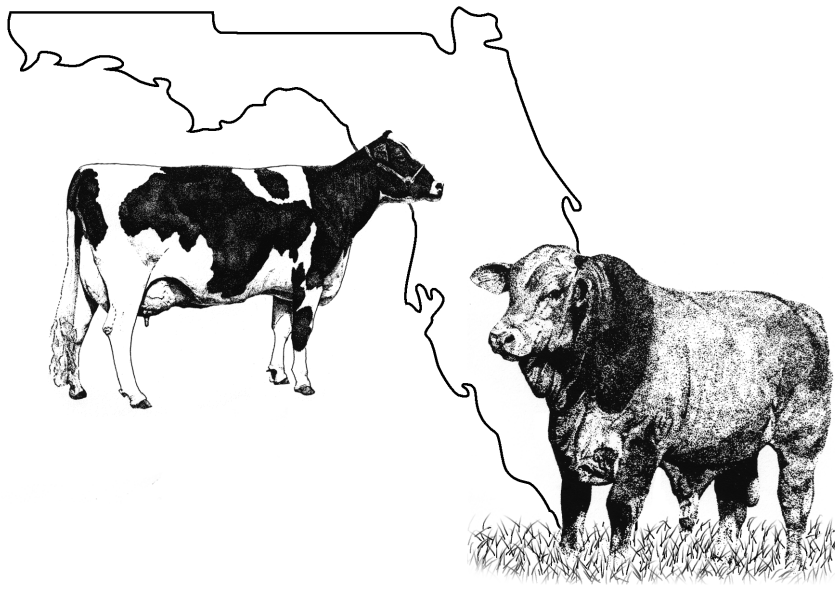
2023 Florida Ruminant Nutrition Symposium

34th Annual Meeting



February 20 - 22, 2023
Best Western Gateway Grand
Gainesville, Florida

PROCEEDINGS

UF UNIVERSITY of FLORIDA
IFAS
Department of Animal Sciences

2023

34th ANNUAL FLORIDA RUMINANT NUTRITION SYMPOSIUM

February 20 - 22, 2023
Best Western Gateway Grand Hotel
Gainesville, Florida

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

Florida Ruminant Nutrition Symposium – February 20 to 22, 2023

Monday, February 20, 2023 - Mini-Symposium sponsored by Balchem Corporation "New Revelations in Transition Cow Nutrition"

2:00 PM **Dr. Clay Zimmerman**, Balchem Corporation. *Welcome and introductions*

2:10 PM **Dr. Mike Van Amburgh**, Cornell University. *"Implications for understanding essential vs. required nutrients"*

2:50 PM **Dr. Barry Bradford**, Michigan State University. *"How do we get the next 5 pounds of milk?"*

3:30 PM ***Refreshment Break***

4:00 PM **Dr. Henry Holdorf**, University of Wisconsin (Purina Animal Nutrition). *"New insights from University of Wisconsin transition cow research"*

4:40 PM **Dr. José Santos**, University of Florida. *"Choline a required nutrient by dairy cows"*

5:20 PM **Dr. Flávio Ribeiro**, Phytobiotics. *"How to cook a Brazilian barbecue"*

5:45 PM ***Poolside Brazilian Barbeque***

Tuesday, February 21, 2023 - Pre-Conference by Church & Dwight *"Make your herd resilient to hidden challenges*"

8:00 AM **Dr. Joel Pankowski**, Arm & Hammer Animal Nutrition and Food Production. *Welcome and introduction.*

8:10 AM **Dr. Sangita Jalukar,** Arm & Hammer Animal Nutrition and Food Protection. *"Preparing the immune system ahead of challenges faced by calves"*

9:00 AM **Dr. Ben Saylor**, Arm & Hammer Animal Nutrition and Food Protection. *"Practices and solutions to* improve *feed hygiene"*

9:50 AM ***Refreshment Break***

10:30 AM **Mike Motta**, Arm & Hammer Animal Nutrition and Food Protection. *"Control environmental pathogens that* silently *steal production"*

11:30 AM ***Buffet Lunch***

Tuesday, February 21, 2023 - Symposium

1:00 PM **Dr. Saqib Mukhtar,** University of Florida. Welcome

1:10 PM **Dr. T. G. Nagaraja**, Kansas State University. *"Beef on dairy* and *liver abscess, what do we know "about it?*

2:00 PM **Dr. Antonio Faciola**, University of Florida. *"Ruminal acidosis, bacterial changes, and lipopolysaccharides"*

2:40 PM ***Refreshment Break***

3:10 PM **Dr. Mike Van Amburgh**, Cornell University. *"Improving precision of diet formulation by describing AA supply on a metabolizable energy basis"*

4:00 PM **Dr. Angela Gonella-Diaza**, University of Florida. *"Maternal methionine supply during the periconceptional period and its impact on calf performance"*

4:40 PM **Vinicius Izquierdo**, University of Florida. *"Impacts of pre- and postpartum heat stress abatement on physiology and performance of grazing Bos indicus-influenced cow-calf pairs"*

5:00 PM **Federico Podversich**, University of Florida. *"Diet-dependent effects of Aspergillus-based prebiotic fed to growing beef cattle"*

5:20 PM ***Welcome Reception***

Wednesday, February 22, 2023 - Symposium

8:00 AM **Dr. Derek Brake**, *University* of Missouri. *"*Limits *to intestinal starch digestion in cattle"*

8:40 AM **Dr. Lance Baumgard**, Iowa State University. *"Revaluating transition cow dogmas"*

9:20 AM **Dr. Stephanie Hansen**, Iowa State University. *"The role of sulfur affecting selenium and copper nutrition in cow calf"*

10:00 AM ***Refreshment Break***

10:30 AM **Dr. Mike VandeHaar**, Michigan State University. *"Breeding cows to do more with less: an update on efforts to improve feed efficiency in the US"*

11:10 AM **Dr. Diwakar Vyas**, University of *Florida*. *"A survey on N efficiency in dairy farms in the USA"*

11:50 AM ***Ruminant Nutrition Symposium Adjourns***

2023 Symposium Speakers

Guests

Dr. Lance Baumgard, Iowa State University
Dr. Barry Bradford, Michigan State University
Dr. Derek Brake, University of Missouri
Dr. Stephanie Hansen, Iowa State University
Dr. Henry Holdorf, University of Wisconsin and Purina Animal Nutrition
Dr. Sangita Jalukar, Arm & Hammer Animal Nutrition and Food Protection
Mr. Mike Motta, Arm & Hammer Animal Nutrition and Food Protection
Dr. T. G. Nagaraja, Kansas State University
Dr. Flávio Ribeiro, Phytobiotics
Dr. Ben Saylor, Arm & Hammer Animal Nutrition and Food Protection
Dr. Mike Van Amburgh, Cornell University
Dr. Mike VandeHaar, Michigan State University

University of Florida

Department of Animal Sciences

Dr. Antonio Faciola
Dr. Angela Gonella-Diaza
Mr. Vinicius Izquierdo
Mr. Federico Podversich
Dr. José E. P. Santos
Dr. Diwakar Vyas

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34th Annual Florida Ruminant Nutrition Symposium

Table of Contents

Title and Presenter
Page

BIOGRAPHIES

Dr. Lance Baumgard is a Distinguished Professor and the Norman L. Jacobson Endowed Professor in Dairy Nutrition in the Department of Animal Sciences at Iowa State University. Before joining Iowa State, Dr. Baumgard was in the faculty of the Department of Animal Sciences at the University of Arizona. Lance received his B.Sc. and M.Sc. in Animal Sciences from the University of Minnesota, and the Ph.D. degree in Animal Sciences from Cornell University. Dr. Baumgard's research focuses on dairy cattle nutrition and metabolism with a major emphasis on nutritional and environmental physiology in cattle. Work by his group has translated novel findings in fundamental metabolic physiology and thermoregulatory metabolism to practical applications in livestock production.

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

Dr. Derek Brake was raised in Marysville, Ohio, and attended The Ohio State University for his B.S. (2006). He completed his M.S. (2009) and Ph.D. (2012) at Kansas State University in ruminant nutrition and nutritional physiology, where he studied the impacts of different flows of amino acids or milk specific proteins on small intestinal starch digestion in cattle. Dr. Brake started his career at South Dakota State University and remained there until he joined the faculty at the University of Missouri in the summer of 2018. Dr. Brake's research has broadly focused on developing a better understanding of how beef and dairy cattle digest, metabolize and ultimately use different nutrients in support of growth or lactation. Dr. Brake is also involved in student mentoring, teaching and service activities. In his "spare time", Dr. Brake has enjoyed operating a 300 head stocker operation on about 600 acres in central Missouri across the past several years.

Dr. Antonio Faciola is an Associate Professor in the Department of Animal Sciences at the University of Florida. Prior to joining the University of Florida in 2017, Dr. Faciola served on the faculty at the University of Nevada for 4 years. Antonio received his B.Sc. and M.Sc. degrees in Animal Sciences from the Federal University of Viçosa, Brazil, the Ph.D. degree in Dairy Science from the University of Wisconsin-Madison, and completed a postdoctoral fellowship at the ARS-USDA U.S. Dairy Forage Research Center in Wisconsin. The goal of his laboratory is to further our understanding of ruminant nutrition to improve the efficiency of nutrient utilization in order to enhance production and minimize environmental impact of livestock. A major emphasis has been on methodological approaches including the dual-flow continuous culture system.

Dr. Angela Maria Gonella is an Assistant Professor of cattle reproduction at the North Florida Research and Education Center and the Department of Animal Sciences at the University of Florida. She received her D.V.M. degree from the University of Applied and Environmental Sciences in Bogota, Colombia, her M.Sc. degree from the National University of Colombia, and her Ph.D. degree from the University of São Paulo in Brazil. Her research interests are diverse and include molecular markers of endometrial and oviductal receptivity, molecular responses to heat stress in the reproductive tract, metabolomic markers of feed and reproductive efficiency, and periconceptional programming. Her extension program focuses on improving the adoption of reproductive technologies and increasing reproductive efficiency in cow-calf operations in Florida.

Dr. Stephanie Hansen is a Professor in Feedlot Nutrition in the Department of Animal Science at Iowa State University. An Iowa native, she earned her B.S. from Iowa State and the M.S. and Ph.D. from North Carolina State University. With 100 peer-reviewed papers and over 10 million dollars in competitive funding, the goal of her research program is to refine mineral requirements of cattle, especially related to optimizing growth and resiliency to stress. She has received early career awards in research from Iowa State University and the American Society of Animal Science. Dr. Hansen is a passionate graduate student mentor and teaches undergraduate and graduate courses in animal nutrition and vitamin and mineral metabolism. She co-hosts podcasts on graduate mentoring (Mentoring Matters) and beef science (Beef Show Podcast). She is also a published fiction author under the name S. L. Hansen.

Dr. Henry Holdorf is a Dairy Nutrition Consultant for Purina Animal Nutrition in Wisconsin. He earned his B.S. and doctorate degrees in Dairy Science from the University of Wisconsin-Madison. During his Ph.D. program, Dr. Holdorf conducted research in the area of choline nutrition and intermediary metabolism in dairy cows.

Vinicius Izquierdo is a Ph.D student in the Department of Animal Sciences at the University of Florida. He received his DVM degree from the Federal University of Pelotas in Brazil in 2019 and the M.Sc. degree beef cattle nutrition and reproduction from the same institution in 2021. Vinicius works under the supervision of Dr. Philipe Moriel at the Range Cattle Research and Education Center in Ona, FL, and his research focuses on nutritional and management practices to mitigate heat stress in grazing *Bos indicus*-influenced beef cattle in tropical and subtropical environments and its consequences to offspring growth, reproduction, and health.

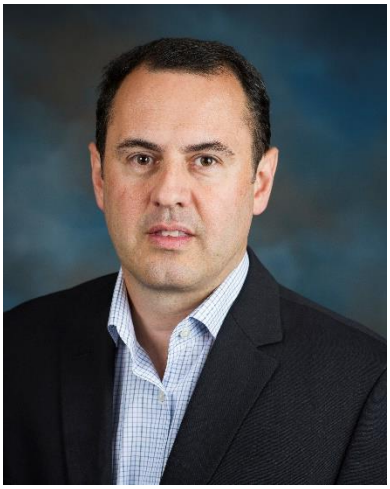
Dr. Sangita Jalukar is a Product Development and Research Coordinator at Church & Dwight, Co., Inc. Dr. Jalukar received her BSc, MSc, and PhD degrees from Maharaja Sayajirao University of Baroda, India, and completed a postdoctoral fellowship in immunology in the College of Medicine at the University of Iowa. Before joining Arm & Hammer, Dr. Jalukar was a Research Scientist and Product Development Coordinator at VI-COR. Dr. Jalukar combines her education and expertise in the use of natural feed additives to improve immunity and health in livestock and poultry.

Mike Motta is the Business Development Manager for the Americas for Arm & Hammer Animal and Food Production. Mike received both his BA and MBA degrees from West Virginia University. Mike has nearly 30 years of experience serving the food and beverage processing and animal health market sectors. As the business development manager, Mike works to bring new products and technologies to the marketplace to provide the latest innovation to customers and key influencers in both animal and food production. Previous to Arm & Hammer, Mike was a Chief Commercial Officer for Novalent Ltd., VP for Business Development at Rochester Midland Corp., VP of North America F&B Division – Zep Inc., and VP of Sales United Kingdom F&B – Diversey. His expertise focuses on environmental treatments to reduce and prevent microorganism outgrowth.

Dr. T. G. Nagaraja is a University Distinguished Professor of Microbiology in the Department of Diagnostic Medicine/Pathobiology in the College of Veterinary Medicine. His research expertise is in gut microbiology of animals, particularly of the rumen of cattle. The investigations have focused on the role of microbes in ruminal function and dysfunction, particularly in animals fed high-grain diets. His research is a blend of basic and applied studies and involves collaborative interaction with Epidemiologists, Food Microbiologists, Molecular Biologists, Production Animal Specialists, Ruminant Nutritionists, and Pathologists. His research has resulted in several patents for the development of vaccines against *Fusobacterium necrophorum* infections in cattle.

Federico Podversich is a Ph.D. candidate in the Department of Animal Sciences at the University of Florida under the mentorship of Dr. Nicolas DiLorenzo working at the North Florida Research and Education Center in Marianna, Florida. Federico received his DVM degree from Universidad Nacional del Litoral in Argentina and his M.Sc. in Animal Sciences from the University of Florida. Before joining University of Florida, Federico completed exchange programs in the School of Veterinary Medicine at the University of Passo Fundo in Brazil and at a Large Animal Clinic in Bayern, Germany. Federico's research focuses on beef cattle nutrition and metabolism with a major emphasis on alternative backgrounding diets and the use of feed additives.

Dr. Flávio Ribeiro is the Business Leader for Ruminants and Swine for Phytobiotics North America. He received his B.Sc. in Animal Science from FAZU in his hometown Uberaba, Brazil. Dr. Ribeiro attended the University of California Davis and received a Post-Graduate Certificate in Animal Sciences. He completed the M.Sc. in Animal Sciences at Iowa State University and the Ph.D. from Texas A&M University with an emphasis in ruminant nutrition and meat science. Dr. Ribeiro spent 10 years in academia as a Postdoctorate Fellow, Research Scientist, and Professor before he joined Phytobiotics. Dr. Ribeiro has a passion for teaching and for the last 13 years he teaches a class on how to cook meat in his famous Brazilian BBQ course at Texas A&M.



José Eduardo P. Santos is a Professor in the Department of Animal Sciences at the University of Florida. He received his DVM degree from São Paulo State University in Brazil, completed the M.Sc. and Ph.D. at the University of Arizona, a clinical residency in Dairy Production Medicine at the University of California Davis, and a sabbatical at SBScibus and the University of Sydney. José spent 8 years in the faculty of the School of Veterinary Medicine at the University of California Davis before moving to the University of Florida in 2008. José is a fellow of the American Dairy Science Association and the American Association for the Advancement of Science.

Dr. Ben Saylor is a Dairy Technical Services Manager for Arm & Hamer Animal and Food Production. Dr. Saylor received his BSc degree in Animal Sciences from the University of Arizona, the MSc in Animal Sciences from Kansas State University and the PhD degree in animal nutrition from the Department of Animal and Dairy Sciences at the University of Wisconsin, Madison. Dr. Saylor specializes on forage quality and conservation and on-farm microbial challenges and their control.

Dr. Mike Van Amburgh is a Professor in the Department of Animal Science at Cornell University where he has a dual appointment in teaching and research. Dr. Van Amburgh received his B.Sc. from The Ohio State University and the doctorate degree from Cornell University. Mike teaches multiple courses and works extensively with the Dairy Fellows Program, and is the advisor for the Cornell Dairy Science Club. The focus of Mike's research program focuses on nutrient requirements of dairy calves, heifers, and lactating dairy cows. A major emphasis has been on the development and update of the Cornell Net Carbohydrate and Protein System (CNCPS).

Dr. Mike VandeHaar received his A.B. degree in biology from Dordt College in Iowa, the M.Sc. and Ph.D. degrees in Nutritional Physiology from the Department of Animal Sciences at Iowa State University. He then moved to the University of North Carolina at Chapel Hill where he completed postdoctoral training in pediatric endocrinology. He joined the faculty of the Department of Animal Sciences at Michigan State University where he is a professor of dairy cattle nutrition and metabolism. The goals of his research program are to improve the efficiency with which dairy cows convert feed to milk, increase lifetime productivity of calves and heifers, and develop practical dairy cattle diet balancing tools

Dr. Diwakar Vyas is an Assistant Professor in the Department of Animal Sciences at the University of Florida. He earned his Ph.D. degree from the University of Maryland in Dairy Cattle Nutrition with a focus on mammary lipid metabolism. Diwakar completed a postdoctoral fellowship at Lethbridge Research and Education Center of Agriculture and Agri-Food Canada working in areas of environmental sustainability and rumen physiology of beef production systems. His research program focuses on optimizing nutritional management to improve production, efficiency of nutrient utilization, and environmental sustainability of livestock production with emphasis on dairy cows and small ruminants.

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[1] Zenobi et al., 2018 J. Dairy Sci. 101 (Suppl. 2): 334, Zenobi et al., 2018 J. Dairy Sci. 101 (Suppl. 2): ii, Zenobi et al., 2018 ADSA, Late-Breaking Original Research, #LB5

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A Nutrition Solution to Reducing Pathogen Shedding and Improving Performance

Sangita Jalukar[1]
Arm & Hammer Animal and Food Production

Introduction

A healthy heifer calf is the idyllic bridge between the past and future. A tremendous amount of work went into getting her dam pregnant, helping her navigate lactation challenges, then guiding her through the dry period, pre-calving and calving events. That calf represents the work that went into building the future success of your dairy.

Disease challenges early in life have an impact on later production. In a study by Renaud et. al., the longer a calf experienced diarrhea in the first four weeks of age the greater was the reduction in weight gain later in the calf's life. When infected calves are compared to healthy calves, the difference was not statistically significant when the calves had scours (Day 5 – Day 21) but was significant after Day 49 and beyond (Renaud et. al.).

It takes a multi-faceted approach to manage calf health and help calves overcome health challenges. Cleanliness is key, starting with a clean, dry calving pen and a transition to a sanitary newborn calf raising facility. Keeping the environment clean and dry with adequate ventilation will set a foundation for calf health.

Keeping the environment outside the calf clean is only part of the effort. It is important to build immunity inside the calf as well, starting with feeding adequate levels of high-quality colostrum at birth. There are nutritional supplements available that help establish and build ideal immune function inside the calf, with a goal of building a calf that's resilient to disease challenges.

Making calves resilient is the key to their long-term growth and performance. Reducing pathogen loads helps improve dry matter intake and drives more effective and efficient weight gain. There are effective nutritional supplements that have proven effective in reducing pathogen load, including customized probiotics and Refined Functional Carbohydrates™ (RFCs™).

This abstract presents benefits of a prebiotic and postbiotic called CELMANAX. CELMANAX contains RFCs produced by proprietary enzymatic hydrolysis of yeast cell walls plus a full dose of yeast culture. The enzymatic hydrolysis liberates the various carbohydrate fractions from the yeast cell walls. RFCs help improve gut health, reduce

[1] Contact at: Arm & Hammer Animal Food Production; Tel (641) 530-1610; E-mail: Jalukar@churchdwight.com.

colonization by certain pathogens and improve immunity for a healthier, more productive animal. Some of the modes of action of RFCs include:

- MOS supports growth of beneficial bacteria like *Lactobacillus* and *Bifidobacterium*
- MOS and Mannose represent a potential binding site for the pili on bacteria such as *E. coli* and *Salmonella*. By agglutinating, or hooking these bacteria together on an MOS molecule, they prevent the pathogen from adhering to and colonizing the intestine.
- Beta glucans support the immune system. Beta 1-3 glucans can also bind mycotoxins. Due to its size, shape and free binding sites, the toxins can be irreversibly bound by this beta glucan. Beta 1-6 glucans bind receptors present on certain immune cells and prime those cells to respond rapidly to a challenge.
- Other RFCs prevent certain protozoa like *Eimeria* and *Cryptosporidium* from attaching to the intestinal wall and causing disease.

Results and Discussion

Dairy calves

An important way to control disease spread from infected calves is to reduce bacterial shedding. An on-farm study was performed on two commercial Wisconsin dairy farms to identify the effectiveness of feeding CELMANAX to reduce bacterial shedding. Calves were housed indoors individually from day 1 through day 6 and then group housed with an automatic feeder until day 56. At day 3 calves were randomized into treatments with about 80 calves per treatment. The study included the following dietary treatments:

- Control
- CELMANAX SCP at 2 g/h/d

Calves were monitored for health, fecal pathogen shedding and average daily gain during the preweaning period. Data was analyzed for overall means and to account for variables including treatment, farm, study week and month and passive transfer status. RFC fed calves had significantly reduced prevalence of *Salmonella* and rotavirus ($p<0.05$) and were 2.5 kg heavier at 50 days of age compared to calves in the control group(Raabis et. al.).

Lucy et. al. evaluated the effectiveness of pre- and postbiotic supplementation to pre-weaned Holstein heifers on body weight gain, diarrhea and shedding of fecal pathogens. There were 450 calves less than 12 hours of age enrolled in the study per treatment. The study lasted 60 days with growth and pathogen shedding monitored. *E. coli* and pathogenic *E. coli* loads were reduced in calves supplemented with RFCs compared to control fed calves ($p< 0.05$). (Starter feed intake was numerically higher for RFC calves. Calf Body weight was higher from day 42 to 56 ($p<0.05$) for the RFC supplemented calves compared to control calves.

Better calf health in the pre-weaning phase not only allows better growth and performance in calves, but it also leads to better lactation performance as well. Calves fed RFCs pre-weaning produced 195 more kg of milk in their first lactation with 13 more kg of fat and 8 kg more protein.

In a 2019 in-house research trial, bull calves were fed Control or CELMANAX supplemented milk replacer followed by common starter diet until 77 days of age. Treatment calves had increased average daily gain ($p<0.05$) and improved feed efficiency ($p<0.01$) compared to control groups. From an economic standpoint, after factoring in the extra days of feed and yardage cost, a profit of $9.68/head was realized.

Beef calves:

The effectiveness of RFCs fed in beef receiving diets has also been evaluated. Benefits analyzed across four different trials showed significant reduction in morbidity and increased average daily gain in three research studies (Ponce et. al, Silva et. al and Danielo et. al.) and improvement in feed efficiency in two studies (Silva et. al and Danielo et. al.). Also, across three different receiving cattle studies, RFC fed cattle experienced fewer cases of Bovine Respiratory Disease (BRD) (Ponce et. al, Silva et. al. and unpublished).

In a study evaluating benefits of CELMANAX supplementation in the pre-conditioning phase opposed to just in receiving diets, six-month-old, weaned steers were fed one of three diets:

- Control for 69 days
- CELMANAX for 69 days (preconditioning and receiving)
- CELMANAX from Day 31 – Day 69 (receiving only)

Results showed that percent morbidity was reduced while average daily gain (kg/d) and gain-to-feed ratio (kg/kg) improved ($p=0.04$) (Silva et. al).

Benefits of RFC supplementation in receiving beef heifer diets has also been reported by Danielo et al. In addition to evaluating performance, they also reported effects of transportation stress on pathogen shedding, and markers of stress and inflammation in the heifers and the effect of RFC supplementation in mitigating these effects. Beef heifers were fed RFCs two weeks post weaning for 60 days and then subjected to shipping stress to simulate a feedlot arrival situation. Total clostridia, *C. perfringens*, *E. coli* and *Salmonella* levels increased following the transport challenge. RFC reduced *C. perfringens*, *Salmonella* and total *E. coli* loads one day following the transport challenge compared to the control group (Danielo et. al.). This led to a conclusion that RFC supplementation to receiving heifers may lead to heavier animals that shed fewer pathogens upon feedlot arrival.

Interleukins and stress hormones were also analyzed in the receiving trial. Control animals had increase in stress hormones, inflammatory cytokines and acute phase proteins indicating an underlying inflammatory status particularly following stress compared to RFC fed animals (Danielo et. al.).

Conclusion

Reducing pathogen shedding and infections in dairy calves improve body weight and help calves transition better post-weaning. These benefits can help improve first lactation productivity. In beef receiving calves, morbidity in receiving animals can be managed with RFCs leading to improvement in body weight, feed efficiency and reduced inflammation and stress. Transportation stress and pathogen shedding was lower in RFC-supplemented animals which may lead to heavier animals that shed fewer pathogens in the feedlot.

References

Danielo, J., K.J. McCarty, J.E. Tipton, R.E. Ricks, N.M. Long. 2020. Effects of post-weaning supplementation of immunomodulatory feed ingredient on body weight and cortisol concentrations in program-fed beef heifers. Dom. Anim. Endocrinol. 72:106427.

Lucey, P. M., I. J. Lean, S. S. Aly, H. M. Golder, E. Block, J. S. Thompson and H. A. Rossow. 2021. Effects of mannan-oligosaccharide and *Bacillus subtilis* supplementation to preweaning Holstein dairy heifers on body weight gain, diarrhea, and shedding of fecal pathogens. J. Dairy Sci. 104:4290–4302.

Renaud, D. L. C. Rot, J. Marshall, and M. A. Steele. 2021 The effect of *Cryptosporidium parvum*, rotavirus, and coronavirus infection on the health and performance of male dairy calves. J. Dairy Sci.104:2151–2163.

Raabis, S., J. Ding and T. S. Ollivet. 2017. The effect of CELMANAX SCP on fecal pathogen shedding, health and performance of preweaned Holstein dairy calves. AABP conference.

Ponce , C. H., J. S. Schutz, C. C. Elrod, U. Y. Anele, and M. L. Galyean. 2012. *E*ffects of dietary supplementation of a yeast product on performance and morbidity of newly received beef heifers. Prof. Anim. Scient. 28:618-622.

Silva, L. G. T., R. F. Cooke, K. M. Schubach, A. P. Brandão, R. S. Marques, T. F. Schumaher, P. Moriel and D. W. Bohnert. 2018. Supplementing a yeast-derived product to enhance productive and health responses of beef steers. Animal: 12:1576–1583.

Preparing the Immune System Ahead of Challenges Faced by Calves

Sangita Jalukar, Ph. D. PAS
Florida Nutrition Conference
Pre-Conference Symposium

1

Importance of calf health

A dairy calf represents the future success of a dairy

Health challenges early in life can often impact mature animals due to:

- Slower growth rate
- Longer time to reach maturity and breeding
- Lower productivity (milk production)
- Lower milk components

2

Long term effects of diarrhea on calf body weight

Table 3. Predicted margins from a repeated-measures linear regression model comparing the weight loss between differing levels of a proportion of days with diarrhea to a baseline of <25% of days in the first 28 d with diarrhea[1]

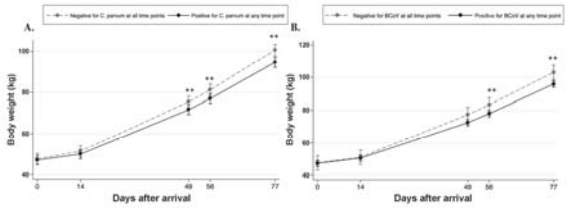
Proportion of first 28 d with diarrhea (fecal score 2 or 3) and day	Predicted weight difference (kg)	95% CI	*P*-value
<25% to 25–30%			
14	−2.12	−6.67 to 2.43	0.36
49	−6.64	−11.42 to −1.87	0.006
56	−5.01	−9.80 to −0.22	0.04
77	−6.24	−11.07 to −1.40	0.01
<25% to 30–38%			
14	−2.82	−7.35 to 1.73	0.23
49	−7.92	−12.69 to −3.15	0.001
56	−8.15	−12.94 to −3.36	0.001
77	−12.71	−17.58 to −7.84	<0.001
<25% to >38%			
14	−4.21	−8.74 to 0.32	0.07
49	−11.88	−16.72 to −7.04	<0.001
56	−9.39	−14.29 to −4.49	<0.001
77	−15.60	−20.57 to −10.63	<0.001

[1]From 198 male Holstein calves housed at a grain-fed veal facility.

Renaud *et. al.* Journal of Dairy Science Vol. 104 No. 2, 2021

3

Long term BW effects of *Cryptosporidium* and coronavirus infections



Renaud *et. al.* Journal of Dairy Science Vol. 104 No. 2, 2021

4

Some of the ways to manage calf health

Calf Management:

- Calving pen cleanliness
- Feed adequate amount (3-4 quarts in first 4 hours and 2 quarts 6-8 hours of life) of good quality colostrum

Nutritional supplements to:

- Reduce pathogen shedding at calving to reduce pathogens in the maternity pen
- Improve passive transfer of immunity from cow to calf
- Help calves manage pathogen exposure
- Improve DMI
- Improve immune response of calves
- Help establish a healthy gut microbial balance

5

A RESILIENT CALF

6

Solutions from ARM & HAMMER

- Customized probiotics (Brand Name CERTILLUS™)
- **Refined Functional Carbohydrate™ (RFCs) (Brand Name CELMANAX™) – Focus of today's talk.**

7

What are RFCs and What do they do?

- Refined Functional Carbohydrates (RFCs) are derived by unique enzymatic hydrolysis of yeast.
- They help to improve gut health, reduce colonization by certain pathogens and improve immunity for a healthier, more productive animal. [1-18]
- Supported by 19 scientific publications in dairy, nine in beef and two in sheep.

8

Refined Functional Carbohydrates (RFCs)

- **MOS** supports growth of beneficial bacteria like *Lactobacillus* and *Bifidobacterium*
- **MOS and Mannose** binds pathogenic bacteria like *E. coli* and *Salmonella*
- **Beta glucans** support the immune system
- **Other RFCs** prevent certain protozoa like *Eimeria* and *Cryptosporidium* from attaching to the intestinal wall and causing disease

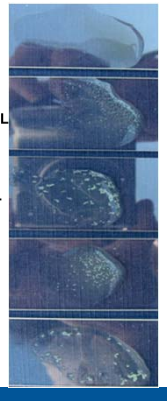
9

Modes of Action of RFCs…

- Reduce pathogen burden
- Support Immune system

10

RFC AGGLUTINATION



Presented at the 2009 ASAS/ADSA Midwestern Section Annual Meeting

11

RFCs Agglutinates E. coli

Embed agglutination video
https://youtu.be/Pi7_QwWiuHg

This video demonstrates how *E. coli* is agglutinated to the MOS component of Celmanax®
Total agglutination time 00:30 seconds

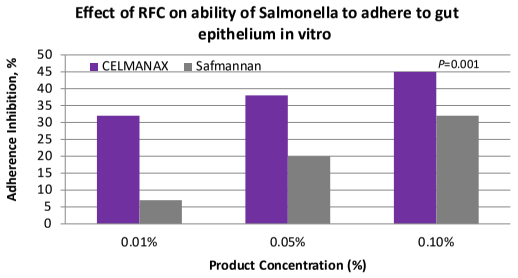
12

Agglutination with field *Salmonella* isolates

Salmonella serotype	Farm ID	Degree of agglutination	Animal type
S. dublin	R286.2	+++	Calf isolates
S. dublin	R286.3	+++	Calf isolates
S. dublin	R299.4	++	Calf isolates
S. dublin	R286.6	++	Calf isolates
S. dublin	R286.7	++	Calf isolates
S. dublin	R286.13	++	Calf isolates
S. dublin	R286.14	++	Calf isolates
S. dublin	R286.16	++	Calf isolates
S. dublin	R287.16	++	Calf isolates
S. dublin	R291.9	++	Calf isolates
S. dublin	R299.1	+++	Calf isolates
S. dublin	R326.2	+++	Calf isolates
S. Heidelberg	Lul1x	+++++	Calf isolates
S. Heidelberg	Lul2x	++++	Calf isolates
S. Heidelberg	Lul3x	++++	Calf isolates
S. Heidelberg	Lul4x	++++	Calf isolates
S. Heidelberg	Lul5x	++++	Calf isolates
S. Heidelberg	Lu21x	++++	Calf isolates
S. Heidelberg	Li34x	+++++	Calf isolates
S. Heidelberg	Li34x	++++	Calf isolates

13

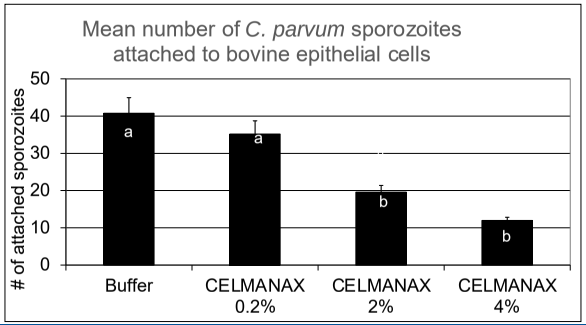
Salmonella attachment inhibition to bovine epithelial cells



Baines *et al.*, 2013. Presented at the Gut Health Symposium in St. Louis.

14

C. parvum attachment inhibition to bovine epithelial cells



15

Immune support in unchallenged ruminant trials.

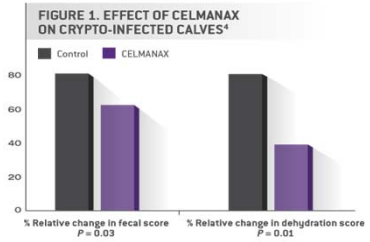
Parameter measured	CELMANAX effect compared to control	Stage of animal	Publication
Interferon gamma	Lower (P<0.05)	receiving beef heifers	Domestic Ani. Endocrinology
Haptoglobin	Lower (P<0.05)	receiving beef heifers	Domestic Ani. Endocrinology
IL-8	Lower (P<0.05)	receiving beef heifers	Domestic Ani. Endocrinology
Serum Cortisol	Lower (P<0.05)	receiving beef heifers	Domestic Ani. Endocrinology
Heterophil phagocytosis of E. coli	Increase at all time points tested (p=0.1)	Transition cows	JDS
IL-6	Lower (P<0.05)	Transition cows	JDS

16

Reduction in bacterial shedding and infections in dairy calves

17

Cryptosporidiosis



Trial 1. Crypto infected calves were fed control or CELMANAX containing milk replacer for 5 days

	Control	Celmanax	P value
Calves positive for Crypto before challenge, %	50	22.7	0.06
Duration of crypto shedding post challenge, d	19.05	15.32	0.02

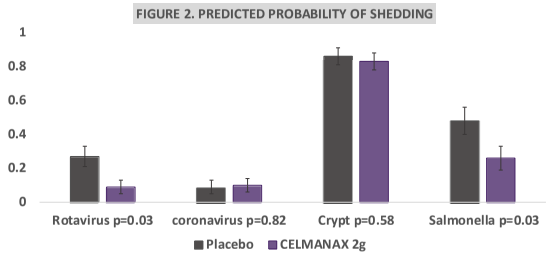
Trial 2. Calves were fed control or CELMANAX containing milk replacer from day1 – 56. On day 6, all calves were challenge with *C. parvum*.

J. Dairy Sci. Vol. 92, E-Suppl. 1

18

Reduced incidence of pathogen shedding in calves

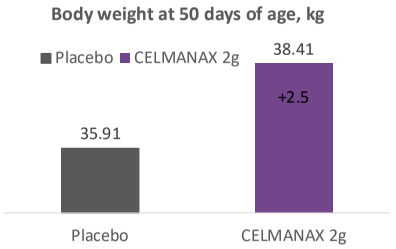
On a commercial farm, 80 calves/treatment were fed control or CELMANAX from day 1-56 days. CELMANAX reduced prevalence of *Salmonella* and rotavirus



Abstract presented at AABP, 2017

19

Less pathogen shedding leads to higher body weights



20

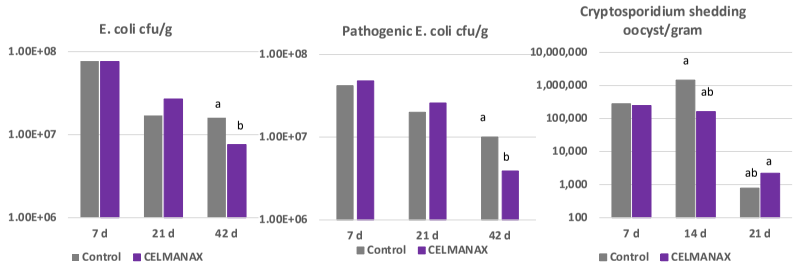
UCD Study Details

- Objective: Evaluate effectiveness of pre- and pro-biotic supplementation to pre-weaned Holstein dairy heifers on bodyweight gain, diarrhea, and shedding of fecal pathogens.
- N = 1,801 Holstein heifer calves <12 hours of age enrolled in study with 450 calves/treatment
- Study duration: 60 days
- Growth and pathogen shedding was monitored

Lucey et. al. JDS Vol. 104 No. 4, 2021

21

Fecal pathogen load



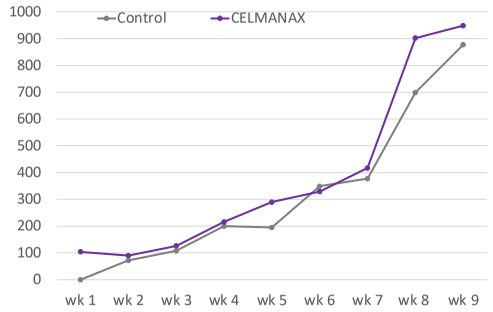
[a,b] $P<0.05$

No treatment differences were noted for load of Clostridium or C. perfringens

Lucey et. al. JDS Vol. 104 No. 4, 2021

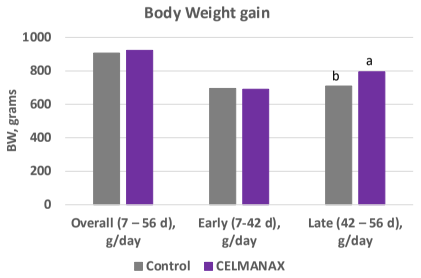
22

Starter feed intake, g/d



23

Improved calf body weight gain

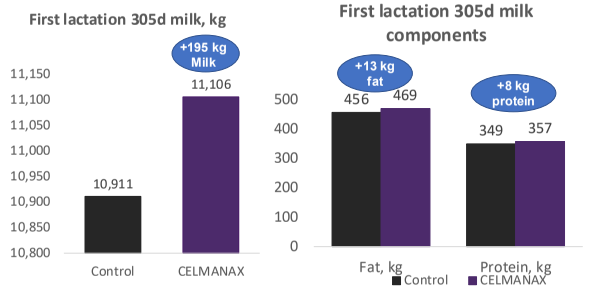


[a,b] $P<0.05$

Lucey et. al. JDS Vol. 104 No. 4, 2021

24

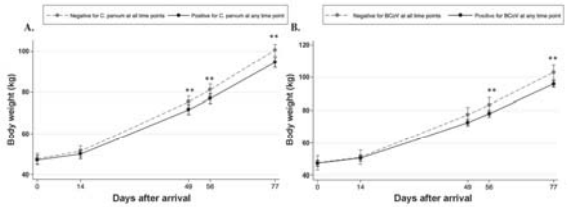
Pre-wean CELMANAX supplementation benefit on first lactation performance



Lucey et. al. Abstract presented at ADSA 2021

25

Long term BW effects of *Cryptosporidium* and coronavirus infections



26

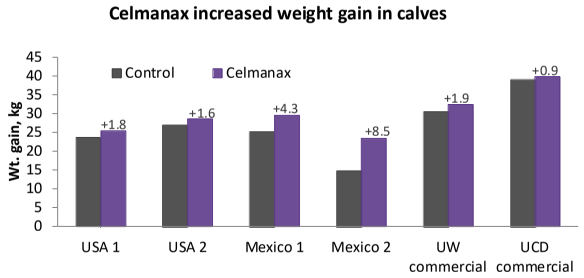
Effect of treatments on performance in veal calves

Effect of treatments on performance			
Parameter	Control	CELMANAX	P-value
n	120	120	
ADG lbs./d, Day 0-56	1.51	1.57	0.42
Feed/Gain, lbs. D 0-56	2.73	2.24	0.13
ADG lbs./d, Day 0-78	1.9	2.03	**0.05**
Feed/Gain, lbs. D 0-78	2.39	2.17	**0.01**

After factoring in the extra days of feed and yardage cost, a profit of $9.68/head was realized for this study done in 2019

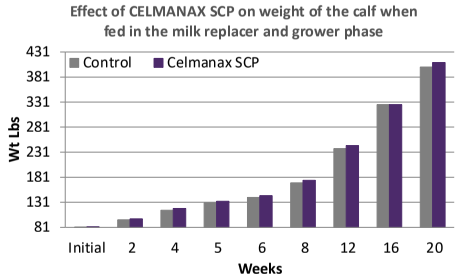
27

Consistency in BW gain in calves on RFC



28

Effect of RFC on body weight when fed in the milk replacer and grower phase



Conclusion: CELMANAX fed calves continued to maintain the higher weight till week 20

29

Beef Calves

30

RFC benefit in beef receiving

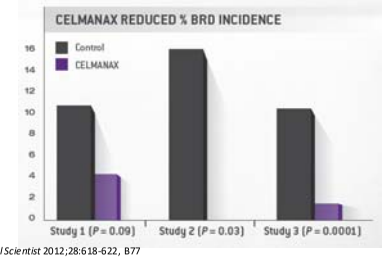
Description	Study 1	Study 2	Study 3	Study 4
Location	Texas Tech	Oreg. State	Clemson	U of MN
Length of study, DOF	35	69	60	34
Replicates	12	7	9	2
Total animals	237	84	72	440
Morbidity reduced (P-value)	0.094	0.03	Not measured	0.0001
ADG increased (P-value)	0.08	0.07	0.04	NS
DMI increased (P-value)	0.022	NS	NS	NS
% Change in F:G v Control (P-value)	-1.6% (NS)	-5.48% (0.08)	-8.9% (0.04)	NS

Ponce et. al. The Professional Animal Scientist 2012;28:618-622
Silva et. al. Animal (2018), 12:8, pp 1576–1583
J. Danielo et al. / Domestic Animal Endocrinology 72 (2020) 106427

31

Success in the receiving period

- In multiple receiving cattle studies CELMANAX fed cattle also experienced fewer cases of Bovine Respiratory Disease (BRD)

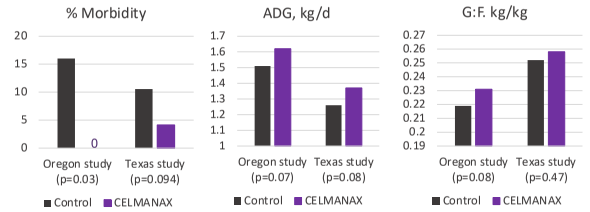


Pance et. al. The Professional Animal Scientist 2012;28:618-622., B77
Schubach et. al. Am. Soc. Ani. Sci, proceedings, Western section 2017 Vol 68 pg 141-146; doi: 10.2527/asasws.2017.0054
U MN study

32

Success in the receiving period

- Two separate studies show CELMANAX supplementation in the receiving phase reduces BRD morbidity and improves ADG and feed efficiency



Oregon study: Silva et. al. Animal: 12:1576–1583
Texas study: Ponce et. al. *The Professional Animal Scientist* 2012;28:618-622.

33

RFC receiving trial

- Receiving heifers fed CELMANAX two weeks post weaning for 60 days and then subjected to shipping stress to simulate feedlot arrival situation.

Parameter	Control, n = 9	CELMANAX , n = 9	
Initial BW, kg	242 ± 8	243 ± 8	0.98
BW gain during trial, kg	40 ± 1	43 ± 1	**0.04**
Feed conversion, kg intake/kg BW gain	14.65 ± 0.39	13.35 ± 0.39	**0.04**

- CELMANAX supplementation improved body weight gain and feed conversion

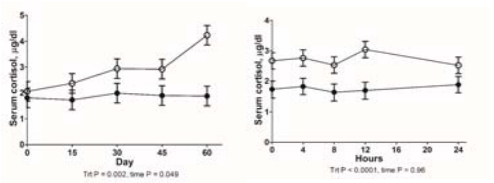
J. Danielo et al. / Domestic Animal Endocrinology 72 (2020) 106427

34

RFC receiving trial

Serum cortisol during feeding period

Serum cortisol during transport stress

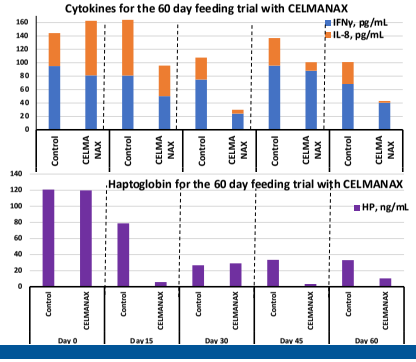


- Total clostridia, *C. perfringens*, total *E.* coli, and *Salmonella* levels increase with a transport challenge in beef heifers
- CELAMANAX reduced *C. perfringens, Salmonella*, and total *E. coli* but not pathogenic E. coli one day following the transport challenge compared to the controls.
- **CONCLUSION**: CELMANAX supplementation to receiving heifers may lead to animals which are heavier and shedding fewer pathogens upon arriving at feedlot

J. Danielo et al. / Domestic Animal Endocrinology 72 (2020) 106427

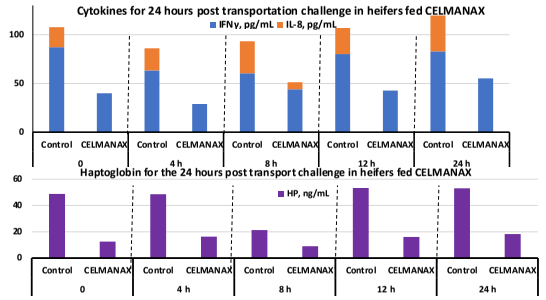
35

Impact of RFC on cytokines during receiving phase



36

Impact of RFC on cytokines during receiving phase (transportation challenge)



37

Take home message

Dairy calves

- Reducing pathogen shedding and infections in calves improves calf body weight, help calves transition better post-weaning, and improves first lactation response.

