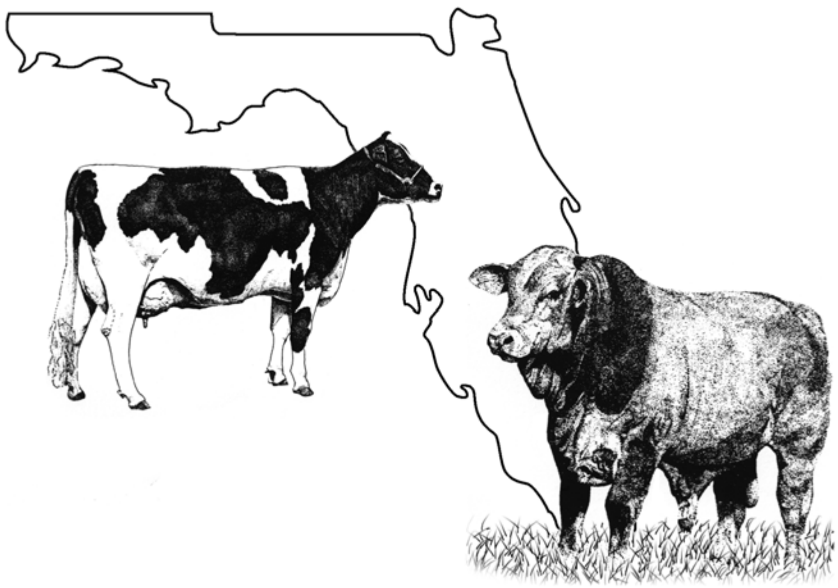
2022 Florida Ruminant Nutrition Symposium

33rd Annual Meeting



May 9 - 11, 2022
Best Western Gateway Grand
Gainesville, Florida

PROCEEDINGS

UF | UNIVERSITY of FLORIDA
IFAS
Department of Animal Sciences

2022

33^{rd} ANNUAL FLORIDA RUMINANT NUTRITION SYMPOSIUM

May 9 to 11, 2022
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 – May 9 to 11, 2022

Monday, May 9, 2022– Mini-Symposium sponsored by Balchem Corporation *"Exploring In Utero influences on Transgenerational Performance"*

2:00 PM **Scott Sorrell**, Balchem Corporation. *Welcome and introductions*

2:05 PM **Dr. Jack Britt**, Jack H. Britt Consulting. *""*

2:45 PM **Dr. Chad Dechow,** Penn State University. *"Epigenetics will change how we manage cattle"*

3:30 PM *Refreshment Break*

3:50 PM **Dr. Jimena Laporta**, University of Wisconsin. *"Phenotypic and Molecular Signatures of Fetal Hyperthermia"*

4:35 PM **Dr. Peter Hansen**, University of Florida. *"Methyl donors and epigenetic regulation of the early embryo"*

5:20 PM **Dr. Clay Zimmerman**. Balchem Corporation. *Summary and wrap-up*

5:45 PM Poolside barbeque

Tuesday, May 10, 2022 – University of Florida Showcase *"Update on Nutrition Research at the University of Florida*"

8:10 AM **Dr. Philipe Moriel**, University of Florida. *"Improving beef progeny performance through developmental programing"*

8:50 AM **Dr. Fernanda Batistel**, University of Florida. *"Methyl donors and fetal and neonatal development"*

9:30 AM *Refreshment Break*

10:00 AM **Dr. Corwin Nelson**, University of Florida. *"Update on vitamin D nutrition for dairy cows"*

10:40 AM **Usman Arshad**, University of Florida. *"Choline: an essential nutrient for dairy cows"*

10:55 AM **Felipe Amaro**, University of Florida. *"Nitrogen efficiency as predictor of production performance in commercial dairy herds"*

11:10 AM **Mariana Nehme Marinho**, University of Florida. *"Assessing feed efficiency and its association with health and reproduction"*

11:30 AM *Buffet Lunch*

<u>Tuesday, February 4, 2020</u> – Symposium

1:00 PM	**Dr. José E. P. Santos**, University of Florida. *Welcome*
1:05 PM	**Dr. John Arthington**, University of Florida. *Department of Animal Sciences update*
1:10 PM	**Dr. Clint Krehbiel**, University of Nebraska. *"Nutrient partitioning during immunological challenge"*
1:50 PM	**Dr. Barry Bradford**, Michigan State University. "*Mechanisms of hypophagia during disease"*
2:30 PM	**Dr. Chanhee Lee**, The Ohio State University. *"Improving N efficiency through diet formulation"*
3:10 PM	*Refreshment Break*
3:40 PM	**Dr. Min Du**, Washington State University. *"Pre- and postnatal muscle and adipose tissue growth in beef cattle"*
4:20 PM	**Dr. Sha Tao**, University of Georgia. *"Environmental effects on calf performance and responses to different feeding programs"*
5:00 PM	*Welcome reception*

<u>Wednesday, February 5, 2020</u> – Symposium

8:00 AM	**Dr. Oscar Queiroz**, Chr. Hansen. *"Methods for silage conservation to improve quality"*
8:40 AM	**Dr. João Vendramini**, University of Florida. *"Forage conservation for winter cow-calf systems"*
9:20 AM	**Dr. Ben Saylor**, Arm & Hammer Animal and Food Production. *"Make your herd more resilient to hidden challenges"*
10:00 AM	*Refreshment Break*
10:30 AM	**Dr. Robin White**, Virginia Tech University. *"The role of animal production on the environment"*
11:10 AM	**Dr. Sara Place**, Elanco Animal Health. *"Beef production and environmental sustainability"*
11:50 AM	Ruminant Nutrition Symposium Adjourns

