feed is "falling" along the wall, and then "rising" more in the center regions of the mixer because of the auger movement. A worn kicker plate does not remove sufficient feed from the wall of the mixer, resulting in improper feed flow and inadequate mixing. Worn augers won't mix properly, while dull or missing knives won't adequately process long forage. Dairies should have regular maintenance programs, measuring the clearance between the kicker plate and the mixer wall, and evaluating augers, knives, and other parts on the mixer. Although the frequency will vary with ingredients, this should be done approximately every 500 loads.

Mix time after the last ingredient. Although it seems to be getting a lot better, many feeders still don't use a timer to monitor mix time after the last ingredient has been added to a load. The best procedure is to utilize the timer function available on most feed management software programs, but external timers (phones, clocks on radios, etc.) can also be used. Most mixers need about 4 ± 1 minutes to properly mix when run at nearly full power (1,700 to 2,000 RPM engine speed). This can be assessed with the TMR sampling procedure discussed above.

Load size. Feed particles mix best when they are falling, or at least dropping, together at the same time. Additionally, shrink increases if load sizes are too large and feed is spilling out of the mixer. Reel auger mixers are notoriously over-loaded. One simple technique we have learned is to simply observe the mixing action of the mixer when a full load of feed is being mixed. Feed should be actively moving in all visible areas of the load of feed.

Levelness of the mixer during mixing. An unlevel mixer can lead to feedstuffs migrating to a region of the mixer, and to feed spilling out of the mixer box. Loads should be level at least during mixing, and preferably at all times. In addition to parking on level ground, sometimes the hitch can be moved up or down to level out the mixer wagon.

Loading position on the mixer box. Why make it any harder on the mixer than necessary? Targeting the loader bucket for the center of the feed mixer assists in uniform feed distribution throughout the mixer more quickly.

Hay/straw quality and processing. Alfalfa hay straw should be processed to less than 3 to 4" and straw to less than 2" to minimize sorting. Another thumb rule is to have the particle size distribution of straw be approximately 1/3, 1/3, 1/3 on the Penn State Particle Separator (Dann, 2012, Personal communication). Most dairies process hay and straw prior to loading to ensure proper particle size and reduce equipment wear on the mixer. If processing is done prior to mixing, ideally the discharge chute of the forage grinder can be set to blow directly into a totally enclosed space to reduce shrink during chopping. Properly maintained knives in a vertical mixer can adequately process forage, and they typically result in less shrink. However, a trade-off that occurs is increased mixer wear. Additionally, the straw or hay bales should be broken apart and premixed with the loader bucket to reduce forage variability.

Loading sequence. Equipment maintenance, load size, and mix time all trump loading sequence, but it too can affect mix uniformity. Loading sequence will depend on mixer type, ingredient type (density, particle size, moisture level and flowability), inclusion level, and convenience of the feeder relative to ingredient location. Generally, lower density and large particle feeds (straw, hay) are loaded first, followed by dry grains, wet by-products, haylage, corn silage, and liquids. Haylage can go in earlier if clumps are present and a longer mix time is desired to try to break down clumps. However, the best way to break down haylage clumps is with a defacer. Sometimes the best loading sequence for a given mixer and set of feedstuffs can only be determined by experimentation.

Liquid distribution. Liquids should be added so that they are dispersed over the central half to two-thirds of the mixer.

Vertical mixer auger speeds. Remember that feed particles mix best when they are falling or actively moving. If the vertical augers are moving too slowly, the feed movement may not be sufficient for feed particles to mix properly. Different companies have designed their equipment to mix at different speeds, but in general TMR consistency will be enhanced when auger speed is increased.

Hay restrictor plate settings in vertical mixers. Restrictor plates force the TMR closer to the auger, enhancing the cutting action of knives. However, they also decrease the mixing action within the mixer. If the mixer is not being used to process forage, then the restrictor plates can be set all the way out on most mixer wagons.

Conclusion

Shrink can cut into a dairy's profitability or deepen its losses, while an inconsistent TMR can impair animal performance. The good news is that often, both can be substantially improved by fine-tuning protocols on the dairy along with targeted

equipment and facility repairs and investments. Review these areas on your dairy, or your clients' dairies, and see where improvements can be made.

Acknowledgements

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SESSION NOTES

Food Safety and Modernization Act: How Will It Affect the Feed Industry?

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Introduction

The Food Safety and Modernization Act (FSMA) was signed into law in January 2011. This law brings a sea change in the Food and Drug Administration's approach to food safety. The bill was introduced and passed by Congress due to the fact that in the US about 48,000,000, or 1 in 6, US citizens suffer from food poisoning annually. Many of these victims are hospitalized and about 3,000 die yearly. Many of these cases will be prevented by a new approach to food safety.