Beef receiving calves

- Morbidity in receiving animals can be managed with RFCs.
- Reduction in morbidity leads to improvement in BW, feed efficiency and lowers inflammation and stress.
- Transportation stress leads to increase in inflammatory cytokines, acute phase protein, and increase in pathogen shedding.
- Transportation stress and pathogen shedding was lower in RFC supplemented animals which may lead to animals which are heavier and shedding fewer pathogens in feedlot phase.

38

Thank you to our research partners!

- University of California, Davis
- University of Wisconsin Vet School
- Clemson University
- Texas Tech University
- Oregon State University
- Contract Research Organizations

39

SESSION NOTES

#ScienceHearted

I'M AN OVER-ACHIEVER.

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

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at 850-865-8355 or
Kemp.Caudill@churchdwight.com.

Innovative Practices and Solutions to Improve Feed Hygiene

Ben Saylor[1]
Arm & Hammer Animal and Food Production

Introduction

Dairy producers are in business for the long haul. Many have survived numerous challenges over the years and are positioned to pass the dairy on to future generations. The most successful dairies have demonstrated resilience, achieving consistent, high-level performance in the face of various obstacles. Resilient dairy producers do what they can to limit the pressures cows face, while conditioning their herd to withstand pressures beyond their control.

Building resilience in cows requires focus in three areas:

- Controlling pathogens within the gastrointestinal tract
- Optimizing rumen function
- Establishing hindgut integrity

Feed hygiene is an often-overlooked area of feed management that can directly affect the three aspects of resilience mentioned above. Hygienic feed is defined as feed that is free of pathogens and toxins that could be detrimental to animal health and performance.

Unfortunately, poor feed hygiene is not an isolated challenge. Pathogens and toxins within feed can interact with numerous other stressors to cause digestive disorders and productivity losses. For example, common challenges like heat stress (Koch et al., 2019), feed restriction (Kvidera et al., 2017), or sub-acute ruminal acidosis (SARA; Emmanuel et al., 2007) have all been shown to increase the permeability of the intestinal epithelium. When the intestinal epithelium is no longer able to protect the host from the environment, pathogens and toxins in the feed enter systemic circulation. As the gastrointestinal tract is home to approximately 75% of the immune system (van der Heijden et al., 1987), this insult can cause an inflammatory response in the host. In addition to the negative effects of pathogens and toxins in the cow, systemic inflammation is known to have a profound energetic cost, >2 kg of glucose per day (Horst et al.; 2021), which competes with more beneficial processes such as milk production and reproduction.

The bad actors threatening feed hygiene

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The total mixed ration (TMR) is the primary source of pathogens and toxins threatening cow health and productivity. These bad actors can be introduced in the field, during storage and feed-out, or throughout the course of feed mixing and delivery.

Clostridia

Clostridia are present everywhere in the environment. These gram-positive, spore-forming anaerobes can have numerous effects on the digestive health of dairy cattle. ARM & HAMMER™ surveyed dairies across the country, analyzing 30,000 fecal samples and 7,000 feed samples, and found that 98.6% of fecal samples and 84.7% of feed samples contained clostridia (Bretl et al., 2022). It was also observed that 78.5% and 33.6% of fecal and feed samples, respectively, contained C. perfringens, a pathogenic species of clostridia known to contribute to hemorrhagic bowel syndrome (HBS) in cattle.

Clostridia found in dairy systems are generally classified into one of two groups:

1. Toxin-producers: The most common toxin-producer is *C. perfringens* which is a known contributor to hemorrhagic bowel syndrome (HBS) in dairy cattle. *C. perfringens* proliferates in the lumen of the intestine to the point that it overwhelms the normal gut microflora (Goossens et al., 2017). It then produces enzymes that cause the breakdown of the mucus layer protecting the intestinal epithelium. Without a functional mucus layer, toxins produced by *C. perfringens* can bind to the intestinal epithelium inducing an immune response. The subsequent inflammation leads to sloughing of the epithelium allowing toxins present in the gastrointestinal tract to enter systemic circulation, ultimately leading to intestinal hemorrhaging and death.

2. Solvent-producers: *Clostridium beijerinckii* and *C. bifermentans* are the most common solvent-producing clostridia found in dairy systems. These organisms produce solvents like acetone, ethanol and butanol which have been hypothesized to negatively impact ruminal fibrolytic bacterial populations and rumen function. Research from ARM & HAMMER has found that inhibiting solvent-producing clostridia in the gastrointestinal tract of dairy cows leads to greater abundance of *Ruminococcus* and *Fibrobacter* spp. in the rumen (Maylem et al., 2013 – Article under review).

Salmonella and *E. coli*

Salmonella and *E. coli* are found in a cow's digestive tract and should not be present in feed. Their presence in silages suggests a poor fermentation or potential manure contamination. If found in the TMR, it is likely that contamination has occurred during feed mixing or delivery. A host of health and performance challenges can be caused by *Salmonella* and pathogenic strains of *E. coli* (Peek et al., 2018).

Yeasts and Molds

Spoilage is often a result of high levels of yeasts and molds in silages and TMR. Counts over 100,000 CFU/g are indicative of spoiled feed (Kung et al., 2018). Yeasts and molds can contribute to poor aerobic stability of silages and TMR and represent a loss of nutrients that can lead to inconsistent intakes and performance. Santos et al. (2014) demonstrated that high counts of yeasts isolated from high-moisture corn can reduce 24 h in-vitro NDF digestibility of a TMR.

Mycotoxins

Mycotoxins are secondary metabolites produced in feeds by various species of molds. A survey in 2012 found that 81% of livestock feed samples collected from the Americas, Europe and Asia tested positive for at least 1 mycotoxin (Rodrigues and Naehrer, 2012). Many mycotoxins are degraded or inactivated by the rumen microbiota (Gallo et al., 2015). However, high ruminal passage rates of modern dairy cows may reduce microbial detoxification (Pantaya et al., 2016).

Results and Discussion

Addressing Feed Hygiene Issues

Dairy producers and nutritionists have two options for addressing feed hygiene issues: limiting the cow's exposure to pathogens and toxins and controlling pathogens and toxins that find their way into the gastrointestinal tract.

Limit exposure to pathogens

Limiting a cow's exposure to pathogens and toxins can be accomplished by optimizing silage fermentation and minimizing contamination during feed mixing and delivery. Fast, efficient silage fermentations create an environment detrimental to pathogen growth. Therefore, adoption of silage management "best-practices" such as harvesting at optimal moisture, use of research-proven microbial inoculants, and thorough packing and covering are all effective strategies for limiting pathogen loads in silages and TMR.

Even if silage is properly stored and fermented, contamination can still occur during silage feed-out as well as during feed mixing and delivery. Here are 10 steps to minimize feed contamination on-farm:

1. Clear debris and spoiled silage off silage pads and feed areas
2. Increase silage feed-out rate and immediately feed defaced silage
3. Feed high-moisture byproducts quickly, empty bays before restocking
4. Keep feed alleys free of debris, scrape at least once daily
5. Clean feed bunks, especially directly under headlocks
6. Don't forget water: Keep waterers clean and clear

7. Feed refusals as soon as possible. Mix batches containing refusals last.
8. Clean TMR mixer once per month
9. Clean pushup blade monthly
10. Clean tires and buckets of all feed payloaders and skid loaders. Avoid handling feed with buckets that have moved manure or dirt.

Control pathogens and toxins inside the cow

Two valuable technologies have been shown to control pathogens and toxins inside the cow. First, *Bacillus* technology has been shown to reduce clostridial loads in the gastrointestinal tract and improve hindgut integrity. Second, Refined Functional Carbohydrates™ (RFCs™) have been shown to bind pathogens and mycotoxins in the gut. Research conducted by ARM & HAMMER involving 77 dairies and 230,000 cows across 18 states looked at the shift in risk for total clostridia and *C. perfringens* before and after feeding *Bacillus.* On average, an 89% increase in the number of fecal samples at low-risk for total clostridia, and a 13% decrease in the number of fecal samples at high-risk for total clostridia was observed following *Bacillus* supplementation. This study also found that *Bacillus* supplementation contributed to a 20% increase in the number of fecal samples at low-risk for *C. perfringens*, and a 26% decrease in the number of fecal samples at high-risk for *C. perfringens*. Other research from ARM & HAMMER has shown that *Bacillus* increase the expression of tight junction proteins in the small intestine and can increase gut barrier integrity (as measured by transepithelial electrical resistance).

Research has also documented the effect of feeding RFCs on agglutination of *Salmonella* and *E. coli.* In one study, the number of unbound *S. Newport, S. enteritidis, S. Dublin* and *S. cholerasius* were all reduced when 20 and 40 mg/ml of RFCs (as CELMANAX™) was fed (Jalukar et al., 2009). In the same study, the number of unbound *E. coli* F18 was reduced in the presence of RFCs. Refined functional carbohydrates have also been shown to reduce epithelial cell damage (as measured by cytotoxicity score) caused by aflatoxin, T-2, DON, zearalenone, and fumonisin B1 (Baines et al., 2014).

Conclusion

Building resilience in cows requires focus on controlling pathogens within the gastrointestinal tract, optimizing rumen function, and establishing hindgut integrity. Feed hygiene is an often-overlooked area of feed management that can directly affect the three aspects of resilience mentioned above. Dairy producers and nutritionists have two options for addressing feed hygiene issues: limiting the cow's exposure to pathogens and toxins and controlling pathogens and toxins that find their way into the gastrointestinal tract. Limiting a cow's exposure to pathogens and toxins can be accomplished by optimizing silage fermentation and minimizing contamination during feed mixing and delivery. *Bacillus* and Refined Functional Carbohydrates have both been shown to control pathogens and toxins inside the cow. Addressing the feed

hygiene issues that silently steal productivity is an essential component of improving the resilience of dairy herds.

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Innovative Practices and Solutions to Improve Feed Hygiene

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Dairy Technical Services Manager
Arm & Hammer Animal and Food Production

2023 Florida Ruminant Nutrition Symposium

1

Presentation Outline

I. Introduction
II. What is feed hygiene and why doe
III. Who are the bad actors threatenin
IV. What can be done to improve feed
   a. Optimize silage fermentation
   b. On-farm strategies to improve feed h
V. How can we control pathogens that find their way to the cow's gastrointestinal tract?
VI. Conclusions

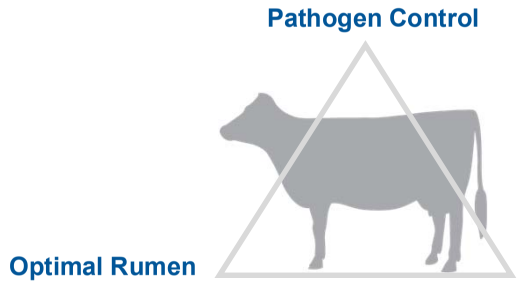
2

Introduction

- The most successful dairies are the ones that are the most *RESILIENT*
- A resilient herd achieves consistent, high-level performance in the face of pressures
- How do we create resilient herds?
  - Limit pressures cows face
  - Create cows that can withstand the pressures we can not control

3

Three pillars of a resilient cow



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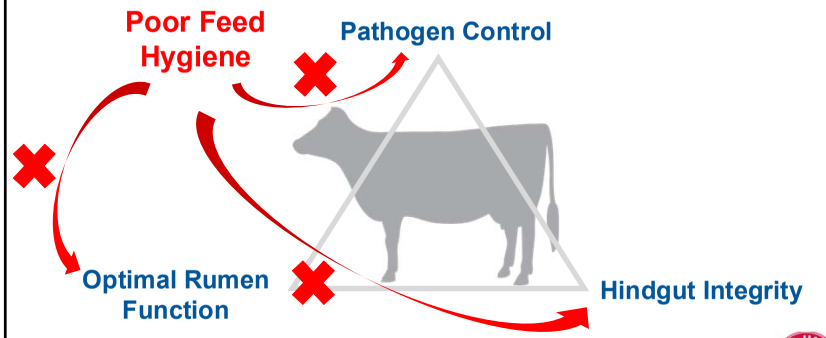
Poor Feed Hygiene Threatens Resilience



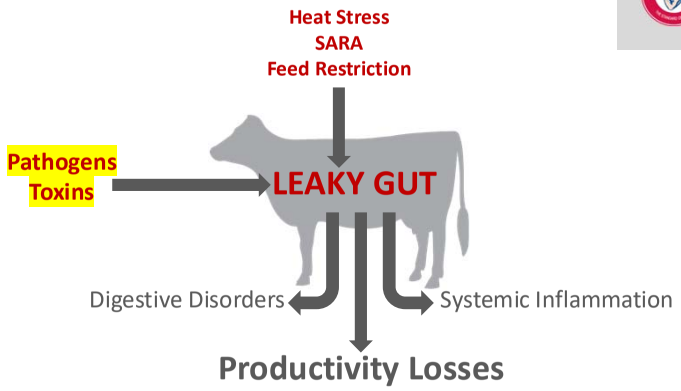
5

What is feed hygiene and why does it matter?

- Hygienic feed is feed that is free of pathogens and toxins that could be detrimental to animal health and performance.
- Poor feed hygiene is **not an isolated challenge.**
- Pathogens and toxins in feed interact with numerous other stressors to cause digestive disorders and productivity losses.

6

A quick example:



Koch et al. (2019); Emmanuel et al. (2007); Kvidera et al. (2017); Horst et al. (2021)

7

Who are the bad actors threatening feed hygiene?

- Toxin-producing Clostridia
- Solvent-producing Clostridia
- *Salmonella*
- *E. coli*
- Yeasts
- Molds
- Mycotoxins

8

Who are the bad actors threatening feed hygiene?

- The TMR is the primary source of pathogens and toxins found in the GIT.
- These bad actors can be introduced in the field, during storage and feed-out, or throughout the course mixing and feed delivery.

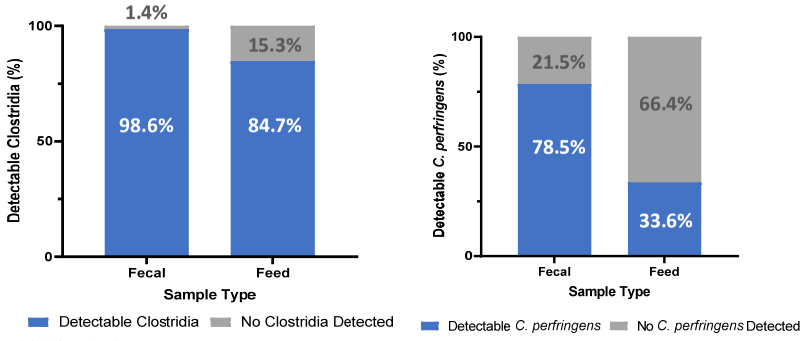
9

The scope and severity of feed hygiene challenges

10

Clostridia

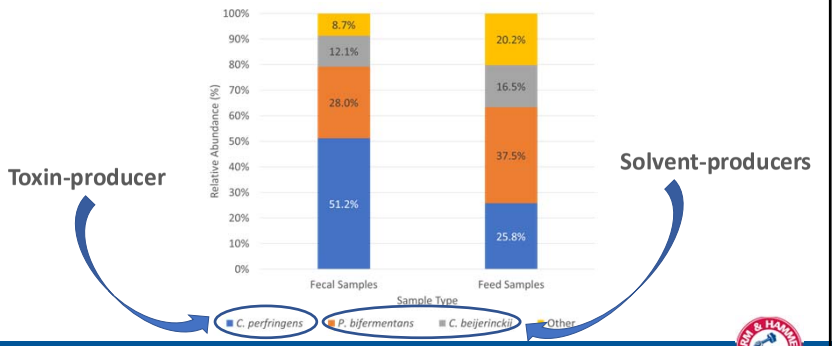
In a survey of more than **30,000** fecal samples and **7,000** feed samples from across the U.S…



Bretl et al. (2022) – ADSA Abstr.

11

Clostridia

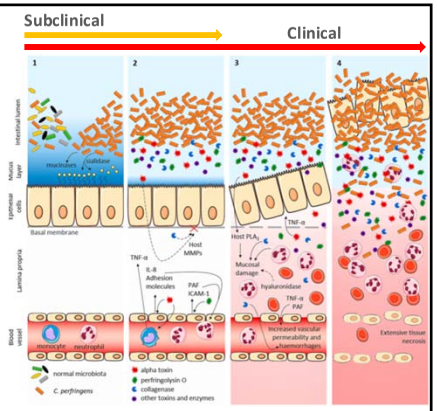


Bretl et al. (2022) – ADSA Abstr.

12

Toxin-producers

- *C. perfringens*
- Known contributor to HBS in dairy cattle

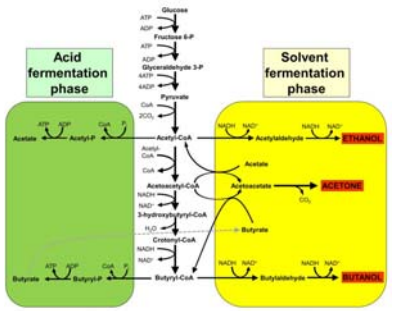


Goossens *et al. Vet Res (2017) 48:9*

13

Solvent-producers

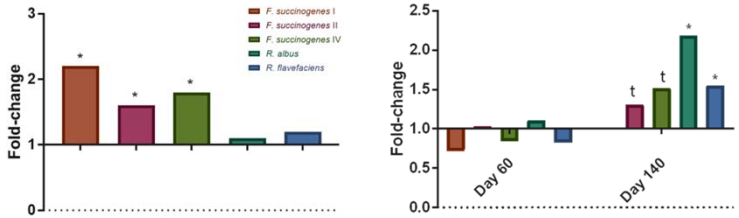
- *C. beijerinckii*, *C. bifermentans*
- Produce solvents like acetone, ethanol, butanol
- Hypothesized to negatively impact rumen function



14

Effect of inhibiting solvent-producing clostridia on ruminal fibrolytic populations

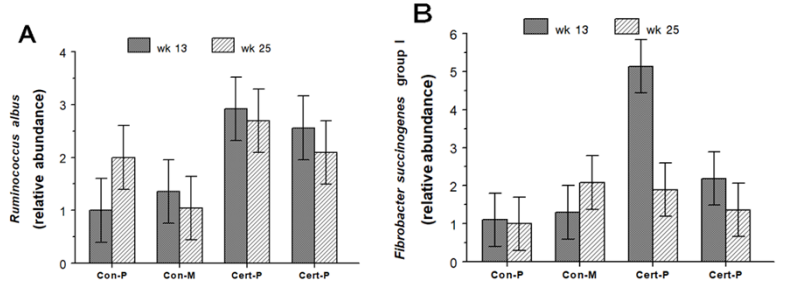
- In 2 field trials, ARM & HAMMER's *Bacillus* technology increased populations of fiber-digesting bacteria in the rumen



*P ≤ 0.05
†0.05 < P ≤ 0.10

15

Effect of inhibiting solvent-producing clostridia on ruminal fibrolytic populations



Trt: *P* = 0.03

Trt: *P* = 0.03

Maylem et al. (2023) – Under Review

16

Outgrowth of Clostridia in TMR

FIGURE 1: Total clostridia enumeration results from Haylage, Corn Silage and TMR samples. Each circle represents an individual sample and black lines represent means (**** = *P*-value<0.0001).

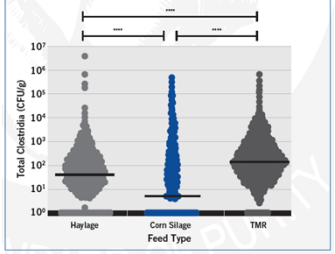
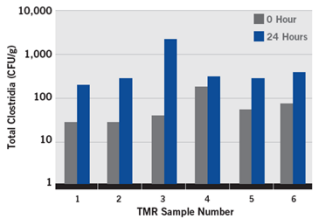


FIGURE 2: Total clostridia enumeration results from TMR samples. Gray bars indicate initial clostridia count and blue bars indicate clostridia counts after 24 hours in a simulated feed out phase environment.



17

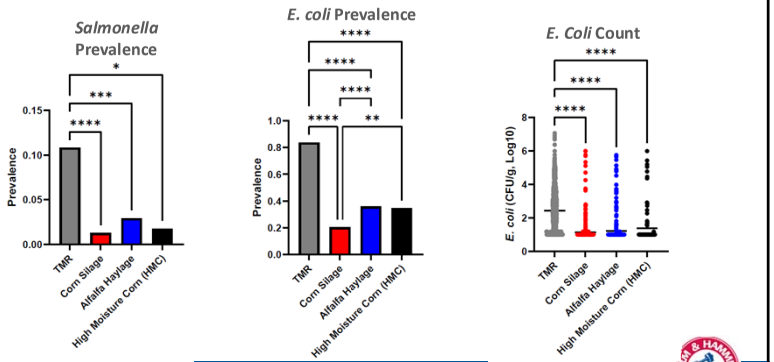
Salmonella and *E. coli*

- *Salmonella* and *E. coli* are found in a cow's digestive tract and should not be present in feed
- Their presence in silages suggests a poor fermentation or potential manure contamination
- Their presence in TMR suggests contamination during mixing or feed delivery
- *Salmonella* and pathogenic strains of *E. coli* can cause a host of health issues

Peek et al. (2018)

18

Salmonella and *E. coli*



P < 0.05

TMR n=1,175 CS n=529 AH n=425 HMC n=110

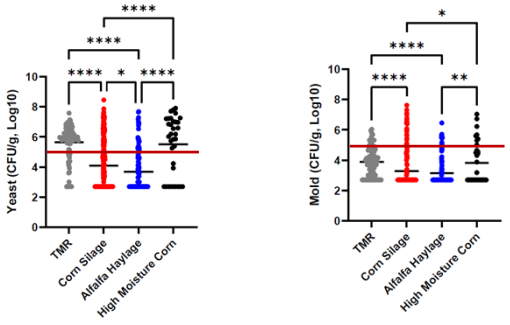
19

Yeasts and Molds

- High levels of yeasts and molds in silages and TMR are indicative of spoilage
- Counts should be < 100,000 cfu/g (ideally < 1,000)
- Y&M contribute to poor aerobic stability (shelf life) of silages and TMR
- Spoilage by yeasts and molds represents a loss of nutrients and can lead to inconsistent intakes and performance

20

Yeasts and Molds

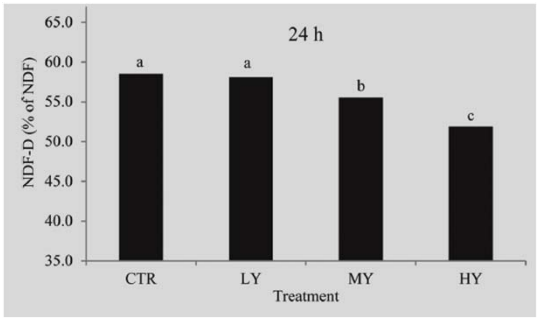


TMR n=98
CS n=272
AH n=132
HMC n=34

Abstr. 2022 Symposium on Gut Health

21

Effect of yeasts on NDF-D



Santos et al. (2015)

22

Mycotoxins

- Mycotoxins are secondary metabolites produced in feeds by various species of molds.
- Survey in 2012 found that **81%** of livestock feed samples collected from Americas, Europe, Asia tested positive for at least 1 mycotoxin (n = 7,049).

Mycotoxin	Effects in ruminants
Aflatoxin	Reduced milk production, impaired immune and ruminal function
T-2	Immunosuppression, infertility, late gestation abortion
DON/Vomitoxin	Gastrointestinal problems and reduced performance
ZEA	Infertility, reduced milk production

Rodrigues and Naehrer (2012); Ogunade et al. (2018)

23

What can be done to improve feed hygiene?

24

What can be done to improve feed hygiene?

- **Option 1:** Limit the cow's exposure to pathogens and toxins
- **Option 2:** Control pathogens and toxins within the GIT

25

Limit the cow's exposure to pathogens and toxins

Strategy #1: Optimize silage fermentation

Strategy #2: Minimize contamination during feed mixing and delivery

26

Optimizing silage fermentation

- Fast, efficient silage fermentations create an environment detrimental to pathogen growth
- Silage management best-practices:
  - Optimal harvest moisture
  - Use of research-proven silage inoculants
  - Fast, thorough packing and covering

27

Effect of homofermentative silage inoculants on feed hygiene

	n	Control mean	Effect size	*P*-value
pH	236	4.10	-0.11	<0.01
Lactic acid, % DM	258	4.67	0.92	<0.01
Yeasts, log cfu/g	86	4.02	0.28	<0.01
Molds, log cfu/g	32	3.00	-0.58	<0.01
Clostridia, log cfu/g	8	3.54	-1.94	<0.01

Adapted from Oliveira et al. (2017)

28

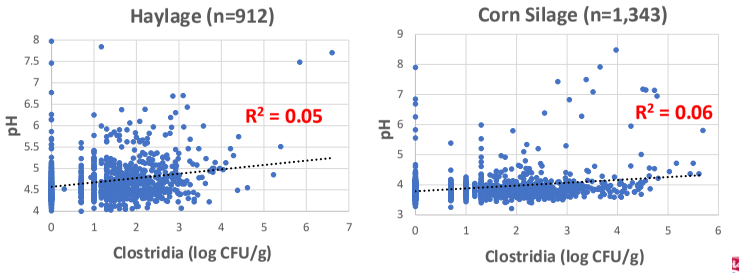
Effect of heterofermentative silage inoculants on feed hygiene

	n	Control mean	Effect size	*P*-value
pH	490	4.13	0.06	<0.01
Lactic acid, % DM	483	4.30	-0.31	<0.01
Acetic acid, % DM	4.94	1.70	1.05	<0.01
Yeasts, log cfu/g	286	4.19	-0.84	<0.01
Molds, log cfu/g	186	2.89	-0.40	<0.01
Aerobic stability, h	241	111.2	82.1	<0.01

Adapted from Arriola et al. (2021)

29

Relationship between silage pH and clostridial counts



30

Minimize feed contamination – 10 Steps

1. Clear debris and spoiled silage off silage pads and feed areas
2. Increase silage feed-out rate and feed defaced silage immediately
3. Feed high-moisture byproducts quickly, empty bays before restocking

31

Minimize feed contamination – 10 Steps

4. Free feed alley of debris, scraping at least once daily
5. Clean feed bunks, especially directly under headlocks
6. Make sure waterers are clean and clear

32

Minimize feed contamination – 10 Steps

7. Feed refusals as soon as possible. Mix batches containing refusals last.
8. Clean TMR mixer once per month.
9. Clean pushup blade monthly.
10. Clean tires and buckets of all feed payloaders or skid loaders. Avoid handling feed with buckets that have moved manure or dirt.



33

Controlling pathogens that find their way to the GIT

34

Controlling pathogens and toxins within the GIT

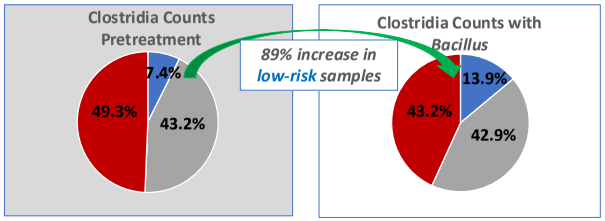
Strategy 1: *Bacillus* technology to reduce clostridial loads in the GIT and improve hindgut integrity

Strategy 2: Refined functional carbohydrates (RFCs) from yeast cell wall to bind pathogens and mycotoxins in the GIT

35

Inhibit Pathogens within the Cow

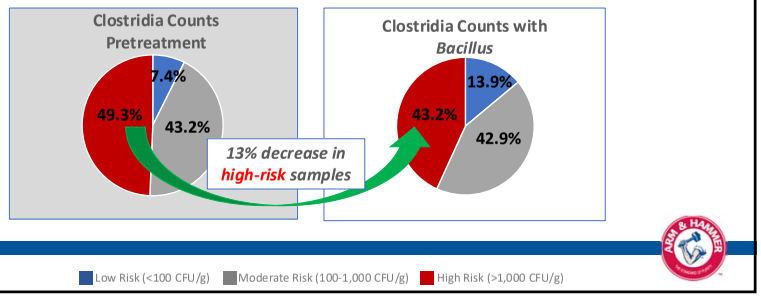
Shift in risk associated with total clostridia before and after feeding *Bacillus* (77 dairies, 230,000 cows, 18 states)



36

Inhibit Pathogens within the Cow

Shift in risk associated with total clostridia before and after feeding *Bacillus* (77 dairies, 230,000 cows, 18 states)



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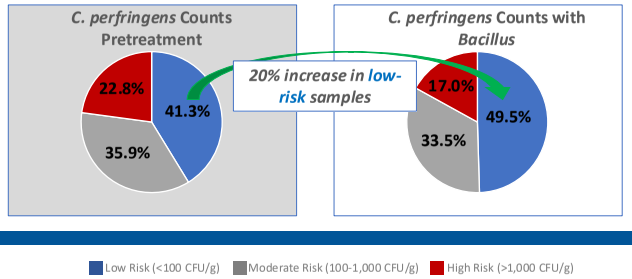
Inhibit Pathogens within the Cow

Shift in risk associated with total clostridia before and after feeding *Bacillus* (77 dairies, 230,000 cows, 18 states)



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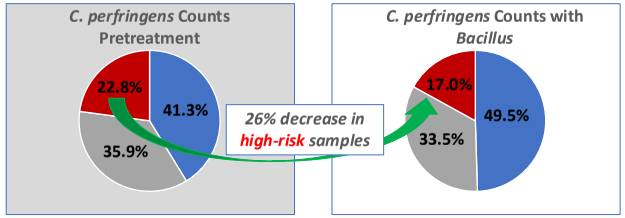
Inhibit Pathogens within the Cow

Shift in risk associated with total clostridia before and after feeding *Bacillus* (77 dairies, 230,000 cows, 18 states)



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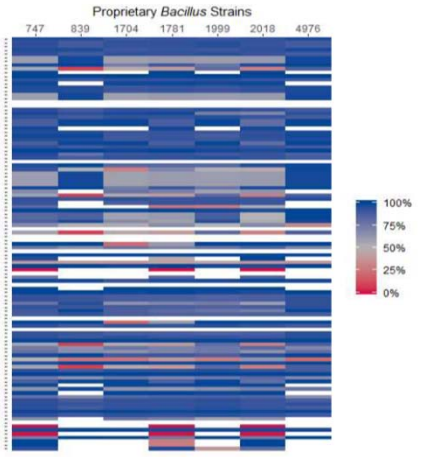
With *Bacillus*, strains matter



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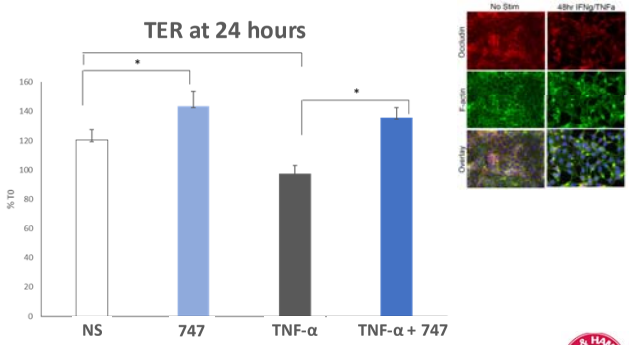
Effect of *Bacillus* feeding on gut barrier integrity



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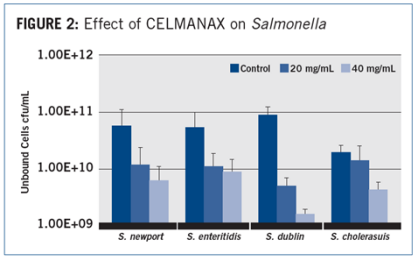
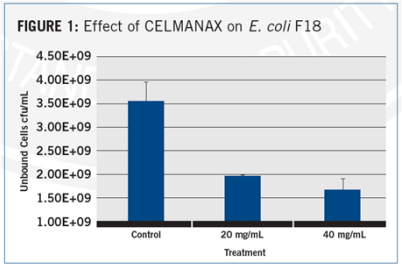
Effect of RFCs on pathogen binding

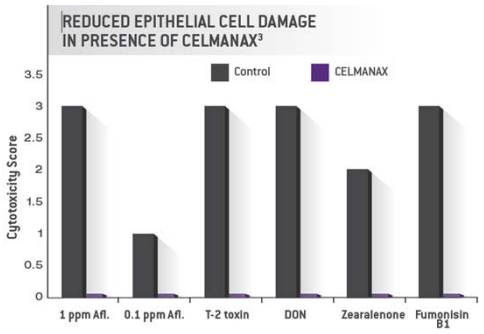




Jalukar et al. 2009. Abstr. Midwest ASAS Mgt.

42

Effect of RFCs on mycotoxin binding



Baines et al. 2014. Abstr. Symposium on Gut Health.

43

Conclusions

- Feed hygiene contributes to the RESILIENCE of a dairy herd
- Exposure to pathogens and toxins can be minimized by:
  - Optimizing silage fermentation
  - Limiting contamination of clean feed on-farm
- Pathogens and toxins in the GIT can be controlled with *Bacillus* technology and RFCs

FEED HYGIENE CHECKLIST

44

SESSION NOTES

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

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To learn more contact
your new rep, Kemp Caudill
at 850-865-8355 or
Kemp.Caudill@churchdwight.com.

Control Environmental Pathogens That Silently Steal Productivity

Mike Motta[1]
Arm & Hammer Animal and Food Production

Introduction

Mastitis, both clinical and subclinical, is the costliest health challenge cows face. In addition, clinical mastitis is the most common cause of adult dairy cow morbidity in the U.S. (NAHMS, 2007).

The highest risk of mastitis infection is in early lactation. The cow has just come through a stressful calving, her udder is adjusting to high levels of milk production, and other factors are associated with higher risk during this period. Mastitis in early lactation is likely to have the greatest economic impact.

Rollin et. al. (2015) estimated the cost of clinical mastitis in the first 30 days of lactation. Direct costs, which include diagnostics, therapeutics, non-saleable milk, vet costs, labor and death loss accounted for $128 per case. Indirect costs, which included losses from future milk production, premature culling, and potential reproduction issues, was much higher at $316 per case. This brought the average total cost to $444 per mastitis case, assuming a milk price of $21/cwt.

Environmental Pathogens Bombard the Udder

Cows face bombardment on a daily basis from environmental pathogens that can initiate mastitis. Mastitis cases caused by environmental pathogens—those pathogens that reside outside of the parlor - account for one-third of all mastitis infections on a dairy. The primary source for these pathogens is dairy bedding. Cows lie down for 10 to 12 hours each day, giving pathogens an opportunity to enter and colonize the teat canal (Tucker et al., 2021). This demands that the outgrowth of environmental pathogens in bedding must be controlled.

Over the past several years the interest in creating a sustainable bedding source has risen. This includes the use of recycled manure, even with the understanding that it can increase the risk pathogenic challenges that can cause mastitis. There are other popular bedding materials, but they can be costly and have a negative impact on equipment. The use of chemical treatments to control pathogens is also an option, but they can be costly and unsafe.

Characterizing Mastitis-Causing Pathogens in Bedding

[1] Contact at: Arm & Hammer Animal Food Production; Tel: (839) 900-1178; E-mail: Mike.Motta@churchdwight.com.

Research conducted by Arm & Hammer Animal and Food Production was focused on characterizing mastitis-causing pathogens in dairy bedding. More than 1,100 bedding samples were collected from dairies across the U.S. Bedding types included:

- Green recycled manure solids
- Digester solids
- Composted manure solids
- Sand
- Other (straw, sawdust, wood shavings, corn stalks)

Sample sources included either unused bedding fresh from the pile or from the stall, collected at the location of the udder.

There were three groups of pathogens that were targeted:

- *E. coli* and total coliforms
- *Klebsiella* group: *Klebsiella, E. coli* and *Shigella*
- Group D streptococci group: *Enterococcus, Streptococcus* and *Staphylococcus*

Within every group, pathogen levels increased from the fresh pile to the stall. The key becomes understanding how to control the regrowth of pathogen populations to protect cows.

Current methods of controlling pathogens in recycled manure solids have drawbacks. Common activities are to pile solids to generate a composting effect or dry solids to reduce moisture content. The baseline pathogen load is reduced through both activities, but it doesn't prevent outgrowth in the stall. There are opportunities to add hydrated lime, but there are risks to employees and, when spread on fields, soil pH can be affected. Acid-based products offer limited protection and pose a significant health risk for employees.

ARM & HAMMER™ sought to identify safe and effective biological applications that reduce outgrowth of mastitis-causing pathogens in recycled manure solids used for bedding. Research examined the use of Bacillus, beneficial, spore-forming bacteria that produce a variety of anti-microbial compounds.

The ability of our proprietary Bacillus strains to inhibit pathogen growth was evaluated against representative isolates of mastitis-causing organisms. The highest-performing strains were selected for inclusion in a bedding application.

The Bacillus strains were tested on dairies bedding with green or composted recycled manure solids. Moisture was removed from the manure using a screw press and product was applied at a single location in the manure processing system. New bedding was applied multiple times per week into deep beds.

On one 700-cow dairy in South Dakota, bedding samples were collected from stalls at locations near the udder prior to and after 100 days of product application. Bulk tank SCC and monthly mastitis events from March through December 2019 (pre-treatment) were compared to those from the same period the following year (treatment period).

There were significant reductions in E. coli and Klebsiella populations between pre-treatment and treatment periods. There was a numerical reduction in total coliforms, a statistical increase in Group D Streptococci, and reductions in Streptococcus and Proteus populations. Bulk tank SCC was reduced 76,000 between pre-treatment and treatment periods in year-over-year comparisons. Also, there were 10 fewer monthly mastitis events on average.

Similar field demonstration studies were conducted on four other commercial dairies. Average counts of E. coli, coliforms, Klebsiella, and Group D Streptococci decreased with bedding treatment. Bulk tank SCC decreased with bedding treatment application on all five demonstration sites, with an average SCC reduction of 56,800 in year-over-year comparisons. There were nine fewer average monthly mastitis events with bedding treatment application on four of the five sites.

Conclusion

The average clinical case of mastitis costs $444. Reducing environmental mastitis requires control of pathogen outgrowth within the bedding in the stalls. Current methods of pathogen control have major drawbacks. *Bacillus* products from ARM & HAMMER can safely and effectively control pathogens in recycled manure solids. Field demonstrations suggest that bedding treatment had the ability to:

- Reduce pathogen loads in bedding
- Reduce bulk tank SCC
- Reduce monthly mastitis cases

Using the Bacillus product to manage pathogen outbreaks results in a net ROI to the dairy. If a 2,000-cow dairy averaging 85 lbs. milk/cow/day, receives a premium of 20 cents per cwt, the annual premium would be nearly $104,000. If the herd had an average clinical mastitis incidence (10% of cows at $444 per case) the annual clinical mastitis cost would be $89,000. Trials have proven that clinical mastitis can be reduced by 25% using Bacillus, which cuts clinical mastitis costs by $22,000. The cost of the Bacillus product is $35,000, which makes an annual estimated net gain of $91,000.

There is additional value inherent in using the Bacillus product. Producers can enhance sustainability and use recycled manure with confidence without buying other bedding products. Reduced infections leads to better cow health, comfort and welfare. Milk production and quality will improve, and along with it the return on investment with greater SCC premiums and reduced cost of mastitis events.

References

NAHMS, 2007. Dairy 2007: Part I: Reference of Dairy Cattle Health and Management in the United States. USDA, Animal and Plant Health Inspection Service, Veterinary Services, Center for Epidemiology and Animal Health, Fort Collins, CO.

Rollin, E., K. C. Dhuyvetter, and M. W. Overton. 2015. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Prev. Vet. Med. 122:257-264.

Tucker, C. B., M. B. Jensen, A. M. de Passille, L. Hanninen, and J. Rushen. 2021. Invited review: Lying time and the welfare of dairy cows. J. Dairy Sci. 104:20-46.

CONTROL ENVIRONMENTAL PATHOGENS THAT SILENTLY STEAL PRODUCTIVITY

Mike Motta
Business Development Manager Americas

Florida Ruminant Nutrition Symposium
February 2023

1

Presentation Outline

I. Understanding Recycled Manure Solids & Dairy Bedding
II. The Cost of Clinical Mastitis
III. Introduction to Environmental Mastitis
IV. Characterizing Mastitis-Causing Pathogens in Dairy Bedding
V. Current Methods of Pathogen Control
VI. The Power of *Bacillus*
VII. Farm A
VIII. Field Demonstration Results
IX. Conclusions
X. Questions and Discussion

2

Understanding Recycled Manure Solids

Used for Dairy Bedding

- By and large, there's an interest in a sustainable approach to bedding across the industry
- Increased interest in using recycled manure, with the understanding it presents pathogenic challenges, risking mastitis
- Other bedding materials can be costly and rough on equipment; i.e. sand
- Some dairies earn premiums for lowering SCC (varies by region)
- Clinical Mastitis (CM) events are costly
- CM has an impact on quality and volume of milk
- CM has an impact on cow health and comfort
- Using chemical treatments to control pathogen outgrowth, can be costly and unsafe

3

The Cost of Clinical Mastitis

- Clinical mastitis = the most common cause of adult dairy cow morbidity in the U.S.
- Rollin et al. (2015) attempted to estimate the cost of clinical mastitis in first 30 days of lactation
- Highest risk period for detection of mastitis is in early lactation
- Mastitis in early lactation likely to have greatest negative economic impact
- Model inputs selected from available literature and field data

NAHMS, 2007; Rollin et al., 2015

4

The Cost of Clinical Mastitis

Model Inputs:

- **Income** (milk price and market cow price)
- **Repro** (21-day pregnancy rate)
- **Value of non-saleable milk** (if fed to calves or if discarded)
- **Nutrition** (feed price, energy density, energy for marginal milk, value of marginal milk)
- **Calving** (# of animals calving per year by lactation)
- **Culling** (value of cows in lactation, culling risk over lactation)
- **Milk production** (per cow 1-30 DIM)
- **Mastitis** (incidence, severity, pathogen type, recurrence risk)

Rollin et al. 2015

5

The Cost of Clinical Mastitis

Direct Costs	Indirect Costs
Diagnostics	Future milk production loss
Therapeutics	Premature culling loss
Non-saleable milk	Future repro loss
Vet service	
Labor	
Death loss	
= $128 / case	= $316 / case

Avg. total cost = $444 / case
@ milk price of $21/cwt

Rollin et al. 2015

6

Introduction to Environmental Mastitis

- Environmental mastitis (EM) accounts for one-third of all mastitis infections on a dairy
- EM is caused by pathogens originating outside of the parlor (in the environment)
- Primary source of these pathogens is in dairy bedding
  - Cows lie down for 10-12 hrs. per day
  - Environmental pathogens can colonize the teat canal
- Reducing cases of EM demands that outgrowth of mastitis-causing organisms in bedding be controlled

Tucker et al., 2021

7

Arm & Hammer Research Trials and Testing

8

Characterizing Mastitis-Causing Pathogens in Dairy Bedding

- Over 1,100 bedding samples from dairies across the U.S.
- Bedding Types
  - Green recycled manure solids (RMS)
  - Digester solids (DigRMS)
  - Composted manure solids (CompRMS)
  - Sand
  - Other (straw, sawdust, wood shavings, corn stalks)
- Sources
  - Fresh Pile (unused)
  - Stall (laid on in pen, collected at location of udder)

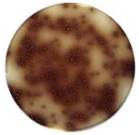
9

Characterizing Mastitis-causing Pathogens in Dairy Bedding

Quantify and characterize target pathogens

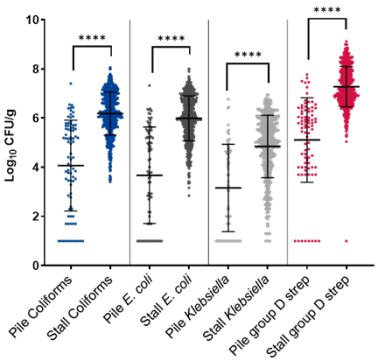
***E. coli* and total coliforms**

***Klebsiella* group** *Klebsiella, E. coli* and *Shigella*



Group D streptococci group *Enterococcus, Streptococcus* and *Staphylococcus*

10



Increasing Pathogen Levels from Fresh Pile to Stall

How do we control this outgrowth?

11

Current Methods of Controlling Pathogens in RMS

- Recycled manure solids (RMS) represent a cost-effective, sustainable bedding source
- Pathogen levels and risk of high SCC and mastitis with RMS challenge current RMS users and prevent future adoption of RMS

12

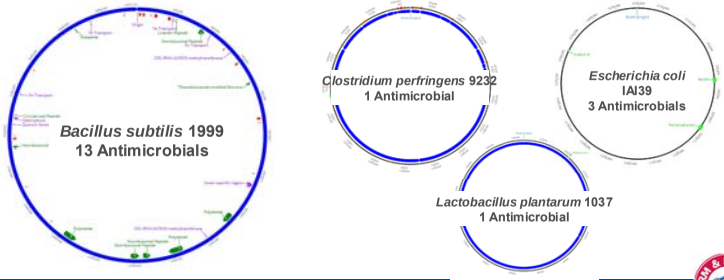
Current Methods of Controlling Pathogens in RMS

- Current methods of controlling pathogens in RMS have drawbacks
  - Drying or composting – baseline challenge is reduced but outgrowth is not prevented
  - Hydrated lime – risks to employee health and soil pH
  - Acid-based products – limited protection and risks to employee health
- ***Our objective was to develop a safe and effective biological application for reducing outgrowth of mastitis-causing pathogens in RMS used as dairy bedding***

13

The Power of *Bacillus*

Bacillus are beneficial, spore-forming bacteria that can produce a variety of anti-microbial compounds.



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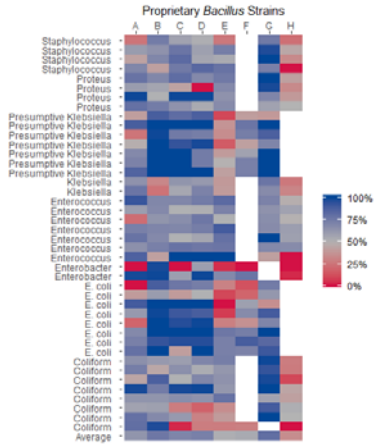
The Power of *Bacillus*

- Inhibition activity of our proprietary *Bacillus* strains was evaluated against representative isolates of mastitis-causing organisms
- Highest-performing strains were selected for inclusion in bedding application



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Target Herds for Initial Investigation

- Dairies bedding with green or composted RMS
- Moisture removed with screw press
- Product applied at single location
- New bedding applied multiple times per week into deep beds

16

Product Application




17

Equipment – Various Formats





18

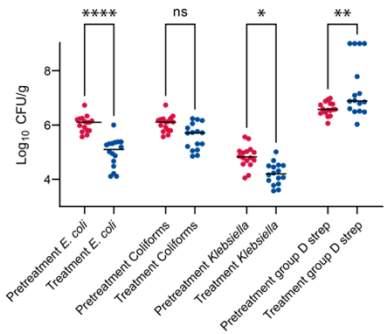
Farm A

- 700-cow dairy in SD
- Bedding with RMS in deep beds
- Bedding samples were collected from stalls (at location of udder) prior to and after 100 days of product application
- Bulk tank SCC and monthly mastitis events from Mar-Dec 2019 (pre-treatment period) were compared to those from Mar-Dec 2020 (treatment period)

19

Farm A

- Significant reductions in *E. coli* and *Klebsiella* populations between pre-treatment and treatment periods
- Numerical reduction in total coliforms
- Statistical increase in Group D Streptococci
  - Reductions in *Streptococcus* and *Proteus* populations



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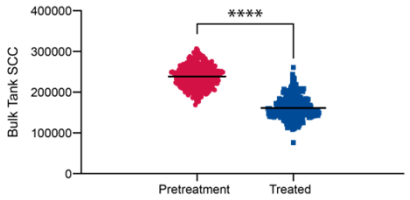
Farm A

- Bulk tank SCC reduction of 76,000 between pre-treatment and treatment periods
- Year-over-year comparison



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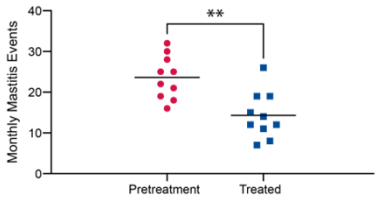
Farm A

- 10 fewer monthly mastitis events on average
- Year-over-year comparison



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Field Demonstration Results

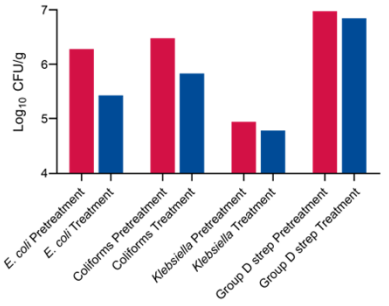
Similar field demonstration studies were conducted on 4 other commercial dairies.