2022 Symposium Speakers

Guests
Dr. Barry Bradford, Michigan State University
Dr. Jack Britt, Jack H. Britt Consulting
Dr. Chad Dechow, Penn State University
Dr. Min Du, Washington State University
Dr. Clint Krehbiel, University of Nebraska
Dr. Jimena Laporta, University of Wisconsin
Dr. Chanhee Lee, The Ohio State University
Dr. Sara Place, Elanco Animal Health
Dr. Oscar Queiroz, Chr. Hansen
Dr. Ben Saylor, Arm & Hammer Animal and Food Production
Dr. Sha Tao, University of Georgia
Dr. Robin White, Virginia Tech
Dr. Clay Zimmerman, Balchem Corporation

University of Florida

Department of Animal Sciences
Felipe Amaro
Usman Arshad
Dr. Fernanda Batistel
Dr. Peter Hansen
Dr. Philipe Moriel
Mariana Nehme Marinho
Dr. Corwin Nelson

Department of Agronomy
Dr. João Vendramini

2022 Florida Ruminant Nutrition Symposium

Speaker Biographies

Dr. Fernanda Batistel is an Assistant Professor in the Department of Animal Sciences at the University of Florida. Previously, she was an Assistant Professor in the Department of Animal, Dairy and Veterinary Sciences at the Utah State University. Dr. Batistel received the BSc in Animal Sciences from the Santa Catarina State University, Brazil, and the MSc in Animal Sciences from the University of São Paulo, Brazil. Dr. Batistel moved to the USA and completed her PhD at the University of Illinois. The focus of Dr. Batistel's research involves how nutrients affects production and metabolism in dairy cattle. Her current research involves the effects of dietary fatty acids on fiber digestion and rumen fermentation and the impact of nutrients on fetal programming.

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 BSc 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. Jack Britt is the owner of Jack H Britt Consulting. Dr. Britt received his PhD from North Carolina State University. He was a professor at Michigan State University, North Carolina State University, and at the University of Tennessee. He served as Interim Head and Associate Dean at North Carolina State University, and Vice President for Agriculture and Executive Vice President and COO at the University of Tennessee. Now, he provides professional consulting to companies involved in agriculture.

Dr. Chad Dechow is an Associate Professor in the Department of Animal Sciences at the Pennsylvania State University. Dr. Dechow received his BSc degree in Animal Sciences from Cornell University, the MSc in Animal Sciences from Penn State University, and the PhD degree in Animal Sciences from the University of Tennessee. The primary focus of his research is on improvements of dairy cow health and well-being through genetic selection programs. His research also focuses on use of records and adoption of technologies to sustain the economic wellbeing of dairy farms.

Dr. Min Du is a Professor and the Funded Chair in Growth Biology in the Department of Animal Sciences at the Washington State University. Dr. Du received his PhD from Iowa State University and completed a postdoctoral fellowship in the Faculty of Medicine at the University of Alberta, Canada. Dr. Du' research focuses on the development of skeletal muscle and adipose tissue. Specifically, his research seeks to explore the epigenetic mechanisms regulating the differentiation of mesenchymal stem cells into myocytes and adipocytes. A component of his research involves nutritional regulation of skeletal muscle and adipose tissue development, epigenetic regulation of stem cell differentiation into myocytes and adipocytes, and fetal development and its long-term effect on offspring performance.

Dr. Peter J. Hansen is a Distinguished Professor and the L.E. "Red" Larson Professor in the Department of Animal Sciences at the University of Florida. Dr. Hansen is known for his research on thermoregulation of cattle, mechanisms of thermal stress induction of cellular damage on bovine embryos, and the identification and characterization of embryokines that regulate development of the preimplantation embryo. His most recent work studies genetic regulation of body temperature and mechanisms by which external cues during prenatal life affect pre- and postnatal phenotypes in dairy and beef cattle.

Dr. Clint Krehbiel is a Professor and Head of the Department of Animal Sciences at the University of Nebraska. Dr. Krehbiel received his BSc and MSc degrees from Kansas State University. He earned his doctorate from the University of Nebraska Lincoln and was a postdoctoral fellow at the U.S. Meat Animal Research Center near Clay Center. Prior to his current appointment, Dr. Krehbiel a faculty member at New Mexico State University and Oklahoma State University. Dr. Krehbiel's research interest is on beef cattle nutrition with a focus on developing methods to improve animal health and efficiency of production.

Dr. Jimena Laporta is an Assistant Professor in lactation physiology in the Department of Animal and Dairy Sciences at the University of Wisconsin. Previously, Dr. Laporta was an Assistant Professor in the Department of Animal Sciences at the University of Florida. Dr. Laporta received her BSc in Biology and MSc in Animal Science in Uruguay, and the PhD degree in Dairy Science from the University of Wisconsin-Madison. Dr. Laporta's research investigates mechanisms involved in mammary gland development and physiology. An important component of her research is to study the underlying molecular mechanisms by which prenatal and postnatal stressors contribute to the programming of offspring's future potential.

Dr. Chanhee Lee is an Assistant Professor of nutrient management in the Department of Animal Sciences, at The Ohio State University. Dr. Lee received his BSc degree from Konkuk University (South Korea), MSc degree from Seoul National University (South Korea), and PhD from the Pennsylvania State University, and completed a postdoctorate at the Lethbridge and Development Centre (Agriculture and Agri-Food Canada, Canada). Dr. Lee's research interests focus on improving production efficiency and reducing the environmental impact of dairy production by increasing efficiency of dietary N utilization

Dr. Philipe Moriel is an Associate Professor in the Department of Animal Sciences at the University of Florida located at the Range Cattle Research and Education Center in Ona, FL. He received his BSc degree in Animal Science from São Paulo State University, Brazil, the MSc from the University of Wyoming, and the PhD in Animal Sciences from the University of Florida. From October 2013 to June 2016, Dr. Moriel was an Assistant Professor and Livestock Specialist with North Carolina State University. In 2016, Dr. Moriel moved to the University of Florida and his research program focuses on nutrition of cows and heifers during gestation and calf nutrition during early stages of pre-weaning phase to modify offspring metabolism and induce long-term consequences to offspring health, growth, and immunity.