The feed industry is regulated by the Food and Drug Administration (FDA). Often people are under the mistaken notion that the US Department of Agriculture has this responsibility, when in fact USDA has no duties related to feed production in the US.

The regulatory oversight of the feed industry is given to FDA in the Food Drug and Cosmetic Act of 1938 as amended. It states the following:

"..(f) The term "food" means
(1) Articles used for food or drink for man or other animals…"

This simple statement gives FDA the legal authority to write and enforce regulations and rules that determine how the feed industry operates.

FSMA brings sweeping changes to food and feed regulation, which has not been updated for over 70 years. If you are interested, the full text of the law can be found at:

http://www.fda.gov/food/guidanceregulation/fsma/ucm247548.htm

Depending on its style, the law is about 80 pages of fine print, which takes a while to go through. This includes many deadline dates for various portions to be put into effect. This process requires FDA to write regulations for each of the points in the law. FDA has fallen behind and has failed to meet many of these dates. FDA has released the Rules for Human Foods. These regulations are about 680 pages long. Since this release, FDA has also released the Animal Feed Rules, which as you might imagine are also quite lengthy they are in the area of 450 pages. As initially released the Animal Feed Rules were virtually the same as those for Human Food. This presents quite a problem, due to the fact that feed mills are vastly different from say a sausage

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making plant. The Human Food Rules contain a lot of rules in regard to sanitizing food contact surfaces that are impossible to perform in a feed mill and do not make any sense.

Following release of the Animal Feed Rules, FDA opened a comment period. The American Feed Industry Association (AFIA) as well as the National Grain and Feed Association (NGFA) submitted detailed and lengthy comments. There were also other industry groups that commented, but these two probably had the largest impact. After reviewing the comments, FDA realized that they pretty much had to go back to the drawing board in regard to Animal Feed Rules. FDA did a lot of editing and incorporated many of the suggestions made by industry, thankfully! They then re-released the rules and opened another comment period. AFIA and NGFA again submitted comments. FDA is under a court order, as a result of a lawsuit buy a consumer group to release the Final Rules by August, 2015. At this point we are not sure what they will look like. Nonetheless, they will no doubt be more practical then the first release. The second comment period was closed on December 15, 2014.

Teeth of FSMA

FSMA changes the FDA food safety process from a reactive to a preventative mode. In the past, FDA could only take action after an event had occurred. Now their efforts will be focused on science-based prevention. It should also be mentioned that in the past FDA could not demand a recall, they could only request it, now they can.

Current Good Manufacturing Practices (cGMP)

A major portion of the rules will be based on Current Good Manufacturing Practices (cGMP). cGMP cover a lot of ground. One of the largest is related to employee training. An example of this may include personal hygiene. Are employees provided with a clean sink to wash their hand in prior to entering the feed plant? Have they been trained in the appropriate method to wash hands? Is this training documented? This may sound silly, but many pet food companies and poultry integrators are bringing in Public Health nurses and other personnel to provide this training. When this, or any other training is given, a written record reporting who was there, the date, who did the training must be created. Every person trained, must sign the roster, creating a record of the fact that they did receive the training. The owner/operator of the facility will be expected to be able to produce these records during an FDA inspection. This is just one example of cGMP, but the record keeping requirements are the same for all training. This specific example may or may not be part of the final rule, however it was part of the first release. There are other issues to keep in mind in regard to personal hygiene. Do you have a written policy on clothing and cleanliness? Many feed mill employees are also part time farmers. Do you allow your employees to enter your feed plant with manure on their shoes? Do you require that your employees have a dedicated pair of shoes that they put on when they arrive at work? Do you have a written policy on illness and infections? Have you trained your employees on this policy? Have you documented it? If a person seems to have the flu,

do you allow them to work? Keep in mind that these calls are up to the owner/operator to make and document. FDA enforcement people have said over and over, “If it is not documented, it did not happen”.

As part of cGMP, each plant will no doubt be required to have a written housekeeping plan. Who cleans what and when is it done? What tools are they provided with? The days of using air hoses to blow dust around are probably over even though this may be the only practical method to remove dust from overhead beams etc. Many pet food plants and some broiler integrators have installed central vacuum systems to remove dust. Each time an area is cleaned, the cleaning must be documented. The person doing the cleaning must sign and initial a sheet with the date showing what cleaning was done. Has each person with cleaning responsibility been trained on how to clean those areas of responsibility? Has the training been documented? Has that employee signed off on the training?

This is just a brief overview of cGMP. There are many rules and most likely the vast majority of feed plants are already operating under these rules. Licensed medicated feed plants have had cGMPs for year. Now, all feed plants will be subjected to cGMPs. In my experience in the feed industry for many years, cGMPs are an essential part of making safe and effective feeds.