Farm ID	Number of cows	State
Farm B	750	MN
Farm C	250	MN
Farm D	800	MN
Farm E	400	WI

23

Field Demonstration Results

- Average counts of *E. coli*, coliforms, *Klebsiella*, and group D streptococci decreased with bedding treatment



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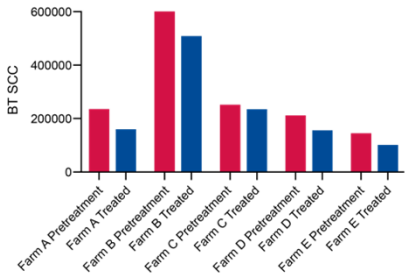
Field Demonstration Results

- Bulk tank SCC decreased with bedding treatment application on all 5 demo sites
- Average SCC reduction = 56,800
- Year-over-year comparison



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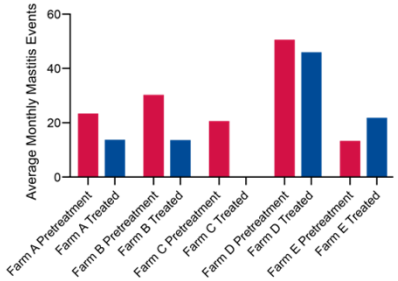
Field Demonstration Results

- Average monthly mastitis events decreased with bedding treatment application on 4/5 trial sites
- Average reduction = 9 events
- Year-over-year comparison



26

Conclusion

27

Conclusions

- Clinical mastitis costs on average $444 per case
- Reducing environmental mastitis requires control of pathogen outgrowth within the bedding in stalls
- Current methods of pathogen control have major drawbacks

28

Conclusions

- Only with ARM & HAMMER™ *Bacillus* can you safely and effectively control pathogens in RMS
- Field demonstrations suggest that bedding treatment has ability to:

Reduce pathogen loads in bedding

Reduce bulk tank SCC

Reduce monthly mastitis cases

29

Overall Value – *Bacillus* Product

ROI Example

SCC Benefit	
# of Cows	2,000
lbs milk/cow	85
Total lbs/day	170,000
Total lbs/year	51,850,000
cwt	518,500
Premium/cwt	$0.20
Total Monthly Premium	8,642
Total Annual Premium	103,700

Cost of Mastitis

Average cost of CM is $444

Industry numbers: Studies show 10% of herd, some show 14%, and some as high as 32%

Using 10% (to be conservative), a herd size of 2,000 has 200 CM cases per year

At $444 per case, for a dairy with 2,000 cows.

Annual CM cost $89K

***Bacillus* Product**

Annual Premium of $104K (from lowering SCC)

Our trial sites have proven we can reduce CM by an average of 25%, (from 200 to 150 cases per year). This cuts CM costs by $22K (25% of $89K)

Cost of *Bacillus* Product is $35K

Annual Est. Net Gain $91K

Est. ROI – 2,000 Cows - Summary

SCC Premium:	+$104K
CM Treatment Savings 25%:	+$22K
Cost of *Bacillus* Product:	$35K
ROI	**$91K**

**ROI = SCC premiums + cost of mastitis savings – Cost of Bacillus Product*

30

Overall Value – *Bacillus* Product

Added Benefits

- Sustainability – No need to buy other bedding materials such as sand. Use recycled manure with confidence
- Cow Health & Comfort – Reduce infections (mastitis)
- Milk Production – Improve quality and volume of milk
- ROI – Improve opportunity to earn premiums for lower SCC and decrease cost of mastitis events

31

SESSION NOTES

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

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To learn more contact
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at 850-865-8355 or
Kemp.Caudill@churchdwight.com.

Beef-on-Dairy and Liver Abscesses: What do we know?

T. G. Nagaraja[1]
Kansas State University

Introduction

Liver abscesses in cattle occur generally because of entry and establishment of pyogenic bacteria into the hepatic parenchyma. Although bacteria can access the liver by several routes, entry of bacteria from the gut, via the portal vein, is by far the most frequent source and route of infection. Liver abscesses occur in all types of cattle, but they are most prevalent and are of greatest economic importance in feedlot cattle, calf-fed Holsteins and beef-on-dairy cattle. Cattle with abscessed livers seldom show any clinical signs and are detected only at the time of slaughter (Nagaraja et al., 1996a). Liver function tests and serological tests targeting *F. necrophorum*- specific antibodies have not shown to be of much diagnostic value (Tan et al., 1994; Macdonald et al., 2017). Changes in blood cell counts and liver function variables in cattle with liver abscesses are consistent with chronic active inflammation, therefore, are non-specific to aid in the diagnosis. Studies have shown that ultrasound scanning of the liver can be used to detect abscesses with limited success (Lechtenberg and Nagaraja, 1991). Ultrasonography may not identify abscess lesions accurately if they are located on the visceral side, in deeper regions of the liver tissue or in a lobe covered by the lung tissue.

Beef-on-Dairy cattle

The use of beef cattle semen to breed dairy cows to produce calves, called beef-on-dairy crosses, for beef production has greatly increased in the past 5 years. Dairy beef production has become an important pillar of the beef industry and plays a key role in contributing to the US beef demand. According to the National Association of Animal Breeders (NAAB), units of beef semen sales from 2017-2021 increased by 260%, largely to inseminate dairy cows to produce beef-on-dairy crosses (NAAB, 2022). The 2016-National Beef Quality Audit Report estimated that 16.3% of fed cattle supply included dairy-influenced cattle (Boykin et al., 2017). The practice increases the value of calves produced from dairies as beef-on-dairy calves have feedlot performance, carcass quality and meat attributes (tenderness, juiciness and flavor) better than calf-fed Holsteins but similar to conventional beef cattle. The beef-on-dairy cattle production system, although varies among calf ranches, typically includes three phases: phase 1 is from birth to approximately 75 days of age with calves housed individually in hutches and fed milk or milk replacer with access to dry feed, phase 2 is calves group housed in pens and fed a diet with varying proportions of roughage and grain until approximately 450 to 600 lbs. body weight, and phase 3 is in a feedlot fed a finishing diet before shipment to harvest.

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A major issue with the beef-on-dairy crosses is high incidence of liver abscesses for which packers exercise a universal discount. Although no published data on the in beef-on-dairy cross cattle exist, there is consensus that the incidence in beef-on-dairy cattle is 2 to 3 times greater than crossbred beef cattle (Foraker et al., 2022). The economic losses are above and beyond those of abscessed livers because of higher occurrence of severely abscessed livers (A+) with extensive adhesions to diaphragm and abdominal viscera. The reason for the higher incidence of liver abscesses is not known although likely explanations include feeding and management practices from birth to harvest, including days on feed at the feed yards, which could be up to 300 to 350 days. Another theory for greater incidence, particularly of the increased severity (A+ abscesses), is that the bacterial flora, qualitatively or based on virulence, of liver abscesses of beef-on-dairy may be different from that of beef breeds. However, there has been no published study that has compared the flora of liver abscesses between beef-on-dairy and beef breeds.

What do we know about liver abscesses?

Almost all the information on etiology, pathogenesis and control of liver abscesses are derived from research conducted with conventional feedlot cattle.

Incidence. The incidence of liver abscesses is highly variable, ranging from a low of 0 to 1 or 2% to a high of 60 to 80%, but the average is about 10 to 20% (Reinhardt and Hubbert, 2015; Amachawadi and Nagaraja, 2016). The wide range in the incidence is reflective of the influence of a number of factors, such as cattle type (feedlot cattle > culled dairy; Calf-fed Holsteins or Beef-on-dairy > crossbreds), gender (steers > heifers), grain type (wheat, barley > corn > sorghum), grain processing (steam flaked, high-moisture corn > dry-rolled corn), roughage type and level (silage > hay; 5 to 7.5% > 12 to 15% roughage level in the diet), season (summer months > winter months) and geographic location (Central region > Pacific Northwest and Northern Plains > Midwestern, Southern Plains and Desert Southwest) (Nagaraja et al., 1996b; Nagaraja and Chengappa, 1998; Amachawadi et al., 2016). The number of abscesses ranges from one to hundreds and the size from pinpoint to over 15 cm in diameter. Historically, liver abscesses are categorized on a scale of 1 to 3 or as A-, A, and A+, respectively (mild to severe), based on the number and size of abscesses. Livers with one or two small abscesses (< 2.5 cm) or abscess scars are scored as 1 or A-), with two to four small or medium-sized abscesses (< 4.5 cm) are scored as 2 or A, and with one or more large or multiple small- or medium-sized abscesses, often with adhesions to adjacent organs are scored as 3 or A+ (Brown et al., 1975).

Economic Importance. Liver abscesses are an economic liability at all levels of beef production − to the producers, the packers, and ultimately to the consumers. Abscesses are the primary cause of liver condemnations in slaughtered cattle. According to the latest National Beef Quality Audit Report (NBQA-2016), 30.8% of livers of slaughtered cattle in the US were condemned and abscesses accounted for 58% of all liver abnormalities (Eastwood et al., 2017). However, the real economic impact of liver abscesses is from reduced animal performance and carcass yield, quality and

value. The impact on cattle performance and carcass attributes is dependent on the severity of abscesses (Nagaraja and Lechtenberg, 2007; Brown and Lawrence, 2010). Generally, liver abscesses with mild abscesses (scored as A- or A) do not have a negative impact on cattle performance and carcass attributes (Brink et al., 1990; Brown and Lawrence, 2010). Cattle with severe liver abscesses (scored as A+) liver abscesses have significantly lower body weight, carcass yield, and dressing percentage, and higher carcass trim, compared to cattle with normal livers or mild liver abscesses (Montgomery, 1985). Gross carcass value analyses have indicated that carcasses with abscesses are less valued than carcasses with normal livers (Brown and Lawrence, 2010). Additionally, an accidental rupture of an abscess and contamination of a carcass with pus will cause interruption in the flow of carcasses along the chain on the slaughter floor, thus costing time and labor (Nagaraja and Lechtenberg, 2007).

Etiology. Liver abscesses are almost always polymicrobial infections with Gram negative anaerobes constituting the predominant flora (Scanlan and Hathcock, 1983; Nagaraja and Chengappa, 1998). All most all studies have concluded that *Fusobacterium necrophorum* is the primary causative agent (Table 1). The second most frequently isolated pathogen is *Trueperella pyogenes* (Scanlan and Hathcock, 1983; Lechtenberg et al., 1988). *Fusobacterium necrophorum,* identified as an animal and human pathogen in the late 1880s, is a Gram-negative, rod-shaped or pleomorphic bacterium (Langworth, 1977). The organism is a normal inhabitant of the rumen and its fermentative role is to utilize lactic acid to produce VFA and breakdown feed and rumen epithelial proteins and amino acids. The organism is also considered to be a major lysine degrading bacterium in the rumen (Russell, 2006; Elwakeel et al., 2013). Ruminal concentration of *F. necrophorum* is low ($< 10^6$), but is greater in cattle fed grain-based diets compared with roughage-based diets (Tan et al., 1994). This is likely to be due to increased lactate availability from the high-grain diet. *Fusobacterium necrophorum* also forms a part of the flora which adheres to the ruminal wall because of its aerotolerance and physiological pH of 7.4 is the optimal pH for its growth. The adhesion has been shown to be mediated by outer membrane proteins (Kumar et al., 2013).

There are two subspecies of *F. necrophorum;* subsp. *necrophorum* and subsp. *funduliforme* (Shinjo et al., 1991). These two subspecies differ in cell morphology, colony characteristics, growth patterns in broth, and most importantly, in the production of virulence factors (Table 1; Tadepalli et al., 2009). Subspecies *necrophorum* is more virulent and thus more frequently encountered in liver abscesses than subsp. *funduliforme,* which tends to occur more often in mixed infections (Table 2; Lechtenberg et al., 1988). The difference in virulence correlates with the difference in virulence factors between the two subspecies, with leukotoxin being the major virulence factor involved in the infection (Tan et al., 1996; Narayanan et al., 2002; Nagaraja et al., 2005). Leukotoxin is an exotoxin, composed of protein that is cytotoxic to neutrophils, macrophages, hepatocytes, and possibly to ruminal epithelial cells (Narayanan et al., 2002). Subsp. *necrophorum* produces more leukotoxin than *funduliforme* (Tan et al., 1992) and isolates from liver abscesses tend to be more leukotoxic than isolates from the rumen; suggesting a selective advantage for high-leukotoxin-producing strains to

survive in the ruminal epithelium and in the liver parenchymal tissue (Tan et al., 1994). Leukotoxin is encoded by a gene designated as *lktA,* which is the second gene in a three-gene operon, *lktB, lktA,* and *lktC* (Narayanan et al., 2001). The subsp. *funduliforme lkt* operon is organized identically to the subsp. *necrophorum* operon. Although the overall sequence similarity of the Lkt proteins is high between the two subspecies (87% and 88%, respectively), the LktA and LktB proteins have significant differences in their N-terminal sequences (Tadepalli et al., 2009). The decreased production of leukotoxin by subsp. *funduliforme* appears to be because of weak promoter activity compared to subsp. *necrophorum* (Zhang et al., 2006). *Trueperella pyogenes* is a Gram positive, rod-shaped and facultatively anaerobic organism, which is frequently isolated as a single or mixed culture from a variety of pyogenic infections in animals (Nagaraja, 2013). The organism exists as a commensal on mucous membranes of the upper respiratory and digestive tracts of animals. The source of *T. pyogenes* of liver abscesses appears to be the ruminal wall and is more frequently isolated from the ruminal wall than the contents (Narayanan et al., 1998). Because it is a facultative anaerobe, its niche is more likely to be the ruminal wall where oxygen is available from the blood circulation in an otherwise anaerobic environment of the rumen. *Trueperella pyogenes* is the second most frequently isolated pathogen in liver abscesses (Tan et al., 1996). The principal virulence factor of *T. pyogenes* is a hemolysin, called pyolysin, which is also cytotoxic to polymorphonuclear cells (Billington et al., 1997; Jost and Billington, 2005). Another species that is frequently isolated from liver abscesses of cattle is *Salmonella enterica* (Amachawadi and Nagaraja, 2015; Amachawadi et al., 2017). Thus far, the predominant serotype of *Salmonella* isolated is a novel serotype, Lubbock, which has been shown to be closely related to the serotype Mbandaka (Bugarel et al., 2015). It is not known whether *S. enterica* is one of the etiologic agents or a secondary invader into an abscess, via lymph or blood, and survived. The plausible hypothesis is that *Salmonella* present in the gut could cross the gut epithelial barrier, most likely in the small or large intestine, to gain access via lymph to the portal circulation, and get filtered by the portal capillary system of the liver to initiate infection. Further studies are needed to determine the importance of *Salmonella* in liver abscesses of cattle.

Additionally, a number of other anaerobic and facultative bacteria including *Bacteriodes* sp., *Clostridium* sp., *Escherichia coli, Klebsiella* sp., *Enterobacter* sp., *Mobilincus* sp., *Pasteurella* sp., *Peptostreptococcus* sp., *Porphyromonas* sp., *Prevotella* sp., *Propionibacterium* sp., *Staphylococcus* sp., *Streptococcus* sp., and many unidentified Gram-negative and Gram-positive bacteria have been isolated from liver abscesses of feedlot cattle (Scanlan and Hathcock, 1983; Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007). In a 16S rRNA genes amplicon sequencing-based bacteriome analysis of the purulent material of liver abscesses (n=48), the predominant phylum and genus identified were *Fusobacteria* (52% of the total sequence reads) and *Fusobacterium,* respectively. Interestingly, the second most dominant phylum was Proteobacteria (14% of the total sequence reads) with *Pseudomonas* as the second most dominant genus The third most abundant genus was *Bacteroides* (Figure 2; Amachawadi et al., 2021). Two other studies have identified that in a proportion of liver abscesses analyzed, phylum Fusobacteria was not dominant and was

supplanted by phyla Proteobacteria and Bacteroidetes (Weinroth et al., 2017; Pinnell et al., 2022).

The bacterial flora of liver abscesses of beef-on-dairy has not been analyzed. However, a study comparing bacterial flora of liver abscesses of calf-fed Holstein steers, which are similar to beef-on-dairy with regard to feeding and management, particularly days on feeding, to that of liver abscesses of crossbred beef cattle have been reported (Amachawadi et al., 2017). Liver abscesses from Holstein steers yielded a higher total number of isolates compared to liver abscesses from crossbred cattle (1,060 vs. 788). *Fusobacterium necrophorum* subsp. *necrophorum* was isolated from all abscesses. The prevalence of subsp. *funduliforme* was 19.1% and was not affected by the cattle type. The prevalence of *Trueperella pyogenes* was higher in crossbred cattle (73.7%) compared to Holstein steers (29.8%). The study concluded that difference in bacterial flora was not the likely reason for higher prevalence and severity of liver abscesses in calf-fed Holstein steers than crossbred beef cattle (Amachawadi et al., 2017).

Pathogenesis. It is generally accepted that ruminal epithelium, damaged by chronic acidity, becomes susceptible to invasion and colonization by ruminal bacteria leading to rumenitis, and the organisms subsequently enter portal circulation to reach the liver (Figure 3; Nagaraja and Chengappa, 1998); thus the term 'acidosis-rumenitis-liver abscess complex'. Smith in 1944 was first to report a study on the ulcerative lesions of the ruminal epithelium and their potential association with liver abscesses in cattle (Smith et al., 1944). The positive association between ruminal pathology and liver abscess incidence was confirmed almost 10 years later by a study reported by Jensen at Colorado State University (Jensen et al., 1954). The study also identified that ruminal lesions occurred because of the damage caused by acidotic conditions of the rumen. A relatively recent study (Rezac et al., 2014) reaffirmed the positive association between ruminal lesions and liver abscesses based on gross pathology data collected from 19,229 cattle originating five commercial feedyards in Texas and one commercial feedyard in Kansas. Ruminal acidosis and subsequent rumenitis as predisposing factors are supported by observations of increased incidence of liver abscesses associated with the following dietary feeding programs:

1. Inadequate roughage in the diet
2. Diets containing rapidly-fermentable grains, such as wheat and barley or processed grains, such as steam-flaked or high-moisture corn
3. Long feeding duration such as that observed with dairy calves raised for beef production

A more direct evidence that *F. necrophorum* in liver abscesses originate from the rumen was obtained by DNA fingerprint analyses of isolates from the ruminal contents, ruminal wall, and liver abscesses of cattle (Narayanan et al., 1997). Restriction fragment length polymorphism analysis of ribosomal RNA genes, called ribotyping, was used to genetically compare isolates from the rumen and liver abscesses of the same animal. In case of *F. necrophorum,* the ribotype patterns of liver abscess isolates were

concordant with those of the corresponding isolates from ruminal walls in eight out of nine sets of samples that were compared. None of the ruminal content isolates matched with the liver abscess isolates. The lack of genetic similarity between ruminal content and liver abscess isolates is because ruminal contents have a number of *F. necrophorum* strains and among those that penetrate the ruminal wall, the strain that survives and colonizes the ruminal wall – essentially an enrichment step – is more likely to reach the liver. The genetic similarity between isolates from liver abscesses and ruminal walls, which suggests a clonal connection, supports the hypothesis that *F. necrophorum* isolates of liver abscesses originate from the rumen.

Although a number of bacteria can enter the ruminal epithelium, *F. necrophorum* is more likely to survive and proliferate because of the protection afforded by the secreted virulence factors. In the epithelium, micro abscesses are formed from which bacterial cells find their way into the blood circulation to enter into the portal blood. Once the organism colonizes the rumen epithelium, penetrates, and enters the portal circulation to reach the liver, the infected epithelial cells of the rumen or the endothelial cells of the hepatic sinusoids can either defend and eliminate the bacteria, tolerate and spread the infection or die. The outcome is generally dependent on the dose and virulence of the organism. A number of bacterial virulence factors contribute to the adhesion, colonization, host defense evasion, spread and tissue damage to cause abscesses. The organism possesses or secretes a number of virulence factors implicated in the pathogenesis, which include leukotoxin, endotoxic lipopolysaccharide (LPS), hemolysin; hemagglutinin, capsule, adhesins or pili, platelet aggregation factor, dermonecrotic toxin, and several extracellular enzymes, chiefly proteases and deoxyribonucleases. All these factors, in concert, contribute to the creation of anaerobic microenvironment in the host tissue, adhesion, colonization, proliferation, establishment of the infection, and destruction of the tissue that lead to the development of abscesses. The two major factors that contribute abscess development are leukotoxin and endotoxic lipopolysaccharide (Table 3).

A question that is often raised is, besides rumen, could hindgut also serve as a source? In cattle fed high grain diets, ruminal microbial and fermentative dysbiosis associated with acidosis have received a great deal of attention (Nagaraja and Titgemeyer, 2007). However, there is evidence that in high grain diet-fed cattle, post ruminal flow of starch results also in hindgut acidosis (loose and frothy feces, increased mucous or mucin cast, etc.; Gressley et al., 2011). The alterations in microbial population and onset of dysbiosis in response to acidotic diets, resulting in losses of richness and diversity and accumulation of toxic products, such as endotoxin, biogenic amines, in the hindgut are similar to that of the rumen (Li et al., 2012; Plaizier et al., 2017). A major difference between the two regions of the gut is that the rumen is lined by a stratified (four layers) squamous epithelial cells compared to a single layer of columnar epithelial cells in the hindgut (Figure 4).

Control. The control of liver abscesses in feedlot cattle has largely with the inclusion of antimicrobial compounds in the feed combined with prudent nutritional management to minimize occurrence of ruminal acidosis and subsequent rumenitis.

Tylosin, a macrolide, is the most effective antibiotic and the most commonly used feed additive (8 to 10 g/ton to provide 60 to 90 mg^{-1} $animal^{-1}$ day^{-1}) in the feedlot. The mode of action of tylosin is believed to be its inhibitory effect on *F. necrophorum* in the rumen, in the liver or both (Nagaraja and Lechtenberg, 2007). A meta-analysis on liver abscess risks of cattle receiving tylosin vs. cattle not receiving tylosin in conventional feeding systems showed that the feeding of tylosin reduced the risk of liver abscesses from 30 to 8% (Wileman et al., 2009). The incidence of liver abscesses in tylosin-fed cattle may be because of the development of resistance in *F. necrophorum* or abscesses caused by bacteria other than *F. necrophorum.* In a study that compared the antimicrobial susceptibilities of bacterial isolates between liver abscesses of cattle that originated from feed yards that fed tylosin or no tylosin, the mean minimum inhibitory concentrations of tylosin to *F. necrophorum* and *T. pyogenes* were not different between the two groups (Nagaraja et al., 1999; Amachawadi et al., 2016). Although tylosin is widely used in the feedlot industry, there is considerable interest in evaluating antibiotic alternatives, such as essential oils and vaccines, to control liver abscesses. Elwakeel at al. (2013) evaluated 5 essential oils (eugenol, vanillin, thymol, guaiacol, and limonene) and of a commercial product, CRINA (DSM Nutritional Products, Parsipanny, NJ) on the growth of *F. necrop*horum and observed that limonene, at 20 or 100 µg/mL, and thymol, at 100 µg/mL, inhibited *F. necrophorum* growth, whereas eugenol, guaiacol, vanillin, and CRINA had no effect. The failure of CRINA to inhibit *F. necrophorum,* was likely because of low concentrations of limonene and thymol in the product. The antimicrobial activity of essential oils is attributed to the disruption of the cytoplasmic membrane of the bacterial cells. In a feedlot study in cattle fed a finishing diet, inclusion of CRINA containing limonene and thymol tended to reduce the incidence of liver abscesses compared to the control, but the difference was not significant (Meyer et al., 2009). Because liver abscess is a bacterial infection and the pathogenicity and virulence factors of *F. necrophorum* have been studied widely for many years, there has been considerable interest and efforts to develop an effective vaccine (Nagaraja and Chengappa, 1998). The use of vaccines has dual benefits; control of liver abscesses and also alleviates public health concerns associated with the continuous use of medically-important antimicrobials in the feed. Thus far two vaccines have reached commercial application. One was a *F. necrophorum* bacterin (Fusogard, Elanco Animal Health) approved for the control of liver abscesses and foot rot in cattle. The second vaccine (marketed as Centurion by Merck Animal Health, Omaha, NE) was a combination of leukotoxoid of *F. necrophorum* and a *T. pyogenes* bacterin, which was shown to reduce the prevalence of liver abscesses in feedlot cattle (Jones et al., 2004). However, this vaccine is no longer commercially available.

Summary and Conclusions

Liver abscesses continue to be of significant economic concern to the feedlot industry. The incidence of total liver abscesses, particularly the severe form (A+), is greater beef-on-dairy and calf-fed Holstein steers raised for beef production than in beef breeds, but reasons are not known. The prevalence of isolation of *Salmonella enterica* in liver abscesses is a novel finding, but the role and the importance need to be investigated. Although tylosin is widely used to control of liver abscesses, the use is

under veterinary oversight. There is considerable interest in evaluating antibiotic alternatives, such as essential oils, probiotics, and vaccines, to control liver abscesses. Leukotoxin, an exotoxin and an outer membrane protein of *Fusobacterium necrophorum* have been the target antigens investigated for the development of vaccines. However, an efficacious vaccine has not been developed yet.

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Table 1. Differences between *Fusobacterium necrophorum* subsp. *necrophorum* and subsp. *funduliforme*

Criteria	**Subsp. *necrophorum* (Biotype A)**	**Subsp. *funduliforme* (Biotype B)**
Cell morphology	Pleomorphic filaments (2 - 100 µm)	Short curved rods (1 - 10 µm)
Colony morphology	Smooth, opaque, and raised with irregular edges	Small, waxy, yellowish, raised, and sticky
Sedimentation in broth (growth)	No	Yes
Phosphatase enzyme	Positive	Negative
Agglutination of chicken erythrocytes	Positive	Negative
Leukotoxin	High	Low or absent
Pathogenicity in mice	+++	+/-

Adapted from Tadepalli et al. (2009).

Table 2. Frequency of isolations of the two subspecies of *Fusobacterium necrophorum* from liver abscesses of cattle

Studies	Cattle type	No. of abscesses cultured	No. of abscesses yielding *Fusobacterium necrophorum* (%)	
			Subsp. *necrophorum*	Subsp. *funduliforme*
Lechtenberg et al., 1988	Feedlot cattle	49	28 (57.1)	23 (47.9)
Nagaraja et al., 1999	Feedlot cattle	77	72 (93.5)	24 (31.2)
Purvis, 2006	Culled dairy cows	57	49 (86)	17 (29.8)
Amachawadi et al., 2017	Cross-bred feedlot cattle	175	175 (100)	38 (21.7)
	Calf-fed Holstein steers	208	208 (100)	35 (16.8)
Herrick et al., 2022	Feedlot cattle	189	151 (79.9)	46 (24.3)
	Culled cattle[a]	91	70 (76.9)	16 (17.6)
Total		**846**	**753 (89.0)**	**199 (23.5)**

[a] Includes culled dairy cows, bulls and beef cows

Table 3. Major virulence factors of *Fusobacterium necrophorum*

Factor and Characteristics	Mechanism of action	Role in pathogenesis
Leukotoxin: A secreted protein of high-molecular weight	Cytotoxic to neutrophils, macrophages, hepatocytes, and ruminal epithelial cells.	Protects against phagocytosis by neutrophils and kupffer cells, damages hepatic parenchyma by the release of cytolytic products.
Endotoxin: Lipo-polysaccharide of the outer membrane	Necrotic effect and induces disseminated intravascular coagulation.	Creates anaerobic micro environment conducive for anaerobic growth.

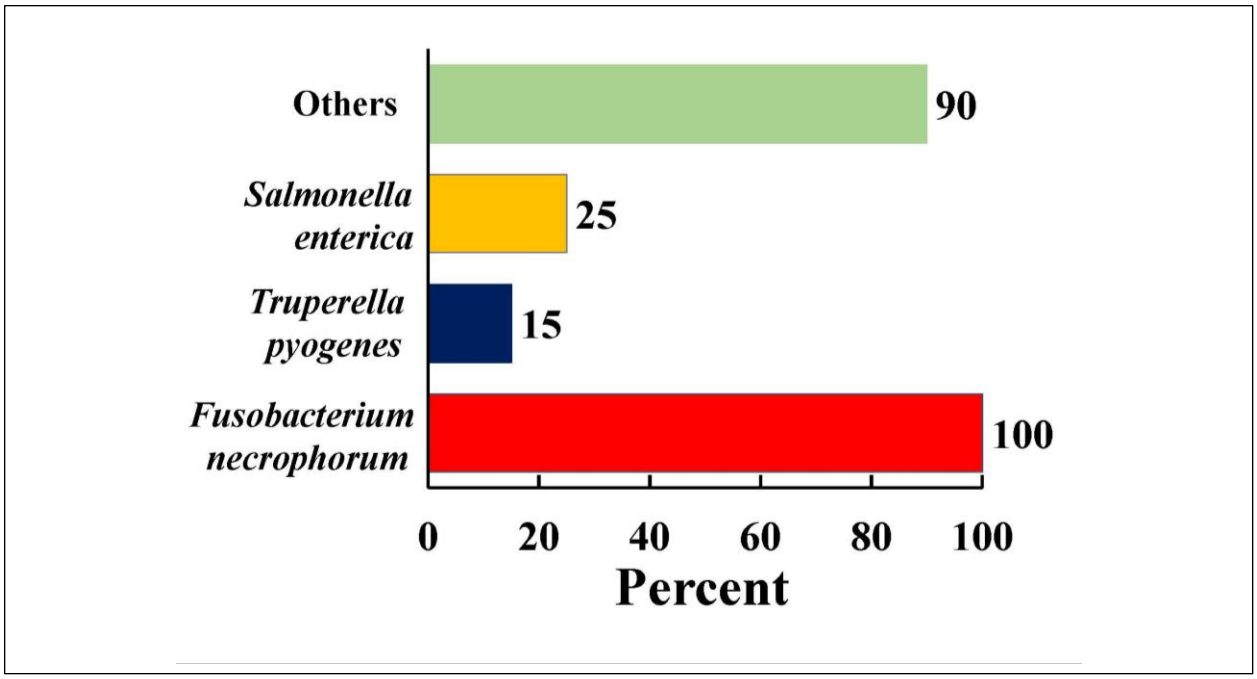

Figure 1. Major etiological agents of liver abscesses of feedlot cattle.

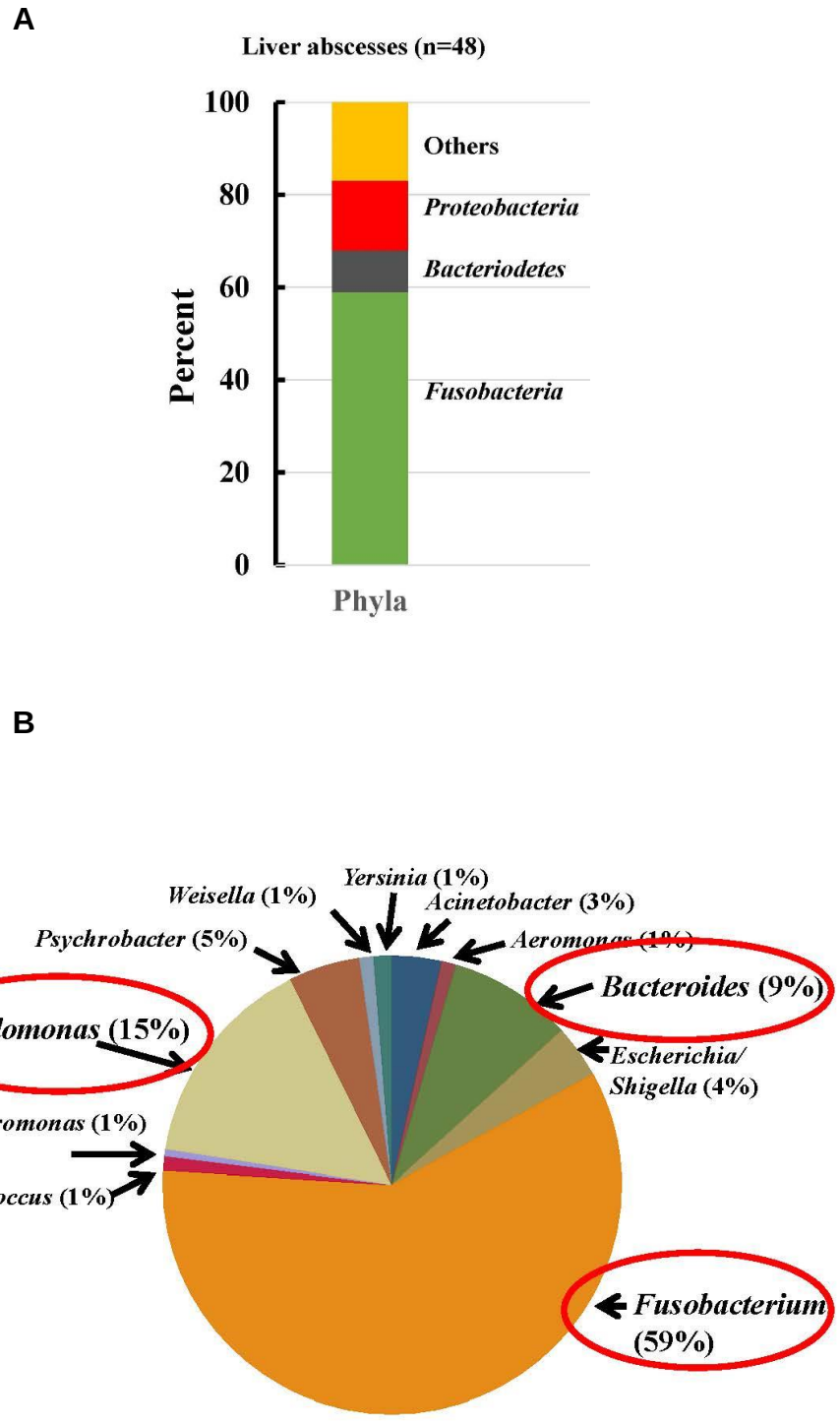

Figure 2. Relative abundance of the bacterial phyla and genera in liver abscesses from cattle high-grain finishing diets (Adapted from Amachawadi et al., 2021).

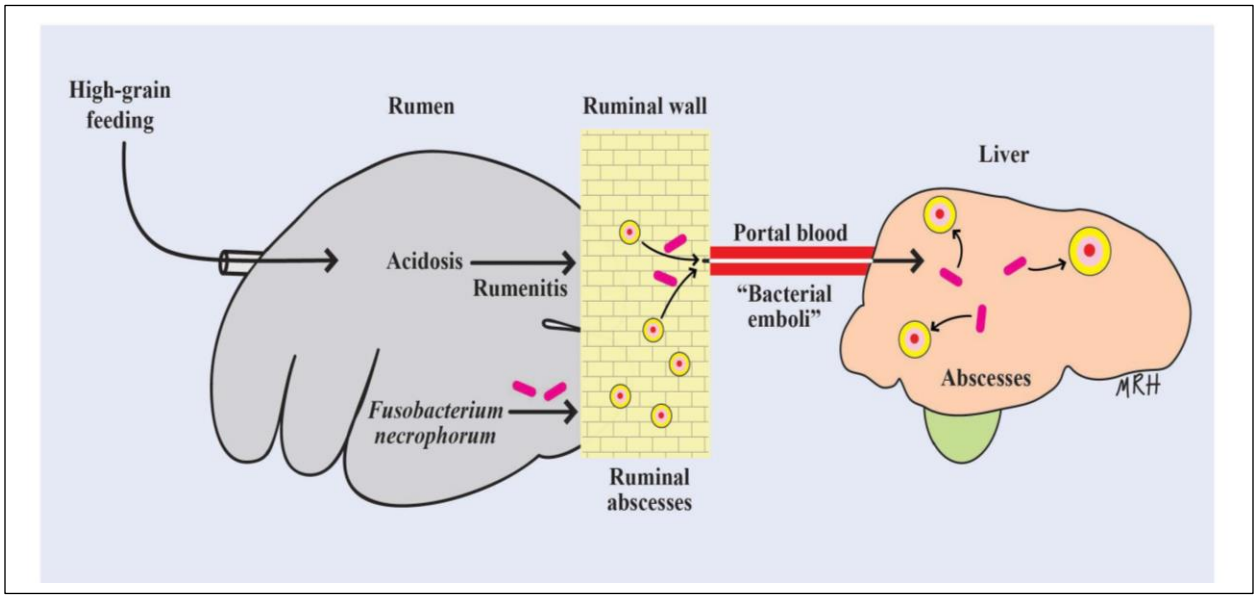

Figure 3. Pathogenesis of liver abscesses in feedlot cattle.

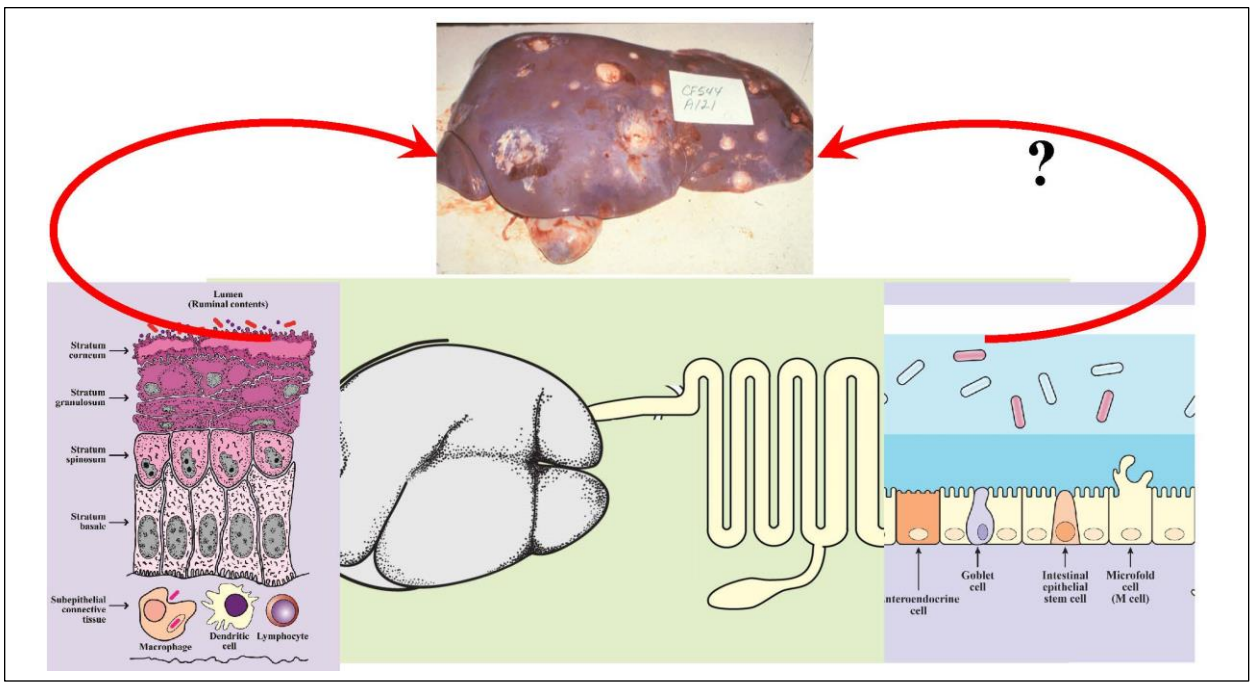

Figure 4. Ruminal vs hindgut as sources of bacterial pathogens (Adapted from Amachawadi and Nagaraja, 2022).

SESSION NOTES

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[1] Zenobi et al., 2018 J. Dairy Sci. 101 (Suppl. 2): 334, Zenobi et al., 2018 J. Dairy Sci. 101 (Suppl. 2): ii, Zenobi et al., 2018 ADSA, Late-Breaking Original Research, #LB5

Ruminal acidosis and lipopolysaccharides: a holistic outlook

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University of Florida

Introduction

Lipopolysaccharides (**LPS**) are outer membrane components of Gram-negative bacteria, that are comprised by three covalently linked regions: the O-antigen, the core oligosaccharide, and the lipid A, whose structure primarily mediates the immunogenicity and the intensity of the intracellular signaling to the host, as well as, the growth of ruminal bacteria (Dai et al., 2020; Sarmikasoglou et al., 2022). More specifically, structural variations in the lipid A, are characterized by diversity in the acylation pattern and are associated with different immunogenicity and growth effects at the host and microbiome, respectively (Sarmikasoglou and Faciola, 2022). Specifically, the hexa-acylated lipid A are found in virulent strains, whereas the penta- and tetra-acylated ones are found in non-virulent or commensal bacterial strains.

With regards to the host responses, the aforementioned structural variation elicits strong or weak activation of the pattern recognition receptors (**PRRs**), especially toll like receptor 4 (**TLR4**) that are located on the host cell membrane. Thus the hexa-acylated lipid As are eliciting strong and the under-acylated lipid As are eliciting weak immune responses (Steimle et al., 2016). Additionally, under-acylated lipid As have been previously reported to antagonize the hexa-acylated ones on host cell receptor binding, thus, LPS produced from bacteria, such as commensals, that express under-acylated lipid As can exhibit weak interaction with the TLR4 and could maintain the homeostasis to the host by inhibiting the severe inflammation from the hexa-acylated ones (Steimle et al., 2019). Several reports indicate that elevated levels of LPS in blood plasma are associated with heart failure, obesity and metabolic diseases, in humans (Fabbiano et al., 2018; Pastori et al., 2022), as well as ruminal acidosis in cattle (Nagaraja and Titgemeyer, 2007).

Concerning the responses of LPS to the ruminal microorganisms, scarce amount of studies have been published in that area; however, their findings concluded that LPS growth effect is evident. More specifically, *E. coli* LPS has been used as a factor to stimulate the growth of *Anaeroplasma abactoclasticum* (*An. abactoclasticum*) strain 6-1 (Robinson et al., 1975) and exhibited stimulatory properties on the growth of *Streptococcus bovis* JB1 (Dai et al., 2020). On the other hand, other studies have been shown that the LPS extraction method could affect any potential stimulatory or inhibitory functions of *E. coli* LPS (Robinson et al., 1975; Sarmikasoglou et al., 2022). For this reason, further research needs to be conducted towards the understanding of the mechanisms associated with LPS effects on ruminal bacteria growth.

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Both the host-host and microbe-host interactions are important to be investigated so we could understand the mechanisms of several metabolic disorders associated with elevated levels of LPS in cattle, such as ruminal acidosis. Ruminal acidosis is a metabolic disorder that occurs when the consumption of rapidly fermentable carbohydrates replaces effective fiber thus causing the excessive accumulation of organic acids (volatile fatty acids and lactate) in the rumen. Ruminal pH ≤ 5.6 is the threshold of ruminal acidosis, where pH 5 – 5.6 is subacute acidosis (**SARA**) and pH below 5 is considered as acute acidosis. Under SARA there is an excessive lysis of ruminal bacteria, thus greater amount of free- LPS is released in the rumen. Under ruminal acidosis, LPS concentration has been reported to be 4- to 16-times greater than normal conditions, contributing to systemic inflammation by disrupting the epithelial barrier function, thus increasing the permeability of ruminal epithelium (Aschenbach and Gäbel, 2000; Meissner et al., 2017; Monteiro and Faciola, 2020), allowing the translocation of LPS into the peripheral tissue (Aschenbach et al., 2019).

In this review, we examined the literature of prevailing mechanisms of host immune tolerance to symbiotic microorganisms in cattle, discuss recent findings regarding LPS growth effect on pure ruminal bacteria cultures, and finally suggest directions for future research towards understanding the association of ruminal LPS with the development of ruminal acidosis.

Structure of Ruminal LPS

Among Gram-negative bacteria species, there is structural diversity on the lipid A region (Coats et al., 2005; Lodowska et al., 2007). Specifically, the number of acyl chains on the lipid A moiety is directly correlated with its ability to induce cytokine production where the hexa-acylated forms are usually the most immunostimulant, contrary to the under-acylated ones (penta- or tetra-acylated) that result in weak host inflammatory responses (Munford and Varley, 2006). Ruminal bacteria composition is determined by several factors, including the diet (Matthews et al., 2019). In general, cows fed high forage diets contain more Gram-negative bacteria, whereas cows fed high grain diets contain more Gram-positive bacteria (Matthews et al., 2019). In the rumen, Gram-negative bacteria are the major source of LPS (Nagaraja and Lechtenberg, 2007).

The presence of LPS in the ruminal fluid is normal since bacterial death and lysis are normal processes that take place during ruminal fermentation; however, under SARA conditions, ruminal LPS concentration is much greater compared to healthy cattle (Nagaraja et al., 1998). In the rumen, the most predominant Gram-negative phylum is Bacteroidetes ~50%, even under SARA conditions (Plaizier et al., 2017) and from previous reports conducted in humans and cows seems that express under-acylated lipid A (d'Hennezel et al., 2017; Sarmikasoglou et al., 2021). More specifically, a preliminary study by Sarmikasoglou et al., 2021 determined the mass of lipid A derived from the rumen microbiome of cows fed Low- and High- forage diets and found that both lipid A sources exhibited dominant mass peaks on the mass range where under-acylated lipid A structures detected (**Figures 1 & 2**). These findings are consistent with previous microbial population studies which suggest that the free ruminal LPS seems to

be produced by non-*E. coli* gram-negative bacteria species (Khafipour et al., 2011). Overall, we found that under healthy conditions the dominant lipid A produced from the ruminal microbiome is under-acylated, contrary to the one produced from the E. coli which is hexa-acylated. Further research needs to focus on elucidating the dominant lipid A acylation pattern of ruminal bacteria under SARA conditions and elaborate on the main contributors of it in specie level.