Dr. Corwin Nelson is an Associate Professor of Physiology in the Department of Animal Sciences at the University of Florida. Dr. Nelson grew up on a dairy farm in East Central Minnesota. After a year of the Farm and Industry Short Course at the University of Wisconsin-Madison, and a couple years of farming, he enrolled at the University of Minnesota-Duluth and earned his BSc in Biochemistry. He moved to Iowa State University where he received his PhD in Biochemistry and Immunobiology. Dr. Nelson completed a postdoctorate in the Department of Biochemistry at the University of Wisconsin-Madison. In 2013, he joined the faculty in the Department of Animal Sciences at the University of Florida. His research focuses on dairy cattle nutrition and the role of nutrients, in particular vitamin D on the immune system in dairy cattle.

Dr. Sara Place is a Chief Sustainability Officer at Elanco Animal Health. Sara completed her BSc degree at Cornell University and the PhD at the University of California Davis. Before joining Elanco, Sara was an Assistant Professor of sustainable beef cattle systems at Oklahoma State University. Sara's area of specialization is sustainable management of livestock production systems focusing on opportunities to improve production efficiency, while reducing the environmental impact and promoting financial sustainability in animal agriculture.

Dr. Oscar Queiroz is the Global Product Manager at Chr. Hansen Animal Health. Dr. Queiroz received his BSc in Agronomy and MSc in Animal Sciences from the University of São Paulo, Brazil, and the PhD in Animal Sciences from the University of Florida. He completed a post-doctorate at the University of Florida and then moved to Argentina as a research coordinator and technical service specialist at Teknal S.A. In 2016, Dr. Queiroz joined Chr. Hansen as a silage specialist in South America and became the global product manager for dairy and beef cattle probiotics and the animal health branch of Chr. Hansen. Dr. Queiroz expertise is on forage quality and conservation and the use of microbial additives to improve silage quality and cattle performance.

Dr. Ben Saylor is a Dairy Technical Services Manager for Arm & Hammer Animal and Food Production. Dr. Saylor received his BSc 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. Sha Tao is an Associate Professor in the Department of Animal and Dairy Sciences at the University of Georgia. He obtained his BSc in Agriculture in 2004 and MSc in Animal Sciences in 2007 at the Henan University of Technology, China. Sha completed his PhD in 2012 at the University of Florida and a post-doctorate also at the University of Florida. Sha's work focuses on the effects of heat stress during the dry period on the mammary gland development, metabolic adaptations to lactation, and calf performance. A component of his research is the use of dietary manipulations during periods of heat stress to evaluate their impact on growth and performance of dairy cattle.

Dr. João Vendramini is a Professor in the Department of Agronomy at the University of Florida. Dr. Vendramini received his BSc degree in agronomy from the University of São Paulo, the MSc degree in Animal Sciences from the same institution, and the PhD in forage management at the University of Florida. He was an Assistant Professor and Forage Specialist at Texas A&M University before joining the University of Florida Range Cattle Research and Education Center, Ona, FL. Dr Vendramini's program is dedicated to forage management with emphasis on sub-tropical production systems. The major area of interest is forage-livestock interface and the impact of forage management on forage production and quality, and animal beef cattle performance.

Dr. Robin White is an Assistant Professor in the Department of Animal and Poultry Sciences at Virginia Tech University. Dr. White received both her BSc and PhD degrees in Animal Sciences from Washington State University and completed postdoctoral studies at Virginia Tech before joining the faculty in the Department of Animal and Poultry Sciences. Dr. White's research focuses on big data analytics with a focus on dairy cattle nutrition and nutritional impacts on digestive efficiency and environmental impact.

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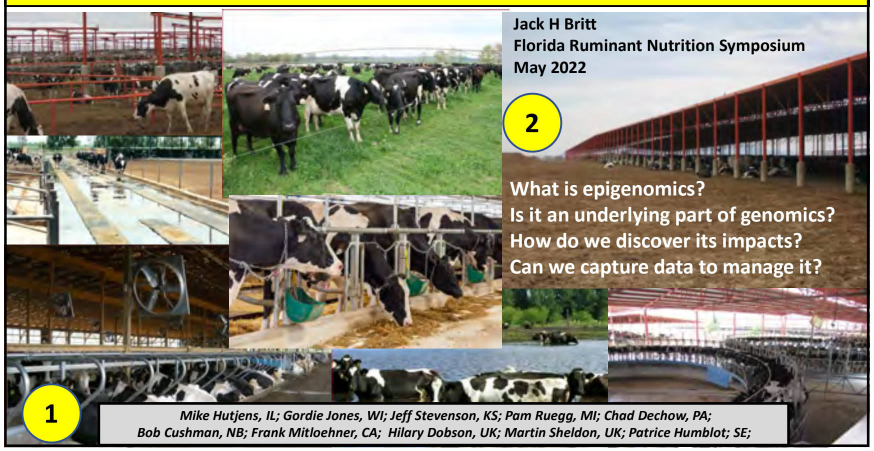
[1] Zenobi et al., 2018 J. Dairy Sci. 101 (Suppl. 2): 334, Zenobi et al., 2018 J. Dairy Sci. 101 (Suppl. 2): ii, Zenobi et al., 2018 ADSA, Late-Breaking Original Research, #LB5

Epigenetics: different environments, different reactions



1

Two definitions:

Epigenetics – *refers to mechanisms <u>that can be</u> <u>transmitted</u> to future generations without changes in DNA sequence* (1)