Hazard Analysis

The major portion of FSMA is based on hazard analysis. There are folks running around telling feed people that they must have a HACCP plan. This is not true. You have to come close though. For those not familiar with HACCP take a look at this website; we do not have time or space to go through it here.

https://en.wikipedia.org/wiki/Hazard_analysis_and_critical_control_points

The most recent proposed rules require the following:

- A written food safety plan;
- Hazard analysis;
- Preventive controls for hazards that are reasonably likely to occur;
- Monitoring;
- Corrective actions;
- Verification; and associated records

The written food safety plan will contain the hazard analysis. In this case the owner/operator will be expected to appoint trained personnel to perform the hazard analysis. Basically, this means that this team will start at the plant gate and review every process throughout the plant until the loaded truck leaves the gate. This will take time and effort. All these activities must be documented. All reasonably foreseeable.

“Hazard means any biological, chemical, physical (including radiological), or physical agent that is reasonably likely to cause illness or injury in animals or humans in the absence of its control.” This quote if from the re-written proposed rule.

http://www.fda.gov/downloads/Food/GuidanceRegulation/FSMA/UCM417131.pdf

That simple sentence covers a lot of ground. The owner/operator will be expected to identify all potential hazards associated with the feed manufacturing and delivery process. The law itself is not prescriptive. The operator is expected to make all the decisions regarding potential hazards. Of course FDA inspectors may not necessarily agree, however, if documentation and justification is complete, the facility will be on solid ground.

Biological hazards will no doubt include things like rodents an insects. Each plant will need to have a documented pest control program. The rodent bait stations will most likely need to be mapped. A written program will need to be in place that identifies how often the bait stations are checked, what specific chemical is used to control pests, and where it is stored if on site. A similar program will have to be in place for insect control. FDA will probably expect windows and doors to be screened to prevent the entrance of pests. They will expect the building perimeter to be clean and weed free. Most likely these issues will be covered by cGMP.

Preventative Controls

§ 507.36 Preventive controls for hazards that are reasonably likely to occur.

For hazards identified in the hazard analysis as reasonably likely to occur:

(a) The owner, operator, or agent in charge of a facility must identify and implement preventive controls, including at critical control points, if any, to provide assurances that hazards identified in the hazard analysis as reasonably likely to occur, will be significantly minimized or prevented and the animal food manufactured, processed, packed, or held by such facility will not be adulterated under section 402 of the Federal Food, Drug, and Cosmetic Act.

It is interesting that FDA mentions critical control points. These are points in a HACCP plan that allow influence on the process to be exerted. While a HACCP plan is not required by FSMA, HACCP is not a new concept to FDA. They have had mandated HACCP requirements for seafood and juice for years. The only reason HACCP is not required, according to an FDA official is that the people who wrote the law were not aware of it.

Preventative controls simply are what will be done to prevent bad things from happening. We have identified the hazards, now we must define and document the process to prevent these hazards from occurring. An example of an identified hazard in may feed plants is the unloading pit. What will you do to prevent feed contamination from the pit? Is the pit always kept covered when not in use? Perhaps the unloading

shed has doors that are kept closed when not being used and the overhead is closed off to prevent birds from roosting.

Monitoring

This is pretty simple. Basically, a method including documentation must be prepared that answers the question: Is what I think is happening really happening?

Maybe a plan a developed that the covering on the receiving pit is checked every 4 hours. Then a document is created, with an initial, documenting that this monitoring function is happening on schedule. This function may include about anything that the operator identified as a hazard that can be prevented. Another example might be measuring and recording the temperature of the mash in the conditioner to be sure it matches a specification you have set as a preventative control.

Corrective Actions

§ 507.42 Corrective actions.
(a) The owner, operator, or agent in charge of a facility must establish and implement written corrective action procedures that must be taken if preventive controls are not properly implemented. The corrective active procedures must describe the steps to be taken to ensure:

(1) Appropriate action is taken to identify and correct a problem with implementation of a preventive control to reduce the likelihood that the problem will recur;

(2) All affected animal food is evaluated for safety; and

(3) All affected animal food is prevented from entering into commerce if the owner, operator, or agent in charge of the facility cannot ensure the affected animal food is not adulterated under section 402 of the Federal Food, Drug, and Cosmetic Act.