Ruminal LPS and Host Interactions

The reticulo-rumen and omasum epithelium consist of a stratified squamous epithelium. Leaflike papillae (~ 15 mm) with characteristic ridges and hollows on their surface are expanded to the lumen and extend their total surface area (Graham and Simmons, 2005). From the luminal surface, morphologically we can distinguish four cell layers; the stratum corneum (**SC**), the stratum granulosum (**SG**), the stratum spinosum (**SS**), and the stratum basale (**SB**) (Steven and Marshall, 1970). The SC is in direct contact with the ruminal and omasal contents and is intensely keratinized (Steele et al., 2011). The highly keratinized areas of SC continuously shed off and regenerates thus providing a dynamic protective barrier between the ruminal contents and the lower living strata (Steele et al., 2016). Still the underlying mechanism on how cells differentiate and migrate from the SB and SS to the SG and finally to SC remain to be elucidated. Lastly, the reticulo-rumen epithelium seems to have less specialized cells thus its ability to secrete antimicrobial compounds or interact with the luminal environment seems limited and remains to be elucidated.

Yet, no established association has been reported between the development of ruminal acidosis and ruminal LPS concentration (Stefanska et al., 2018); however, PRRs, such as TLRs have been reported to be expressed in cells within the bovine ruminal tissue (Malmuthuge et al., 2012). More specifically, previous studies found that primary rumen epithelial cells (**REC**) exhibit greater expression of genes associated with TLRs, such as TLR2, and TLR4, as well as, proinflammatory cytokines, such as IL1β, TNFα, and CXCL8, after stimulation with *E. coli* LPS (Zhang et al., 2016; Kent-Dennis et al., 2020), indicating the presence of TLR4s on their cell membranes. Since the total ruminal LPS is not structurally equivalent to *E. coli*-LPS, primarily, because the former exhibits under-acylated (low endotoxic) and the latter hexa-acylated (high endotoxic) lipid A structures (Sarmikasoglou et al., 2021), further investigation on the immunopotential of ruminal microbiome derived LPS to REC is on demand.

Considering that, our lab conducted a study where primary REC were exposed for 6 h to ruminal LPS and evaluated their immune responses. Ruminal LPS was collected from two-cannulated beef steers that have been adapted to a grain-induced acidosis diet two weeks before rumen fluid collection. On the last day of this two-week period, the ruminal fluid was collected and centrifuged three times in succession to acquire the bacterial pellet, as it has been previously described by Sarmikasoglou et al., (2022).

A modified hot-phenol extraction was utilized to extract LPS from ruminal bacteria obtained from the rumen-cannulated steers as described previously by Sarmikasoglou

et al., (2021). Briefly, to isolate total LPS from ruminal fluid, the bacterial pellet was boiled and then treated with 90% phenol. The aqueous layer was then transported into a regenerated cellulose dialysis membrane for further dialysis against Milli-Q until phenol was not detectable at 260 nm in Milli-Q. Dialyzed samples were then treated with 5 mM $MgCl_2$ followed by 20 µg/mL Dnase I for 2 h at 37 °C to degrade contaminating DNA. After, 20 µg/mL Rnase H was added for 2 h at 37 °C, to degrade contaminating RNA and, lastly 30 mg/mL Proteinase K was added to degrade any proteins. The preparation was then lyophilized and crude LPS mass was determined. After lyophilization, dry samples were resuspended into Milli-Q water and the supernatant was treated with 50 mM acetic acid, 95% ethanol and transferred into ultracentrifuge tubes and then spun for 8 h at 4 °C. The LPS gels were resuspended in endotoxin-free water and lyophilized to determine the dry weight of pure LPS. To confirm the purity of ruminal-derived LPS, the final products were visualized with the Pierce™ Silver Stain Kit in accordance with the manufacturer's instructions. In all cases, the Pierce™ Silver Stain Kit indicated a purity identical to that of LPS purified from pure bacterial isolates.

With regards to the primary REC culture, we collected rumen tissue from 6 yearling steers (approximately 10-mo-old) group housed in an outdoor, dry-lot pen. Steers had *ad libitum* access to water and bermudagrass hay. Approximately 15 min post slaughtering, ruminal tissue from ventral sac was excised and washed with ice-cold Ca^{2+}- and Mg^{2+}-free phosphate buffered saline solution (**PBS**) containing antibiotic-antimycotic cocktail. Antibiotic-antimycotic cocktail composed of 400 U/mL penicillin, 400 µg/mL streptomycin, 1 µg/mL amphotericin B and nystatin, 240 U/mL, as final concentrations, respectively. Then the washed ruminal tissue submerged into the same solution and kept in ice until further REC isolation.

Isolation and cultivation of REC was done essentially as it has been previously described (Kent-Dennis et al., 2020). Ruminal papillae were cut off at their base, chopped into small pieces and washed with Ca^{2+}- and Mg^{2+}-free PBS containing an antibiotic-antimycotic cocktail. Papillae small pieces were subjected to serial trypsinization using a trypsin-EDTA solution. Papillae were agitated in the trypsin-EDTA solution at 37°C and the resulted supernatant was collected and replaced with fresh solution every 30 min. The process was repeated 6-times in total, and only the 3 to 6 fractions were separately strained through sterile gauze, pooled and resuspended in M199 cell culture media, 15% fetal bovine serum, 1X GlutaMAX™, 20 mM HEPES, and an antibiotic-antimycotic cocktail. Cell suspensions were seeded into 60-mm cell culture dishes coated with bovine collagen and placed in an incubator with constant 37°C and 5% CO_2 humidified atmosphere. The following day cells were washed with PBS containing Ca^{2+}- and Mg^{2+} and fresh media was added. The M199 cell culture media was replaced with minimum essential media (**MEM**) containing 10% fetal bovine serum, 1X GlutaMAX™, and an antibiotic-antimycotic cocktail. The MEM media was then replaced every other day.

Before the start of the experiment, we validated that the isolated RECs are not highly contaminated with fibroblasts. A threshold of acceptable fibroblast contamination

was that REC cultures would exhibit < 10% of CD90-positive cells, similar to previous studies (Kisselbach et al., 2009). We cultured the isolated RECs from each REC-donor for 10 d and then a subset of cells from each REC-donor was trypsinized to detach the cells and resuspend them in PBS- 0.25% BSA solution. Then the cells resuspended again to PBS-0.25% BSA solution. After the cells were filtered through a 40μm cell strainer and the filtrate was centrifuged to pellet the cells. The cell pellet was resuspended in 3 mL PBS-0.25% BSA. Then 1 mL of cells was mixed with 1 μL FITC conjugated mouse anti-human CD90 and left on ice for 20 min, washed with 10 mL of PBS-0.25% BSA solution. The cell pellet was resuspended in 1 mL PBS-0.25% BSA solution and loaded to flow cytometer. Percentage of fibroblast contamination was then determined by flow cytometry using an Attune NxT flow cytometer based on a minimum of 10,000 events, and data analysis was performed with FlowJo version 10.0.7. All of our isolated RECs (n = 6) exhibited < 10% of CD90-positive cells. Mice fibroblasts used as positive control and exhibited 14% CD90+ cells.

The isolated REC were exposed to nonpyrogenic water (**CON**), 20 μg/mL of *E. coli* O111:B4 LPS (**E. COLI**), 10 μg/mL of total ruminal-LPS (**RUM10**), 20 μg/mL of total ruminal-LPS (**RUM20**) and 40 μg/mL of total ruminal-LPS (**RUM40**) for 6 h.

More specifically, the continuous exposure of REC to LPS treatments was for 6 h, with 3 technical replicates per treatment, and 1 plate for each biological replicate of isolated REC. Following exposure to LPS treatments, 1 well for each treatment was trypsinized and cells were immediately analyzed for viability using propidium iodide staining to determine the percentage of dead cells with a flow cytometer. The remaining 2 wells for each treatment were lysed in 1 mL of Trizol and stored at −80°C until further RNA extraction.

With regards to the results, no effects were found on cytotoxicity assay; however, we observed that all ruminal LPS treatments did not upregulate the genes related to inflammation, contrary to the *E. coli* LPS that upregulated the genes associated with innate immune response. Therefore, we can conclude that the immuno-potential of ruminal LPS is lower to *E. coli* LPS, which further supports our previous findings that structurally, ruminal LPS differs from the *E. coli* LPS (Sarmikasoglou et al., 2021).

Ruminal LPS and Ruminal Microorganisms Interactions

To sustain the microbial community and its functions, metabolic interactions develop among the different microorganisms that reside in the ruminal and intestinal microbiomes. As a general rule, previous reports have shown that bacterial strains that are more abundant in the rumen are able to grow faster compared to strains that are less abundant and that the less abundant strains' growth is stimulated by the addition of growth factors, such as greater supply of certain B vitamins (van Glyswyk et al., 1992). More specifically, some ruminal microorganisms develop a commensal interaction where, some ruminal bacteria produce metabolites that are required for the growth of others. For example, l,4-naphthoquinone (Gomez-Alarcon et al., 1982) and heme (Caldwell et al., 1965) are produced by some ruminal microorganisms as by-products

but are required for the growth of other fermentative species such as *Succinivibrio dextrinosolvens*, and *Bacteroides ruminicola* (Wolin et al., 1997).

Other interactions pertain to nitrogen utilization, carbohydrate fermentation, as well as amensalistic interactions. Amensalistic interactions appeared when the metabolite of an organism is inhibitory to the growth of another (M. Alexander et al., 1972). Amensalistic interactions in the rumen are limited and mostly referred to the chitinolytic bacteria such as *Ruminococcus flavefaciens* and *R. albus* (Stewart et al., 1992; Bernalier et al., 1993), as well as to the bacteriocin-producing bacteria such as *S. bovis* (Iverson and Millis, 1976; Odenyo et al., 1994).

To the same extent our team has been interested in investigating the potential effects of ruminal LPS on the growth of bacteria associated with the development of ruminal acidosis. At first, we conducted a study dosing 200,000 EU of *E. coli* LPS to the growth media of the lactate producing bacteria (S*. bovis and Selenomonas ruminantium*) and the lactate utilizing bacterium (*Megasphaera elsdenii*), and found that the *E. coli* LPS stimulate the growth of the lactate producers. Then we implemented the same study by dosing ruminal LPS, where we found that ruminal LPS suppress the growth of lactate producers and that the *E. coli* LPS stimulatory effect should be further elucidated between different strains. Despite the fact that the mode of action that allows ruminal LPS to suppress the growth of ruminal bacteria remains to be elucidated, our findings suggest a potential amensalistic interaction between the LPS derived from the ruminal microbiome and the lactate-producing bacteria *S. bovis* JB1 and *S. ruminantium* HD4.

Those studies validate the idea that ruminal LPS plays a role in the development of ruminal acidosis by affecting the growth of ruminal bacteria associated with it. This hypothesis; however, has not been tested empirically since little consideration has been given to the role of LA composition and the effects of its potential microbe-microbe interactions.

Conclusions

In summary, our experimental holistic approach identified that mixed ruminal LPS (***i***) exhibit under-acylated lipid A structures contrary to hexa-acylated lipid A, typically expressed by commercially available LPS, such as from *E. coli*, (***ii***) suppress the expression of genes associated with inflammation, and (***iii***) slow down the growth and/or decreased the production of total OAs, acetate, and lactate in lactate -producing bacteria (*Se. ruminantium* HD4, *S. bovis* JB1), and do not affect lactate-utilizing bacterium (*M. elsdenii* T81). Overall, we can conclude that ruminal LPS seems to mitigate, and not exacerbate, the development of ruminal acidosis, potentially by weakly stimulating the inflammatory response of REC and suppressing the growth of bacteria associated with it. Lastly, further research should focus on the potential effects of diet on the expression of different lipid A acylation patterns in ruminal LPS, and the evaluation of the underlying mechanisms that allow ruminal LPS to suppress the growth of ruminal lactate producing bacteria.

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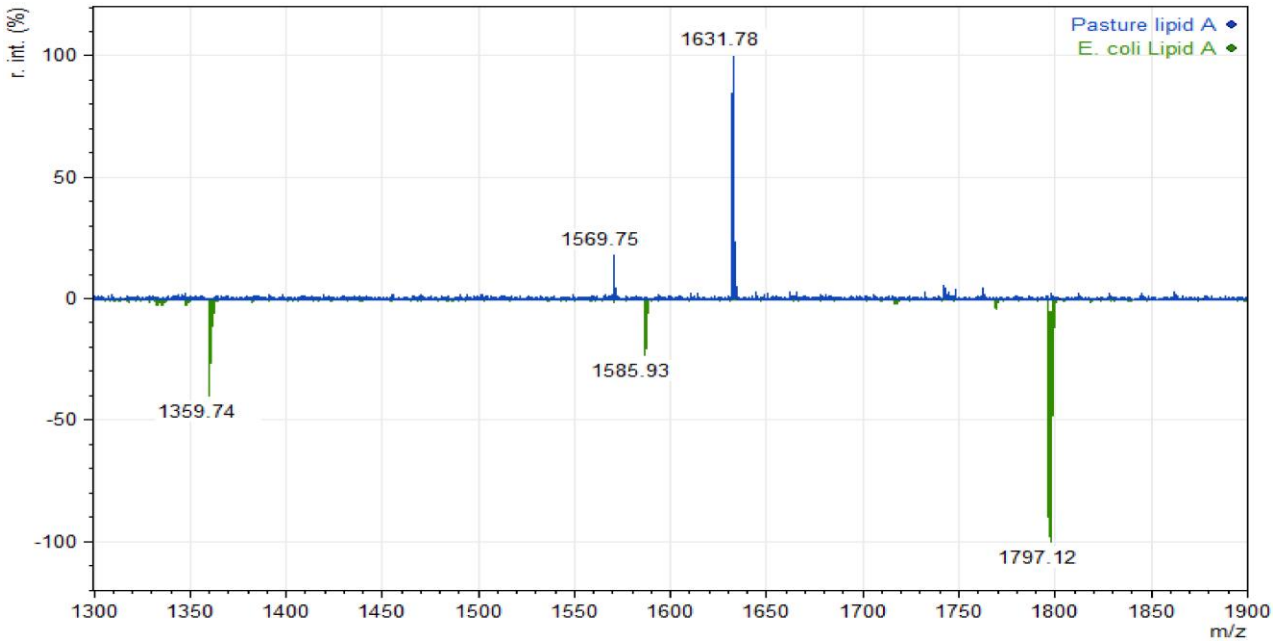

Figure 1. Comparison of MALDI-TOF MS profiles of TMR and E. coli lipid A. In comparison to lipid A from E. coli standard (bottom), lipid A from Low forage-fed cow (top) exhibits tetra-acylated structure. m/z = mass-to-charge ratio.

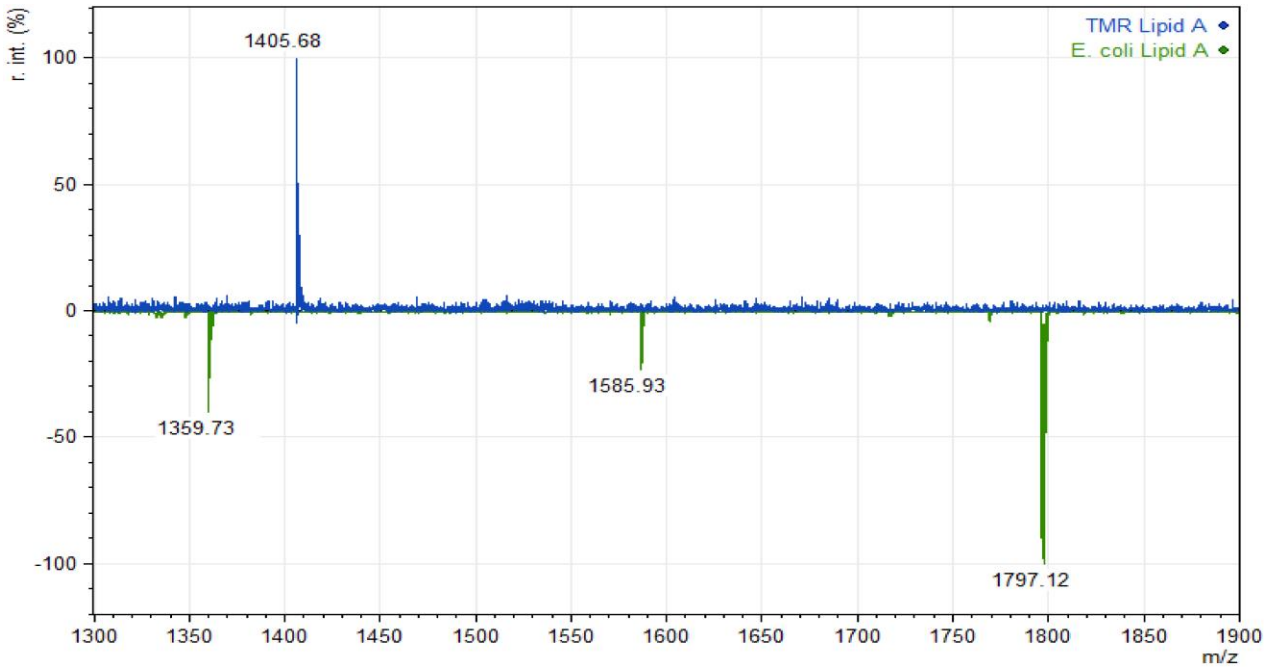

Figure 2. Comparison of MALDI-TOF MS profiles of pasture and E. coli lipid A. In comparison to lipid A from E. coli standard (bottom), lipid A from High forage-fed cow (top) exhibits penta-acylated structure. m/z = mass-to-charge ratio.

SESSION NOTES

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Modeling and Integrating Metabolizable Energy and Protein Supply and Requirements in Lactating Dairy Cattle to Optimize Nitrogen Utilization

Mike Van Amburgh[1], Andres Ortega, and Andrew LaPierre
Cornell University

Introduction

Improving the prediction of supply and use of metabolizable energy (ME) and protein (MP) is dependent on several factors that can be measured routinely or predicted with reasonable precision. The prediction of ME is dependent on factors such as total feed intake, the chemical composition of the feed consumed, and ruminal and post-ruminal digestibility and the cost of metabolism of excess nitrogen (N). The prediction of MP is dependent on the same factors, although MP is more complex as it is highly dependent on the quantity, profile and digestibility of amino acids (AA) that escape the rumen, whereas substrates for ME can be absorbed anywhere along the GIT. Recognizing how those substrates are partitioned differ as they are absorbed farther down the GIT. Feed protein is one of the most expensive macronutrients in dairy cattle diets and overfeeding degradable protein relative to rumen requirements results in excessive N losses to the environment (Huhtanen and Hristov, 2009). Efficient use of feed N can be achieved by first meeting the requirements of the rumen microbial population, followed by balancing diets to meet the AA requirements of the cow. To decrease competition for quality protein that could otherwise be fed to humans, dairy cattle are fed byproducts of human food production, thereby converting waste products into highly valuable milk protein and other nutrients.

To frame the thesis of this paper, the modeling approach used in Cornell Net Carbohydrate and Protein System (CNCPS) v7 will be utilized to describe the relationships and accounting necessary to integrate ME, MP and AA supply and requirements (Tylutki et al., 2008; Higgs, 2014; Van Amburgh et al., 2015; Higgs et al, 2023). There are at least five major steps necessary to improve the prediction of MP and AA supply and requirements in a lactating and dry cow. Those five areas are the use, characterization and application of crude protein, recycled urea and endogenous protein, intestinal digestibility and determining first limiting nutrients through integration of AA requirements with ME supply. This paper will focus on the integration of AA with ME and provide some examples of cattle responses to diets formulated in this manner.

Concept of first limiting nutrient and integration of ME and AA

Using the information presented so far, the AA supply can be more accurately described which then allows for calculations of requirements on a more refined basis.

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Requirements for each individual essential AA (EAA) in the CNCPS v7 are predicted for processes that are quantified by the model (maintenance, lactation, pregnancy, growth) and subsequently divided by the efficiency of transfer to that process to give the total AA requirement (O'Connor et al., 1993; Fox et al., 2004). The efficiency of transfer could also be thought of as the additional requirement for each AA relative to the requirements quantified by the model. Such processes include oxidation across the gut or in other tissues, anaplerotic requirements, synthesis of non-essential AA, gluconeogenesis, etc. (Lapierre et al., 2005; Lapierre et al., 2006; Lemosquet et al., 2010; Lobley, 2007). The apparent efficiency of AA use for any given diet can be calculated by dividing model predicted amino acid requirement (AAR) by amino acid supply (AAS), which can be variable, and typically decreases as AAS increases relative to either AAR or metabolizable energy (Hanigan et al., 1998).

This decrease in apparent efficiency of AA use represents AA being increasingly used for purposes other than those quantified or described by the model. If the utilization of each AA for every process in metabolism could be adequately quantified, the term 'efficiency of use' would become obsolete as it would be 100% (there would be no additional requirement above model predictions). The ability of cows to direct AA to other uses demonstrates the interactions among different nutrients and is an example of the metabolic flexibility that allows productivity to be maintained across a wide range of nutrient inputs and supply (Lobley, 2007). The pertinent question for ration balancing is: what level of additional AA supply is required above the predicted requirements for milk protein synthesis and body protein requirements to maximize productivity and minimize AA wastage? The answer to this question is going to differ among models as supply and requirements are calculated in different ways.

The optimum supply of EAA in v7 was estimated similarly to Doepel et al. (2004) using a dataset of studies that infused AA into the abomasum, duodenum, or intravenously and fitted a logistic curve (Higgs, 2014). The optimum supply of each EAA was defined as the point in which a logistic curve was approaching plateau most rapidly (Lysine example; Figure 1). This point is similar to the breakpoint in the segmented linear model used in the NRC (2001) but is further integrated with the ME supply to describe the relationship based on the energy driven demand for AA and not just as a percent of protein. The efficiency of use of model predicted AAR to AAS for each AA in v7 are in Table 1. The impact of energy supply on the utilization of AA was also investigated by regressing the efficiency of use of AAR and AAS against AA supply relative to total ME (Lysine example; Figure 2). Interestingly, the optimum supply of Met and Lys estimated using this approach was 15.1% and 5.7% of EAA, respectively, which is similar to results found in other studies that used different approaches (Rulquin et al., 1993; Schwab, 1996; Schwab et al., 1992). However, under these circumstances, no relationship was observed between the 'efficiency' of AA use when AA supply was expressed relative to MP supply, but a strong relationship was observed when AA were expressed relative to ME supply which agrees the findings of Van Straalen et al. (1994). These data suggest that when balancing diets, it might be more appropriate to consider AA supply relative to ME which is an approach used in swine (NRC, 2012). Establishing requirements for monogastric animals is less complicated than in ruminants as the true

AA supply is more easily determined (Lapierre et al., 2007). With the available AA infusion study data and the updated techniques described previously in this paper, AA requirements in the ruminant animal are becoming both more accurate and precise. To extend the comparison of non-ruminant to ruminant, the predicted Lys requirement for a lactating sow in the NRC (2012) model is 2.72 g Lys/Mcal ME which is similar to the 3.03 g Lys/Mcal ME calculated in this study for dairy cows. Likewise, the recommended ratios for each EAA and Lys are similar in the dairy cow and sow except for Met and His (Table 1). These data suggest, as improvements are made to the predictions of true AA supply in dairy cows, consideration of the approach used to balance AA in other species where AA supply is more easily determined could provide opportunities to improve productivity and the efficiency of nutrient use.

Table 1. Efficiency of use and optimum supply of each EAA relative to total EAA, ME and Lys.

AA	Efficiency of use	% EAA	g AA/ Mcal ME	Lys:AA Dairy[1]	Lys:AA Swine[2]
Arg	0.55	10.2%	2.04	1.49	1.85
His	0.70	4.5%	0.91	3.33	2.50
Ile	0.61	10.8%	2.16	1.40	1.78
Leu	0.67	17.1%	3.42	0.89	0.89
Lys	0.62	15.1%	3.03	1.00	1.00
Met	0.53	5.7%	1.14	2.66	3.71
Phe	0.53	10.7%	2.15	1.40	1.82
Thr	0.53	10.7%	2.14	1.41	1.49
Trp	0.58	2.9%	0.59	5.16	5.33
Val	0.62	12.4%	2.48	1.22	1.15

[1] Optimum Lys:EAA ratio for the data set used
[2] Optimum Lys:EAA ratio for a lactating sow (NRC, 2012)

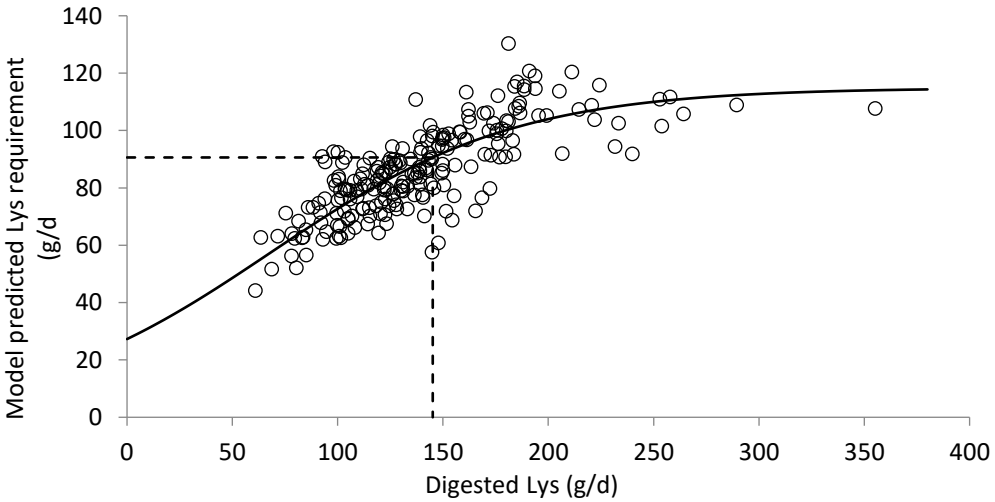

Figure 1. Logistic fit of model predicted Lys requirement and Lys supply. The dashed line represents the optimum ratio of Lys requirement and Lys supply.

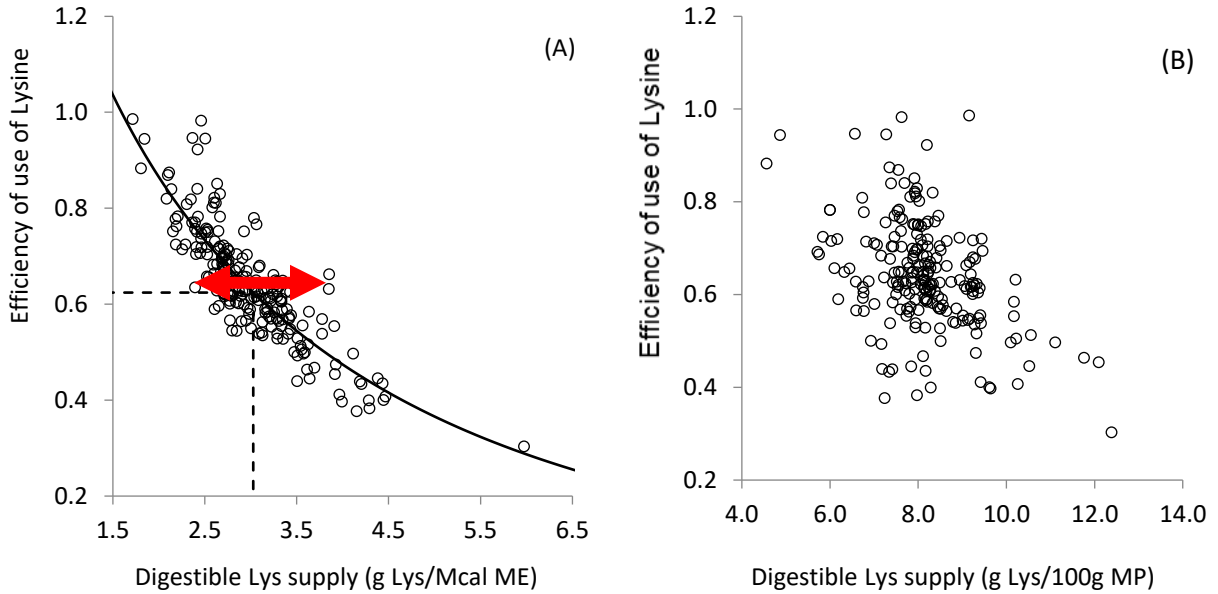

Figure 2. Relationship between model predicted Lys efficiency of use and Lys supply relative to ME (A) or MP (B). The dashed line in (A) represents the Lys supply at the optimum ratio of model predicted Lys requirement and supply. No significant relationship was determined in (B).

Studies Conducted Using This Approach

Four studies have been conducted using this approach where all EAA were evaluated (Higgs et al., 2023; LaPierre et al., 2019, 2020) or Lys and Met were evaluated using this approach through CNCPS v6.55 (Benoit et al., 2021). In all cases, the EAA were formulated on a gram basis per megacalorie ME.

The data from the LaPierre et al. (2019) were based on previous results exploring AA balancing in lactating dairy cattle (Higgs, 2014; Higgs and Van Amburgh, 2016). Findings from the initial study suggest an optimal requirement of each EAA at a given level of metabolizable energy (Table 1) however, variation exists around data, creating ambiguity about their accuracy (Figure 2; red arrow depicting the range in data). To confirm the values, three diets were formulated to be isocaloric and excess in energy to prevent a first-limiting effect on animal performance. The only differences in these diets were in the level of EAA fed, creating differences in the ratios of EAA to metabolizable energy. The Neutral diet (NEU) was formulated to match the optimal ratios determined by Higgs (2014) and Higgs and Van Amburgh (2016), whereas the Positive (POS) and Negative (NEG) control diets were formulated to be one standard deviation above and below the optimal ratio for each EAA (Table 1). One hundred and forty-four (n=144) Holstein cows [26 primiparous and 118 multiparous; 2.9 ± 1.4 lactations; 92 ± 24 DIM at enrollment] were enrolled in a 114 day longitudinal study. Cattle were housed in a freestall setting at stocking density of 100%. Each pen was fed TMR once daily at approximately 0600 h and pens were targeted for 5% refusal rate (Table 2). All nine

pens were fed the POS diet during a 14-day covariate period and randomly assigned to one of three diets described above for the remaining 100 d.

Table 2. Ingredients and chemical composition of experimental diets

Ingredient, % DM	Negative[1]	Neutral	Positive
Corn silage	51.49	51.49	50.40
High moisture ear corn	9.43	9.46	9.93
Triticale	7.25	7.25	7.98
Corn grain	6.38	6.42	5.95
Soybean meal	8.16	5.55	2.72
Soybean hulls	9.25	3.84	2.83
SoyPLUS[2]	--	0.91	3.59
Canola	1.81	9.17	6.31
Urea	0.62	0.51	0.51
Smartamine M[3]	--	0.04	0.05
Smartamine ML[4]	--	--	0.07
Blood meal	--	--	3.08
Energy Booster	0.73	0.73	0.91
Dextrose	1.63	1.63	2.18
Minerals and Vitamins	3.26	2.90	3.15
Chemical components[6], % DM			
CP	14.04	14.75	15.95
SP, % CP	42.93	40.29	37.33
Ammonia, % SP	13.53	14.57	12.67
ADICP, % CP	5.68	5.86	5.46
NDICP, % CP	15.01	15.47	18.66
Acetic acid	0.45	0.45	0.46
Propionic acid	0.02	0.02	0.02
Lactic acid	2.57	2.58	2.61
Sugar	3.95	4.06	3.90
Starch	29.82	29.31	29.30
Soluble fiber	6.01	5.55	5.05
ADF	20.79	19.96	19.77
NDF	32.39	31.03	31.36
Lignin, % NDF	8.06	9.65	8.73
$uNDF_{240}$, % NDF	25.50	29.09	28.73
Ash	6.60	6.92	6.57
EE	3.49	3.61	3.78
Metabolizable Energy, Mca/kg	2.58	2.60	2.61

[1] Negative = balanced for ME (assuming 45 kg ECM), all EAA scaled one standard deviation below ideal EAA ratio according to Higgs (2015); Neutral = balanced for, all EAA scaled to ideal EAA ratio according to Higgs (2015) ; Positive = balanced for ME, all EAA scaled one standard deviation above EAA ratio according to Higgs (2015)

[2] SoyPLUS (West Central Cooperative, Ralston, IA) rumen protected soybean meal

[3] Smartamine M (Adisseo USA Inc, Alpharetta, GA) rumen protected Met (100% AANt)

[4] Smartamine ML (Adisseo USA Inc, Alpharetta, GA) rumen protected Lys (75 % AAN) and Met (25% AAN)

[6] Chemical components are expressed as % DM unless stated. SP = soluble protein; ADICP = CP insoluble in acid detergent; NDICP = CP insoluble in neutral detergent; WSC = water soluble carbohydrates; uNDF240 = undigested NDF after 240 hours of in vitro fermentation; EE = ether extract.

Table 3. Effects of treatment diets on milk production, intake, body weight and body condition scores

	Negative[1]	Neutral	Positive	SEM	Treatment
Intake and milk yield, kg/d					
Dry matter intake	27.9	28.2	28.5	0.27	0.98
Energy correct milk yield[2]	40.5[a]	43.7[b]	44.8[b]	0.57	<0.01
Milk yield	36.8[a]	39.8[b]	40.8[b]	0.47	<0.01
True protein yield	1.13[a]	1.26[b]	1.28[b]	0.01	<0.01
Fat yield	1.53[a]	1.62[ab]	1.67[b]	0.03	<0.01
Lactose yield	1.77[a]	1.91[b]	1.97[b]	0.03	<0.01
Milk composition, %					
True protein	3.09[a]	3.17[b]	3.14[b]	0.02	<0.01
Fat	4.20	4.12	4.14	0.06	0.64
Lactose	4.78	4.82	4.81	0.02	0.31
MUN	10.5[a]	11.4[b]	13.8[c]	0.14	<0.01
Body weight and condition					
Initial body weight, kg	691.5	692.7	697.5	4.27	0.83
Final body weight, kg	721.2	718.2	723.3	3.26	0.09
Body weight change, kg/wk	2.26	2.03	2.53	0.33	0.58
Initial BCS, 1-5 Scale	2.90	2.86	2.84	0.02	0.75
BCS, 1-5 scale	2.88[a]	2.92[b]	2.93[b]	0.01	0.01
CNCPS v.7 Parameters					
Feed efficiency	1.48[a]	1.55[b]	1.59[b]	0.02	<0.01
Intake of MP, g/day	2656.6[a]	2974.4[b]	3207.5[c]	162.4	0.02
Nitrogen use efficiency	0.282[a]	0.300[b]	0.299[b]	0.003	<0.01

[1] Negative = All EAA scaled one standard deviation below ideal EAA ratio according to Higgs (2014); Neutral = All EAA scaled to ideal EAA ratio according to Higgs (2014); Positive = All EAA scaled one standard deviation above EAA ratio according to Higgs (2014). All diets balanced and in excess of ME.

[2] Estimated according to Tyrrell and Reid (1965)

The cattle fed the NEU dietary treatment produced similar levels of energy corrected milk and yield similar production of fat components when compared to cattle fed the POS treatment (Table 3). The productivity of the cattle was similar even though the difference in crude protein of the two diets was over 1 unit, suggesting that cattle fed the NEU diet were at least as productive with their N supply as cattle fed the POS diet. Evaluation of MUNs indicate that the excretion of urea nitrogen was higher in the POS diet over the NEU diet, suggesting either that NEU cattle may have had a more balanced profile of EAA or that they were less wasteful with the N given to them. Cattle fed the NEG likely had a deficient supply of EAA as their production and feed efficiency was lower than either the NEU or POS cattle. Further analysis of the data collected from this experiment, coupled with model evaluation through CNCPS v.7, will help to reinforce our hypothesis that the optimum digestible EAA supply relative to ME generated by Higgs (2014) were within the range of true requirements for lactating cattle.

For the second study, 192 Holstein cows (2.68 ± 1.37 lactations; 85 ± 26 days in milk; 672.2 ± 82.5 kg BW) were blocked in pens of 16 (n=12) by parity, days in milk, body weight, and previous lactation performance as part of randomized block design. Each pen was fed TMR once daily at approximately 0630 h where pens were fed in the same sequence and targeted for a 5% refusal rate. All cattle were fed a common diet for a one-week acclimation period followed by a one-week covariate period in which baseline samples were taken to be used in the statistical analysis. Dietary treatments included a 2 x 2 factorial design with two levels of dietary starch (23% [LS]and 29% [HS] DM) and two levels of essential amino acid supply (100% [100] and 105% [105] of the optimum grams of EAA per Mcal/ME requirement according to Higgs and Van Amburgh (2016) (Table 4). Diets were formulated using CNCPS v7 which predicts EAA requirements similar to Doepel et al. (2004) and Lapierre et al. (2007) but expresses requirements relative to ME (Higgs and Van Amburgh, 2016). Given the emphasis towards the evaluation of N and EAA efficiency of use, all diets were formulated to be isocaloric; however, diets did vary in the ingredients that supply energy and EAA. High starch (HS 100 and HS 105) diets were formulated with higher levels of starch containing ingredients, with a majority being a highly digestible steam flake corn, allowing for an increased pool size of fermentable starch in the rumen. To match the caloric density of the HS diets, the low starch diets (LS 100 and LS 105) were supplemented with a high palmitic form of Energy Booster (MSC Company, Dundee, IL), which did increase the level of fatty acids consumed by those cattle. Rumen unsaturated fatty acid load (RUFAL) was formulated to be similar in all four diets. Protein feeds were evaluated for intestinal digestibility using the Ross Assay (Gutierrez-Botaro et al., 2021) to predict intestinally digestible N for more accurate predictions of EAA supply. Further, updated EAA profiles for commonly fed feeds determined within our lab (Van Amburgh et al., 2017) were implemented within the model to improve EAA supply predictions.

Table 4. Formulated EAA supply relative to megacalories of metabolizable energy

	Grams EAA:Mcal ME				
Essential Amino Acid	Higgs (2016)[1]	LS 100[2]	LS 105	HS 100	HS 105
Arginine	2.04	2.79	2.94	2.72	2.84
Histidine	0.91	1.12	1.16	1.10	1.19
Isoleucine	2.16	2.15	2.25	2.11	2.16
Leucine	3.42	3.18	3.37	3.20	3.32
Lysine	3.03	2.95	3.09	2.95	3.09
Methionine	1.14	1.11	1.18	1.11	1.18
Phenylalanine	2.15	2.09	2.21	2.06	2.12
Threonine	2.14	2.01	2.08	1.99	2.07
Tryptophan	0.59	0.60	0.62	0.59	0.61
Valine	2.48	2.34	2.43	2.30	2.39

[1] Optimum supply of EAA per Mcal ME according to Higgs et al. (2014)
[2]LS 100= Low starch, 100% EAA requirements; LS 100= Low starch, 105% EAA requirements; HS 100= High starch, 100% EAA requirements; HS 105= High starch, 105% requirements

In this study the production of milk protein increased as cows were fed the HS diets, supported by an increased supply of EAA; however, those cows also consumed

significantly more feed, which would provide for both more glucogenic substrates and greater microbial yield, which would supply even greater EAA. This improvement in milk protein output by the increase in EAA supply in the HS 105 diets occurred while decreasing the efficiency of N utilization compared to the other diets (Table 5). In contrast, cows fed the HS 100 diet had the highest level of N efficiency compared to other treatments and a reasonable but slightly lower milk protein output by approximately 50 g/d. This data supports the hypothesis that greater glucogenic substrates support greater milk protein synthesis and further indicate the optimum EAA values per unit of ME are reasonable but there are some EAA that are required at higher levels to support the energy signaling for greater protein synthesis. Nichols et al. (2018) recently presented similar findings where the post-ruminal supplementation of glucogenic precursors improved milk N efficiency at both a low level of MP supply (75% of requirements) and higher level of MP supply (120% of requirements). Given that we were not able to fully meet the balanced requirements for all the EAA at the 105% level, these small deficiencies might explain why the milk protein response was not greater than observed and that milk N efficiency was decreased. Further work to evaluate this interaction between glucogenic supply and milk protein synthesis will have to ensure that all EAA requirements are effectively met. However, this data does suggest the optimum requirements as described by Higgs and Van Amburgh (2016) is a good starting point in formulation of EAA supply relative to ME for lactating dairy cattle. Improvements in milk fat output and feed efficiency for cattle fed the low starch diets should not be disregarded given the improved efficiency of use for N in the HS 100 diet. A body of literature exists that describes similar improvements in feed efficiency when diets are supplemented with lipogenic nutrients, and more work is needed to evaluate the effect of fat and fatty acid supplementation when diets are balanced for EAA.

In the last study, the focus of the project was monensin levels and milk composition using the concept of a “modern diet” or updated dietary concepts since the approval of Rumensin in the marketplace. With the LaPierre et al., (2019, 2020) studies, the EAA data were re-analyzed in both CNCPS v7 and v6.55 and the Lysine and Methionine levels on a gram/Mcal basis were back calculated in v6.55 to be v7 equivalent. Diets in the monensin study were formulated to meet or exceed nutrient demands for high producing lactating dairy cows using CNCPS (v6.55; Van Amburgh et al., 2015). Methionine and lysine were balanced using the latest information on requirements and supply as generated in the studies of LaPierre et al. (2019) where amino acid requirements are described on a gram per unit of ME basis (Higgs and Van Amburgh, 2016). For diet formulation, the methionine requirement was set at 1.19 g methionine per Mcal ME and lysine was set at 3.21 g per Mcal ME (or 2.7 times the grams methionine). All diets consisted of (DM basis) 34.9 % corn silage, 19.4 % grass haylage, 18 % corn meal, 6.8 % soybean meal, and 21 % pre-mix containing monensin (Purina Animal Nutrition, Caledonia, NY). Treatments were 0 g/ton monensin (CON), 11 g/ton monensin (R11), 14.5 g/ton monensin (R14.5), and 18 g/ton monensin (R18) on a DM basis, and monensin intake was formulated to be 305 mg/d, 404 mg/d, and 515 mg/d for R11, R14.5, R18, respectively.

Lactation performance results are in Table 6. We observed a numerical increase in DMI in the R18 group compared to CON, R11, and R14.5 (27.7 vs. 26.9, 26.8, and 26.7 kg/d, respectively). Monensin treatment tended to have a quadratic effect on DMI (P = 0.10) where R11 and R14.5 had slightly decreased DMI compared to CON, but DMI increased in the R18 group. This finding is not consistent with previous studies as increasing dietary monensin has been associated with either no change or a slight decrease in DMI (Akins et al., 2014; Hagen et al., 2015), although Recktenwald et al. (2014) reported a trend for increased DMI in cows fed monensin compared to none in diets high and low in starch and protein content. Milk yield was not affected by monensin treatment in agreement with experiments of Alzahal et al. (2008) and Hagen et al. (2015) (Table 4). The lack of an adaptation period for the CON group following the covariate diet of 11 g/ton monensin was predicted to decrease the ability to detect treatment effects because we observed a decrease in milk yield in the CON group compared to all monensin treated groups from wk 4 to 9 (data not shown) indicating cows were still adjusting to the removal of monensin in the beginning 3 wk of the experimental period. This is consistent with lactose production data as we observed a decrease in lactose yield in the CON group compared to all monensin treated groups following wk 3 of the experimental period (data not shown). In agreement, Akins et al. (2014) reported an increase in milk yield in cows fed monensin from wk 4 to 12, but not from wk 1 to 3, suggesting cows were still adapting to monensin changes in the diet.

Although non-significant, ECM, FCM, and SCM all increased with monensin treatment compared to CON likely from the increase in milk component production in the monensin fed groups (Table 6). Previously, experiments by He et al. (2012) and Martinez et al. (2009) found monensin had no significant effect on component corrected milk yield. We observed an average 7 kg/d increase in ECM and FCM yield compared to actual milk yield across all treatment groups, and a 3.5 kg/d increase in SCM yield, again likely a result of the diet formulation of higher EAA levels, modest fat levels and strong rumen fermentation conditions. No significant treatment effects were observed for milk fat concentration or yield; however, milk fat percentage increased numerically with increasing monensin concentration (4.60, 4.67, 4.71, and 4.66 for CON, R11, R14.5, and R18 respectively; Table 7). The numerical increase in milk fat was most likely an effect of monensin on de novo FA synthesis as there was a linear increase (P < 0.05; Table 5) in de novo and mixed fat content with increasing levels of monensin. Previous research has shown monensin decreases milk fat concentration with increasing monensin levels (Dubuc et al., 2009; Duffield et al., 2008b), while others (Martinez et al., 2009; McCarthy et al., 2018) have reported no effect on milk fat. More recently, monensin has been shown to interact with other dietary factors such as starch content and unsaturated oils to reduce milk fat, rather than causing milk fat depression independently (McCarthy et al., 2018).

The increase in de novo and mixed FA synthesis and yield in mid- to late lactation dairy cattle was an interesting and exciting observation and one that is not well documented. The increase in de novo and mixed FA through the feeding of monensin could be due to a couple different substrate supplies. Monensin is known to increase the supply of propionate and under certain conditions, propionate can be part of an

initiation sequence where synthesis of acyl chains from carbon atoms could potentially lead to incorporation into chain elongation of FA (Palmquist, 2007). In addition, with increased propionate, there will be greater glucose and capacity for reducing equivalents which means increased NADPH +H supply which would allow for an increase in the FA synthase reaction allowing for production and elongation of FA. The protein sparing effect of monensin could increase the supply of certain amino acids, including the branched chain amino acids and their conversion to branched chain volatile FA and these could serve as precursors for chain elongation for chain lengths less than 16 carbons (Massart-Leen et al., 1981; Ha and Lindsay, 1990; Liu et al., 2018). Diets were not formulated to contain high quantities of fat; thus, it is possible that with lower exogenous FA, there was less competition for certain enzymes related to glycerol production and utilization, but de novo FA synthesis could be increased. Finally, it is also possible, that some of the fat content and yield was related to the supply of methionine and lysine. In the current study, the methionine and lysine were supplied at what we believe are closer to the true requirements and, with the DMI observed, the metabolizable methionine level was approximately 85 g/d and the lysine levels were approximately ≥225 g/d, levels much higher than typically fed. This data would suggest that overcoming the limitation of at least two essential amino acids (EAA) allowed for greater milk fat synthesis in these cows. There is emerging data to suggest there is a link between mTOR signaling, EAA, and the regulation of milk fat synthesis (Li et al., 2016; Nichols et al., 2020).

Overall, the milk and component yield of these mid- to late lactation cattle was high and unprecedented suggesting the conditions of evaluating monensin feeding in cattle fed more contemporary diets was achieved. Increasing the supply of monensin had no significant effects on milk yield, DMI, or production efficiencies; however, some of that lack of difference is likely due to shift from a covariate period with monensin feeding to a control diet where monensin was removed and an inadequate adjustment period. We observed a positive response to monensin treatment with linear increases in de novo and mixed FA concentration which resulted in enhanced milk fat yield. This indicates monensin can be fed at higher concentrations to achieve high milk component yields in lactating cows fed contemporary diets optimized for component yield, and more research is warranted to understand the relationship between monensin and ruminal FA synthesis, especially the de novo and mixed FA.