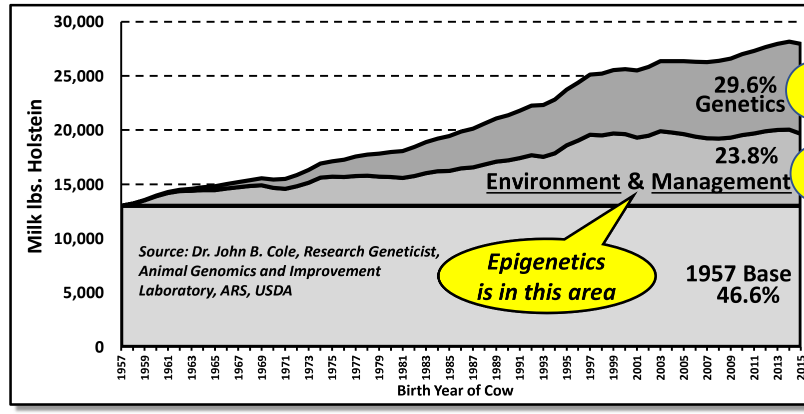
Epigenomics – *refers to mechanisms that can affect cells in the current generation [for example stem cells], <u>but may or may not be transmitted</u> to future generation* (2)

NIH Roadmap Epigenomics Mapping Consortium: http://www.roadmapepigenomics.org/

For purposes of this program, **epigenetics refers to both** heritable changes in gene activity and expression (in the progeny of cells or of individuals) and also stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable.

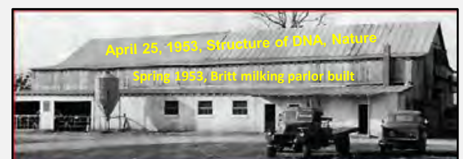
2

Milk yield increases attributed to genetics and environment & management



3

Genetic expression – from then to now

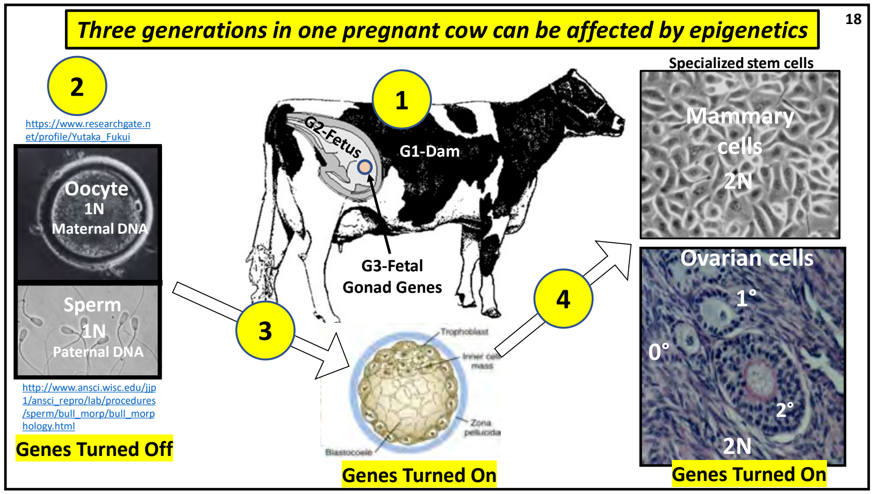


1

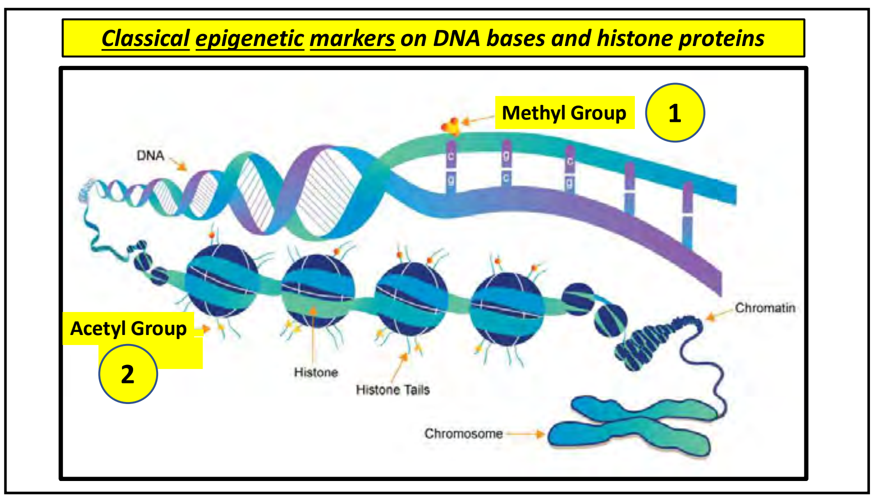
2 3

DNA (gene) *transcription* ⬇	**DNA, *methyl*-DNA, *acetyl*-Histones**
m-RNA *translation* ⬇	**m-RNA, *m*-RNA, RNAi, microRNA, lncRNA**
Protein (function)	***"Expression" of DNA affected many ways***