In this case, the operator must have a documented plan in place that describes what actions will be taken, should a preventative control fail. For example, should something fall into the receiving pit while unloading, what will be done to prevent feed being manufactured at that time, that may be adulterated according to the FDA definition of adulteration, from leaving the plant. Here is how FDA defines adulterated:

(a) Poisonous, insanitary, etc., ingredients

(1) If it bears or contains any poisonous or deleterious substance which may render it injurious to health; but in case the substance is not an added substance such food shall not be considered adulterated under this clause if the quantity of such substance in such food does not ordinarily render it injurious to health.[1]
(2)

(A) if it bears or contains any added poisonous or added deleterious substance (other than a substance that is a pesticide chemical residue in or on a raw agricultural commodity or processed food, a food additive, a color additive, or a new animal drug) that is unsafe within the meaning of section 346 of this title; or

(B) if it bears or contains a pesticide chemical residue that is unsafe within the meaning of section 346a (a) of this title; or

(C) if it is or if it bears or contains
(i) any food additive that is unsafe within the meaning of section 348 of this title; or
(ii) a new animal drug (or conversion product thereof) that is unsafe within the meaning of section 360b of this title; or

(3) if it consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food; or

(4) if it has been prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health; or

(5) if it is, in whole or in part, the product of a diseased animal or of an animal which has died otherwise than by slaughter; or

(6) if its container is composed, in whole or in part, of any poisonous or deleterious substance which may render the contents injurious to health; or
(7) if it has been intentionally subjected to radiation, unless the use of the radiation was in conformity with a regulation or exemption in effect pursuant to section 348 of this title.

Again, this is a very broad definition and it covers lots of issues. So the goal of this program of hazard analysis and preventative controls is to prevent adulteration of feed.

Verification and Records

Finally, the operator must verify that all these efforts are working. Basically this mean that the system must be tested at scheduled and documented intervals. Ways must be found and described as to how the system will be challenged to see if it is working. Again it needs to be pointed out that the law and the rules are not prescriptive. The responsibility to come up with these programs and plans lies with the owner/operator. FDA reserves the right to disagree if they feel an important component has been left out or is address in a non-appropriate fashion.

Conclusion

FSMA brings major changes to how the feed industry will do business going forward. The final Animal Feed Rules will be published in August 2015. FDA released the proposed rules once and the comments were so detailed, and the suggested changes so important, that they basically rewrote the rules and released them again. Following that comment period which ended in December 2014, FDA is performing edits again. They have said they will not open these rules for any further comments. What the final rules look like remains to be seen.

While firms will not be required to have a full blown HACCP plan they will need everything else. Hazard analysis, preventative control, cGMPs and records will be key.

When FDA shows up at your door, you will have 24 hours to produce the requested records. Thus it is critical that these records be organized and that more than one person knows where they are and how to access them. Some of the rules suggest that companies will be responsible for nutrient excesses or deficiencies. This could have a large impact on consultants who own the formulations. If a company is making a feed that a consultant requests and it is deficient in say selenium, it is possible that that consultant will be liable for producing an “adulterated” feed.

Employee training and the appropriate documentation will be critical. If something results in an adulterated feed and FDA can show that the person involved was not trained that company is going to have a problem.

Document everything. Keep the documents organized so that they can be accessed while the FDA inspector is on site.

This is a shallow treatment of FSMA and its impact on the feed industry. If your company has not started implementing FSMA requirements as we know them today, you had better get going. FDA has the legal authority to enforce FSMA already, even though the final rules are not released. It will be incumbent on the industry to stay calm in the face of an FDA inspection by an inspector who may not be well trained. FDA has indicated that it is working hard to train inspectors but they are not sure they have the financial resources to get it done quickly enough. An excellent cGMP program will answer many of the needs of FSMA by elimination of hazards. My advice is be prepared and have great records and a detailed written food safety plan.

National Grain and Feed has a great document available for purchase that will help you get started.

The HACCP Approach To Feed Quality Assurance…What it Entails.
National Grain and Feed Association
1250 Eye Street, N.W., Suite 1003, Washington DC, 20005
Phone: (202) 289-5388

While a HACCP plan is not required, this document will provide lots of help in meeting FSMA requirements without establishing critical control points. In fact critical control points are about the only thing left out of FSMA rules.

Another program is offered by AFIA. They call it SAFE FEED/SAFE FOOD. FDA has indicated that firms that are SF/SF-certified will most likely meet all their FSMA requirements. Information on this program can be found here: http://safefeedsafefood.org/main/home.cfm

This is probably the simplest way to meet FSMA demands.

In addition there are consultants in the industry who will help you get your programs in order.

One last point in regard to enforcement. In that past, FDA has pretty much gone after consent decrees as its enforcement procedure. The FDA Secretary has ordered that FDA now go after direct criminal proceedings against the CEO or whoever is in charge. This can result in jail terms, in fact they have already gotten convictions of several corporate officers as a result of FSMA. This process has a way of getting the guys in charge very interested in FSMA compliance. Arrest and jail time is not precedent setting. Cases like this have gone to the US Supreme Court who have ruled the CEO deserved to go to jail.

SESSION NOTES