Summary

To better describe AA supply and requirements and develop approaches to formulate diets closer to meeting the requirements, several steps have been taken to improve the predictions. These approaches provide solutions to offset bias in calculations and provide new insights into how to evaluate AA requirements on an energy allowable basis consistent with monogastric species. It is anticipated that actualizing all of these approaches will allow for lower N feeding and more efficient diets that result in lower cost and less environmental impact of dairy cattle.

Table 5. Effects of treatment diets on milk production, intake, body measurements, and efficiencies

	Treatments					P-Values				
	LS 100[1]	LS 105	HS 100	HS 105	SEM	Starch	AA	Starch*AA	Time	Starch*AA*Time
Intake and milk production, kg/d										
Dry matter intake	26.65	26.35^{y}	28.95^{x}	28.34	0.66	0.01	0.54	0.83	0.73	0.54
Energy correct milk yield[2]	48.31^{a}	48.90^{ab}	48.02^{a}	49.48^{b}	0.33	0.66	0.01	0.20	0.01	0.65
Milk yield	43.81^{a}	43.92^{a}	43.46^{a}	45.73^{b}	0.26	0.01	0.01	0.01	0.01	0.06
True protein yield	1.35^{a}	1.37^{a}	1.42^{b}	1.47^{c}	0.01	0.01	0.01	0.09	0.01	0.25
Fat yield	1.83^{ab}	1.86^{b}	1.77^{a}	1.79^{ab}	0.02	0.01	0.32	0.72	0.01	0.21
Lactose yield	2.16^{a}	2.17^{a}	2.14^{a}	2.26^{b}	0.01	0.01	0.01	0.01	0.01	0.85
Milk composition, %										
True protein	3.08^{a}	3.13^{a}	3.27^{b}	3.25^{b}	0.02	0.01	0.56	0.02	0.01	0.01
Fat	4.20^{b}	4.26^{b}	4.09^{b}	4.00^{c}	0.04	0.01	0.72	0.07	0.19	0.04
Lactose	4.94	4.93	4.93	4.94	0.007	0.59	0.68	0.79	0.01	0.85
MUN, mg/dL	10.3^{a}	13.9^{b}	11.9^{c}	11.2^{d}	0.15	0.01	0.01	0.01	0.01	0.02
Fatty acid composition, %										
De Novo	25.0	25.3	26.9	26.7	0.27	0.01	0.90	0.38	0.01	0.01
Mixed	43.1	42.6	43.9	42.8	0.37	0.13	0.04	0.42	0.01	0.01
Preformed	31.5^{a}	30.9^{ab}	29.0^{c}	30.0^{bc}	0.38	0.01	0.54	0.03	0.01	0.76
Body measurements										
Initial body weight, kg	676.0	678.9	680.2	686.1	12.5	0.65	0.72	0.90	---	---
Final body weight, kg	698.5	698.2	696.1	690.7	2.95	0.09	0.34	0.39	---	---
Initial BCS, 1-5 Scale	2.93	2.89	2.83	2.88	0.02	0.02	0.90	0.11	---	---
Final BCS, 1-5 scale	3.00	2.96	3.01	3.00	0.04	0.56	0.50	0.63	---	---
Rumination, min/day	655.0	646.3	634.5	610.4	16.2	0.09	0.31	0.64	0.24	0.35
Efficiencies										
Feed Efficiency	1.65^{a}	1.66^{a}	1.59^{b}	1.54^{c}	0.02	0.01	0.03	0.01	0.01	0.01
ECM Feed Efficiency	1.82^{a}	1.85^{a}	1.75^{b}	1.67^{c}	0.02	0.01	0.05	0.01	0.01	0.01
Milk N:feed N, %	32.2^{ab}	32.0^{a}	32.5^{b}	31.2^{c}	0.20	0.31	0.01	0.04	0.01	0.01

[1]LS 100= Low starch, 100% EAA requirements; LS 100= Low starch, 105% EAA requirements; HS 100= High starch, 100% EAA requirements; HS 105= High starch, 105% requirements. [2]Estimated according to Tyrrell and Reid (1965)

Table 6. Effect of increasing dietary monensin concentration on lactation performance

	Diet[1]					*P*-value[2]			
Item	CON	R11	R14.5	R18	SEM	Linear	Quad	Trt	Trt x week
Days in milk[3]	190	168	193	184	7.2	-	-	-	-
Monensin, mg/d	0	384	465	589	-	-	-	-	-
DMI, kg/d	26.9	26.8	26.7	27.7	0.31	0.29	0.09	0.22	< 0.01
Milk, kg/d	39.3	39.9	39.7	39.6	0.34	0.48	0.38	0.69	< 0.01
Fat, %	4.60	4.67	4.71	4.66	0.04	0.16	0.40	0.38	0.16
Fat, kg/d	1.79	1.83	1.85	1.83	0.02	0.15	0.52	0.40	< 0.01
Protein, %	3.35	3.37	3.36	3.39	0.02	0.15	0.89	0.41	< 0.01
Protein, kg/d	1.30	1.33	1.33	1.33	0.01	0.13	0.46	0.41	< 0.01
Lactose, %	4.63	4.65	4.63	4.63	0.01	0.98	0.27	0.51	< 0.01
Lactose, kg/d	1.82	1.85	1.84	1.84	0.02	0.34	0.50	0.71	< 0.01
MUN, mg/dL	8.96[a]	10.24[b]	9.61[ab]	9.52[ab]	0.28	0.12	0.04	0.05	< 0.01
PUN, mg/dL	9.11	9.13	9.04	8.89	0.17	0.42	0.42	0.72	< 0.01
ECM[4], kg/d	46.0	46.9	47.1	46.8	0.50	0.17	0.47	0.46	< 0.01
3.5% FCM[5], kg/d	46.0	46.9	47.2	46.8	0.53	0.19	0.51	0.49	< 0.01
SCM[5], kg/d	42.5	43.3	43.5	43.2	0.46	0.17	0.41	0.42	< 0.01
BW, kg	692	691	694	693	2.1	0.74	0.67	0.83	0.26
BW change, kg/d	0.16	0.27	0.16	0.44	0.09	0.07	0.33	0.08	-
BCS[6]	2.93[a]	2.93[a]	3.04[b]	2.93[a]	0.40	-	-	< 0.01	-
Rumination, min/d	647	645	639	641	6.2	0.40	0.91	0.77	0.01

[a-b]Means within a row differ with different superscripts ($P < 0.05$).
[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin
[2]Week effect for all estimates ($P < 0.01$).
[3]Average of experimental period.
[4]Calculated according to Tyrell and Reid (1965).
[5]Calculated according to NRC (2001).
[6]Largest standard deviation of treatment means.

Table 7. Effect of increasing dietary monensin concentration on de novo, mixed, and preformed fatty acid production

	Diet[1]					*P*-value[2]			
Item	CON	R11	R14.5	R18	SEM	Linear	Quad	Trt	Trt x Wk
Total FA, g/100 g milk	4.33	4.39	4.43	4.37	0.04	0.22	0.34	0.41	0.31
De novo[3]									
g/100 g milk	1.13	1.16	1.17	1.16	0.01	0.05	0.32	0.17	0.35
g/d	438	452	458	454	6.3	0.06	0.46	0.21	0.06
g/100 g FA	26.1	26.4	26.2	26.3	0.11	0.24	0.54	0.41	< 0.01
Mixed[4]									
g/100 g milk	1.85	1.88	1.91	1.90	0.02	0.02	0.79	0.10	0.07
g/d	720	737	753	746	11.8	0.09	0.76	0.28	< 0.01
g/100 g FA	42.8	42.9	43.0	43.1	0.18	0.25	0.66	0.64	< 0.01
Preformed[5]									
g/100 g milk	1.34	1.35	1.36	1.33	0.02	0.95	0.27	0.61	< 0.01
g/d	520	527	533	521	7.1	0.61	0.28	0.54	< 0.01
g/100 g FA	31.0	30.7	30.8	30.6	0.21	0.15	0.98	0.46	< 0.01
Chain length	14.57	14.54	14.54	14.54	0.01	0.02	0.27	0.08	< 0.01
Level of unsaturation	0.235^{a}	0.231^{ab}	0.227^{b}	0.227^{b}	0.002	<0.01	0.94	0.01	< 0.01
Fatty acids									
16:0, g/100 g milk	1.79^{y}	1.81^{xy}	1.85^{x}	1.83^{xy}	0.02	0.02	0.74	0.09	0.07
16:0, g/d	695^{y}	712^{xy}	728^{x}	720^{xy}	9.6	0.02	0.67	0.09	< 0.01
18:0, g/100 g milk	0.36	0.36	0.37	0.36	0.01	0.80	0.33	0.60	< 0.01
18:0, g/d	140	142	145	141	2.3	0.35	0.26	0.32	< 0.01
18:1 *cis*-9, g/100 g milk	0.79	0.79	0.79	0.78	0.01	0.91	0.59	0.86	< 0.01
18:1 *cis*-9, g/d	305	308	311	306	4.0	0.57	0.42	0.66	< 0.01

[a-b]Means within a row differ with different superscripts ($P < 0.05$).
[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin.
[2]Week effect for all estimates ($P < 0.01$).
[3]C4 to C14 (Barbano and Melilli, 2016).
[4]C16, C16:1, and C17.
[5]Greater than or equal to C18.

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Maternal methionine supply during the periconceptional period and its impact on calf performance

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Introduction

There is growing evidence of the role of parental nutrition and environmental conditions, from periods before conception and throughout gestation, in offspring development. Barker's hypothesis of "fetal origins" or "fetal programming" holds that the origins of chronic diseases in later life lie in fetal responses to the intrauterine environment (Barker and Osmond 1986). Specifically, it suggests that the origin of chronic diseases in adulthood results from fetal adaptations to malnutrition. Epidemiological studies in human and animal models have highlighted the critical role of intrauterine nutrition and environment in programming development. (Almond and Currie 2011, Broadhead et al., 2019).

Epigenetic mechanisms are important regulators of gene expression that can potentially be heritable without altering the DNA nucleotide sequence (Waterland and Michels, 2007; Sutton et al., 2017). These mechanisms include DNA methylation, chromatin remodeling, and non-coding regulatory RNAs (Allis and Jenuwein 2016, Sutton et al., 2017; Wei et al., 2017, Lacal et al., 2018; Reynolds et al., 2019;). Epigenetic mechanisms can change the function of a gene by changing its expression (transcription to mRNA and translation to protein), but do not involve changes in the DNA sequence. These mechanisms are essential during gametogenesis, embryonic development, and subsequent cell differentiation. It is through epigenetic mechanisms that pluripotent cells commit to different fates and sustains the expression of different sets of genes to give rise to a new cell group. For example, embryonic cells containing the same DNA have the ability to become skin, liver, or mammary gland cells. This is because they employ epigenetic mechanisms that favor the expression of some genes and repress the expression of others, giving rise to cells with completely different functionality. Furthermore, some important developmental events depend on epigenetic factors, such as X chromosome inactivation in females and regulation of imprinted genes for which only one parental allele is expressed in the offspring (Jin et al., 2011; Moore et al., 2013; Smallwood and Kelsey, 2012).

In addition to their role in normal cell differentiation, epigenetic marks can be modified by environmental factors (i.e., nutrition, exposure to drugs or pollutants, stress, etc.), and they are often referred to as “cell memory.” DNA methylation is a heritable

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epigenetic mark involving the covalent transfer of a methyl group to the C-5 position of the cytosine ring of the DNA by the DNA methyltransferases (Moore et al., 2013). This mechanism occurs within the One-carbon (1C) metabolism, which comprises a series of interlinking metabolic pathways that include the methionine and folate cycles that are central to cellular function, providing 1C units (methyl groups) for the synthesis of DNA, polyamines, amino acids, creatine, and phospholipids. One-carbon metabolism functions as a key biochemical conduit between the parental environment and epigenetic regulation of early development. Therefore, modifications of the functionality of the 1C metabolism could lead to long-term consequences in postnatal life.

Several models that aim to understand the importance of developmental programming have been studied in different species, including rodents (Kwong et al., 2000), sheep (Sartori et al., 2020), and cattle (reviewed in Broadhead et al., 2019). Data has shown that the effects of developmental programming could be beneficial or detrimental; this will depend on the intrauterine environment and on the environment during postnatal life (Reynolds and Caton, 2012). In livestock, data has shown the importance of the periconceptional period for embryonic development because alteration during this phase could impact postnatal development (Van Eetvelde et al., 2017). The periconceptional period is defined as the period before and after the time of conception. In mammals, DNA methylation levels during the periconceptional period are well established. Male and female DNA methylation levels are high before fertilization occurs. However, after fertilization, DNA demethylation occurs during the first stages of embryogenesis. Once the embryo reaches the blastocyst stage and cell-linage determination, DNA methylation is established (Smallwood and Kelsey, 2012).

Folate, choline, methionine, and betaine are important for the methionine cycle to generate S-adenosylmethionine, which is essential for different metabolic processes, including DNA methylation (Cronje, 2018). Estrada-Cortes et al. 2021 described an experimental model where 1.8 mM of choline chloride or control was added in the in vitro culture media during early embryo development (day 1 to day 7 after fertilization). On day 7.5, embryos were transferred, and after calving, the postnatal phenotype of the calves was determined. Calves from the choline treatment were heavier at birth and at 205-adj weaning weight. Also, Peñagaricano et al. (2013) supplemented rumen-protected methionine (RPM) in dairy cows from calving until embryo flushing. After superovulation and embryo flushing, the embryonic transcriptome was determined. They found several differentially expressed genes when comparing embryos collected from cows supplemented or not with RPM. Some of those genes were related to embryonic development and immune response. Next, Acosta et al. (2016) reported that using RPM during the periconceptional period in Holstein cows decreased methylation levels in blastocyst compared to the cows that did not receive RPM. All this data proves that 1. Components of the 1-carbon cycle play an important role in early embryo development, probably due to alterations in DNA methylation and gene expression. And 2. Some of these components, when feeding into the diet (rumen protected), influence the embryo and offspring development.

However, there is no evidence of the effect of feeding RPM during the periconceptional period on gene expression or DNA methylation status of the embryo and the progeny in beef and dairy cattle. Also, there is no evidence of the effect of RPM on post-natal and post-weaning performance in female calves in beef cattle. Our hypothesis is that feeding RPM during the periconceptional period will program bovine gestation in a manner that enhances fetal and postnatal growth. Next, we will describe a series of studies conducted on beef cattle to understand the role of RPM supplementation during the periconceptional period in postnatal life.

Experiment 1

This experiment aimed to determine the plasma concentration of methionine after RPM supplementation. Grazing, dry, non-pregnant cows (n=10) were individually supplemented for six consecutive days with 1 pound of cottonseed meal and 0.25 lbs. of minerals, containing or not 15 gr of RPM (Smartamine; Adisseo Alpharetta, GA). Blood samples were collected 6 hours after feeding each day to determine plasma methionine concentration. Data were analyzed using PROC MIXED of SAS. Plasma methionine concentration doubled (Con = 14.57 ± 1.29 μM; RPM = 31.18 ± 1.73 μM; $P \leq 0.01$) in the RPM group as early as 24 h after initiation of supplementation and remained elevated until the end of the study. This dose of RPM (15 gr per animal per day) was chosen for Exp. 2.

Experiment 2

One hundred and fourteen Brangus-Angus crossbred beef lactating cows (age= 4.9 ± 0.2 years) were enrolled in this experiment. Animals were blocked in two blocks for management purposes (Block A n = 50; Block B n = 64). Both blocks received a forage-based diet (Table 1). Two treatments were supplemented for 14 days, starting 7 days before the artificial insemination (AI) and continuing until 7 days after AI (Figure 1) using an individual feeder (Super SmartFeed - Automated Supplement; C-Lock Inc; Rapid City, South Dakota, USA). Supplemented treatments consisted of Control (454 gr of corn gluten) and RPM (454 gr of corn gluten and 15 gr of RPM; Smartamine M, Adisseo). Cows were randomly assigned to the treatments within groups (Control n = 56, and RPM n = 58). Estrus was synchronized with a 7-d Co-synch + CIDR protocol, and split AI with sexed semen to produce females was conducted in all cows (Beef reproduction task force, 2023). Ten days after AI, clean-up bulls were introduced to the groups until the end of the 90-day-breeding season. Cows with low treatments intake recorded by the Super SmartFeed were excluded from the experiment.

Blood sample collection: Blood samples were collected 30 and 60 days after artificial insemination. Samples were collected from the jugular vein into evacuated tubes containing EDTA (BD Vacutainer). After collection, tubes were placed immediately on ice and transported to the laboratory. Blood samples were centrifuged for plasma harvesting. Plasma was aliquoted into 2 mL tubes and stored at -80°C until pregnancy-associated glycoprotein (PAG) concentration analysis.

Ultrasonography: Pregnancy diagnosis was conducted 30, 60, and 120 days after artificial insemination by ultrasonography (Esaote ultrasound, MyLab Delta Vet, with 10-5 MHz transducer). Embryo and fetal measurements were conducted by ultrasonography 30 and 60 days after artificial insemination. On day 30, amniotic vesicle diameter, amniotic vesicle circumference, embryo length, and abdominal cavity were measured. On day 60, eye cavity diameter, whiter-rump length, head length, and transversal head distance were measured.

Post-natal collection: After calving, lactating cows were located together in the pasture and fed Bermuda grass hay. A total of 40 female and male calves (Control = 19; RPM = 21; 6 males and 34 females) were considered for birth weight analysis. Body weights were collected within 24 hours of birth. Later only female calves were considered for further analyses. Female calves (Control = 16; RPM = 18) suckled their dams until weaning (~8 months of age). Body weight, whiter height, body length, and heart girth were measured from 2 months of age until weaning. Adjusted 205-day weaning weight was calculated using the formula [weaning weight – birth weight)/days of age at weaning] × 205 + birth weight. At seven months of age, liver and subcutaneous adipose tissue biopsies were collected from female calves. After collection, tissue samples were rinsed with sterile PBS solution and immediately snapped frozen in liquid nitrogen. Samples were kept at -80°C freezer until analysis. Tissue samples were used for total RNA extraction and submitted to RNA sequencing using Illumina platforms. Bioinformatic analysis was conducted to determine differences in gene expression. Pathway enrichment analysis was conducted using IPA software (Qiagen).

Post-weaning: At weaning, all heifers were allocated together in a pasture and received a forage-based diet. Post-weaning data started to be collected one month after weaning. Every two weeks, from ~280 days of age, body weight, whiter height, body length, and heart girth were collected.

Statistical analyses: Data were analyzed using the GLIMMIX procedure of SAS. Continuous data were tested for normality of the residues and homogeneity of variances and transformed when necessary. Post-natal and post-weaning body weight, wither height, heart girth, and body length were analyzed as repeated measurements.

Results

Gestational traits: Pregnancy per artificial insemination (P/AI) was 50% and 55% in Control and RPM groups, respectively. There were no statistical differences in PAG's concentration at day 30 (Control: 16.68 ± 0.96 ng/mL; RPM: 16.54 ± 0.96 ng/mL) and day 60 of pregnancy (Control: 10.24 ± 0.83 ng/mL; RPM: 10.16 ± 0.83 ng/mL). Additionally, all embryo and fetal measurements were not different between treatments except for amniotic vesicle circumference, which tends to be higher in RPM than in control (P = 0.06; Table 2).

Post-natal traits: A treatment by sex interaction was observed (P=0.04) in birth weight. Male calves (Control, n = 3; RPM, n = 3) from cows fed RPM showed greater birth weight than those from the control group (Control = 31.9 ± 2.3 kg; RPM = 41.4 ± 2.3 kg), but no difference was observed in females (n=34; Control = 31.3 ± 1.03 kg; RPM = 33.3 ± 1.0 kg). Later only female calves were considered for further analyses. Body weight, body length, and heart girth were not different ($P > 0.05$) between treatments at 60, 120, 180, and 240 days (Figure 2). There was an effect of treatment (P = 0.03) on wither height, where RPM group had increased height at days 60 and 120. No difference ($P = 0.71$; Control: 199.02 ± 5.2 kg; RPM: 201.57 ± 4.65 kg) was observed between treatments for 205-day adjusted weaning weight.

Gene expression: Differences in gene expression were considered when P < 0.01 and *log 2-fold change* of < -1.5 and >1.5. There were 129 genes downregulated and 24 genes upregulated in the liver of RPM group. Enrichment analysis showed that the top canonical pathways were related to the inhibition of immune system function in the liver of RPM group. In the adipose tissue samples, six genes were upregulated, and 22 were downregulated in the RPM group. Enrichment analysis showed enrichment of extracellular matrix and cellular response to extracellular stimulus, and fibroblast growth factor binding enriched in the RPM group.

Post-weaning traits: No differences ($P > 0.05$) in body weight and length were observed between treatments after weaning. However, a treatment-by-age interaction was observed ($P = 0.007$) in heart girth, having a larger heart girth in the RPM group at days 336 and 350. The wither height tended to differ between treatments (treatment effect, $P = 0.08$; Interaction, P = NS), where the RPM group presented higher wither height at days 294, 308, 336, 350, and 364.

Conclusions

Here we summarized the preliminary results of an ongoing experiment. Feeding 15 gr of RPM 7 days prior and 7 days after the artificial insemination increased the amniotic vesicle size but did not influence any other variable measured during gestation. Also, male calves from the RPM group presented higher birth weights; however, we did not find any differences in the female calves. Further research must evaluate the potential sexual dimorphism in response to RPM in the periconceptional period.

After birth, only female calves remain in the study. RPM group presented increased wither and withered heights on several time points. We found several genes that were affected by treatment in liver and adipose tissue samples. Interestingly, in both tissues, we found a larger number of downregulated genes in the RPM than in the control group.

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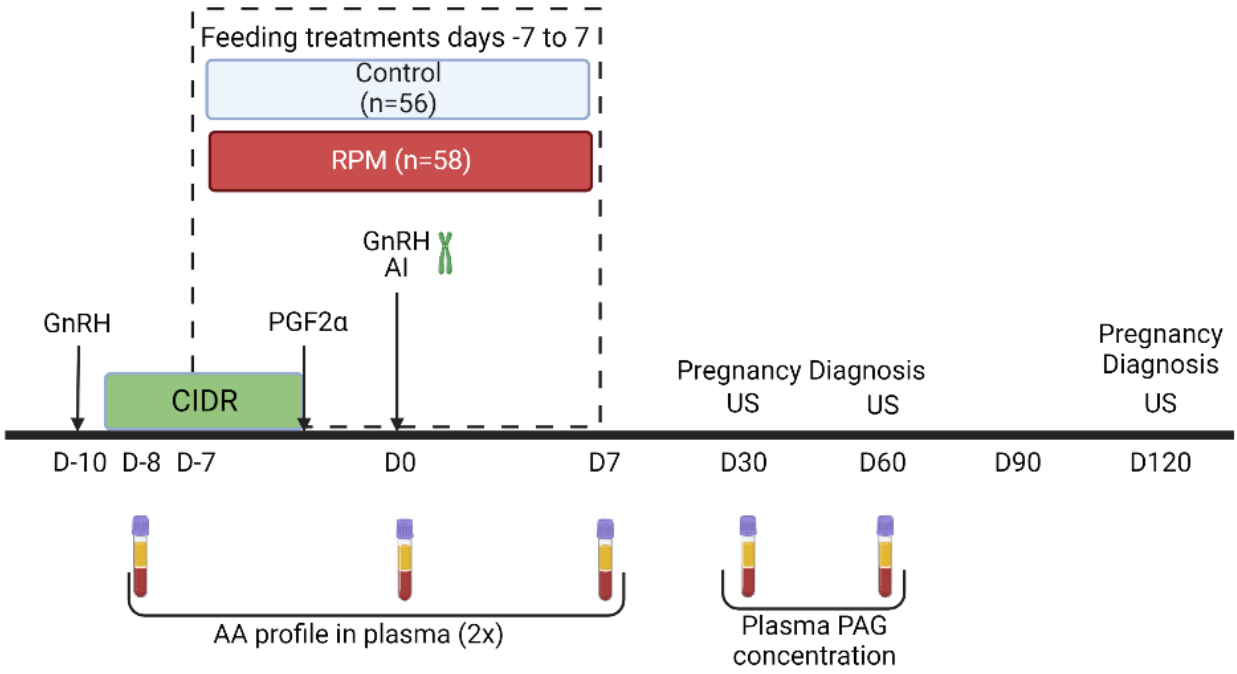

Figure 1 Schematic design of the experimental treatments and 7-d Co-synch + CIDR split-time AI sexed semen protocol.

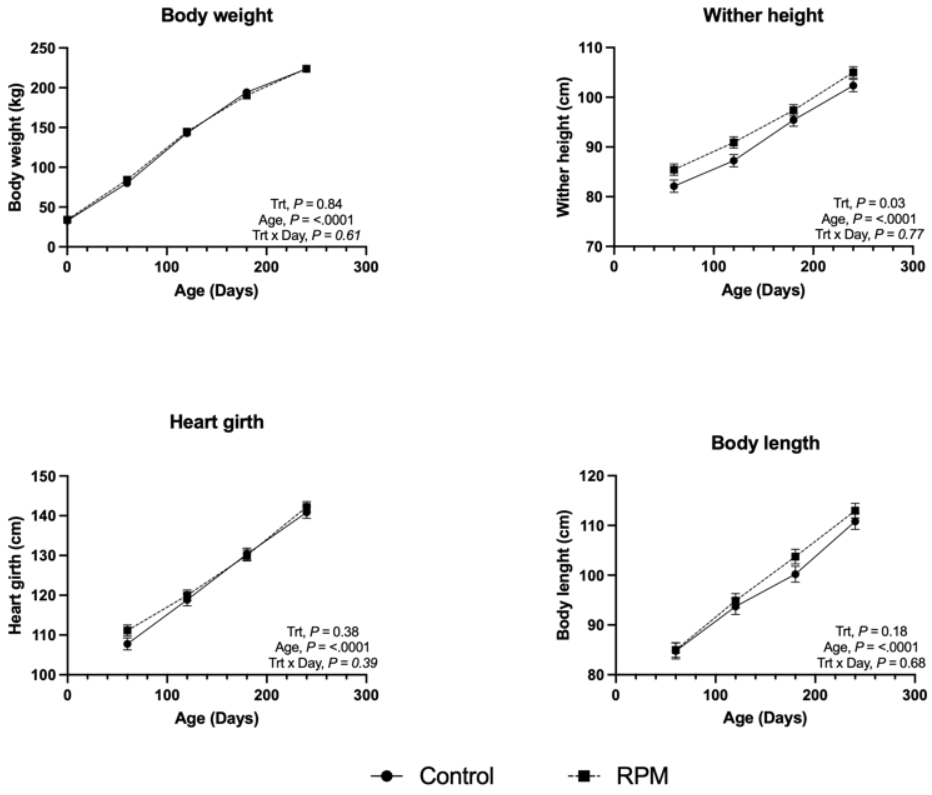

Figure 2. Post-natal measurements in female calves from birth to weaning.

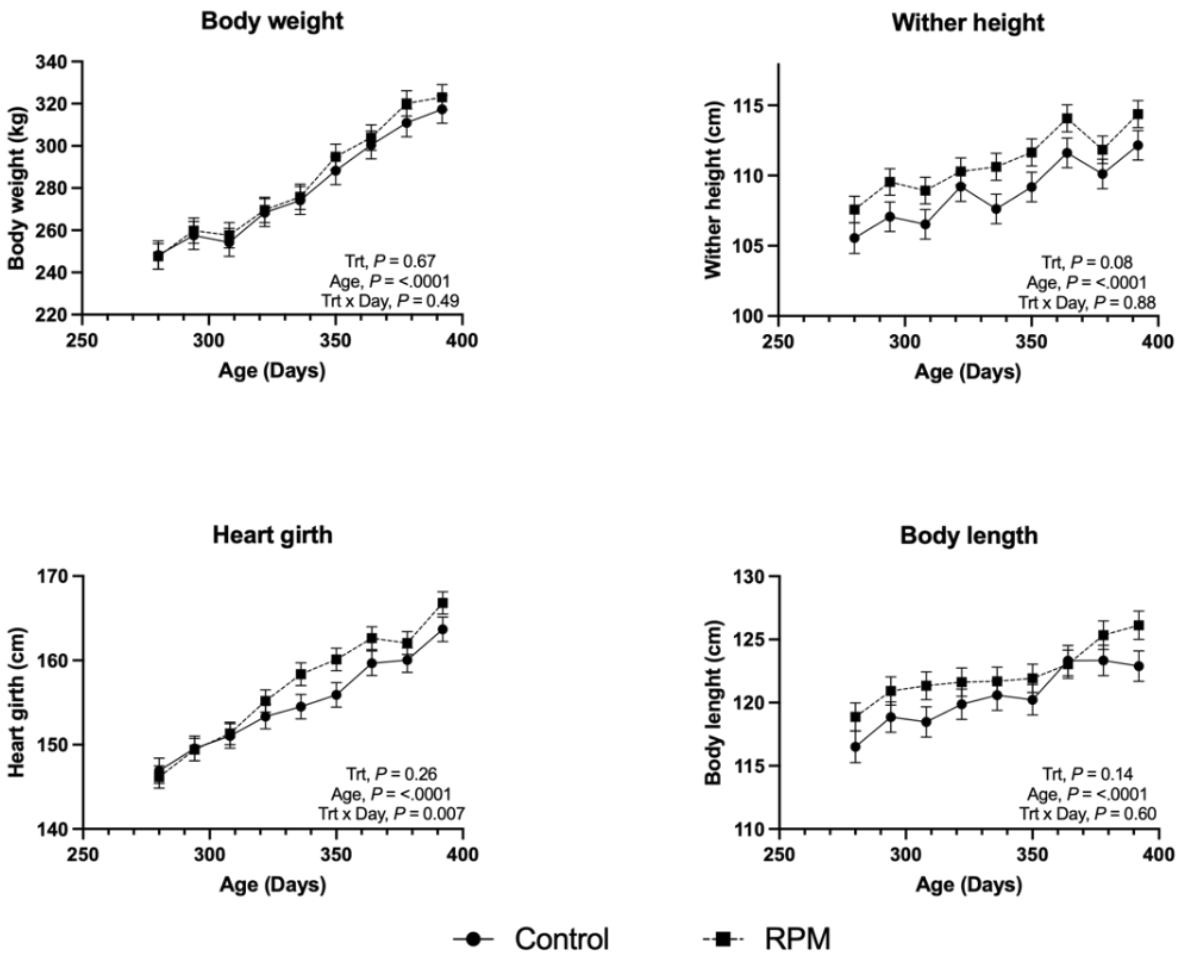

Figure 3. Post-weaning measurements in female calves.

Table 1. Ingredient and nutrient composition of the diet (dry matter basis)

Ingredient, %	
Corn silage	30.17
Gin trash	33.67
Cotton seed meal	6.44
Corn gluten feed	28.69
Minerals	1.02
Nutrient, %	
Crude protein	14.70
Neutral detergent fiber	37.30
Acid detergent fiber	28.10
Ether extract	3.80
Ash	5.96
NE_m, Mcal/lb	0.66
NE_g, Mcal/lb	0.40

Table 2. Embryo and fetal measurements at 30 and 60 days of pregnancy

	Control	RPM	*P*-value
Day 30			
Amniotic vesicle diameter (mm)	10.96 ± 0.24	11.48 ± 0.23	0.13
Amniotic vesicle circumference (cm^2)	0.88 ± 0.04	0.99 ± 0.04	0.06
Embryo length (mm)	10.97 ± 0.41	10.98 ± 0.40	0.98
Abdominal cavity (mm)	5.88 ± 0.17	6.04 ± 0.17	0.51
Day 60			
Eye cavity diameter (mm)	5.97 ± 0.29	5.55 ± 0.32	0.28
Wither-tail length (mm)	41.47 ± 1.44	41.80 ± 1.22	0.86
Head length (mm)	26.77 ± 0.55	27.23 ± 0.53	0.55
Head transversal (mm)	19.76 ± 0.49	20.65 ± 0.45	0.34

SESSION NOTES:

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Impacts of pre- and postpartum heat stress abatement on physiology and performance of grazing Bos indicus-influenced cow-calf pairs

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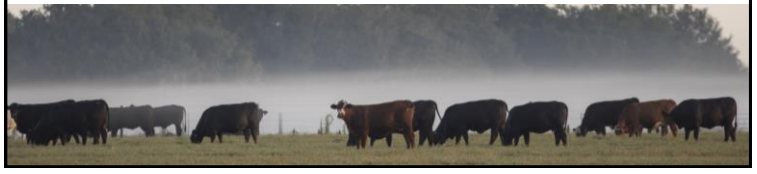
February 21, 2023



1

Introduction

- Grazing *Bos indicus*-influenced beef cattle during chronic periods of severe heat and humidity conditions
  - *Internal body temperature increases*
  - *Cattle respond with different behavioral, physiological, cellular, and molecular signatures* (Lees et al., 2019; Moriel et al., 2017)
- Heat mitigation strategies needed for grazing beef cattle

2

Introduction

- Gestational heat stress programs offspring postnatal life
  - *Reduced fetal growth and birth weight*
  - *Weaning weights decreased in dairy calves*
  - *Offspring performance is impaired* (Tao et al., 2019; Laporta et al., 2018, 2020)
- Variety of outcomes among different species

3

Hypothesis & Objectives

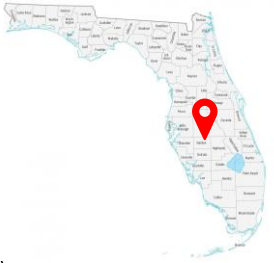
Hypothesis: The use of heat abatement strategies during late gestation and early postpartum will reduce body temperature but increase body condition score at calving and enhance postnatal growth and immune function of their progeny.

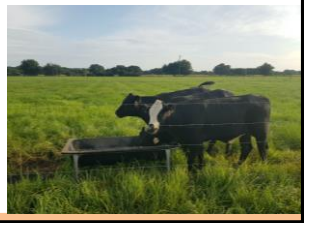
Objectives: To evaluate the impacts of **heat abatement during pre- and postpartum** on prepartum **body temperature**, **body condition score** change, **physiological measurements** of heat-stressed *Bos indicus*-influenced beef heifers, and **postnatal growth** and **immunocompetence** of their progeny.

4

Experimental Design

- Range Cattle Research and Education Center
  - July 2021 to March 2022
- 64 pregnant Brangus heifers (21 months)
  - BW: 454 ± 37 kg
  - BCS: 6.3 ± 0.28
- 1 of 16 bahiagrass pastures (4 heifers/pasture)
- Treatments (8 pastures/treatment)

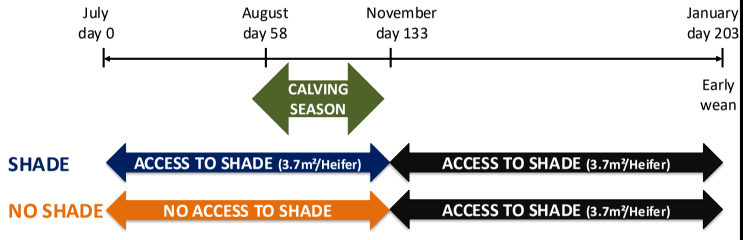




5

Experimental Design

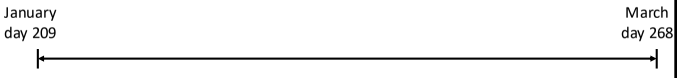
Maternal Treatment



6

Experimental Design

Offspring Management



- 52 Calves (119 ± 19 d of age)
- 1 of 16 drylot pens (3 to 4 calves/pen)
  - Same distribution of the maternal treatment
- Soybean hulls-based diet (3.25% DM of BW)
  - CP: 21.0%
  - NDT: 71.5%

7

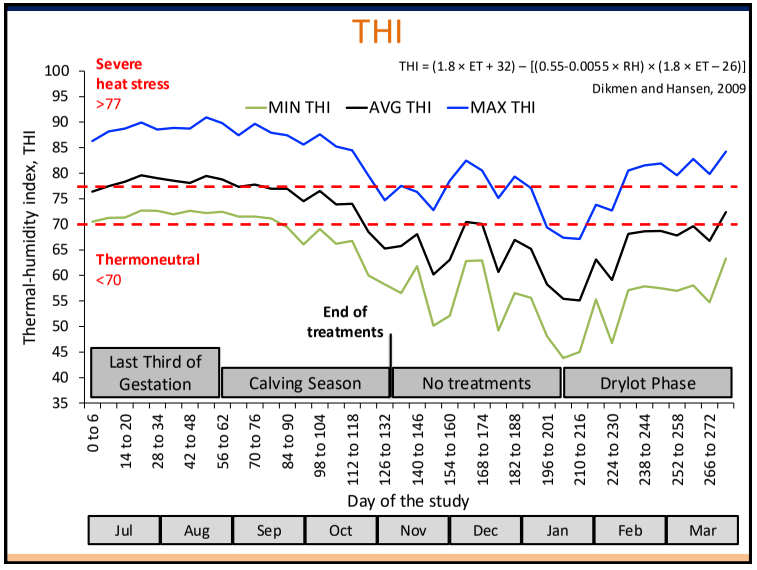
Days on Treatment and Forage Evaluation

Shade = Access to shade from day 0 to 133
No Shade = No access to shade from day 0 to 133

	Shade access			P-value
	NSH	SH	SEM	Shade
Days on treatment				
Pre-partum	80	86	4.0	0.29
Post-partum	53	47	4.0	0.29

Shade × Day $P \geq 0.12$	Shade $P \geq 0.32$	Day $P \leq 0.0001$	Day of the study					P-value
			0	35	70	120	SEM	Day
Herbage mass, kg DM/ha			2550b	2248a	4659c	4418c	126.5	<0.0001
Herbage allowance, kg DM/kg BW			1.42a	1.24a	2.65b	2.65b	0.089	<0.0001
Crude protein, % of DM			15.0c	11.8b	11.5b	9.4a	0.28	<0.0001
IVDOM, %			49.4d	39.3c	35.5b	31.5a	0.62	<0.0001

8



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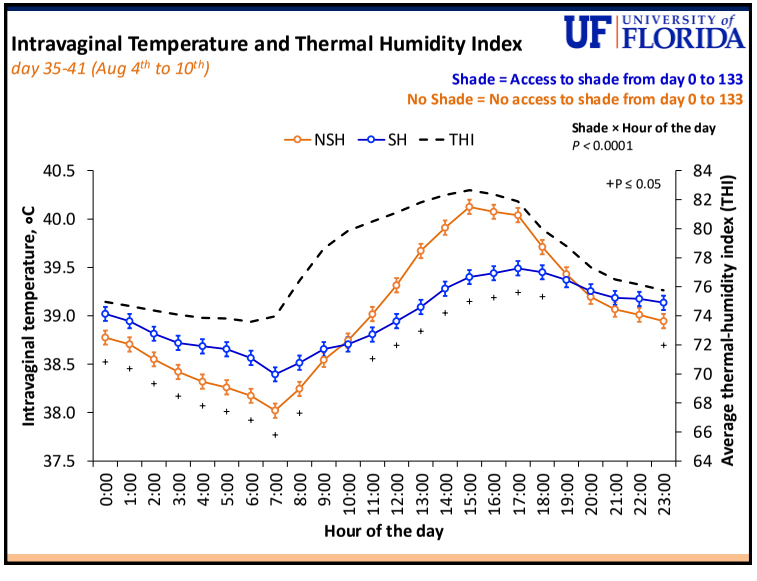

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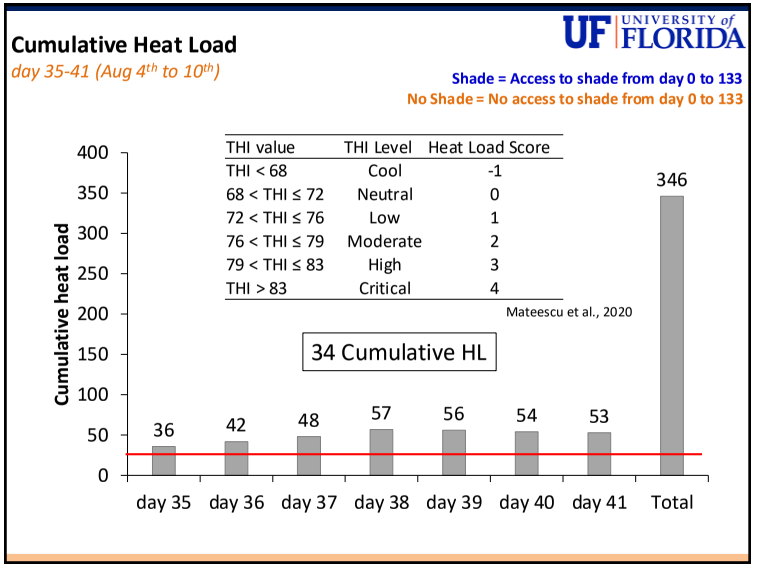

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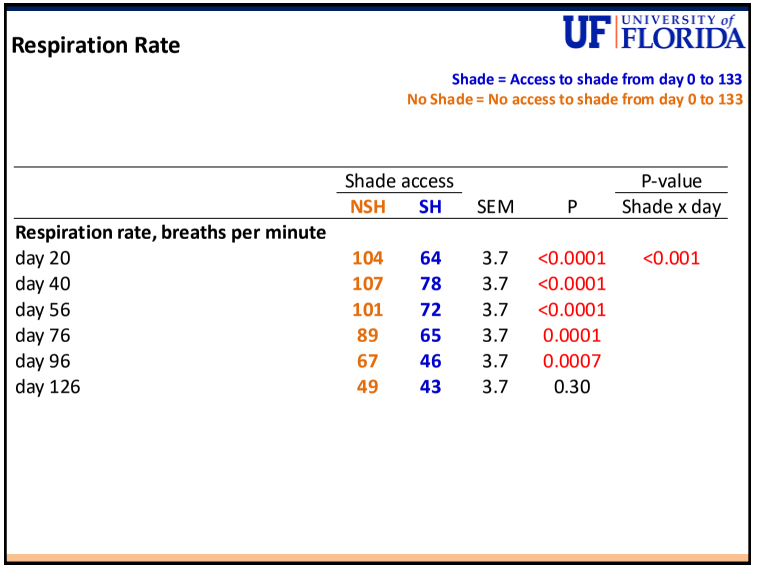

Respiration Rate

Shade = Access to shade from day 0 to 133
No Shade = No access to shade from day 0 to 133

	Shade access				P-value
	NSH	SH	SEM	P	Shade x day
Respiration rate, breaths per minute					
day 20	104	64	3.7	<0.0001	<0.001
day 40	107	78	3.7	<0.0001	
day 56	101	72	3.7	<0.0001	
day 76	89	65	3.7	0.0001	
day 96	67	46	3.7	0.0007	
day 126	49	43	3.7	0.30	

12

Activity Report

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Shade = Access to shade from day 0 to 133
No Shade = No access to shade from day 0 to 133

Shade × Hour of the day × Day
P ≥ 0.12

	Shade access				P-value
	NSH	SH	SEM	P	Shade × hour
Standing under shade, % of total heifers					
1:00 PM	0	46.9	2.88	<0.0001	<0.0001
5:00 PM	0	12.0	2.88	0.005	
Standing outside shade, % of total heifers					
1:00 PM	70.3	5.2	3.88	<0.0001	<0.0001
5:00 PM	30.7	13.5	3.88	0.004	
Laying under shade, % of total heifers					
1:00 PM	0	32.3	4.22	<0.0001	<0.0001
5:00 PM	0	7.8	4.22	0.21	
Laying outside shade, % of total heifers					
1:00 PM	9.4	0.5	2.49	0.02	0.01
5:00 PM	14.6	5.7	2.49	0.02	

13

Activity Report

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Shade = Access to shade from day 0 to 133
No Shade = No access to shade from day 0 to 133

Shade × Hour of the day × Day
P ≥ 0.12

	Shade access				P-value
	NSH	SH	SEM	P	Shade × hour
Drinking, % of total heifers					
1:00 PM	3.6	0	1.15	0.13	0.22
5:00 PM	2.6	1.6	1.15	0.53	
Grazing, % of total					
1:00 PM	15.6	11.5	3.43	0.40	0.10
5:00 PM	51.0	59.4	3.43	0.09	

14

Intravaginal Temperature and Thermal Humidity Index

day 126-132 (Nov 3rd to 9th)

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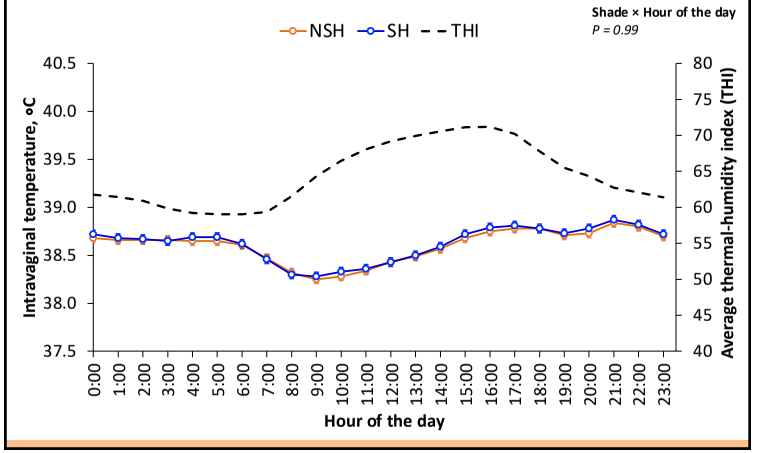
Shade = Access to shade from day 0 to 133
No Shade = No access to shade from day 0 to 133



15

Cumulative Heat Load

day 126-132 (Nov 3rd to 9th)