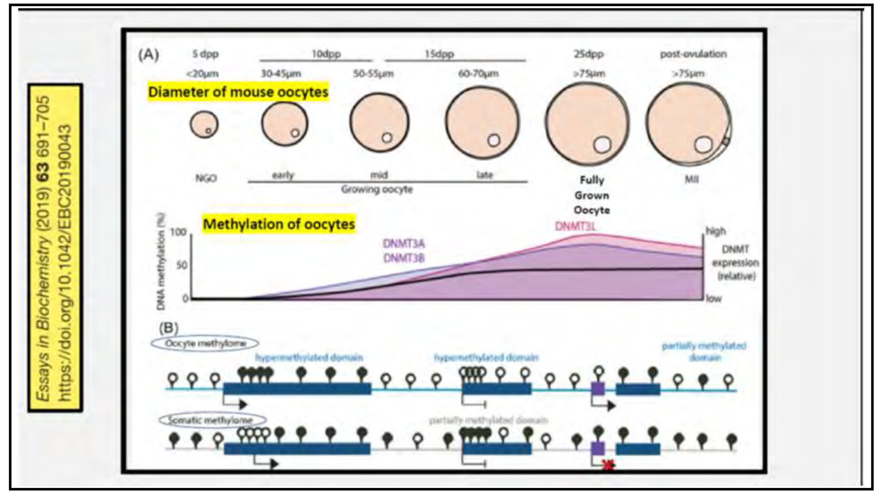
4


Three generations in one pregnant cow can be affected by epigenetics
18
2
https://www.researchgate.net/profile/Yutaka_Fukui
Oocyte
1N
Maternal DNA
Sperm
1N
Paternal DNA
http://www.ansci.wisc.edu/jjp1/ansci_repro/lab/procedures/sperm/bull_morp/bull_morphology.html
Genes Turned Off
1
G2-Fetus
G1-Dam
G3-Fetal
Gonad Genes
3
4
Genes Turned On
Specialized stem cells
Mammary
cells
2N
Ovarian cells
1°
0°
2°
2N
Genes Turned On

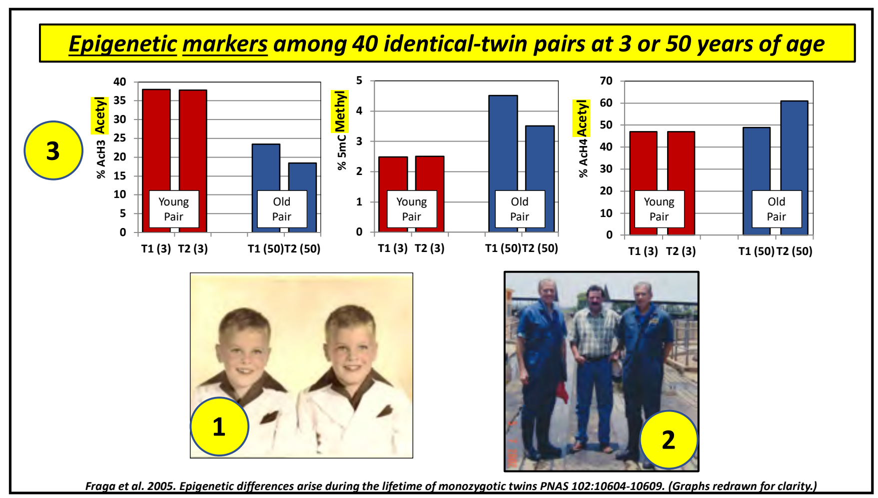
5


Classical epigenetic markers on DNA bases and histone proteins
Methyl Group
1
DNA
Acetyl Group
2
Histone
Histone Tails
Chromatin
Chromosome

6


Essays in Biochemistry (2019) 63 691–705
https://doi.org/10.1042/EBC20190043
Diameter of mouse oocytes
Methylation of oocytes
DNMT3A
DNMT3B
DNMT3L
Fully
Grown
Oocyte

7


Epigenetic markers among 40 identical-twin pairs at 3 or 50 years of age
3
% AcH3 Acetyl
Young
Pair
Old
Pair
T1 (3) T2 (3)
T1 (50) T2 (50)
% 5mC Methyl
% AcH4 Acetyl
1
2
Fraga et al. 2005. Epigenetic differences arise during the lifetime of monozygotic twins PNAS 102:10604-10609. (Graphs redrawn for clarity.)

8

Epigenetics alters gene expression by restricting enzyme access to DNA strands

1 Normal

t a a a g c

a t g c c g

Relaxed DNA Helix

2 Epigenetic (methyl, acetyl or other)

a t t a g a c a c g g c

Restrained DNA Helix

9

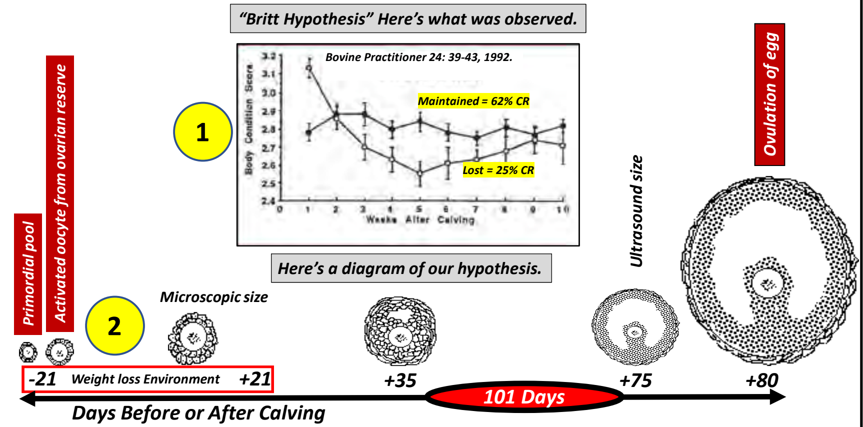
Examples of potential epigenetic-like effects

1. Milk fresh cows 4X for 3-4 wks. postpartum → Greater yield from 2X milking remainder of lactation
2. Greater BCS loss 3 wks. postpartum → Lower fertility at TAI at 80 days postpartum
3. IVF and MOET for producing embryos → Poorer health & underperforming yields
4. Heat stress during 6 wks. before birth → Lower yields for at least next 3 generations of daughters
5. Fetal development during lactation → Lower yield and longevity than expected

10

Example: How does weight loss affect fertility of a cow's eggs?

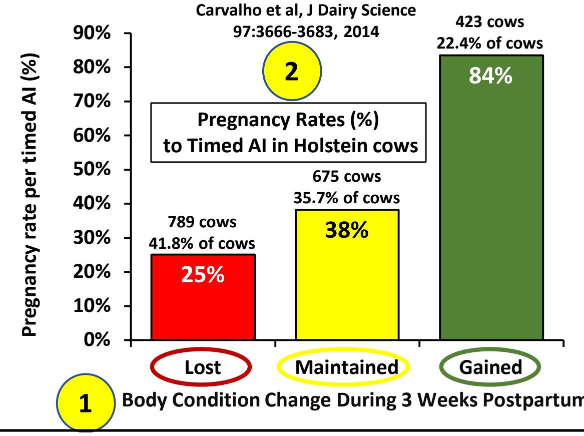
"Britt Hypothesis" Here's what was observed.