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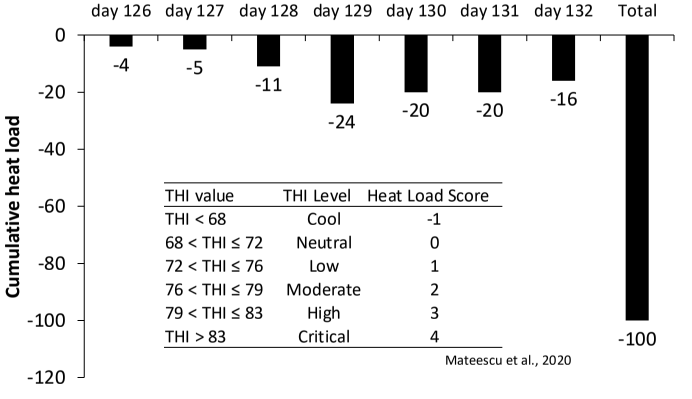
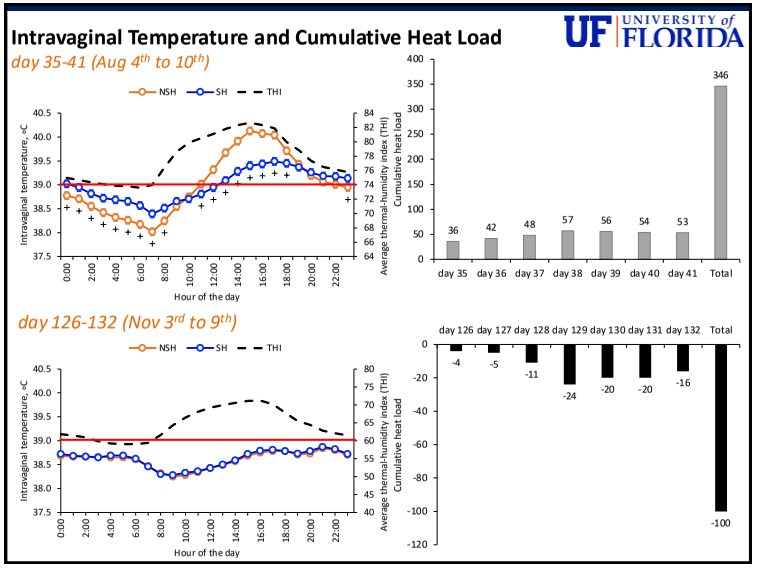
Shade = Access to shade from day 0 to 133
No Shade = No access to shade from day 0 to 133



THI value	THI Level	Heat Load Score
THI < 68	Cool	-1
68 < THI ≤ 72	Neutral	0
72 < THI ≤ 76	Low	1
76 < THI ≤ 79	Moderate	2
79 < THI ≤ 83	High	3
THI > 83	Critical	4

Mateescu et al., 2020

16



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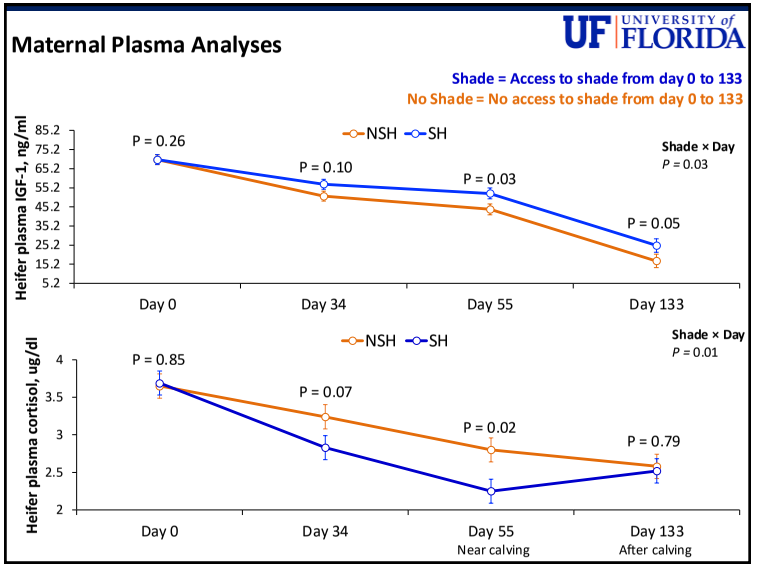

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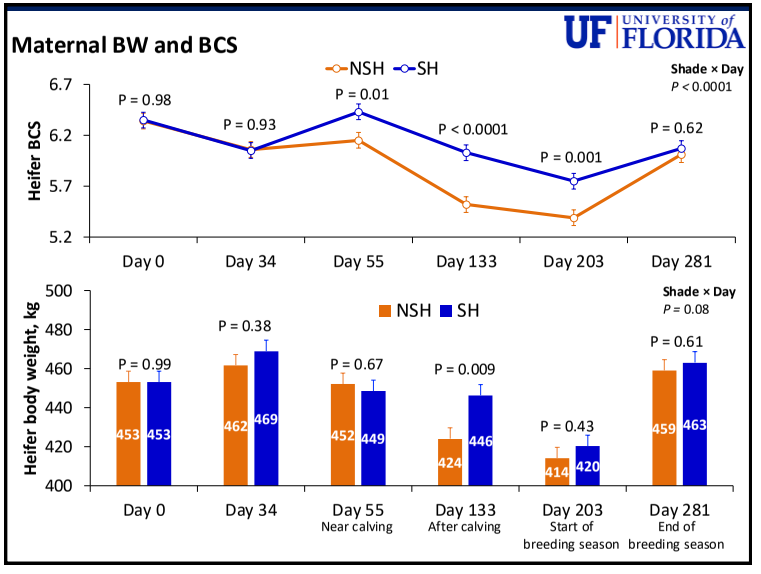

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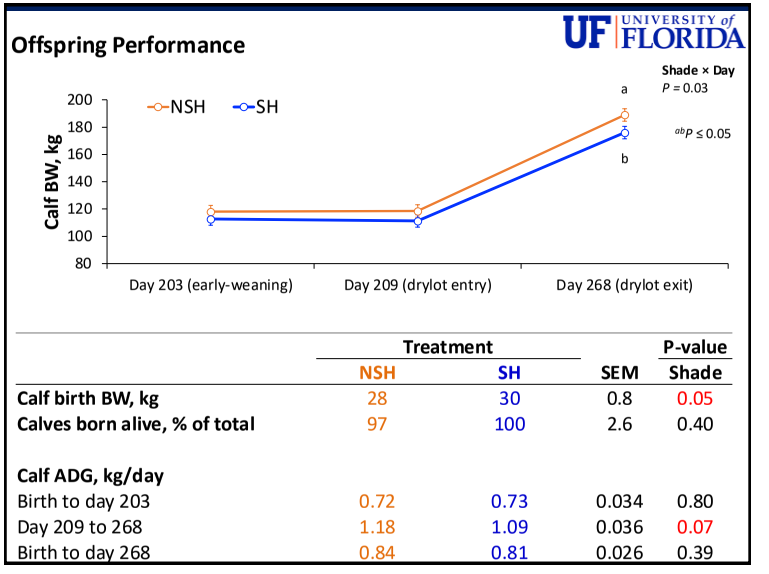

	Treatment			P-value
	NSH	SH	SEM	Shade
Calf birth BW, kg	28	30	0.8	0.05
Calves born alive, % of total	97	100	2.6	0.40
Calf ADG, kg/day				
Birth to day 203	0.72	0.73	0.034	0.80
Day 209 to 268	1.18	1.09	0.036	0.07
Birth to day 268	0.84	0.81	0.026	0.39

20

Offspring Performance

	Treatment			P-value
	NSH	SH	SEM	Shade
Intake day 209 to 268, kg				
Hay DM	25	22	1.2	0.17
Concentrate DM	277	262	9.4	0.29
Total DM	302	284	9.3	0.21
Intake day 209 to 268, % of BW				
Hay DM	0.28	0.27	0.018	0.61
Concentrate DM	3.10	3.10	0.019	0.93
Total DM	3.38	3.37	0.267	0.72
Gain:Feed	0.23	0.23	0.005	0.65

21

Offspring Plasma Analyses

	Maternal treatment			P-value	
Item	NSH	SH	SEM	Shade	Shade × day
Plasma cortisol, ug/dL	2.43	2.42	0.149	0.93	0.15
Plasma Hp, mg/mL	0.405	0.468	0.0235	0.06	0.80

	Maternal treatment				P-value	
Item	NSH	SH	SEM	P	Shade	Shade × day
BRSV						
Seroconversion, % of total						
Day 222	76.9	50.0	8.57	0.02	0.23	0.01
Day 236	69.2	50.0	8.57	0.10		
Day 268	80.2	96.2	8.57	0.19		
Serum titers, log2						
Day 222	2.00	1.31	0.285	0.08	0.26	0.09
Day 236	1.85	1.27	0.285	0.15		
Day 268	2.69	3.00	0.291	0.44		

22

Offspring Plasma Analyses

	Maternal treatment			P-value	
	NSH	SH	SEM	Shade	Shade × day
BVDV-1					
Seroconversion, % of total					
Day 268	76.9	76.9	8.88	1.00	0.44
Serum titers, log2					
Day 268	3.84	4.43	0.632	0.67	0.69
IBR					
Seroconversion, % of total					
Day 268	63.7	53.9	8.97	0.24	0.97
Serum titers, log2					
Day 268	1.63	1.42	0.234	0.41	0.94
PI-3					
Seroconversion, % of total					
Day 268	100	96.2	3.86	0.47	0.82
Serum titers, log2					
Day 268	8.76	8.19	0.513	0.57	0.83

23

Conclusions

- The access to shade during pre- and postpartum alleviates the effects of heat stress by reducing intravaginal temperature, decreasing respiration rates and modifying the behavior of pregnant heifers
- Heat abatement increased heifer body condition score and plasma IGF-1 during late gestation, increased calf birth body weight, reduced calf post-weaning growth, and had minor changes to calf humoral immune function

24

SESSION NOTES


inbra
nutrição animal

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DIET-DEPENDENT EFFECTS OF AN ASPERGILLUS-BASED PREBIOTIC FED TO GROWING BEEF CATTLE

Podversich Federico [1], Tarnonsky Federico [1], Medeiros da Silva Gleise[1], Schulmeister Tessa [1], Vargas Martinez Juan[1], Heredia Daniella[1], Araceli Maderal [1], Chebel Ricardo[2], Ipharraguerre Ignacio [4], Gonella Angela [1], Dubeux Jose [1], and DiLorenzo Nicolas *[1]

[1]North Florida Research and Education Center, University of Florida, Marianna, FL, 32446
[2]College of Veterinary Medicine, University of Florida, Gainesville, FL, 32611
[4]Institute of Human Nutrition and Food Science, Christian-Albrechts-University, Kiel, Germany

1

Introduction

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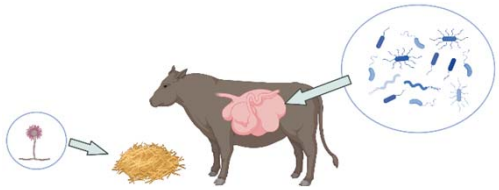
Prebiotic: **a substrate that is selectively utilized by host microorganisms conferring a health benefit (Gibson et. al., 2017).**

***Aspergillus oryzae*: aerobic multicellular fungus, that is thought to provide unidentified growth factors to the ruminal microorganisms (Martin and Nisbet, 1990).**



2

MODE OF ACTION OF *ASPERGILLUS ORYZAE*

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DIRECT EFFECTS ⇨ **CONSISTENTLY OBSERVED**

SOURCE OF GROWTH FACTORS

ESTIMULATES THE GROWTH AND ACTIVITY OF RUMINAL:

- ❑ BACTERIA:
  - ❑ Cellulolytic: *Ruminococcus flavefaciens, Fibrobacter succinogenes*
  - ❑ Lactate utilizer: *Megasphera elsdenii, Selenomonas ruminantium*
- ❑ FUNGI: *Neocallimastix frontalis*

? Some direct effects remain unknown… (room for research)

Source: Beharka et. al., 1991; Beharka and Nagaraja, 1993; Frumholtz et. al., 1989; Kreikemeier et. al., 1997; Nisbet and Martin, 1993 ab; Schmidt et. al., 2004; Waldrip and Martin, 1993

3

MODE OF ACTION OF *ASPERGILLUS ORYZAE*

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INDIRECT EFFECTS ⇨ **LESS CONSISTENT**

- ❑ Stabilization of ruminal pH
- ❑ Enhanced
  - ❑ Digestibility of nutrients and fiber degradation
  - ❑ Microbial crude protein synthesis
  - ❑ Intestinal mucosal barrier
- ❑ Reduced
  - ❑ Heat stress and systemic inflammation
  - ❑ Small intestine permeability

Source: Beharka et. al., 1991; Beharka and Nagaraja, 1993; Caton et. al., 1993; Chiquete 2009; Frumholtz et. al., 1989; Gomez-Alarcon, 1990; Kaufman et. al., 2021; Rius et. al., 2022; Sun et. Al., 2016; Wiedmeier et. Al., 1987.

4

MODE OF ACTION OF *ASPERGILLUS ORYZAE*

PHENOTYPICAL RESPONSES ⇨ **HIGHLY VARIABLE**

- Enhanced
  - Body weight gain
  - Feed conversion
  - Milk yield
- Reduced
  - Days to weaning
  - Subacute ruminal acidosis
  - Fecal water loss
- Altered mineral bioavailability

Source: Beharka et. al., 1991; Chiquette, 2009; Nickels and Relling, 2017; Podversich et. Al., 2022; Rius et. al., 2022; Sallam et. al., 2019; Sucu et. al., 2018; Zerby et. al., 2011.

5

Diet-dependence ➔ possible explanation for variable responses

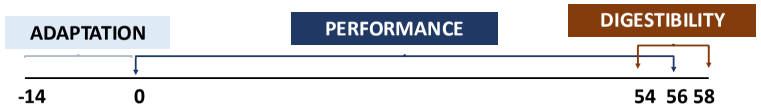
- **OBJECTIVE:**
  To evaluate the effects of feeding either sorghum silage or byproducts-based backgrounding diets supplemented or not with an Aspergillus oryzae based prebiotic (AOP; Amaferm, BioZyme Inc., St. Joseph, MO) on growth performance, nutrient digestibility, and feeding behavior of beef heifers

- **HYPOTHESIS:**
  The inclusion of AOP may alter animal performance, nutrient digestibility, and feeding behavior in a diet-dependent manner

6

EXPERIMENTAL DESIGN

- Generalized randomized block design
- 2 x 2 factorial arrangement:
  - DIET: Sorghum silage based vs. Byproducts based
  - ADDITIVE: AOP (targeting 2 g/d) vs. Control
    - Sorghum Control (SC)
    - Sorghum AOP (SA)
    - Byproducts Control (BC)
    - Byproducts AOP (BA)



7

MATERIAL AND METHODS

- PERFORMANCE
  - 21 Heifers/trt (84 heifers total)
  - 3 pens/trt (12 pens total)
  - BW every 14 d
  - Intake recorded daily (GrowSafe)
  - Chewing activity (SCR collars)
- DIGESTIBILITY
  - 10 heifers/trt (40 heifers total)
  - 2 pens/trt (8 pens total)
  - Feed collected twice daily, d 54 – 57
  - Fecal collected twice daily, d 55 – 58 (rectal grab)
  - iNDF as internal marker, ruminally incubated for 288 h
- Location: NFREC – Marianna, FL

8

MATERIAL AND METHODS – Diets and Ingredients

	BYPRODUCTS	SORGHUM
Ingredient Inclusion as % DM		
Sorghum silage	-	89.00
Distillers grains, dehydrated	8.00	8.00
Citrus pulp, pelleted	63.00	-
Cotton gin trash (burrs)	21.00	-
Liquid supplement[1]	5.00	-
Premix (CTL vs. AOP)	2.00	2.00
Vitamin-mineral supplement[2]	1.00	1.00
Formulated Energy and CP%		
CP, % DM	12.99	12.90
NEg, Mcal/kg of diet DM	0.93	0.95

[1] Custom-formulated liquid molasses (Quality Liquid Feeds, Dodgeville, WI).
[2]"VITAMINDE" (Flint River Mills, Brainbridge, GA)

AMAFERM PREMIX*:
Byproducts:
99.0% DDGS
1.0% Amaferm

Sorghum:
98.8% DDGS
1.2% Amaferm

PREMIX CONTROL:
100% DDGS

*Amaferm targeted intake:
~ 2 g/hd/d

9

MATERIAL AND METHODS – Analyzed composition of the diets

	BYPRODUCTS		SORGHUM	
	Control	AOP	Control	AOP
DM, as % of as fed	89.9	90.0	50.5	49.5
OM, % DM	91.4	91.0	93.7	93.4
CP, % DM	14.1	13.4	11.5	12.5
NDF, % DM	31.8	32.5	54.4	54.1
ADF, % DM	23.1	23.1	31.6	31.5
Starch, % DM	4.0	4.6	16.9	14.7
Ash, % DM	8.64	8.97	6.26	6.59

Nutrient concentration in DM basis

10

STATISTICAL ANALYSIS

- Generalized randomized block design with a 2 x 2 factorial arrangement of treatments
- MIXED Procedure of SAS (SAS Institute Inc., Cary, NC)
- Experimental unit: heifer
- Model: fixed effects of AOP, Diet, their interaction, and pen nested within the interaction of AOP and Diet
- Initial BW was tested as a covariate, remaining in the model when significant
- Significance: $P \leq 0.05$; Tendencies $0.10 > P > 0.05$
- Outliers were considered when > 2 SD for G:F (performance) or DMD (digestibility)

11

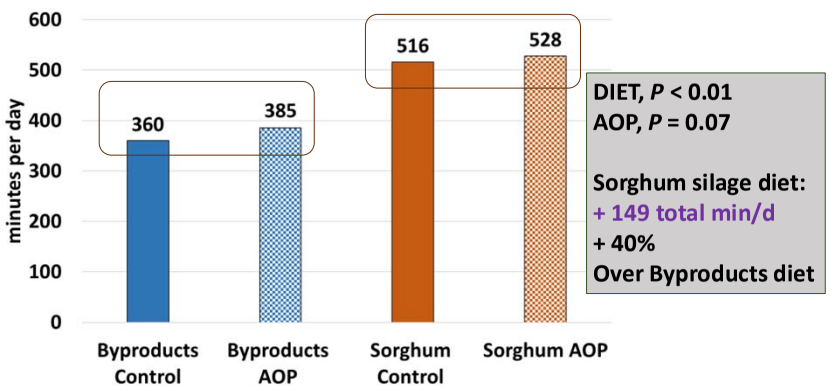
RESULTS - PERFORMANCE

	BYPRODUCTS		SORGHUM		SEM	*P*-value		
	Control	AOP	Control	AOP		DIET	AOP	DIET×AOP
Initial BW (d 0), kg	330.6	329.3	329.4	328.7	9.72	0.93	0.97	0.92
Final BW (d 56), kg	392.9	396.3	366.3	367.9	10.25	<0.01	0.93	0.80
Amaferm intake, g/d	0	2.1	0	2.2	-	-	-	-
ADG, kg	1.11	1.20	0.66	0.70	0.036	<0.01	0.54	0.09
DMI, kg/d	10.7	10.3	8.9	9.2	0.28	<0.01	0.91	0.18
DMI, as % of BW	2.99	2.85	2.53	2.65	0.076	<0.01	0.91	0.11
G:F, (kg/kg)	0.104^{b}	0.120^{a}	0.081^{c}	0.073^{c}	0.004	<0.01	0.36	<0.01
RFI, (kg)	0.30	-0.34	-0.16	0.28	0.234	0.72	0.69	0.03
NEg, Mcal/kg DM	0.88^{b}	1.01^{a}	0.81^{bc}	0.75^{c}	0.036	<0.01	0.37	0.01

[a, b, c] Different superscript $P < 0.05$
RFI = No diff. when means were separate

12

RESULTS – CHEWING ACTIVITY



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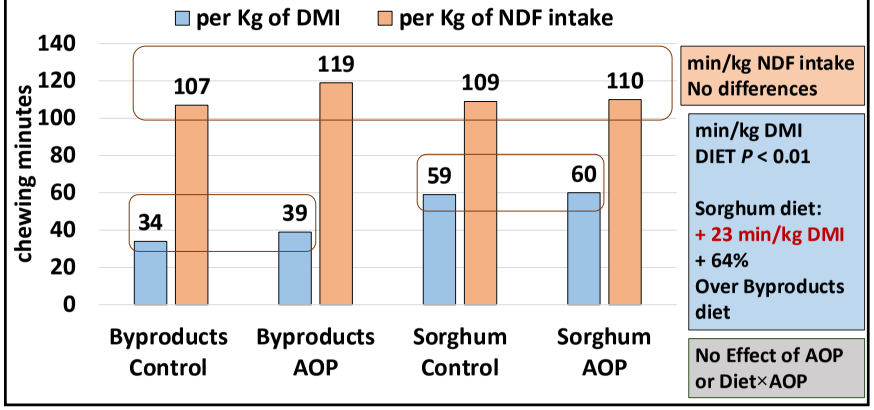
RESULTS – CHEWING ACTIVITY



14

RESULTS – CHEWING ACTIVITY

	BYPRODUCTS		SORGHUM		SEM	*P*-value		
	Control	AOP	Control	AOP		DIET	AOP	D*A
Bunk visits, visits/d	51.8	54.7	74.8	76.9	3.37	<0.01	0.47	0.91
Visit duration, min/visit	3.2	3.2	2.6	2.7	2.93	0.01	0.60	0.71
Bunk visit duration, min/d	145.5	143.7	160.4	168.9	4.78	<0.01	0.48	0.28
Intake, g DM/visit	234.0	237.0	152.9	155.4	12.1	<0.01	0.82	0.98
Intake, g DM/min of visit	72.2	73.4	59.1	57.6	2.36	<0.01	0.96	0.56
Head-down duration, min/d	62.9	60.3	72.3	71.2	3.79	0.01	0.64	0.84

15

RESULTS – APPARENT TOTAL TRACT DIGESTIBILITY

	BYPRODUCTS		SORGHUM		SEM	*P*-value		
	Control	Amaferm	Control	Amaferm		DIET	AMA	DIET×AOP
DMI, kg/d	11.30	10.10	9.99	9.80	0.65	0.22	0.30	0.44
DMI, % BW	3.01	2.70	2.69	2.83	1.18	0.57	0.64	0.20
Digestibility								
DM, %	62.5[a]	58.1[b]	47.6[d]	54.2[c]	1.03	<0.01	0.30	<0.01
OM, %	65.8[a]	61.7[b]	49.8[d]	55.9[c]	0.99	<0.01	0.33	<0.01
NDF, %	44.8[b]	40.4[c]	47.7[b]	54.6[a]	1.19	<0.01	0.33	<0.01
ADF, %	44.0[b]	39.6[c]	46.8[b]	52.7[a]	1.17	<0.01	0.53	0.01
CP, %	48.7[a]	42.5[b]	29.0[c]	43.0[b]	1.53	<0.01	0.01	<0.01
Starch, %	92.5	88.0	77.8	79.6	4.44	0.01	0.09	0.48

[a, b, c] Different superscript, $P < 0.05$

16

CONCLUSIONS

- Based on ADG, the sorghum silage-based diet proved to be a viable alternative for backgrounding replacement heifers
- Chewing activity differed between diets measured in total min/d and min/kg DMI, however heifers exhibited similar min/kg NDF intake
- Heifers fed the sorghum-based diet had more visits to the feed bunks, spent less time, and consumed less feed/visit than heifers fed the byproducts-based diet

17

CONCLUSIONS

- Inclusion of *Aspergillus*-based prebiotic:
  - Improved gain-to-feed ratio by 15% in the byproducts-based diet, without altering G:F in the sorghum silage-based diet
  - Improved overall digestibility of the sorghum silage-based diet, with the opposite effect in the byproducts diet
- These results confirm the premise that AOP affects animal responses differently depending on basal diet
- Further research should be conducted to determine ruminal and metabolic changes associated with the observed results

18

SESSION NOTES

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Limits to intestinal starch digestion in cattle

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Introduction

Perhaps the greatest ecological niche of cattle and other ruminants is their ability to use nutrients from fibrous feeds that are largely intractable to digestion from mammalian enzymes. Pregastric fermentation facilitates the capture energy from cellulose. Yet, fermentation of fiber for dietary energy has several limitations, which inhibit the efficiency of energy utilization in comparison to aerobic respiration. Cellulose principally serves as a structural moiety in plants and is often less densely concentrated in feeds in comparison to nonstructural plant carbohydrates (e.g., starch). Typically, for enough energy to be digested from cellulose a relatively large amount of biomass must be consumed. Additionally, pregastric fermentation has inherent heat losses (i.e., energy loss) and some end-products of fermentation do not serve metabolically useful purposes (e.g., methane). And methane contributions are proportionally greater in cattle fed large amounts of forages in comparison to cattle fed large amounts of nonstructural plant carbohydrates. Starch and other non-fiber carbohydrates, however, are more densely concentrated in feeds, result in proportionally less pregastric methane production, and may be digested in the postruminal alimentary tract. Consequently, non-fiber carbohydrates often provide a more efficient and economical method to provide dietary net energy to cattle in comparison to fermentation of fiber when non-fiber carbohydrates are abundant and less costly.

Glucose absorbed from nonstructural plant carbohydrates in the small intestine can improve amounts of circulating glucose concentrations that can improve performance, health and reproduction in cattle. Also, small intestinal digestion of starch provides between 30 to 42% more energy than starch fermented in the rumen (Owens et al., 1986, McLeod et al., 2001; Brake and Swanson, 2018). Yet only 5 to 20% of dietary starch consumed by cattle is digested postruminally (Harmon, 1992). Ostensibly, limits in small intestinal digestion of nonstructural carbohydrates have resulted in large efforts to shift the site of starch digestion from the small intestine to the rumen. These efforts generally lead to greater energy intake from nonstructural carbohydrates because the capacity for ruminal fermentation of nonstructural carbohydrates is apparently unlimited, but albeit less efficient. Furthermore, increases in rates and amounts of organic acid production that result from ruminal fermentation of nonstructural carbohydrates predisposes cattle to increased incidences of morbidity that can have long-term deleterious impacts. Additionally, physical and chemical processing of grains to increase ruminal fermentation of nonstructural carbohydrates involves increases in energy expenditure to produce beef and milk, increases machinery requirements, and

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creates greater dependence on fossil fuels. Coordinately, development of novel technologies or feed formulations able to ameliorate limits in small intestinal starch digestion could improve sustainability and health of beef and dairy cattle fed corn-based diets.

Benefits of increasing small intestinal starch digestion in cattle could also provide immediate and large improvements in revenue to United States beef and dairy producers. Huntington et al. (2006) reported that in 2005 the United States produced over 253 billion kilograms (i.e., 10 billion bushels) of grain. They (Huntington et al., 2006) approximated that 50% of this production was consumed by animals and 15% of animal consumption was accounted for by nearly 14 million finishing cattle in the United States. Because starch encompasses 72% of dry matter in corn grain and is the primary grain fed to finishing cattle, Huntington et al. (2006) concluded that significant economic improvements may be achieved through marginal improvements in the efficiency of conversion of corn grain to saleable beef from finishing cattle. Specifically, these authors (Huntington et al., 2006) calculated that if corn grain was valued at $0.10/kg, a 1% improvement in efficiency of conversion of corn grain to beef would result in annual reduction of feed costs by $23 million for finishing cattle. Currently, the United States produces over 348 billion kilograms of corn grain (NASS, 2023). Using more recent reports (NASS, 2023) for amounts of beef cattle in feedlots (25,842,000 head) together with current corn grain prices ($0.27/kg) and assuming that the same proportion of corn grain is used for finishing cattle as that estimated by Huntington et al. (2006), we calculate that under current economies a 1% improvement in efficiency of conversion of corn grain to beef would yield a savings of nearly $70 million to the United States feedlot industry. Yet, current feeding strategies seem unable to facilitate complete digestion of dietary starch that flows to the small intestines.

Some unique aspects to digestion and absorption of nutrients in cattle

Current limitations in small intestinal starch digestion seem to be related to several unique differences in postgastric digestive physiology that are seemingly unique to cattle. Comparatively, cattle and other ruminants are late-comers in the evolution of mammalian digestive physiology, and their digestive system represents a phylogenetic peak of complexity in comparison to nonruminant animals. Yet, little is known of the mechanisms that control digestion and nutrient absorption in the small intestines of ruminants. The dearth of information related to factors that control small intestinal digestion and absorption of nutrients in ruminants is unfortunate, because absorption of nutrients from the small intestine can provide much greater amounts of energy to support physiologically productive purposes in comparison ruminal fermentation.

Ruminants are not born with an immediate ability to support pregastric fermentation. Correspondingly, neonatal ruminants require nutrients and digest food similarly to nonruminants. Ingestion of solid food and cessation of suckling initiates development of pregastric fermentation and results in the greatest modifications in digestive physiology observed among any domesticated animal. Like other nonruminants, expression of enzymes that contribute to digestion of nonstructural plant carbohydrates are relatively

modest immediately after birth, but in 1 to 2 weeks after birth secretion of enzymes from the pancreas and expression of enzyme activity along the small intestinal epithelium increase rapidly (Guilloteau et al., 2009). Activity among enzymes needed to hydrolyze nonstructural plant carbohydrates are responsive to nutritional stimuli in neonates (Zoppi et al., 1972), and activity of these enzymes in calves seems to evolve in an ontogenic way by more than 2,400% in the first 30 days of life (Guillloteau and Le Huerour-Luron, 1996). Yet even though preruminant calves demonstrate a large capacity for small intestinal carbohydrate digestion that is responsive to changes in luminal nutrient flows, capacity for small intestinal digestion of nonstructural carbohydrate digestion is substantially reduced in cattle after development of pregastric fermentation (Owens et al., 1986; Huntington, 1997; Brake and Swanson, 2018).

Responses of α-glycohydrolase secretions to dietary protein and amino acids

It is likely that the primary factor currently limiting small intestinal digestion of starch is suboptimal capabilities for hydrolytic cleavage of polysaccharides and oligosaccharides (Mayes and Orskov, 1974; Orskov, 1976; Kreikemeier and Harmon, 1995). Regulation of brush border α-glycohydrolases appears to be conserved between ruminants and nonruminants; however, unique differences in expression of α-glycohydrolase expression in the small intestinal epithelium and development of pregastric fermentation may not allow for adequate expression of these enzymes in ruminants because of altered postgastric nutrient flows. Sucrase-isomaltase is expressed to a much greater extent than maltase-glucoamylase in nonruminants and is thought to be the primary enzyme that hydrolyzes small chain oligosaccharides in these species (Van Beers et al., 1995). Ruminants, however, have no measurable sucrase activity in the small intestine (Siddons, 1968), which provides strong evidence that the evolutionary divergence of ruminants has led to some level of altered small chain oligosaccharide digestion (Harmon, 1992). In fact, ruminants commonly express greater activity of maltase-glucoamylase throughout the small intestine (Russell et al., 1981; Janes et al., 1985; Kreikemeier et al., 1990), and it may be that small chain oligosaccharide digestion is primarily controlled by this enzyme in ruminant small intestines.

Basal levels of expression of all the brush border α-glycohydrolases is developmentally imprinted early on in fetal development (Van Beers et al., 1995). Yet there is also strong evidence that general regulatory mechanisms can influence brush border α-glycohydrolase expression in nonruminants (Van Beers et al., 1995). Goda et al. (1983) reported that decreased starch intake by rats led to rapid decreases in maltase-glucoamylase and other α-glycohydrolase activities. Bustamente et al. (1986) and Morrill et al. (1989) observed significant increases in brush border α-glycohydrolase activities that corresponded to increased starch intake in rats. Interestingly, starvation increased expression of jejunal sucrase-isomaltase, but not lactase in rats (Nsi-Emvo et al., 1994). Further, when rats were re-fed, a band of enterocytes migrating up the intestinal villi with upregulated sucrase-isomaltase existed (Nsi-Emvo et al., 1994). These data have been interpreted to suggest that enterocyte regulation of brush border α-glycohydrolases is only capable of occurring in developing stem cells located in the

intestinal crypt, and that mature enterocytes are incapable of differentially expressing α-glycohydrolases. It is yet to be clearly defined whether these apparent mechanisms for regulation brush border α-glycohydrolase expression in nonruminant enterocytes are controlled at the transcriptional level.

To date there are no reports that indicate that cattle can increase small intestinal brush border α-glycohydrolase activity in response to greater luminal flows of small chain oligosaccharides; however, several authors (Russell et al., 1981; Khatim and Osman, 1983; Kreikemeier et al., 1990; Bauer et al. 2001) have reported the relative activities of small intestinal brush border α-glycohydrolases along the small intestine. Taniguchi et al. (1995) studied nutrient fluxes across splanchnic tissues with either ruminal or postruminal supply of casein when cornstarch was provided abomasally. These authors (Taniguchi et al., 1995) observed that as postruminal protein supply increased a concomitant increase occurred for glucose release across both the portal drained viscera and total splanchnic tissues leading to increased circulating glucose levels. These improvements in circulating glucose were related to a nearly 50% improvement in N retention by these cattle. Richards et al. (2002) directly measured small intestinal cornstarch digestibility with titrated levels of casein provided abomasally and reported linear increases in small intestinal organic matter and starch digestibility with increasing abomasal casein. Guimaraes et al. (2007), reported that brush border maltase activities were increased approximately 179% when casein was infused postruminal to cattle that had developed pregastric fermentation. We recently completed a study that evaluated impacts of changes in postruminal nutrient flows on small intestinal brush border α-glycohydrolase activity in cattle (Trotta et al., 2020). In that experiment, greater luminal flows of casein increased activity of maltase and glucoamylase and tended to increase isomaltase activity in comparison to increases in luminal flows of glutamic acid or cornstarch alone for a relatively long period of time (i.e., > 42 days). Ostensibly, activities of digestive enzymes in the small intestines of cattle are responsive to postgastric nutrient flow, but greater flows of protein rather than starch may elicit an augmented digestive response.

When Harmon (2009) reviewed the available data, he concluded that a major nutritional factor affecting small intestinal hydrolytic capacity in cattle was energy provided from postruminal protein flows. However, some of our data (Figure 1) indicate that luminal nutrient sensing likely impacts adaptations in small intestinal digestion among cattle rather than changes in metabolizable energy supplies from different nutrient sources to the small intestine. Despite strong evidence that luminal nutrient sensing modulates small intestinal digestion in nonruminants there is nearly no data on the mechanisms that modulate small intestinal digestion in cattle even though several recent reports have indicated that cattle express mRNA for chemosensory molecules in small intestinal tissues (Moran et al., 2014; Doherty et al., 2015; Fan et al., 2020).

Our laboratory has completed several measures of small intestinal starch disappearance in response to changes in postgastric nutrient flows in cattle that have developed pregastric fermentation. In our initial investigations (Brake et al., 2014a) we observed that responses to greater postruminal flows of casein in small intestinal starch

digestion were rapid and appeared to achieve a steady state after 6 days. In a following experiment that was designed to determine if responses in small intestinal starch digestion to greater postruminal nutrient flows from casein were in direct response to amino acids, we observed that changes in small intestinal starch digestion were similar between casein and an amino acid analog of casein (Brake et al., 2014b). In that experiment, we also observed that greater luminal flows of glutamic acid alone increased small intestinal starch digestion to a greater extent than casein or the amino acid analog of casein (Brake et al., 2014b).

These observations led us to investigate if responses in small intestinal starch digestion in cattle to greater postruminal flows of glutamic acid were responsive to different amounts of glutamic acid flowing to the duodenum (Blom et al., 2018). To facilitate some additional measures of nutrient balance, measures of small intestinal starch digestion were collected across a 12 day period (Blom et al., 2018). In that experiment (Blom et al., 2018), we measured linear increases in small intestinal starch digestion to greater postruminal flows of glutamic acid, and that casein (a positive control) and glutamic acid both increased small intestinal starch digestion.

Modulation of responses in small intestinal starch digestion to amino acids

We recently completed work on a more extensive effort to determine how changes in postruminal nutrient flows in cattle influenced pancreatic and brush border enzyme activity (Trotta et al., 2020) together with impacts of increases on small intestinal starch digestion on energy and nutrient balance and effects on changes in body composition in growing calves (Acharya et al., 2023). In that experiment, infusions were provided for a greater period (60 days) than our previous studies to allow for measures of changes in body composition. Measures of small intestinal starch digestion were obtained from composite samples of digesta collected 42 to 45 days after steers were continuously provided greater duodenal flows of casein, glutamic acid or cornstarch alone. Based on our previous observations, we anticipated that measures of small intestinal starch digestion would be increased by greater postruminal flows of casein and glutamic acid in that experiment (Acharya et al., 2023). As expected, measures of small intestinal starch digestion were increased nearly 27% in response to greater postruminal flows of casein; however, greater postruminal flows of glutamic acid had no impacts on measures of small intestinal starch digestion after 45 days of infusion (Acharya et al., 2023). And our measures of brush border enzyme activity (Trotta et al., 2020) appeared to be in close agreement with our measures of small intestinal starch digestion (Acharya et al., 2020)

The lack of response to greater postruminal flows of glutamic acid but a positive response to casein was surprising. When we plotted measures of small intestinal starch digestion in response to greater postruminal flows of casein, glutamic acid or cornstarch from our experiments across time (Figure 1), there appears to be evidence that responses to greater postruminal flows of glutamic acid become refractory but that responses to casein do not.

Glutamate is the primary anaplerotic substance for small intestinal epithelium (El-Kadi et al., 2009), and others (Harmon, 2009) have speculated that responses in small intestinal starch digestion to greater postruminal flows of casein in cattle that have developed pregastric fermentation are in response to greater energy supplies to small intestinal tissue from protein. Refractory responses to greater glutamic acid flows seem to suggest that mechanisms other than greater supplies of metabolizable nutrients are responsible for increases in small intestinal starch digestion to cattle. Indeed, our group has observed that increasing concentrations of glutamic acid result in increased secretions of cholecystokinin, a potent gastrointestinal peptide that stimulates secretion of the enzyme responsible for the initial step of starch digestion in the small intestine, from duodenal explants (Doherty et al., 2015). Interestingly, cholecystokinin secretions were also synergistically enhanced by inosine 5'-monophosphate. Inosine 5'-monophosphate potentiates cellular receptor responses to glutamate by allosterically binding savory or umami taste receptors. Thus, these data support that changes in postgastric nutrient flows impact secretion of hormones in cattle that influence small intestinal starch digestion through methods other than increases in metabolizable nutrient flows. Furthermore, we have also used quantitative PCR to measure translation of taste receptor proteins in duodenal tissues collected from a subset of the steers in our most recent study (n = 3, 3, and 4 for control, casein and glutamic acid, respectively; Acharya et al., 2023). The number of tissues available for measures of translation of chemosensory molecules in duodenal tissue do not allow for appropriate tests of differences between treatments; however, these preliminary data (Figure 2) seem to suggest that postruminal flows of nutrients resulted in altered translation of these molecules and that sensory signals are involved in adaptations of small intestinal digestion in cattle. An understanding of these mechanisms could provide a greater understanding of the mechanisms that regulate the digestive physiology in the small intestines of cattle.

Impacts of increases in starch digestion on glucose utilization in ruminants

Glucose from greater small intestinal starch digestion in cattle must either be oxidized, used for tissue gain (McLeod et al., 2006), or support lactogenesis (Overton and Waldron, 2004). Shifting site of starch digestion to the small intestine can increase glucose assimilated from the diet into circulation. We have observed that rates of glucose appearance were more than 50% greater when small intestinal starch digestion was increased in response to greater postruminal flows of casein (Figure 3).

McLeod et al. (2001) reported that increases in retained energy from abomasal infusions of partially hydrolyzed starch were entirely lipid accretion (McLeod et al., 2001) and calculated that 35% of increases in lipid accretion were associated with alimentary tissues. Generally, contribution of glucose carbon to lipid accretion in cattle are largely thought to contribute to de novo fatty acid synthesized and deposited in intramuscular fat (Smith and Crouse, 1984). However, others (Nayananjalie et al., 2015) did not observe differences in rates at which glucose was used for de novo fatty acid synthesis in different adipose tissues (e.g., subcutaneous, intramuscular, alimentary).

Alternatively, we observed that increases in small intestinal starch digestion in cattle in response to greater postruminal flows of casein tended ($P = 0.11$; Table 1) to increase rates of de novo fatty acid synthesis in longissimus dorsi (i.e., intramuscular fat) but not alimentary or subcutaneous adipose tissue ($P \geq 0.88$); however, differences in de novo fatty acid synthesis rates were not different when expressed on an equal metabolic body weight basis.

Conclusions

Capacity for small intestinal starch digestion appears to be limited in cattle, which is unfortunate because small intestinal starch digestion has potential to increase dietary net energy and glucose assimilated from the diet when compared to ruminal fermentation of starch. Increases in dietary net energy or glucose assimilated from the diet can simultaneously improve efficiency of production and provide greater amounts of substrate important to production of intramuscular fat or lactose. Interestingly, greater postruminal flows of high-quality protein (i.e., casein) and glutamic acid can increase small intestinal starch digestion in cattle; however, effects of greater postruminal flows of glutamic acid appear to be transient whereas response to greater postruminal flows of casein do not. Indirect observations seem to indicate that response in small intestinal starch digestion to greater postruminal flows of casein or glutamic acid are modulated, at least in part, by postgastric nutrient sensing mechanisms and are not completely in response to greater supplies of metabolizable nutrients to small intestinal tissues. A greater understanding of the regulatory mechanisms that control small intestinal starch digestion in cattle could allow for novel diet formulation or development of small molecules with potential to create large opportunities to enhance the efficiency with which cattle use nutrients from feed for production of beef or milk.

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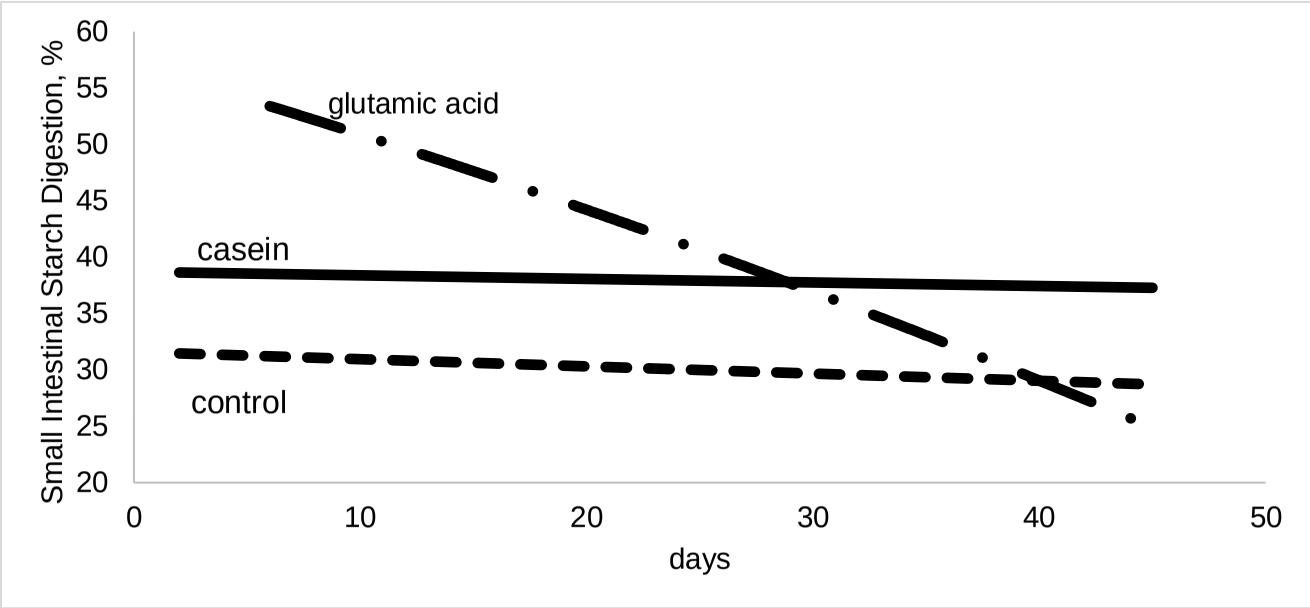

Figure 1. Changes in measures of small intestinal starch digestion to different luminal nutrient flows over time

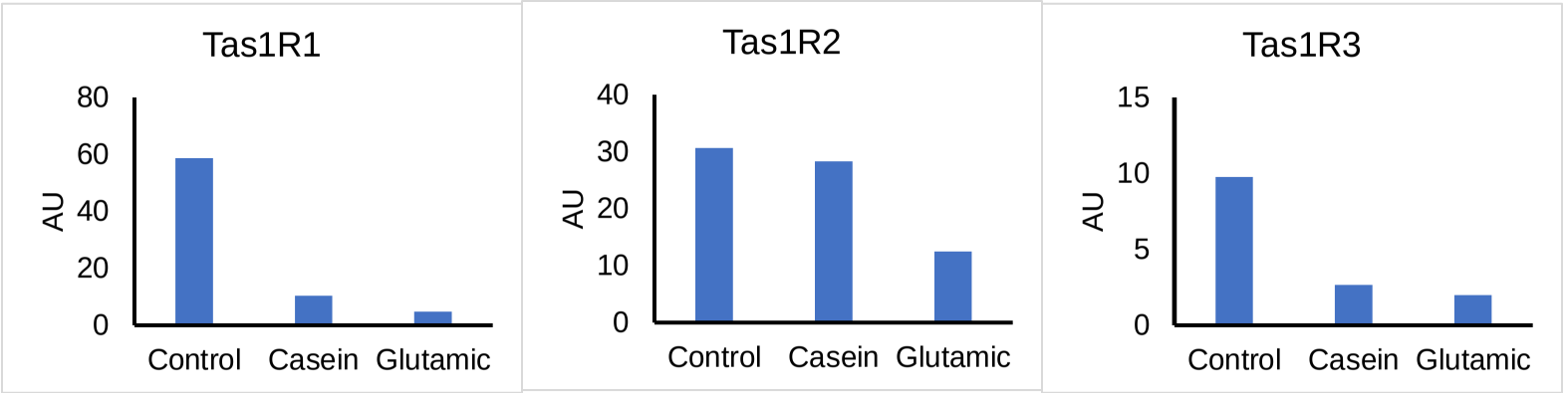

Figure 2. Response in taste receptor translation in duodenal epithelium to changes in luminal nutrient flows.

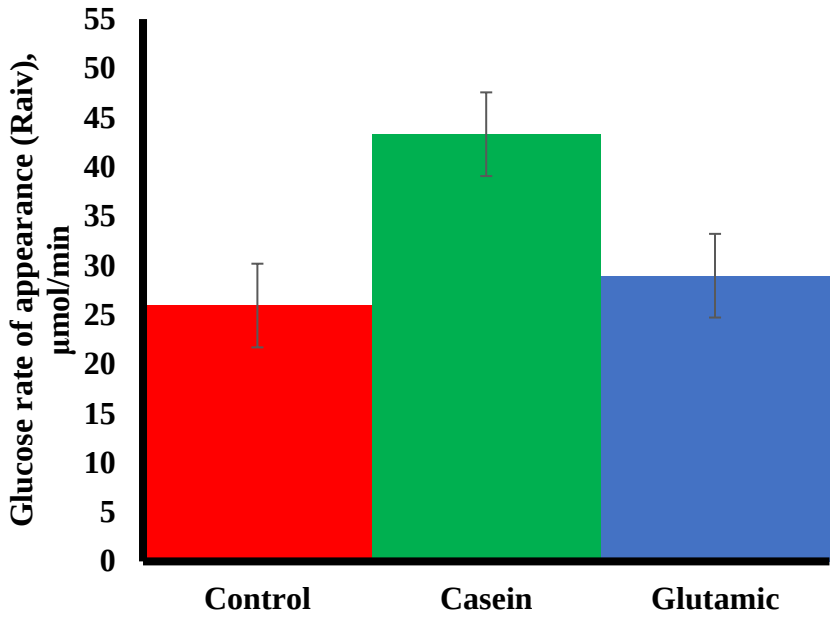

Figure 3. Effect of duodenal infusion of casein or glutamic acid on glucose rate of appearance (µmol/min) in steers receiving 1.5 kg of duodenally infused raw cornstarch, P < 0.01; SEM = 4.24

Table 1. Effect of duodenal infusion of casein or glutamic acid on palmitate fractional synthesis rate in steers receiving 1.5 kg of duodenally infused raw cornstarch.

	Treatment				
Adipose Site	Control	Casein	Glutamic	SEM	*P*-value
% per h					
Omental	0.0468	0.0412	0.0400	0.015	0.88
Longissiumus dorsi	0.0399[a]	0.0738[b]	0.0510[a]	0.015	0.11
Subcutaneous	0.0540	0.0488	0.0559	0.016	0.89
% per h/MBW					
Omental	0.0007	0.0002	0.0007	0.0006	0.26
Longissiumus dorsi	0.0011	0.0012	0.0012	0.0007	0.99
Subcutaneous	0.001	0.000007	0.001	0.0006	0.22

[a,b] Means in rows with different superscripts tend to differ ($P < 0.15$)

SESSION NOTES

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Challenging the dogma of subclinical diseases in dairy cattle

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Introduction

Optimizing cow health and productivity during the transition period represents a significant hurdle to the dairy industry. During early lactation inadequate nutrient consumption is coupled with increasing milk energy output; a scenario that creates a negative energy balance (NEB; Drackley, 1999). Therefore, milk yield during NEB is prioritized by alterations in carbohydrate, lipid, protein, and mineral metabolism. Traditionally, excessive adipose tissue mobilization, the ensuing hyperketonemia and the magnitude of hypocalcemia were thought to be the pathological foundation of transition cow problems and immunosuppression. However, high producing healthy cows may also present high NEFA, hyperketonemia and transient subclinical hypocalcemia. These are key homeorhetic adjustments that cows employ to prioritize milk synthesis at the expense of tissue accretion. Further immune activation also markedly influences metabolism and mineral trafficking, and these adjustments are utilized to prioritize an activated immune system. Thus, an inflamed cow also has a very similar bioenergetic and mineral metabolism footprints as a high producing healthy cow. We believe that altered NEFA, ketones, and calcium are due to one of two reasons: 1) high producing healthy cows are naturally adjusting metabolism during NEB to emphasize milk synthesis, or 2) unhealthy cows in which metabolic alterations reflect immune activation and subsequent hypophagia. The difference in these two models is more than an academic debate, since this nuance has immense economic implications for the producer.

Correlation is Unequal to Causation

Dairy cow lactation maladaptation has extensively been researched for more than five decades and this is primarily because the incidence of health problems is highest in the first two months of lactation. The periparturient period certainly has the dynamic variations in bioenergetics (NEFA, glucose, ketones, insulin, glucagon, BUN, etc.) and minerals (Ca and P) during lactation. Importantly, these temporal patterns are often occurring while negative health events are detected. Correlation and causality are sometimes incorrectly assumed to be equal in regard to the events that occur during the transition period and are claimed to be inevitable rather than coincidental. Most of the assumptions have been largely based on associations and not cause-and-effect relationships garnered from controlled and intervening experimentation. Even from a relationship perspective, assessing the strength or robustness of the associations is difficult due to variability in analysis and statistical methods. In particular, different

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metabolite thresholds are biasedly set for different outcomes and time points among observational studies. Additionally, inconsistent association metrics (e.g., odds ratio, relative risk, hazard ratio) are used to assess these relationships. The inconsistency and inaccuracy of using correlation to interpret causation creates suspect on-farm decision-making and unnecessary farm expenses. More detailed description of this area is covered herein, see our recent review (Horst et al., 2021).

Traditional Dogmas

Long-standing tenets describe a causal role of hypocalcemia, increased NEFA, and hyperketonemia in the incidence of transition diseases and disorders (Figure 1). Hypocalcemia has traditionally been considered a gateway disorder leading to ketosis, mastitis, metritis, displaced abomasum, impaired reproduction, and decreased milk yield (Curtis et al., 1983; Goff, 2008; Martinez et al., 2012; Chapinal et al., 2012; Riberio et al., 2013; Neves et al., 2018a,b). The proposed mechanisms by which hypocalcemia leads to these ailments include impaired skeletal muscle strength and gastrointestinal motility (Goff, 2008; Oetzel, 2013; Miltenburg et al., 2016; Goff, 2020), decreased insulin secretion (Martinez et al., 2012, 2014), and the development of immunosuppression (Kimura et al., 2006). Like hypocalcemia, increased NEFA and hyperketonemia are presumed causative to illnesses such as DA, retained placenta, metritis, reduced lactation performance, poor reproduction, and an overall increased culling risk (Cameron et al., 1998; LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010; Chapinal et al., 2011; Huzzey et al., 2011). Excessive NEFA mobilization and the affiliated increase in hepatic lipid uptake, triglyceride (TG) storage, and ketone body production has been traditionally believed to be the driving factor leading to ketosis and fatty liver (Grummer, 1993; Drackley, 1999). Additionally, elevated NEFA and ketones are thought to compromise immune function (Lacetera et al., 2004; Hammon et al., 2006; Scalia et al., 2006; Ster et al., 2012) and suppress feed intake (Allen et al., 2009). Thus, the magnitude of changes in NEFA, BHB and Ca have traditionally been purported as predictors of future performance.

Culling Trends

A cow's entire lactation and the opportunity to have an additional lactation are heavily dependent on how successfully she adapts throughout the transition period. There is a disproportionate amount of health care and culling that occurs within 60 days after parturition. Minimizing large increases in NEFA and hyperketonemia and preventing subclinical hypocalcemia have been a key strategy in an attempt to improve overall herd health (because the dogma is that they are causal to disease). However, despite our industry's endeavors (medically treating for hyperketonemia and subclinical hypocalcemia), herd health has arguably not improved with time (Table 1). The question then needs asking: "are we attempting to fix the wrong problem"?

Inflammation in the Transition Period

Regardless of health status (Humblet et al., 2006), increased inflammatory biomarkers are observed in nearly all cows during the periparturient period (Ametaj et al., 2005; Humblet et al., 2006; Bionaz et al., 2007; Bertoni et al., 2008; Mullins et al., 2012). The magnitude and persistency of the inflammatory response seems to be predictive of transition cow performance (Bertoni et al., 2008; Bradford et al., 2015; Trevisi and Minuti, 2018). During the weeks surrounding calving, cows are exposed to a myriad of stressors which may permit endotoxin entry into systemic circulation and thereby initiate an inflammatory response (Khafipour et al., 2009; Kvidera et al., 2017c; Barragan et al., 2018; Proudfoot et al., 2018; Koch et al., 2019). The frequency and severity of these inflammation-inducing insults presumably determine the level of inflammation that follows (Bertoni et al., 2008; Trevisi and Minuti, 2018). Common origins of endotoxin entry include the uterus (metritis) and mammary gland (mastitis). Additionally, we believe the gastrointestinal tract may contribute as many of the characteristic responses (rumen acidosis, decreased feed intake, and psychological stress) occurring during the transition period can compromise gut barrier function (Horst et al., 2021).

Although an overt inflammatory response is present around calving, numerous reports have described a reduction in immune competence during this time (Kehrli et al., 1989; Goff and Horst, 1997; Lacetera et al., 2005). Traditionally, hypocalcemia and hyperketonemia have been primary factors considered responsible for periparturient immunosuppression (Goff and Horst, 1997; Kimura et al., 2006; LeBlanc, 2020), however, recent evidence suggests this is more complex than originally understood and that the systemic inflammatory milieu may be mediating the immune system to become “altered” and not necessarily “suppressed” around calving (Trevisi and Minuti, 2018; LeBlanc, 2020). Whether or not the “immune incompetence” frequently reported post-calving is causative to future illnesses or is a consequence of prior immune stimulation needs further attention.

The Importance of Glucose

To adequately recognize the connection between inflammation and transition period success, an appreciation for the importance of glucose is a prerequisite. Glucose is the precursor to lactose, the milk constituent primarily driving milk volume through osmoregulation (Neville, 1990). Approximately 72 g of glucose is required to synthesize 1 kg of milk (Kronfeld, 1982). A variety of metabolic adaptations take place in lactating mammals including increased liver glucose output and peripheral insulin resistance which allows for skeletal muscle to have increased reliance upon lipid-derived fuel (i.e., NEFA and BHBA) to spare glucose for milk synthesis and secretion by the mammary gland (Baumgard et al., 2017). The immune system is also heavily reliant on glucose when activated. The metabolism of inflammation (discussed below) has its own unique metabolic footprint to direct glucose toward the immune system. Consequently, when the onset of inflammation and lactation coincide, glucose becomes an extremely valuable and scarce resource.

Ketogenesis occurs when glucose is in short supply. This can come from a combination of factors including lack of substrate (i.e., reduced feed intake and ruminal fermentation) or high glucose utilization by other tissues (i.e., the immune system or mammary gland). When glucose demand is high, the TCA cycle intermediate oxaloacetate leaves the cycle to supply carbon for gluconeogenesis (Krebs, 1966). Oxaloacetate is also the molecule that combines with acetyl CoA (the end-product of adipose-derived NEFA) to allow the TCA cycle to continue progressing. If the TCA cycle is limited in its progression due to lack of oxaloacetate, acetyl CoA enters into ketogenesis. The link between onset of lactation, immune system activation, and lack of glucose leading to ketogenesis may help explain the metabolic footprint of a poorly transitioning dairy cow.

Metabolism of Inflammation

Inflammation has an energetic cost which redirects nutrients away from anabolic processes (see review by Johnson, 2012) and thus compromises productivity. Upon activation, most immune cells become obligate glucose utilizers via a metabolic shift from oxidative phosphorylation to aerobic glycolysis (not anaerobic glycolysis typically learned about in biochemistry classes), a process known as the Warburg effect. This metabolic shift allows for rapid ATP production and synthesis of important intermediates which support proliferation and production of reactive oxygen species (Calder et al., 2007; Palsson-McDermott and O'Neill, 2013). In an effort to facilitate glucose uptake, immune cells become more insulin sensitive and increase expression of GLUT3 and GLUT4 transporters (Maratou et al., 2007; O'Boyle et al., 2012), whereas peripheral tissues become insulin resistant (Poggi et al., 2007; Liang et al., 2013). Furthermore, metabolic adjustments including hyperglycemia or hypoglycemia (depending upon the stage and severity of infection), increased circulating insulin and glucagon, skeletal muscle catabolism and subsequent nitrogen loss, and hypertriglyceridemia occur (Filkins, 1978; Wannemacher et al., 1980; Lanza-Jacoby et al., 1998; McGuinness, 2005). Interestingly, despite hypertriglyceridemia, circulating BHB often decreases following LPS administration (Waldron et al., 2003a,b; Graugnard et al., 2013; Kvidera et al., 2017a). The mechanism of LPS-induced decreases in [BHB] has not been fully elucidated but may be explained by increased ketone oxidation by peripheral tissues (Zarrin et al., 2014). Collectively, these metabolic alterations are presumably employed to ensure adequate glucose delivery to activated leukocytes.

Energetic Cost of Immune Activation

The energetic costs of immunoactivation are substantial, but the ubiquitous nature of the immune system makes quantifying the energetic demand difficult. Our group recently employed a series of LPS-euglycemic clamps to quantify the energetic cost of an activated immune system. Using this model, we estimated approximately 1 kg of glucose is used by an intensely activated immune system during a 12-hour period in lactating dairy cows. Interestingly, on a metabolic body weight basis the amount of glucose utilized by LPS-activated immune system in mid- and late-lactation cows, growing steers and growing pigs were 0.64, 1.0, 0.94, 1.0, and 1.1 g glucose/kg

$BW^{0.75}$/h, respectively; Kvidera et al., 2016, 2017a,b, Horst et al., 2018, 2019). A limitation to our model is the inability to account for liver's contribution to the circulating glucose pool (i.e., glycogenolysis and gluconeogenesis). However, both glycogenolytic and gluconeogenic rates have been shown to be increased during infection (Waldron et al., 2003b; McGuinness, 2005) and Waldron et al. (2006) demonstrated that ~87 g of glucose appeared in circulation from these processes. Furthermore, we have observed both increased circulating glucagon and cortisol (stimulators of hepatic glucose output) following LPS administration (Horst et al., 2019) suggesting we are underestimating the energetic cost of immunoactivation. The reprioritization of glucose trafficking during immunoactivation has consequences as both are considerable glucose-demanding processes. Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake: this coupling of enhanced nutrient requirements with hypophagia decreases the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus, wool, etc.).

Inflammation and Metabolic Disorders

The periparturient period is associated with substantial metabolic changes involving normal homeorhetic adaptions to support glucose sparing for milk production. Early lactation dairy cows enter a normal physiological state during which they are unable to consume enough nutrients to meet maintenance and milk production costs and typically enter negative energy balance (NEB; Drackley, 1999; Baumgard et al., 2017). During NEB, cows mobilize NEFA in order to partition glucose for milk production in a homeorhetic strategy known as the "glucose sparing." However, increasing evidence suggests that chronic inflammation may be an additional energy drain that initiates the sequence of these disorders (Bertoni et al., 2008; Eckel and Ametaj, 2016) and this is supported by human, rodent, and ruminant literature which demonstrate effects of lipopolysaccharide (LPS) and inflammatory mediators on metabolism and hepatic lipid accumulation (Li et al., 2003; Bradford et al., 2009; Ilan et al., 2012; Ceccarelli et al., 2015). We and others have demonstrated that cows which develop ketosis and fatty liver postpartum have a unique inflammatory footprint both pre- and post-partum (Ohtsuka et al., 2001; Ametaj et al., 2005; Abuajamieh et al., 2016; Mezzetti et al., 2019; Figure 3). Because the activated immune system has an enormous appetite for glucose, it can exacerbate a glucose shortage by both increasing leukocyte glucose utilization and reducing exogenous gluconeogenic substrates by inhibiting appetite. Reduced DMI is a highly conserved response to immune activation across species (Brown and Bradford, 2021) which can further increase NEFA mobilization and hepatic ketogenesis (Figure 3).

Inflammation and Subclinical Hypocalcemia

Subclinical hypocalcemia (SCH) remains a prevalent metabolic disorder afflicting ~25% of primiparous and ~50% of multiparous cows in the United States (Reinhardt et al., 2011). Although no overt symptoms accompany SCH, it has been loosely associated with poor gut motility, increased risk of DA, reduced production performance (i.e., milk yield and feed intake), increased susceptibility to infectious disease, impaired

reproduction, and an overall higher culling risk (Seifi et al., 2011; Oetzel and Miller, 2012; Caixeta et al., 2017). Recent reports indicate that the severity of negative health outcomes observed in SCH cows appears dependent on the magnitude, persistency, and timing of SCH (Caixeta et al., 2017; McArt and Neves, 2020). For example, Caixeta et al. (2017) classified cases as either SCH or chronic SCH and observed more pronounced impairments on reproductive performance with chronic SCH. Similarly, McArt and Neves (2020) classified cows into 1 or 4 groups based on post-calving Ca concentrations: normocalcemia (>2.15 mmol/L at 1 and 2 DIM), transient SCH (≤ 2.15 mmol/L at 1 DIM), persistent SCH (≤ 2.15 mmol/L at 1 and 2 DIM), or delayed SCH (> 2.15 mmol/L at 1 DIM and ≤ 2.15 mmol/L at 2 DIM). Cows experiencing transient SCH produced more milk and were no more likely to experience a negative health event when compared to normocalcemic cows, whereas the opposite (i.e., higher health risk and hindered productivity) was observed in cows experiencing either persistent or delayed SCH. Clearly not all cases of SCH are equivalent; in fact, transient hypocalcemia appears to be correlated with improved “health” and productivity and this may explain why inconsistencies exist in the relationship between SCH and reduced productivity and health (Martinez et al., 2012; Jawor et al., 2012; Gidd et al., 2015). However, it remains unclear why, despite successful implementation of mitigation strategies, SCH remains prevalent, why SCH is associated with a myriad of seemingly unrelated disorders, and what underlying factors may be explaining the different “types” of SCH.

Impressively, immune activation was originally hypothesized by early investigators to be involved with milk-fever (Thomas, 1889; Hibbs, 1950), but until recently (Eckel and Ametaj, 2016) it has rarely been considered a contributing factor to hypocalcemia. Independent of the transition period, we and others have repeatedly observed a marked and unexplainable decrease in circulating calcium following LPS administration in lactating cows (Griel et al., 1975; Waldron et al., 2003; Kvidera et al., 2017b; Horst et al., 2018, 2019; Al-Qaisi et al., 2020). Infection-induced hypocalcemia is a species conserved response occurring in humans (Cardenas-Rivero et al., 1989), calves (Tennant et al., 1973; Elsasser et al., 1996;), dogs (Holowaychuk et al., 2012), horses (Toribio et al., 2005), pigs (Carlstedt et al., 2000) and sheep (Naylor and Kronfeld, 1986). Additionally, hypocalcemia occurs in response to ruminal acidosis in dairy cows (Minuti et al., 2014). It is unlikely that cows (even those that are presumably “healthy”) complete the transition period without experiencing at least one immune stimulating event and we are likely underestimating its contribution to postpartum hypocalcemia. In summary, it is probable that immune activation is at least partially explaining the incidence of SCH in the postpartum period. It is intriguing to suggest that cases of delayed, persistent, and chronic SCH recently described by Caixeta et al. (2017) and McArt and Neves (2020) may be related to the severity of the periparturient inflammatory response. This hypothesis may explain why these cases of SCH are associated with reduced health, as these may represent direct consequences of immune activation rather than simply decreased Ca.

In addition to SCH, there are on-farm milk-fever situations that are biologically difficult to explain. For example, even while strictly adhering to a pre-calving calcium

strategy, there remains a small percentage (~<1%) of cows that develop clinical hypocalcemia. Additionally, reasons for why a mid-lactation cow develops milk-fever are not obvious. Further, there appears to be an undecipherable seasonality component to clinical hypocalcemia in the southwest and western USA that coincides with the rainy season. Inarguably, there remain some aspects of Ca homeostasis that continue to evade discovery.

Conclusions

New evidence and thinking around inflammation are challenging the traditional dogmas surrounding hypocalcemia, elevated NEFA, and hyperketonemia as the causative factors in transition cow disease. We suggest, based upon the literature and on our supporting evidence, that activation of the immune system may be the causative role in transition cow failure (rather than the metabolites themselves) as inflammation markedly alters nutrient partitioning and these metabolites as a means of supporting the immune response (Figure 3). More research is still needed to understand the causes, mechanisms, and consequences of immune activation and how to prevent immune activation or support its efficacy to provide foundational information for developing strategies aimed at maintaining productivity.

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Table 1. National Animal Health Monitoring Systems

Culling Reason	NAHMS (1996)	NAHMS (2002)	NAHMS (2014)
Voluntary Reasons	21.3	19.3	21.1
Reproduction	25.3	26.5	24.2
Mastitis	25.1	25.9	24.4
Injury	4.1	6.0	5.2
Death	3.8	4.8	4.2
Disposition	0.9	0.9	-
Lameness	14.2	16.3	16.8
Other	3.9	4.1	-

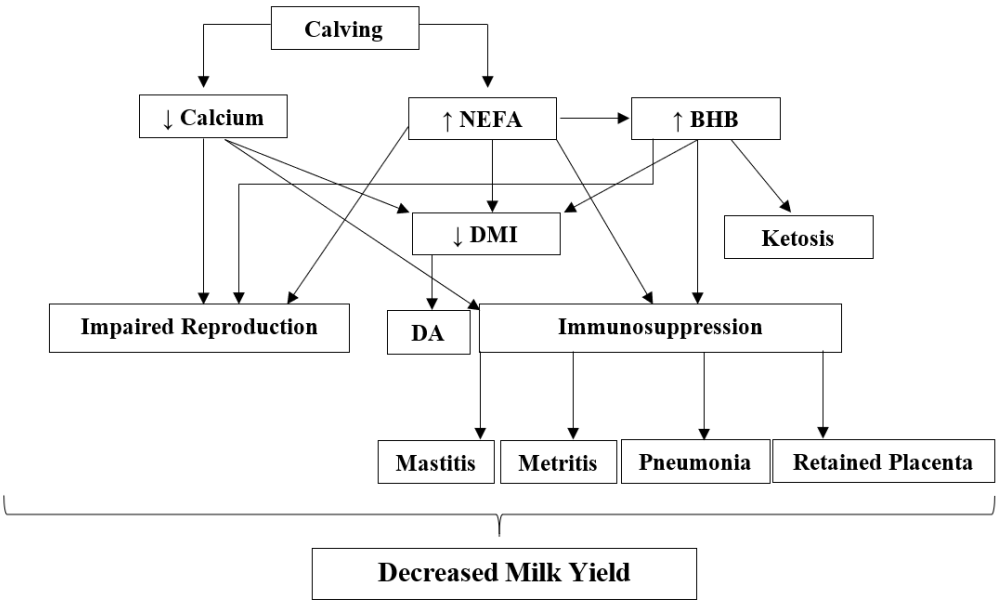

Figure 1. Traditional mechanisms by which hypocalcemia and increased NEFA and ketones are thought to cause poor transition cow health and performance.

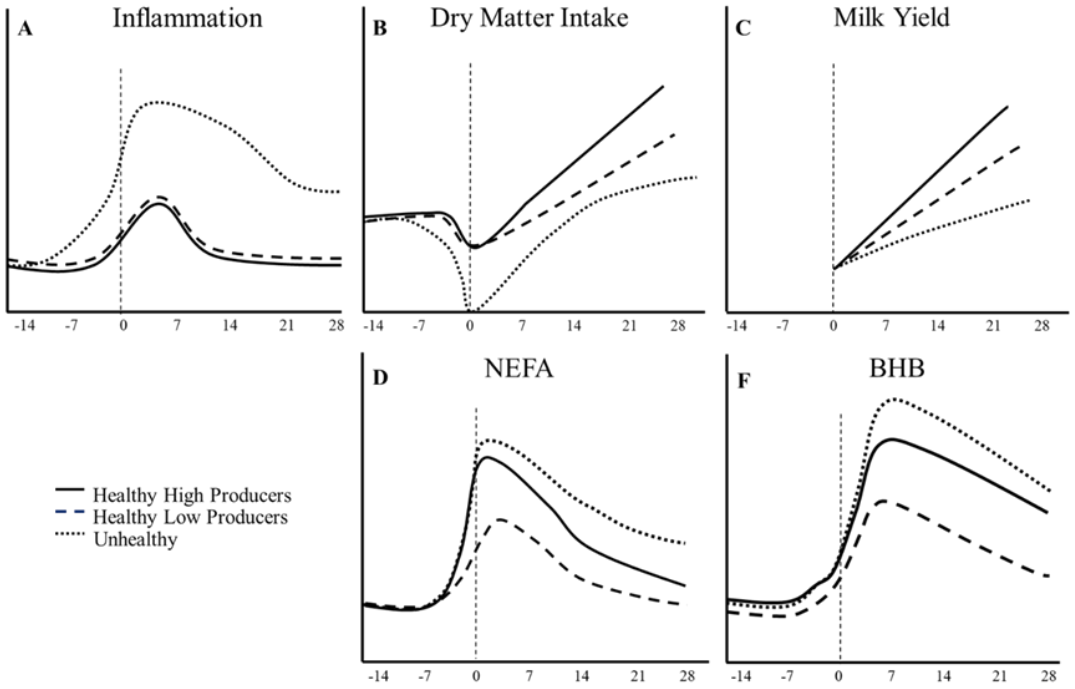

Figure 2. Transition period patterns inflammation (A), dry matter intake (B), milk yield (C), NEFA (D) and BHB (F) in healthy high producers (solid line), healthy low producers (dashed line) and unhealthy (dotted line).

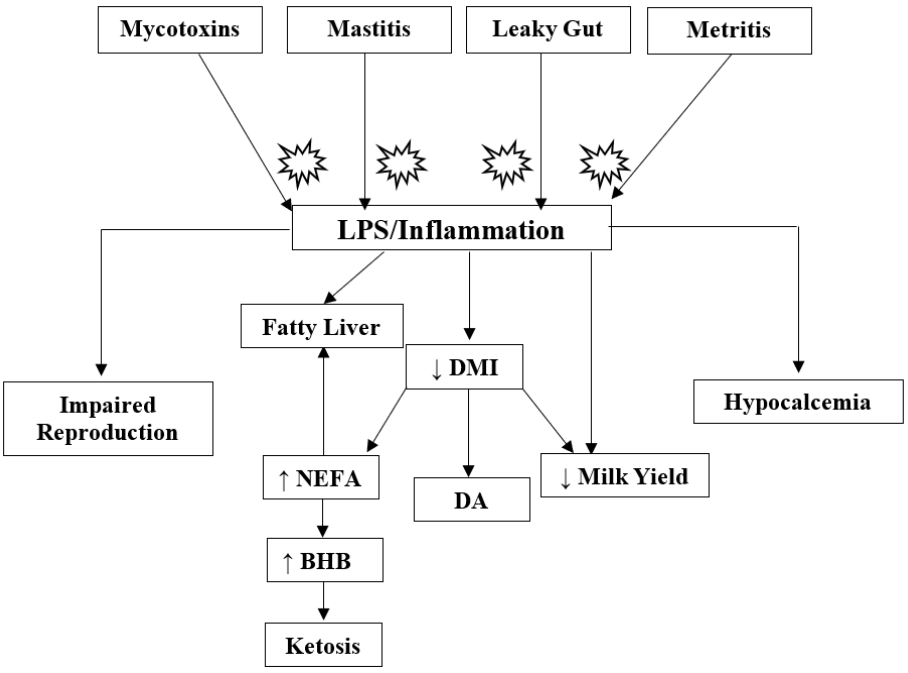

Figure 3. Potential downstream consequences of immune activation. In this model, decreased feed intake, hypocalcemia, excessive NEFA, hyperketonemia and hepatic lipidosis are not causative to poor transition cow performance and health, but rather a reflection of prior immune stimulation.

SESSION NOTES

The role of sulfur affecting selenium and copper nutrition in cow-calf

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Sources of S

Beef cattle have a maximum tolerable concentration of 0.30-0.50% dietary sulfur (**S**), which can easily be exceeded depending on the feed (NASEM, 2016). The maximum tolerable concentration varies depending on the specific source of S and composition of diet. Sarturi et al. (2012) demonstrated that concentration of rumen degradable S is of more concern than total S amount because rumen undegradable S is not readily reduced to sulfide and therefore does not contribute to ruminal hydrogen sulfide gas production. This means feeds that include more rumen degradable S have more impact on animal health than do feeds higher in rumen undegradable S. Inorganic S compounds, such as ammonium sulfide, provide more rumen degradable S. Ethanol coproducts such as distillers grains may be high in rumen degradable S, because ethanol plants use sulfuric acid to control pH during processing. Furthermore, sulfate concentrations of water used during production also impacts S concentrations of distillers grains (Schingoethe et al., 2008). Because of these processes, S content of ethanol coproducts can be extremely variable. For example, Buckner et al. (2011) found S content in distillers grains across six different ethanol plants in Nebraska varied widely within each plant and between each plant. The rumen microbiome can eventually adapt to the presence of high S, decreasing hydrogen sulfide formation and lessening S toxicity risk. Highly variable S content of the diet prevents this adaptation and creates greatest risk for cattle deaths from excess S.

Other feedstuffs high in S include corn gluten feed, molasses, and alfalfa hay (NASEM, 2016). In general, the more crude protein a feedstuff has, the more S will be present in the form of S amino acids. The S available for microbial reduction to sulfide depends on the rumen degradability of the protein (NASEM, 2021). For example, alfalfa hay has an estimated 0.28% dietary S (NRC, 1996). Crude protein availability of alfalfa hay varies widely depending on factors such as plant maturity at harvest, handling, and storage (Lacefield, 1988); consequently, the availability of the S present also depends on these factors. Brome hay has a slightly lower concentration of S than alfalfa (NRC, 1996), but availability of S depends on similar factors as alfalfa. Molasses has an estimated S concentration of 0.64% DM (NASEM, 2021), and 65-77% of this S is available for reduction by bacteria (Bouchard and Conrad, 1973). Further, brassica vegetables are high in S and crude protein, and both are rapidly degraded in the rumen (de Evan et al., 2019). Because rumen degradable S content of most forages is typically not as high as ethanol coproducts, cattle fed high forage diets have tolerable concentrations of dietary S closer to the 0.5% threshold (NASEM, 2016).

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Depending on the region, water can be a major contributor to total S intake. Gould et al. (2002) analyzed forage and water samples from randomly selected cow-calf operations across 23 states to estimate total S intake per animal on each operation. They found that 11.5% of operations had an estimated S intake ≥ 0.4% DM. Drought increases the risk of S toxicity from water. During periods of drought, water sulfate becomes more concentrated; the southeastern United States was found to be most susceptible to drought-induced increases in water sulfate concentration (Xie et al., 2019). This means drought can result in increased S intake in cattle. Because water sulfate is already in solution, it is reduced to sulfide rapidly in the rumen, meaning there are significant risks associated with increased S intake from water sulfate (NASEM, 2021). It is important to consider all S sources and potential variation among dietary S concentrations when determining cow S intake.

S Antagonism of Cu

Ruminants exposed to high dietary S experience decreased Copper (**Cu**) retention (van Ryssen et al., 1998; Spears et al., 2011; Richter et al., 2012; Pogge and Hansen, 2013). In the rumen, sulfuric compounds are readily reduced to sulfide, which results in Cu antagonism. This can occur directly or in tandem with Molybdenum (**Mo**) (Suttle, 2010). Sulfide can directly bind Cu, forming insoluble Cu sulfide, thus decreasing bioavailability of Cu (López-Alonso and Miranda, 2020). Sulfide can also bind Mo, forming thiomolybdates (Suttle, 2010). These compounds form tightly bound complexes with available Cu in the rumen, rendering it indigestible throughout the entire digestive tract (Suttle, 1974). If no ruminal Cu is available for thiomolybdates to bind, they are absorbed into the blood and inhibit Cu function within tissue (Gould and Kendall, 2011). Therefore, when thiomolybdate formation is a risk, at least some soluble Cu in the diet is essential to bind thiomolybdates and prevent their absorption. Clarke and Laurie (1980) found at high S:Mo ratios and pH 6.5, trithiomolybdate is most prevalent, and as pH decreases, tetrathiomolybdate becomes more dominant. Tetrathiomolybdate has the most affinity for Cu (Gould and Kendall, 2011); thus, as rumen pH decreases, thiomolybdate-Cu binding becomes more active. This becomes important given that high concentrate diets decrease rumen pH (Calsamiglia et al, 2008). Further, iron (**Fe**) can exacerbate Cu antagonism through binding Cu and S, thus decreasing Cu available for thiomolybdate binding (Suttle, 2010). This increases thiomolybdate absorption and Cu antagonism within tissues. **Figure 1** illustrates the binding and antagonism of S, Mo, and Fe on Cu in the rumen.

Symptoms of Cu Deficiency

Dietary copper requirements depend on concentrations of antagonists such as S and Mo (NASEM, 2016). Provided concentrations of S remain below 0.25% and Mo below 2 mg/kg DM, 10 mg Cu/kg DM should be satisfactory (NASEM, 2016). Various enzymes, cofactors, and reactive proteins depend on Cu to function. These Cu-dependent compounds play important roles in reproduction, bone development, connective tissue development, and pigmentation (Suttle, 2010). Cu's role in tissue

growth is especially important during fetal development. Cattle with liver and plasma Cu concentrations of 20 mg/kg DM and 0.50 mg/L, respectively, are considered deficient (NRC, 1996). Unless severely deficient, plasma Cu concentration is not as valuable as liver in determining overall Cu status (Claypool et al., 1975). This is because the liver maintains plasma Cu at relatively stable concentrations unless liver Cu concentrations become too depleted (Herdt and Hoff, 2011). Because of this, moderate Cu deficiency can be difficult to detect without conducting liver biopsies. Moderate deficiency in pregnant animals may result in fetal malformation and death, as connective tissue disorders that may arise from Cu deficiency impairs fetal cardiac and bone development (Tinker and Rucker, 1985). Further, severely Cu deficient cattle may exhibit impaired growth and immune function, diarrhea, osteoporosis, and joint problems (Gooneratne et al., 1989). One of the earliest signs of Cu deficiency is loss of hair pigmentation (NASEM, 2016). It is important to note that, although moderate Cu deficiency may not present obvious symptoms, it may still result in impaired growth and reproductive function (Hidiroglou, 1979). Visible symptoms of Cu deficiency can also be related to other illnesses; therefore, it is important to monitor intake of Cu and its antagonists to predict potential for Cu deficiency.

Strategies to Overcome S Antagonism of Cu

If a herd is at risk of Cu deficiency due to high dietary S or Mo, various Cu supplementation strategies are available to ensure maintenance or repletion of Cu status. In most cases, symptoms of Cu deficiency can be resolved by supplementation (Gooneratne et al., 1989). Injectable trace mineral supplements provide the most rapid repletion (Hartman et al., 2018), which may be beneficial to rapidly improve severe Cu deficiency. Injectable trace mineral supplements are not a permanent fix to deficiency, so other supplementation strategies should be considered in partnership with trace mineral injection to prevent deficiency from occurring again. Organic sources of Cu such as amino acid bound Cu, are often more available for absorption than inorganic sources because they are insoluble in the rumen and avoid antagonist binding (Spears, 2003). However, it is still important to feed rumen soluble, inorganic sources of Cu available for thiomolybdates to bind in order to prevent unbound thiomolybdates from entering the bloodstream and inhibiting Cu directly within tissue (Black and French, 2004). **Figure 2** from Hartman et al. (2018) shows the injectable trace mineral supplement is most effective at rapidly increasing liver Cu, but by day 28, it is similar to the inorganic-organic Cu blend supplement. Further, Hartman et al. (2018) demonstrated that the cattle given the inorganic Cu supplement alone took the longest to reach similar liver Cu concentrations as the other treatments. Tribasic Cu chloride is insoluble in the rumen and may therefore be more available for absorption in the presence of ruminal antagonists (Spears et al., 2004). If cattle do not have high sulfur or molybdenum intake, inorganic Cu sources such as Cu sulfate are adequate to prevent deficiency (Spears et al., 2004). Both organic and inorganic sources of Cu are commonly available in salt-mineral premixes, or they can be added to the total mixed ration (Smart et al., 1992). Cu oxide needles can also be used to provide a slow release of Cu over time from the rumen; however, they have been shown to decrease forage utilization due to the antimicrobial properties of the Cu being released (Arthington, 2005). Further, these

boluses can cause rapid increases in liver Cu concentration and thus should be used with caution (Hansen and Messersmith, unpublished). Because Cu requirements are largely dependent on antagonists, it is important for producers to consider antagonistic pressure when choosing a repletion strategy to avoid Cu toxicity.

Caution Against Over-Supplementation of Cu

Though vital, supplementing too much Cu can be fatal. The maximum tolerable concentration of Cu is 40 mg/kg DM; anything near or above this concentration can cause excess Cu to accumulate in the liver (NASEM, 2016). The exact maximum tolerable Cu concentration is dependent on the presence of antagonists in the diet (NASEM, 2016). Relative to other species, ruminants have little ability to excrete excess Cu (López-Alonso and Miranda, 2020). Because the ruminant liver stores most excess Cu, there are no physiological signs of overfeeding Cu until hemolytic crisis occurs, during which Cu is suddenly released from the liver in large amounts (NASEM, 2016). It can take months of overfeeding Cu to get to this point. In fact, feeding slightly less than 40 mg Cu/kg DM over extended periods of time has been found to result in unsafe levels of Cu accumulating in the liver (Bradley, 1993). During hemolytic crisis, red blood cells rupture, hemoglobin is excreted in urine, and widespread necrosis occurs (NASEM, 2016). Many of these effects are caused by the high levels of oxidative metabolites that are released from the liver during necrosis (Gummow et al., 1991). Death occurs between 12 and 72 hours after the onset of hemolytic crisis (Bradley, 1993); therefore, by the time clinical symptoms appear, it is often too late to correct the issue. Because plasma Cu concentrations are well regulated, a liver biopsy is the only exact indicator of excess Cu (López-Alonso and Miranda, 2020). If excess Cu is found, producers can decrease dietary Cu fed and feed Cu antagonists until liver Cu concentrations are in the safe range of 125-600 mg/kg DM (Kincaid, 1999).

S Antagonism of Se

Selenium (**Se**) is an essential mineral for cattle, incorporated into selenoproteins in selenocysteine. Selenoproteins such as glutathione peroxidase and thioredoxin reductase support antioxidant function. Selenium is a unique nutrient because federal guidelines limit how much can be supplemented, up to of 3 mg of Se/cow/day allowed. This is because while Se supports biological functions ranging from reproduction to immunity, it can also be quite toxic at relatively low concentrations in the diet. The NASEM (2016) recommendation for dietary Se for all classes of beef cattle is 0.1 mg Se/kg DM, while the maximum tolerable concentration is 2 mg Se/kg DM.

Selenium may enter a cow’s diet through supplementation in the form of organic or inorganic Se, injectable Se, or as a rumen bolus, strategies which will be discussed below in detail. Selenium is incorporated into selenomethionine in plants (higher order organisms like cattle cannot do this) and varies tremendously depending on soil Se concentrations. In areas such as Florida, sandy soils often do not hold Se and thus plants and subsequently grazing cattle can be quite Se deficient. Similarly, Se deficient soils are common in many areas of the U.S., including Wisconsin, the Pacific NW and

the NE. Other areas may have the opposite challenge, where soil Se is high and Se accumulating plants thrive. This can lead to incidences of Se toxicity.

When dietary S is increased, through feed or water sources of sulfate, Se absorption may be decreased. Sulfur and Se share very similar chemical structures, which is why Se can replace S in the amino acids cysteine and methionine to form selenocysteine (critical for selenoproteins) or selenomethionine. The similarity in sulfate and selenate results in competition for the same transporter in the intestine. Selenate is actively absorbed in the small intestine via the same transporter as sulfate (SLC13A1), and this is the likely point of antagonism by which high S diets decreases Se status. While the specific S-Se transporter interaction has not been extensively examined in ruminants, mineral transporters are well conserved, meaning the literature examining this interaction in other species is likely very relevant to the field of cattle nutrition.

In comparison to Cu, our understanding of the impact of excess S on Se metabolism is limited. Most of the work has been with dairy cows, sheep or feedlot cattle as models. Apparent absorption and balance of Se linearly decreased as S increased from 0 to 0.4% added S (as Ca and Mg sulfate) in dairy cows (Ivancic and Weiss, 2001). Fecal Se increased and urinary Se decreased as S increased, further supporting the assertion that high S decreases Se absorption. The authors also showed that feeding supplemental S over 0.2% reduced Se status of cows even when fed 0.3 mg supplemental Se as Na selenate (Ivancic and Weiss, 2001).

Hartman et al. (2018) utilized Red Angus growing steers to examine the effects of additional S (0.3% added from calcium sulfate) in a corn-silage based diet. The total diet contained 0.48% S, and 2 mg supplemental Mo/kg DM. After feeding the antagonistic diet, with no additional Se for 90 days, liver Se was dramatically decreased (2.0 v. 1.22 mg/kg DM) compared to steers receiving a non-antagonist diet that also included 0.1 mg Se (sodium selenite). Because the antagonist treatment also did not receive supplemental Se (the authors were trying to decrease TM status prior to a repletion period), it must be noted this decrease could be from added S antagonizing Se absorption and/or from lesser amounts of dietary Se. Plasma Se was also decreased in the antagonist treatment, but not as severely as liver (135 vs. 128 ug/L). As described in the Cu section, repletion with injectable TM (Multimin90) most quickly increased liver Se, with the organic (SelPlex-Se)/inorganic blend being next most effective and feeding 150% NASEM (2016) requirements from all inorganic Se taking the longest.

Symptoms of Se Deficiency

Selenium deficiency in the cowherd may manifest in a few different ways. One of the most obvious symptoms attributable to Se is retained placenta. If a producer notes increased incidence of retained placenta, plasma Se should be analyzed, and if necessary, liver Se. Additionally, because vitamin E and Se have overlapping and synergistic roles in antioxidant function in the body, vitamin E supplementation and status should also be examined. A cowherd deficient in vitamin E will draw more on Se to support function in the body, and vice versa. Another likely antioxidant role of Se is in

support of immune function, supporting many different immune cell types such as neutrophiles (NASEM, 2021). Another symptom of Se may be “non-thrifty” newborn calves. These calves may not want to suckle or struggle to suckle. This is because Se deficiency impairs proper muscle function and development, and calves born to Se-deficient cows have underdeveloped throat muscles, causing them to struggle to suckle. In severe cases, calves may be born dead and the veterinarian may diagnose white muscle disease. This appears as white striations in the skeletal and cardiac muscle and affected calves die within a few days of birth because of heart failure (NASEM, 2021).

Strategies to Overcome S Antagonism of Se

Producers have a variety of options by which they may supplement Se to cows, which requires an understanding of how inorganic and organic Se are handled in the body. Inorganic Se such as sodium selenite is the most common form of Se added to cow diets. This Se is absorbed via the aforementioned selenate/sulfate transporter, and in the liver eventually is converted to selenocysteine to enter the selenoprotein pool. Organic Se is in the form of selenomethionine and does not utilize the sulfate transporter for absorption in the small intestine. Rather, it will enter the bloodstream via the methionine transporter in the gut. Because of this, selenomethionine also readily crosses placental and mammary barriers. Selenomethionine can directly enter the body’s amino acid pool, and may be incorporated into muscle or milk protein in place of methionine. In this case, the Se is not used to support Se functions for the cow, but may be used in the future, if that selenomethionine is mobilized. Selenomethionine can be metabolized in the liver to selenide and eventually into selenocysteine, but cannot go directly to selenocysteine. Once in the form of selenocysteine, it can help form selenoproteins and be used in support of Se-dependent functions.

Two alternatives to supplement Se to cows are injectable or bolus. Injectable may be forms such as Mu-Se, which include vitamin E, or Multimin90, which includes Cu, Zinc (**Zn**) and Manganese (**Mn**) in addition to Se. Our laboratory and others have extensively studied Multimin90, and we have examined the effects of this injectable on Se status of cattle fed high S (and Mo) diets. As shown in **Figure 3**, similar to the results on Cu, injectable Se most quickly recovered liver Se concentrations, while inclusion of organic Se (**Sel-Plex**) increased liver Se by d 28 and supplementing 150% NASEM (2016) from inorganic achieved similar liver Se by d 42. This reinforces that there are many strategies to overcome S-antagonism of trace minerals, and producers should chose the one that fits their timeline, labor and economic needs.

Caution Against Over-Supplementation of Se

Cattle readily store Se in the liver, which can lead to toxicity risk. Thus, producers should use caution against supplementing Se to cows from multiple sources. For example, incorporation of an inorganic Se at maximal feeding allowance, plus an injection or bolus, plus an organic source is generally not advisable.

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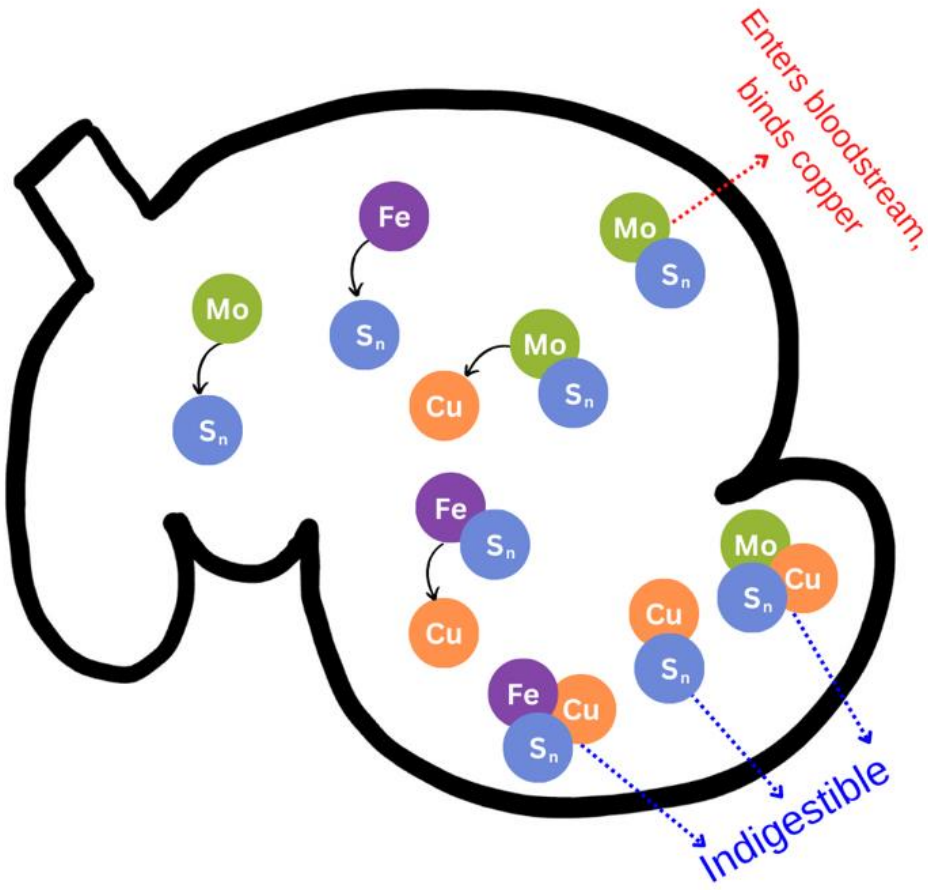

Figure 1. A simplified illustration of S, Fe, and Mo antagonism of Cu in the rumen.