11

The Britt Hypothesis: 22 years later…

Carvalho et al, J Dairy Science 97:3666-3683, 2014



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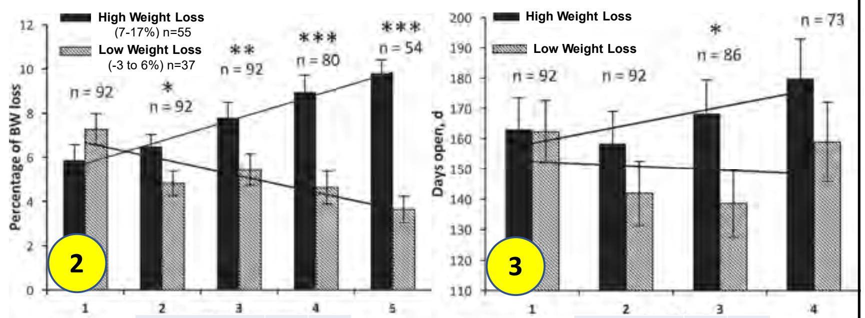
1

Do cows have to lose more weight naturally to have high yields?

M. Zachut and U. Moallem. 2017. J. Dairy Sci. 100 (4) 3143–3154



	High	Low
Avg 305 d yield, lbs.	86.7	85.8
Milk fat, %	3.40	3.46
Milk protein, %	3.09	3.15

	High	Low
Days open	167	150
Concep rate all, %	32.5	42.1

13

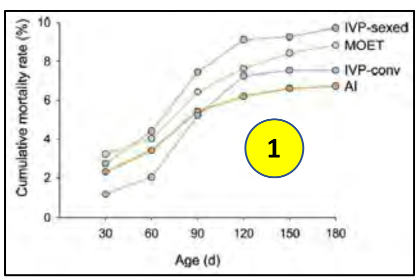
Sensors, robotics & artificial Intelligence to manage epigenetics

1

Maximizing weight gain before and after weaning using automated milk and starter feeders.

15

Manipulating embryos may cause epigenetic developmental effects



2

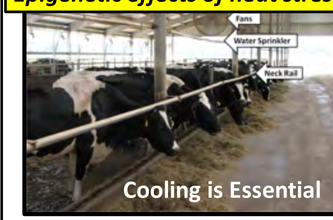
	Estrus & A.I.	I.V.P. Conve'l	I.V.P. Sexed	MOET
Gestation length, d	276.3	276.5	276.2	275.5
Birth weight, lbs.	85	87	86	85
Wt. first breeding, lbs.	757	772	782	762
Projected 305 milk, lbs.	24,207	24,081	23,577	24,158

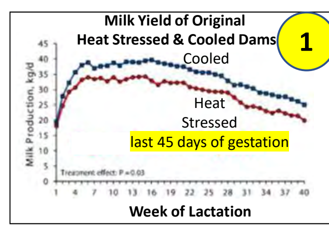
L. G. B. Siqueira, S. Dikmen, M. Sofia Ortega, and Peter J. Hansen. 2017. J. Dairy Sci. 100:5899–5908

14

Epigenetic effects of heat stress

19

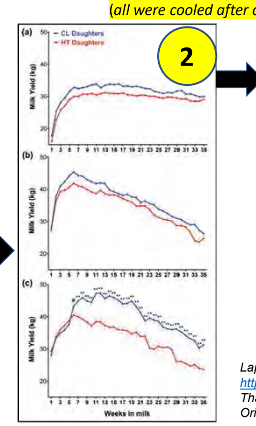


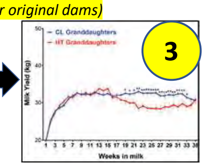


Daughters of Original Heat Stressed and Cooled Dams

Granddaughters of Original Heat Stressed and Cooled Dams

(all were cooled after original dams)





Laporta, et al J. Dairy Sci.
https://doi.org/10.3168/jds.2020-18154
Thanks to Jeff Dahl for providing Original graphic.

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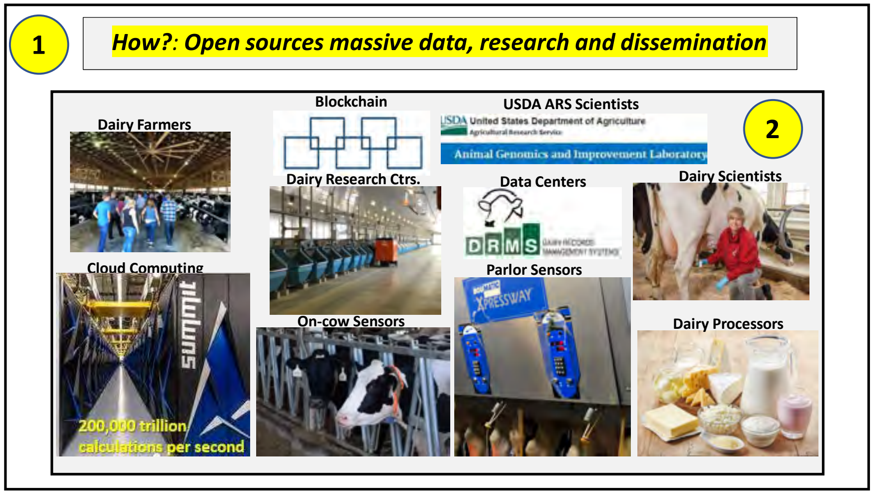
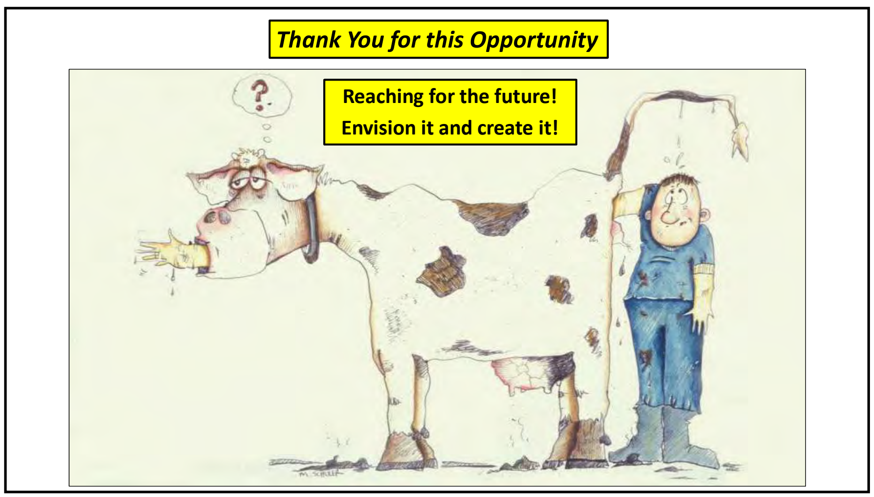

1
How?: Open sources massive data, research and dissemination
Blockchain
USDA ARS Scientists
USDA United States Department of Agriculture
Animal Genomics and Improvement Laboratory
2
Dairy Farmers
Dairy Research Ctrs.
Data Centers
DRMS
Dairy Scientists
Cloud Computing
summit
200,000 trillion calculations per second
Parlor Sensors
XPRESSWAY
On-cow Sensors
Dairy Processors

17


Thank You for this Opportunity
Reaching for the future!
Envision it and create it!
?

18

Epigenetic considerations from a dairy breeding and management perspective

PennState College of Agricultural Sciences

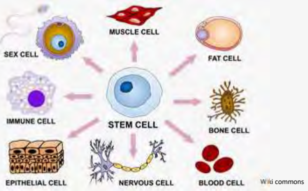
Chad Dechow
Associate Professor of Dairy Genetics

Selz-Pralle Aftershock 3918
214 lbs / 24.9 gallons
PTAM = -127 lbs

1

Topics

- **Beyond the code**
- **Breeding perspective**
  - Advanced breeding programs, hybrid vigor, transgenerational transmission
- **Management**



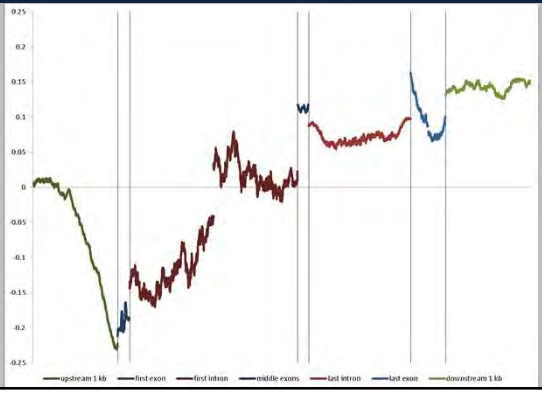
2

Same DNA sequence. Different package?

Chemical modifications to the genome alter gene expression

3

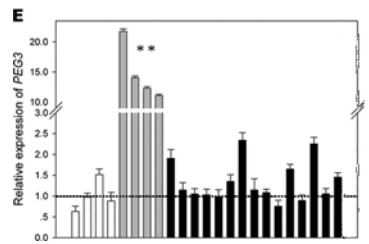
DNA methylation and gene expression



4

Aberrant epigenetic changes & dead clones

- **Donor cell must be epigenetically reprogrammed**
  - From differentiated to totipotent
- **Normal vs dead cloned calves**
  - Multiple genes and tissues
- **Aborted fetuses**
- **Placental abnormalities**



Su et al. doi.org/10.1016/j.theriogenology.2010.11.045.

5

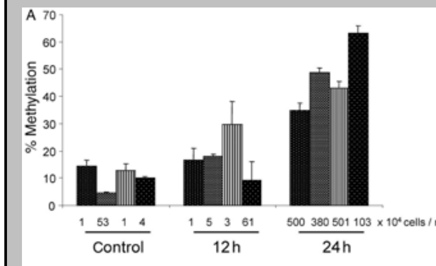
Mastitis & αS1 Casein

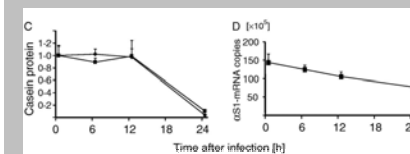
- **Casein production reduced during mastitis**
  - Particularly coliform
- **3 quarters infused with pathogen**
  - E-coli
  - Staph aureus
- **Methylation of αS1 Casein regulatory regions**

Vanselow et al., 2006: Journal of Molecular Endocrinology (2006) 37, 463–477

6

E-Coli & Casein





Vanselow et al., 2006: Journal of Molecular Endocrinology (2006) 37, 463–477

7

Differential methylation in blood

8

Epigenetic programming

- Essential to proper embryonic and fetal development
- Directs cell response to environmental state
- Variation among cows evident
- Implications for genetic & breeding programs?