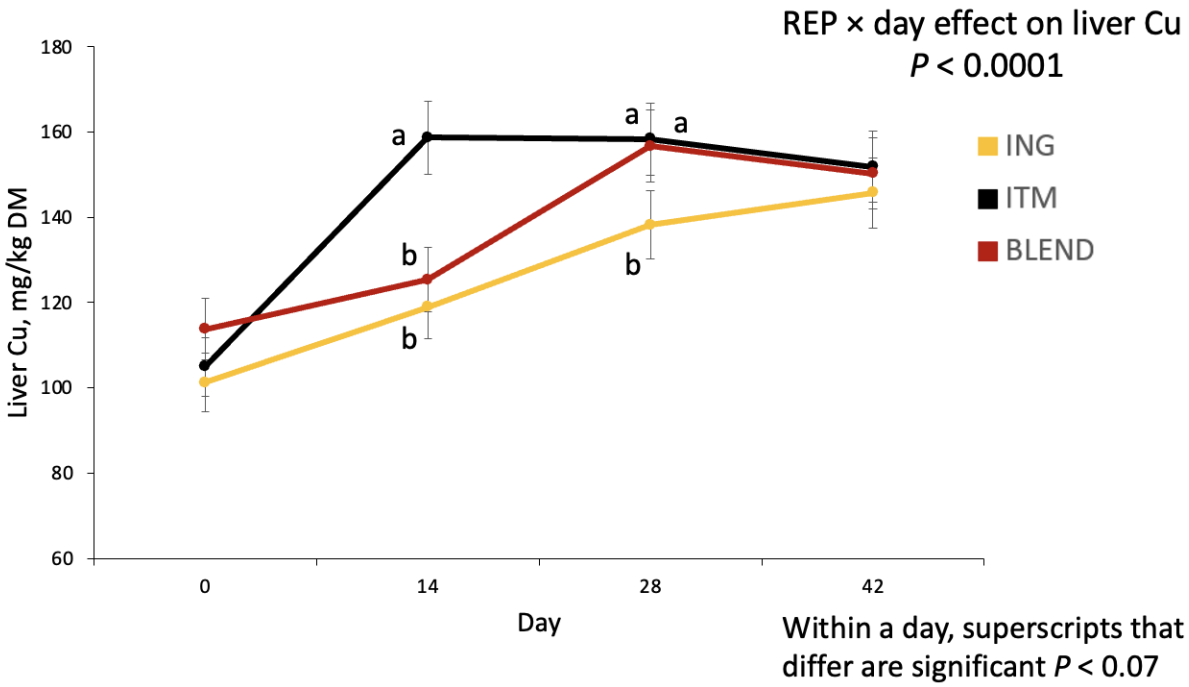

Figure 2. Effect of trace mineral repletion strategy (**REP**) on liver Cu concentrations following a 90 d depletion period that included high S. ING is 150% of NASEM (2016) dietary trace mineral supplementation from only inorganic sources. ITM is a Multimin90 injection on top of 100% NASEM (2016) trace mineral supplementation from inorganic sources. BLEND is 150% of NASEM (2016) dietary trace mineral supplementation, from 25% organic (Availa Cu, Mn, Zn and Sel-Plex Se) and 75% inorganic sources (originally published by Hartman et al. (2018).

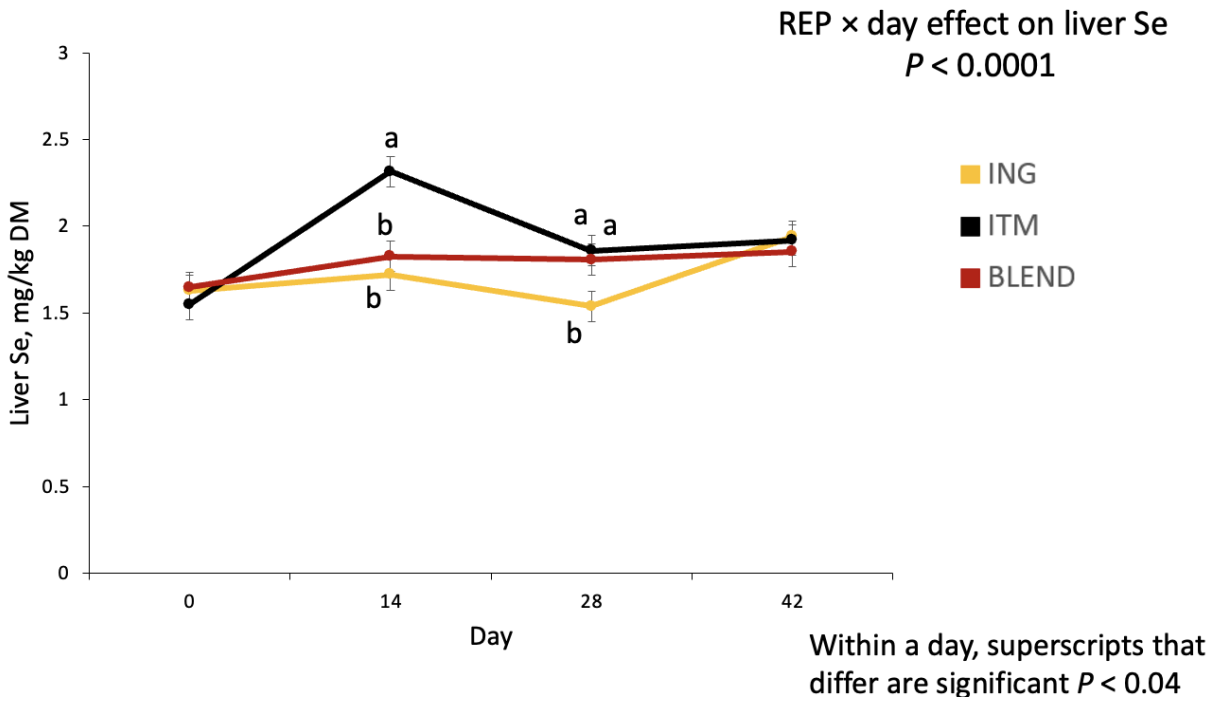

Figure 3. Effect of trace mineral repletion strategy (**REP**) on liver Se concentrations following a 90 d depletion period that included high S. ING is 150% of NASEM (2016) dietary trace mineral supplementation from only inorganic sources. ITM is a Multimin90 injection on top of 100% NASEM (2016) trace mineral supplementation from inorganic sources. BLEND is 150% of NASEM (2016) dietary trace mineral supplementation, from 25% organic (Availa Cu, Mn, Zn and Sel-Plex Se) and 75% inorganic sources (originally published by Hartman et al. (2018).

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Breeding cows to do more with less: an update on efforts to improve feed efficiency in the USA

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Michigan State University

Introduction

Feed accounts for half the costs on most dairy farms. Thus, cows with greater feed efficiency, meaning those cows that need less feed for each pound of milk they produce, are likely to be more profitable. Cows that are more efficient also need less land per pound of milk and will produce less waste per pound of milk. They might also produce less methane per pound of milk. Thus, feed efficiency is a trait well worth considering as a breeding goal, but, until now, it has never been a trait we have focused on in our breeding goals. Instead, we have focused on more milk per cow, and feed efficiency has increased along with increased milk. With the 2021 version of the US Net Merit, we now focus specifically on feed efficiency as a selection trait.

Since the 1990s, we have known that feed efficiency is a heritable trait, based on work from Europe (Veerkamp et al., 1995). The problem, however, was that we did know then and still do not know the feed intake of individual cows on most commercial farms, and we need feed intake to calculate feed efficiency. Traditionally, to estimate the genetic breeding value of new dairy sires, we used data from thousands of his daughters, compared to their herdmates. Thus, direct selection for feed efficiency was simply impossible. The advent of genomics has changed that. Genomics enables us to make faster progress for existing traits, like milk protein or fat yield, and to breed for new traits, like feed efficiency. To do that, however, we need a reference population of cows with known phenotypes for feed efficiency and known genotypes. We can then determine the relationship of each individual marker in a cow's genome to the trait of interest and make equations that relate the genotype and phenotype. We then take that equation and apply it to new cows based on their genotype to predict their phenotype. For an excellent review of genomic selection, see Eggen (2012).

In 2010, we started a project in the U.S., with Michigan State University and the University of Wisconsin as leaders, to study the genomics of feed efficiency. Our team has both nutritionists and geneticists. We were able to first obtain major funding from the UDSA National Institute of Food and Agriculture, and we now have funding from the U.S. Foundation for Food and Agriculture Research and the U.S .Council for Dairy Cattle Breeding (CDCB). Other team members include scientists from Iowa State University, the University of Florida, the UDSA Animal Genomics Improvement Lab in Maryland, and the CDCB. Our goal is to measure feed efficiency on thousands of cows

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in research herds and develop a database of feed efficiency phenotypes and genotypes that can be used to develop genomic breeding values for feed efficiency.

After 12 years of work, we have 7500 cows in our database to serve as our reference population for estimating feed efficiency breeding values, with ~800 more cows added every year. This is the largest database of measured cow feed intakes in the world for developing predictions of feed efficiency; it is housed by the US CDCB. We also are collaborating with Canadian scientists as part of the Resilient Dairy Genome Project (http://www.resilientdairy.ca/). Currently, our CDCB database includes over 1,000 additional feed efficiency phenotypes calculated from Canadian data. More cows are available from other countries. The more cows in the database, the more reliable the estimate breeding values for feed efficiency will be.

Our efforts to collect data have now led to changes in dairy cattle selection. In December 2020, feed efficiency was added to the US Net Merit Index (**NM$**), as a new trait called Feed Saved (**FS$**). Feed Saved is a term first coined by dairy geneticists in Australia (Pryce et al., 2015), and both Australia and the Netherlands were already using predicted breeding values for feed efficiency of dairy cattle before 2020.

Feed Saved is a trait composed of two parts. First, FS$ considers body weight (**BW**) and the feed saved when a cow is smaller and needs less feed for maintenance, so a greater proportion of her feed is used for milk. Second, FS$ considers a calculation known as Residual Feed Intake (**RFI**) and the feed saved when a cow is more efficient at digesting and metabolizing nutrients as she makes milk and meets her maintenance needs. Cows with a negative RFI eat less feed than predicted based on BW, milk production, parity, BW change, days-in-milk, and the intake of their cohorts on the same diet at the same time at the same location (**Figure 1**).

As long as the resulting daughters from bull or cow with a positive breeding value for FS$ produce at least as much milk, with the same protein and fat content, as herdmates, they will be more efficient at turning feed into milk. To better understand Feed Saved, let's consider its two parts separately: Cow body size and RFI.
The points to remember are:

1. Feed is saved when cows are smaller but continue to produce as much milk. They produce more milk per unit of body weight.
2. Feed is saved when cows have lower residual feed intake.
   They eat less than expected based on their milk production, body weight, and body weight change.

Breeding for smaller cows

Cows, like all animals, need some feed every day for maintenance, just to stay alive. The amount of feed energy needed for maintenance is directly related to the cow's body weight. For years, we have been breeding and managing cows for greater milk production. As cows eat more feed, a greater proportion of their feed intake is used

for milk and a smaller proportion is used for maintenance. This is commonly called the "Dilution of maintenance". Today's dairy cows produce 5 times more milk than their predecessors 80 years ago, and, although they are also a little larger and they eat more, their feed efficiency had doubled due to the dilution of maintenance.

Based on analysis of the cows in our dataset, we discovered that the maintenance cost penalty assigned to larger cows in NM$ was only half of what it should be. The recent revision of the Nutrient Requirements of Dairy Cattle (NAMEM, 2021; NASEM was formerly known as NRC) also increased the maintenance cost of dairy cattle. In nutrition, we predict maintenance costs as a function of a cow's "metabolic body weight", or BW in kg to the 0.75 power. In the 2001 version of the Dairy NRC, the net energy requirement for maintenance was 0.08 x metabolic BW. In NASEM 2021, it was increased to 0.10 x metabolic BW, and the NASEM 2021 cited evidence that the requirement might be even higher than that. Perhaps as we have selected for cows that make more milk per pound of BW, we have also selected for cows that are more metabolically active and just need more calories for maintenance. Over the past 100 years, the average BW for Holsteins, as well as Jerseys, has increased. Our data says it is time to reverse that trend!

The penalty in NM$ for larger BW includes extra feed expenses incurred by large animals during the rearing and dry periods, as well as added housing costs, but is slightly offset by the fact that large cows receive credit for greater salvage and calf values. In the US, the expenses and income associated with BW are based on the body weight composite (**BWC**), which is comprised of five linear type traits: stature, strength, body depth, dairy form, and rump width. The new Feed Saved trait incorporates all net costs associated with BWC. Our dataset has enabled new calculations to relate BWC and its associated traits to a cow's BW.

Breeding for negative Residual Feed Intake

When cows eat feed to obtain nutrients for maintenance or milk, they must first digest and metabolize feed ingredients to the metabolites that are actually used by cells for maintenance and milk synthesis. When considering the energy flow of feed, the cow must convert the "Gross Energy" of its feed to "Net Energy" (**Figure 2**). Some cows are more efficient at this than others. Those with a positive RFI eat more than expected; we cannot justify their greater intake, so they are less efficient. In contrast, cows with a negative RFI eat less than expected; thus, they are more efficient. The biological basis of RFI is not well understood, but as we breed for cows with negative RFI, we are breeding for better digestive ability, less turnover of body tissue, a more efficient liver or mammary gland, or some combination of these. We might even be breeding for a lower maintenance requirement per unit of metabolic BW and reversing the trend for increased maintenance per unit of metabolic BW that has occurred over the past 50 years.

To measure RFI, we consider 3 energy needs of a cow when computing her expected DMI: 1) the energy secreted as milk, 2) the energy required for maintenance

as predicted based on BW, and 3) the change in body energy (growth or body condition change) based on changes in BW, as shown in **Figure 1**. We compute the RFI of each cow by comparing her actual DMI with the expected DMI of a cow of equivalent BW, milk yield, milk composition, BW change, days-in-milk, and parity with other cows in a cohort, where a cohort is cows fed the same diet at the same place at the same time. RFI is the deviation, or residual, from the expected intake based on the cohort – this is the same concept as the deviation from herdmate that we've used in routine genetic evaluations of other key traits for decades. Residual Feed Intake is always just a ranking of cows in a group or cohort.

In the US, our RFI reference dataset is based on measures of milk yield, milk composition, BW, body condition score, and DMI for 42 or more days in mid-lactation. We chose mid-lactation (between 50 and 200 days-in-milk), because that's when cows are in peak lactation, and when their BW and body condition are relatively stable.

Our studies have shown that the heritability of RFI is 17% (Tempelman et al., 2015), making it more heritable than feet/leg type traits (15%) and only slightly less heritable than milk yield (20%). So, it is clear that we can make progress on this trait. To keep genomic evaluations for RFI, and thus Feed Saved, up to date, we must continue measuring feed intake on individual cows every year for the reference population. This will continue to require significant investments of time, money, labor, and technology on research farms, but the resulting information can be used to compute the Predicted Transmitting Ability (**PTA)** for feed efficiency of all cows, bulls, heifers, and calves in the national population.

Will selecting for Feed Saved have any negative consequences?

Because selective breeding will have long-term effects on the dairy cow population, it is critical that selection for Feed Saved does not decrease health or fertility. In addition, our current reference population is composed almost entirely of cows fed total mixed rations relatively high in grain and housed in confined settings. In the future, cows may be fed diets with less starch than our reference. All data to date indicate that breeding for cows with negative RFI or lower body weight composite will have no negative effects. Although DMI and RFI are highly correlated, the value of selecting against RFI, instead of against DMI, is that RFI is phenotypically not correlated with important traits like milk production or BW change.

We found a high correlation of the RFI rankings for cows fed high or low starch diets (Potts et al., 2015), for cows fed high or low forage diets (Mangual et al., 2016), and for cows fed diets with sufficient or marginally deficient protein (Liu and VandeHaar, 2020). In addition, Florida studies show that selecting against RFI based on measurements in mid-lactation has no negative effects on health or fertility and may even benefit reproductive performance (Nehme Marinho et al., 2021; Nehme Marinho and Santos, 2022). Currently, the CDCB website (uscdcb.com/feed-saved/) shows that genetic correlations for RFI with pregnancy rate, productive life, and disease resistance traits are close to zero (less than 10%).

Selection against BWC should benefit health and fertility. Van Raden et al. (2018) showed that BWC was genetically correlated negatively with health (-0.26 with health index), productive life (-0.10), and livability (-0.14) and was not correlated (or slightly negatively correlated) with calving ability (-0.07), daughter pregnancy rate (-0.05), and conception rates (-0.01). The only possible negative effect of selecting against BWC is that BWC was negatively correlated with somatic cell score (-0.10), which might indicate larger, taller cows have less mastitis; however, overall health was better for smaller cows.

Incorporating Feed Saved into Net Merit

We expect Feed Saved to assist dairy producers in breeding cows of moderate size that can convert consumed feed into milk and body tissue even more efficiently than they do now. Said another way, it will help dairies avoid breeding cows that waste feed in achieving and maintaining excessive body size or waste too much energy as feces, gas, urine, and heat.

Mathematically, the formula for Feed Saved has values of -138 for PTAs for BWC and -1 for PTAs for RFI; thus, larger values of Feed Saved are desirable. Currently, the standard deviation of PTA values for Feed Saved is about 109 pounds per lactation, so significant genetic variation exists between animals (**Figure 3**). In 2020, Feed Saved PTAs of all evaluated Holstein bulls ranged from -453 to +594 pounds of feed per lactation and the range for the top 100 NM$ bulls was -183 to +395 pounds per lactation. An example of how Feed Saved works is shown in **Figure 4**.

As previously mentioned, the genetic correlation of Feed Saved with milk production is near zero, due to the way RFI is computed, and correlations with health and fertility traits are close to zero – these traits will be monitored closely to ensure that gains in feed efficiency are not accompanied by losses in health, fertility, or longevity. Reliabilities of Feed Saved are currently lower than desired due to the small size of the genomic reference population for RFI. At this time, we expect average reliabilities of Feed Saved to be 28% for young, genome-tested bulls and 38% for progeny-tested bulls. As additional data are accumulated, reliabilities will increase. Current heritabilities are 19% for RFI and 40% for BWC.

Because feed costs are so important in dairy production, the economic value of Feed Saved is quite large, and the relative economic weight for incorporating this new trait in the Lifetime Net Merit Index **(NM$)** is about 21% (roughly 40% for BWC and 60% for RFI). Net Merit will continue to focus on increasing milk protein and fat yields, but our expectation is that addition of Feed Saved into NM$ in the coming years will provide an extra $8 million per year in net profit to U.S. dairy farmers, and these gains will accumulate over time. The current weighting in NM$ is shown in **Table 1**.

Introduction of routine CDCB genomic evaluations for Feed Saved is a big step forward, and the result of a decade of university research. Our work is not finished,

though. Beyond adding roughly 800 new cows to the genomic reference population each year, we are collaborating with international partners who can contribute cows to a larger global reference population, developing proxies to predict DMI from inline milk analysis systems, wearable sensors, and computer vision algorithms, and carrying out intensive nutrition and physiology studies that will advance our understanding of metabolic regulation, methane emissions, health, and fertility. This work will advance continued improvements in the efficiency and sustainability of the dairy industry.

Questions for the future

One of the major values of ruminants in the food chain is that they can convert poor quality foods into high quality foods for people. As we select cows, we need to make sure they can efficiently digest fiber. Our work so far suggests that more efficient cows will digest fiber as well as less efficient cows (Potts et al., 2015). As cows digest fiber, however, they also produce methane. Methane emissions are a growing concern. More work is needed to understand the relationship of feed efficiency and methane emissions and to determine if we can select for cows that emit less methane per unit of milk without impairing fiber digestibility.

Finally, culled dairy cows and dairy bulls enter the beef stream, so as we consider goals in dairy cattle breeding, we should consider our linkages to the beef industry. Fewer dairy cows will likely mean we need more beef cows. Should we keep striving for higher production, resulting in fewer dairy cows, if we will need more beef cows in their place? On individual farms, more milk per cow might be financially beneficial, but at the national level, this tradeoff seems pointless, especially if higher producing cows need more grains and have more health or fertility problems. Perhaps we should put more emphasis on efficiency, fertility, and health traits and less on production. In addition, modern cows seem to have higher maintenance requirements. If breeding for higher production results in increased maintenance costs, maybe it is time to put less emphasis on production and more emphasis directly on feed efficiency.

Summary and Conclusion

Feed efficiency (**FE**) of dairy cattle can be increased through improvements in nutrition, management, and selection. Increasing the energy-corrected milk (**ECM**) production per cow generally decreases the proportion of feed used for maintenance and thus enhances FE. Because the maintenance requirement of cows is highly correlated with body weight, for a given level of ECM, smaller cows have greater FE. Even after accounting for production per unit of BW, some cows use feed more efficiently than others. These efficient cows have a negative residual feed intake, meaning that their observed feed intake is less than their predicted intake based on BW, milk production, parity, BW change, days-in-milk, and the intake of their cohorts on the same diet at the same time at the same location. Our Genomics of Feed Efficiency Consortium has been working together since 2010 to amass a dataset of 7500 RFI phenotypes. Our group is comprised of scientists from Michigan State University, the University of Wisconsin, Iowa State University, the University of Florida, the USDA

Animal Genomics and Improvement Laboratory, and the US Council on Dairy Cattle Breeding. With our dataset, we have developed equations to relate the genotype of a cow with her RFI phenotype. We also were to more accurately predict body weight based on Holstein type traits and to predict how much extra feed larger cows need to support their maintenance requirements. In 2021, these relationships were combined to form a new trait called Feed Saved. Feed is saved when cows have smaller BW and when they have negative RFI. This new trait is now part of the Net Merit Index at about 20% of the total index. Selection using the new NM$ will result in cows that produce more milk fat and protein, are healthier and more fertile, have smaller BW, and use feed more efficiently.

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Table 1. Weighting of traits in the Net Merit Index in selected years since its introduction in 1971.

	1971	2018	2021
Milk Yield	52	-1	0
Fat Yield	48	27	22
Protein Yield		17	17
Productive Life		12	15
Udder Composite		7	3
Feet/legs Composite		3	1
Daughter Pregnancy Rate		7	5
Conception Rate (HCR + CCR)		3	2
Calving Ability (CA$)		5	3
Somatic Cell Score		-4	-3
Health trait subindex		2	2
Livability (LIV + HLIV)		7	5
Early first calving			1
Body Weight Composite		-5	-9
Residual Feed Intake			-12

*Highest ranked traits highlighted in yellow.
*A negative value indicates selection against the trait.
*Feed Saved is the combination of the inverse of Body Weight Composite and Residual Feed Intake.

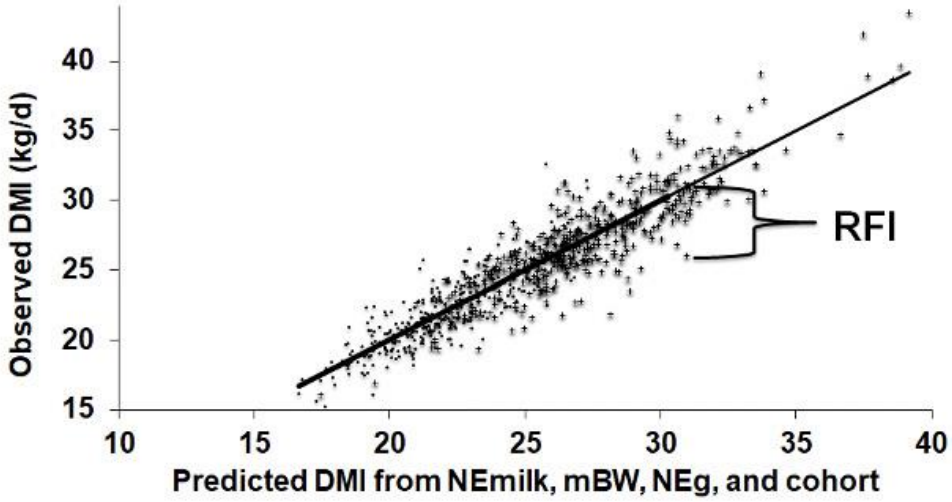

Figure 1. Illustration of residual feed intake (**RFI**), where RFI represents the difference between observed dry matter intake (**DMI**) and expected DMI. Energy needs and expected DMI are based on milk energy output, body weight, body weight change, parity, and days-in-milk within a cohort of animals fed the same diet at the same place and time.

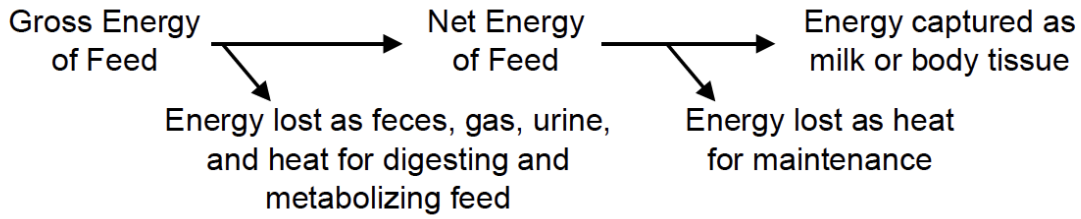

Figure 2. Energy flow in a cow. Selecting for Feed Saved related to Residual Feed Intake (**RFI**) will improve the conversion of Gross Energy to Net Energy, whereas selecting for Feed Saved related to body weight will improve the proportion of Net Energy that is captured in milk instead of being used for maintenance.

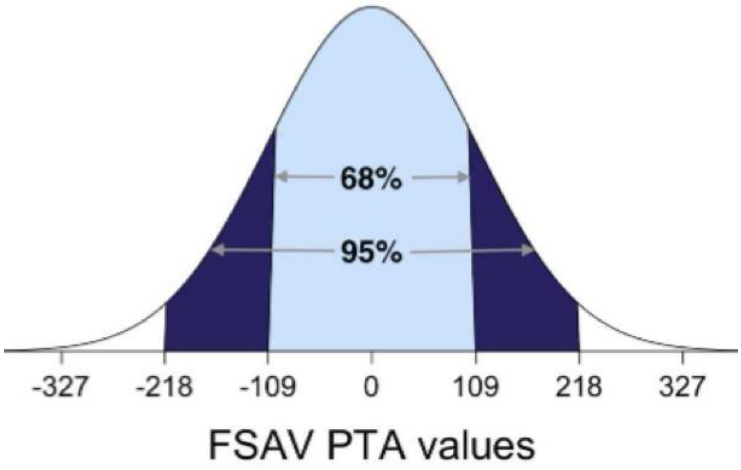

Figure 3. Distribution of Feed Saved Predicted Transmitting Ability (**PTA**) in pounds of feed per lactation for modern Holsteins. One standard deviation in the dataset is 109 lb. About 30% of all cows are below -109 lb per lactation or above 109 lb per lactation. Significant gains can be made.

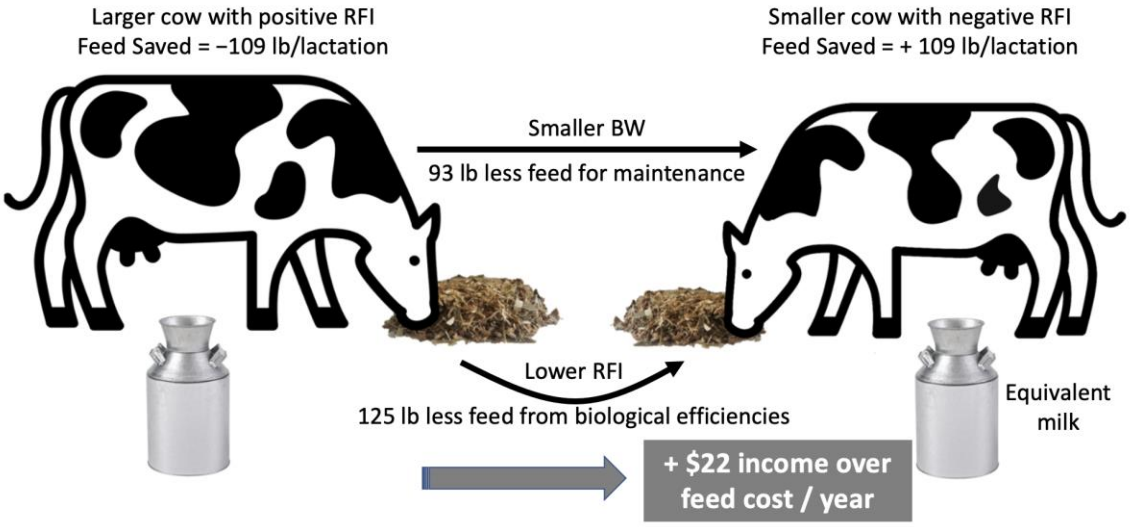

Figure 4. Example of feed savings from smaller body weight and lower residual feed intake (**RFI**). Based on one standard deviation (SD) in Feed Saved in the current Holstein population and on the current weighting in Net Merit, we expect that a cow at 1 SD above the average for Feed Saved will eat 218 lb less feed per lactation than a cow at 1 SD below the average. Of this 218 lb, 93 lb will be associated with a smaller BW, and 125 lb will be associated with a lower RFI. At a feed cost of 10 cents / lb, this is $22 greater income over feed cost per year. Given that a typical cow will eat 15 to 20,000 pounds of feed per year, this is only a 1% reduction in feed cost per year. However, the change is permanent and will accumulate with generations.

SESSION NOTES

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A survey on N efficiency in dairy farms in the USA

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University of Florida

Introduction

The growing environmental concerns from the US dairy industry has lead researchers to focus on nutrient management for more efficient utilization of available resources and reduce environmental emissions of greenhouse gases and volatile organic compounds (Place and Mitloehner, 2010). Milk nitrogen (N) efficiency, defined as conversion of dietary N into milk N, is typically low (20-35%; Chase et al., 2009) in lactating dairy cows. Most of the dietary N is lost in feces and urine and N is considered one of the major pollutants from dairy production systems (Noftsger et al., 2005).

Nitrogen efficiency is a crucial aspect in the successful operation of commercial dairy farms. Therefore, improving nitrogen efficiency at commercial dairy farms is not only important for the health of the herd and the productivity of the farm, but also for the protection of the environment. Studies have reported that NE ranges from 16 to 40% (Chase, 2004; Powell et al., 2010; Fadul-Pacheco et al., 2017), implying that most of the dietary N consumed by a dairy cow is excreted in manure, contributing to excess N to the environment (Castillo et al., 2000; Bouwman et al., 2013). Nitrogen efficiency less than 20% should be considered very low while 30-35% is above average and greater than 35% is considered excellent NE. However, most on-farm measurements of NE is in range between 20 and 30% (Table 1). Powell et al. (2010) observed greater NE on confinement dairies compared with grazing-based dairies perhaps due to strategic use of concentrates, and other diet supplements and precisely balanced rations. Besides the environmental concerns, low NE may negatively impact animal performance.

Studies have shown that lactating dairy cows and herds with low NE (~22%) have lower milk yield and profitability when compared with high NE cows and herds (32.8 and 36%, respectively; Calsamiglia et al., 2010; Fadul-Pacheco et al. 2017). In addition, the wide range of NE observed across herds and experiments may be the result of differences in diet composition and farm management suggesting great potential for improvement. Therefore, studying the relationships between NE and production parameters of lactating dairy cows, and identifying dietary strategies to improve NE of dairy herds may contribute to reduction of the environment impact of the dairy industry and towards increasing milk production and farm profitability. By optimizing the use of nitrogen inputs and minimizing waste, dairy farmers can improve the sustainability of their operations and ensure that they are able to meet the growing demand for dairy products while minimizing their environmental footprint.

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Because of the inverse relationship between dietary CP and NE, feeding high CP diets lowers NE in lactating dairy cows (Colmenero and Broderick, 2006; Huhtanen and Hristov, 2009; Calsamiglia et al., 2010). Additionally, NE increases by feeding diets with greater non-fibrous carbohydrates and greater energy due to greater efficiency of N utilization by rumen microbes (Broderick, 2003; Calsamiglia et al., 2010). Hence, strategies to improve NE in dairy cows are usually focused on dietary manipulations that aim to lower dietary CP concentration and improve yields of milk and milk protein (Dijkstra et al., 2011). Remarkably, increased N intake has little effect on fecal N output in dairy cows, as most of the excess N is excreted through urine (Colmenero and Broderick, 2006) resulting in a linear positive association between N intake and urine N (Kebreab et al., 2002). Huhtanen and Hristov (2009), using a meta-analytical approach concluded that feeding low CP diets to lactating dairy cows is the most efficient dietary strategy to reduce N losses in dairy systems, and that increasing milk production but not dietary CP, could also increase NE; however, the effect is considerably smaller.

Efficiency of N utilization is related with dry matter intake (DMI), dietary CP, milk yield and milk protein concentration. The range of NE observed across dairy herds probably reflects differences in diet composition along with animal factors such as stage of lactation and parity. Therefore, investigating the relationships between NE and performance of lactating dairy cows, and the effect of main dietary nutrients on NE of dairy herds may contribute to a better understanding of factors influencing NE and may provide directions for improving NE in commercial dairy herds.

Survey of Commercial Dairy Farms

We surveyed 28 dairy farms across the US in our study. Farms were located in Central Valley (CA; n=13), Texas Panhandle (TX; n=10) and North Central and Central Florida (FL; n=5) and were sampled between June 2020 and March 2021. Farms surveyed had to keep records of daily feed offered and refusals at the pen level. In addition, individual cow milk yield and milk fat and protein percentage were required. Additionally, diet ingredient composition from each pen was needed for feed ingredient sampling and diet reconstitution in the laboratory. Dairy herds were composed of Holstein cows. Farms were visited ± 4 d relative to the DHI milk test day for dietary ingredients, TMR, and refusal sampling. A second visit was scheduled for data collection from farm management software (DairyComp305, n=22; DHI-Plus, n=3; PCDART, n=3) and feeding software (EZfeed, n=16; FeedWatch, n=10; handwritten spreadsheet, n=2). Herds were housed in free stall barns with dry lot access (n= 11), exclusively dry lots provided with shade (n=10) and free stall barns (n=7). Diet composition was obtained from farm management or feeding software. A total of seventy-four different diets were used at the dairy farms surveyed in this study. In some dairy farms (CA=6, TX=7, and FL=3), the concentrate ingredient composition was confidential; hence, mixed concentrate was sampled instead of individual ingredients. Data were analyzed by ANOVA with linear mixed models using the MIXED procedure of SAS (version 9.4; SAS Institute). Statistical models included the fixed effects of NE as linear (NE_{LIN}; NE) and quadratic covariates (NE_{QUAD}; NE × NE), cow parity (P; primiparous vs. multiparous cows), lactation stage (LS; early vs. mid- vs. late lactation),

and interactions (NE_{LIN} × P; NE_{QUAD} × P; NE_{LIN} × LS; and NE_{QUAD} × LS. Pen within farm, and location (state) were used as random effects. A stepwise backward elimination method was used to remove all non-significant *(P* < 0.10) interactions including NE_{QUAD}.

Based on the descriptive statistics in Table 2, the mean herd and pen sizes were 2516 and 248 cows, respectively. Cow parity averaged 2.15, the percentage of primiparous pens was 30.2%, whereas the multiparous pens represented 69.8% (data not shown). Days in milk averaged 164, and the proportions of early, mid-, and late lactation pens were 26.3, 37.9 and 35.8%, respectively. Milk urea-N was available in 137 pens analyzed and averaged 12.5 mg/dL. Nitrogen efficiency averaged 27.8% ranging between 14.2 and 46.7% for minimum and maximum NE, respectively. Nitrogen efficiency agrees with the values observed in other studies carried out under commercial dairy farm conditions. Fadul-Pacheco et al. (2017) reported average 29% NE in Canadian dairies, Powell et al. (2010) reported average 26% NE when summarizing data from commercial dairy herd studies, while Chase (2004), reported average 28.8% NE when summarizing data from 46 dairy farms in New York state. The range between minimum and maximum NE in the current study (14.2 to 46.7%) was wider compared to the range observed in the studies mentioned above (16 to 40%). In the present study, NE was estimated from each pen of the commercial dairy farms used for data collection; however, in previous studies (Chase, 2004; Powell et al., 2010; Fadul-Pacheco et al. 2017), NE was averaged for each dairy farm including all lactation stages, and parities within the farm. In addition, the wide ranges in NE across dairy farms and experiments have been suggested as a consequence of animal variations, diet composition, and farm management (Calsamiglia et al., 2010), which implies latent opportunities to improve NE of dairy farms through animal breeding, diet refinement, and improved farm management.

All production parameters evaluated were associated with NE; however, the associations between NE and yields of ECM, and 3.5FCM were dependent on cow parity. Milk yield was associated with the NE_{LIN} (*P* < 0.01), P (*P* < 0.01), LS (*P* < 0.01), and NE_{QUAD} (*P* < 0.01); however, none of the other interactions were significant. Figure 1 shows the negative quadratic association between NE and milk yield for early, mid-, and late lactation pens. Energy corrected milk was associated with the NE_{LIN} and NE_{QUAD} (*P* < 0.01), LS (*P* = 0.02), and the interaction NE_{QUAD} × P (*P* = 0.02), while P and its interaction with NE_{LIN} was not associated with ECM. Similarly, 3.5FCM was associated with NE_{LIN} and NE_{QUAD} (*P* < 0.01), LS (*P* < 0.01), and the interaction NE_{QUAD} × P (P = 0.02), while no parity effects were observed (*P* = 0.80). The interaction between NE_{QUAD} and P, implies that greater NE yielded smaller increases in ECM and 3.5FCM for primiparous pens compared to multiparous pens (Figure 2A and Figure 2B, respectively). The quadratic effect observed between NE and milk yield in our model resulted in lower milk yield for low NE (18%) when compared with medium and high NE (28 and 38%, respectively), regardless of the stage of lactation. However, the difference in milk yield between medium and high NE was much lower suggesting the possibility of optimal NE for maximizing milk yield between this range. Similarly, Colmenero and Broderick (2006) reported a quadratic increase in milk yield with feeding incremental levels of dietary CP concentration ranging from 13.5 to 19.4%, in their study, maximum

milk yield was achieved at 16.7% CP. Comernero and Broderick (2006) observed that NE linearly decreased with increasing levels of dietary CP, similar to the results observed in this study. At very lower dietary CP levels, and consequently high NE (beyond 40%), inadequate availability of metabolizable protein and subsequently intestinally absorbable AA, particularly methionine and lysine, may limit yields of milk and milk protein in dairy cows (NRC, 2001; Cabrita et al., 2011). Instead, at low NE, most likely achieved because of increased dietary CP concentration, milk production does not seem to improve beyond certain levels (17%; Colmenero and Broderick, 2006), however, milk yield responses to dietary CP might be variable depending on the source of CP used in the diets, most likely because of differences in MP and AA profile of ingredients (Ipharraguerre and Clark, 2005, Cabrita et al., 2011). In addition, to prevent the onset of possible hyperammonemia with greater concentrations of CP in the diet, NH_3 must be converted to urea, which is less toxic, in the urea cycle. The conversion of NH_4^+ and HCO_3^- to carbamoyl phosphate is the first step of the urea cycle and consumes energy in the form of ATP. Although limited research has been done in this area, Milano et al. (2000) reported that sheep under a sustained oversupply of ammonia (2.4-fold the basal concentration) had higher liver O_2 consumption rates than control, in addition, Reed et al. (2017) reported a reduction in milk gross energy for cows fed excess N, and a linear positive association between excess RDP and heat production was also reported in their study, these findings may indicate greater oxidation and energy loss when cows are fed excess N, however, the energy cost of hepatic urea synthesis was considered minor relative to other metabolic processes (Reynolds, 2005). Furthermore, there is a cost associated with the urinary loss of N in derivation of metabolizable energy (NASEM, 2021), which also may contribute to the decreased in milk yield observed in the current study.

Since there was no interaction between NE and P or LS, optimal NE for maximizing milk yield was 34.7%, regardless of early-, mid- or late lactation. Furthermore, because of the main effect of LS, maximum milk yield response was 39.6, 42.4 and 34.4 kg/d for early, mid-, and late lactation pens, respectively. Early lactation dairy cows have been reported to have greater feed efficiency (milk yield/DMI, kg/kg) compared with cows in later stages of lactation because of depressed DMI and greater milk production (VandeHaar et al., 2016). Since NE in dairy cows usually follows feed efficiency trends (Marinho et al., 2021), the lack of interaction effect between NE and LS was unexpected. Peak lactation is observed between 4 to 8 weeks post-partum (NASEM, 2021); however, we observed greatest milk yield for mid-lactation pens compared with early and late lactation pens. We believe the stratification criteria used for pen LS classification (DIM < 105 for early lactation pens) may have affected our results as very early lactation pens (DIM < 14) were included in this group and it may have brought average milk yield down for the early-lactation group.

Based on the results from this survey, we conclude that NE can be used as performance indicator in commercial dairy herds and 34.7% was observed as optimal NE for maximizing milk yield in commercial dairies. In addition, multiparous cows are more efficient at increasing ECM and 3.5FCM with increasing NE probably due to additional protein requirements for growth in primiparous cows.

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Table 1. Feed to milk N use efficiencies on dairy farms (Adapted from Powell et al., 2010).

N input range, g/cow/day	Nitrogen utilization efficiency range (%)	Source
512-666	26-33	Powell et al., 2006
289-628	22-29	Kebreab et al., 2001
200-750	21-32	Castillo et al., 2000
496-897	21-36	Chase, 2004
838-1360	16-24	Aarts et al., 2000
468-668	22-36	Fadul-Pacheco et al., 2017

Table 2. Descriptive statistics of 285 pens used in our study[1]

Item	n	Mean	Minimum	Maximum	SD
Herd	28	2516	320	5462	1614
Pen size	285	248	24	597	110
Parity	285	2.15	1	4.41	0.88
DIM	285	164	7	393	86
Milk yield, kg/d	285	37.0	16.2	62.5	8.1
3.5% FCM[2], kg/d	285	40.2	18.2	65.5	7.6
ECM[3], kg/d	285	39.7	18.0	65.0	7.3
Protein, %	285	3.30	2.71	4.3	0.33
Fat, %	285	4.08	3.33	5.42	0.46
Protein yield, kg/d	285	1.21	0.54	2.01	0.23
Fat yield, kg/d	285	1.49	0.69	2.37	0.28
MUN, mg/dL	137	12.5	6.70	28.7	3.41
N intake, kg/d	285	0.68	0.28	0.98	0.11
Milk N, kg/d	285	0.19	0.086	0.317	0.04
NE[4], %	285	27.8	14.2	46.7	4.95

[1]The data set contained information from 70,461 lactating dairy cows from 28 dairy farms in CA (n = 13), TX (n = 10), and FL (n = 5). The values presented on this table are means from each pen used in the statistical models.
[2]3.5% FCM = [0.4324 × milk yield (kg/d)] + [16.216 × fat yield (kg/d)].
[3]ECM = 0.327 × milk yield (kg/d) + 12.95 × fat yield (kg/d) + 7.2 × protein yield (kg/d).
[4]Nitrogen efficiency; NE = [milk yield (kg/d) × milk true protein (%)/6.38]/[DMI (kg/d) × diet CP (%)/6.25] × 100.

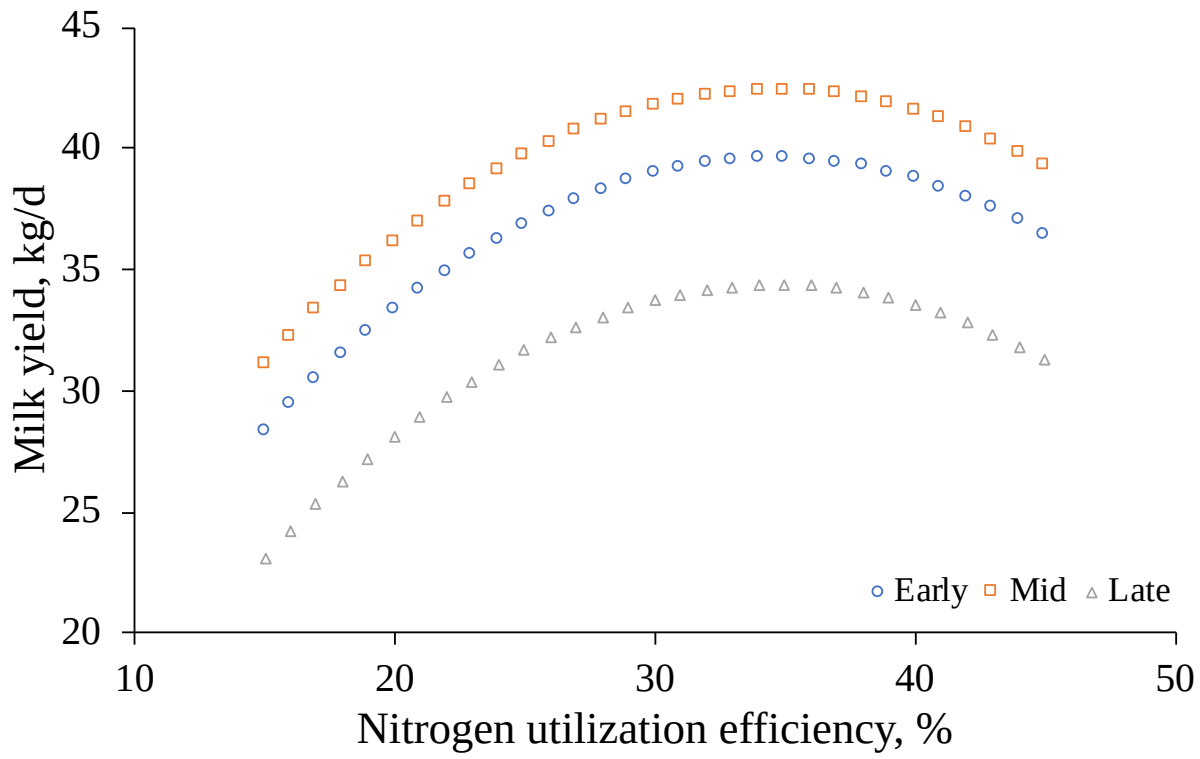

Figure 1. Daily milk yield in early, mid-, and late lactation dairy cows according to nitrogen utilization efficiency (NE). NE_{LIN} ($P < 0.01$), NE_{QUAD} ($P < 0.01$), parity ($P < 0.01$), and lactation stage ($P < 0.01$).

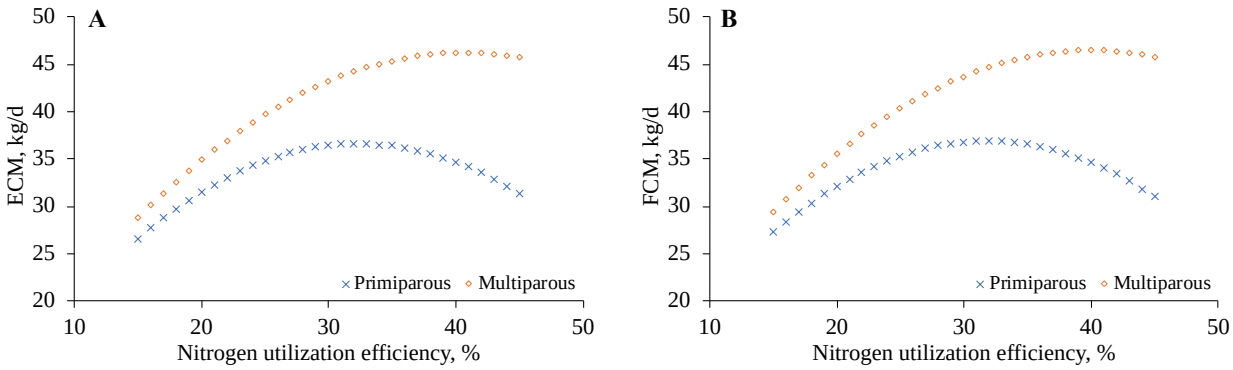

Figure 2. Daily milk yield in primiparous and multiparous dairy pens according to nitrogen utilization efficiency (NE). A) Energy corrected milk. Nitrogen utilization efficiency as linear covariate ($P < 0.01$), NE as quadratic covariate ($P < 0.01$), parity ($P = 0.78$), lactation stage ($P = 0.02$), and NE × NE × P ($P = 0.02$). B) 3.5% fat corrected milk. Nitrogen utilization efficiency as linear covariate ($P < 0.01$), NE as quadratic covariate ($P < 0.01$), parity ($P = 0.80$), lactation stage ($P < 0.02$), and NE × NE × parity ($P = 0.02$).

SESSION NOTES