9

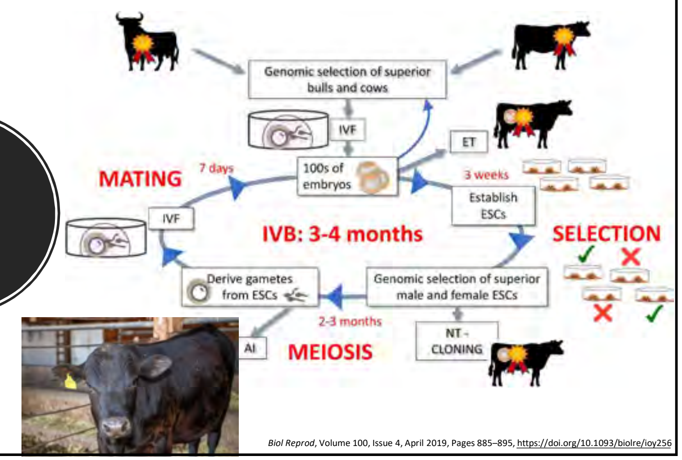
Implications for breeding programs

- Advanced breeding programs
- Crossbreeding
- Transgenerational selection

© Courtesy of Pioneer Hi-Bred International, Inc.

10

In vitro breeding (IVB)

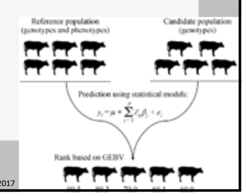


Biol Reprod, Volume 100, Issue 4, April 2019, Pages 885–895, https://doi.org/10.1093/biolre/ioy256

11

Current hurdles or unknowns

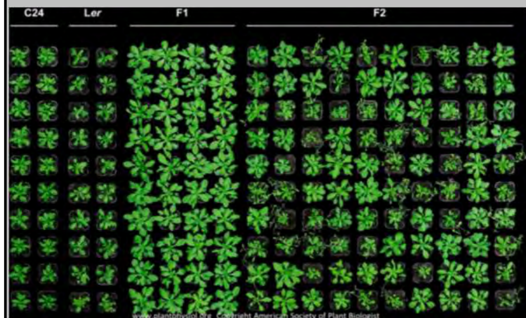
- Number of generations between reference population and genetic nucleus
- Effects of epigenetic (re)programming
  - Developmental abnormalities
  - Normal, but altered state from reference population



Kang, 2017

12

Epi-alleles contribute to hybrid vigor in Arabidopsis



- Parental allele from one strain alters methylation pattern in alternate strain
- Hybrid vigor generally reduced after first generation
- Researchers able to stabilize hybrid vigor by targeting altered pathways

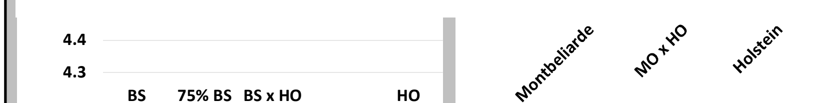
PNAS 108:6:2617-2622, PNAS 109:3570-3575 PNAS 112: E4959–E4967

13

Hybrid vigor for production

Is the epigenome responsible for hybrid vigor in animals?

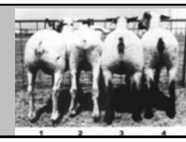
Can we learn to induce/retain hybrid vigor state in later crosses or purebreds?



14

Mutation altering epigenetic state

Genotype Maternal / paternal	Phenotype
+ / +	Normal
+ / C	Hypertrophy
C / +	Normal
C / C	Normal



- **Imprinted genomic region**
  - High level of DNA methylation
- **Facilitated by DNA sequence mutation**
  - Reduced methylation
  - Increased gene expression
- **Genomic selection implications**

Aphrodite Kallipygos, The National Archaeological Museum of Naples, Italy.

15

Transgenerational epigenetic inheritance?

- **Intergenerational:**

  pregnant granddam – dam – offspring
  - Observed in many species to varying degrees
  - Environmental insult has effects on grand-progeny
- **Transgenerational:**

  non-pregnant granddam – dam – offspring

  great-granddam – granddam – dam - progeny
  - Observed in plants and some experimental species
  - Existence controversial in mammals

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Genetics and breeding

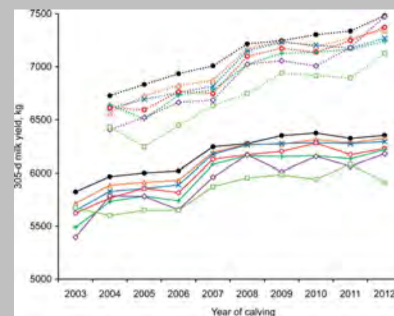
- **Learning to program cells will unlock advanced breeding program possibilities**
- **Describing epigenetic profiles may help unlock “non-additive” effects**
  - Targeted breed and line crossing
- **Select animals with favorable epigenetic states**

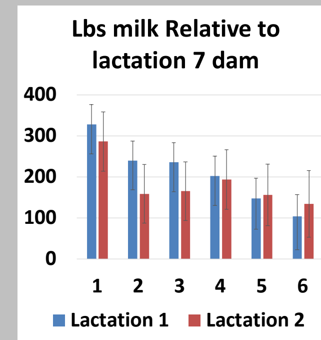
17

Can we manage cows to induce a favorable epigenetic state?

18

Younger dams favored





Storli et al., JDS 97:6242

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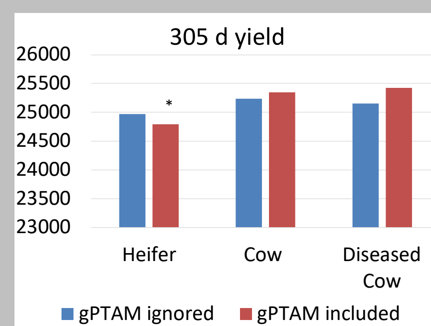
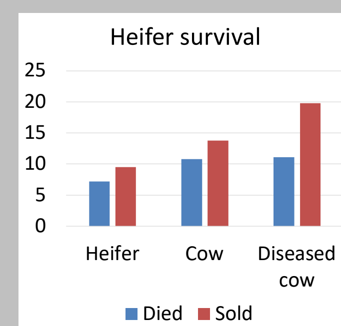
Florida results

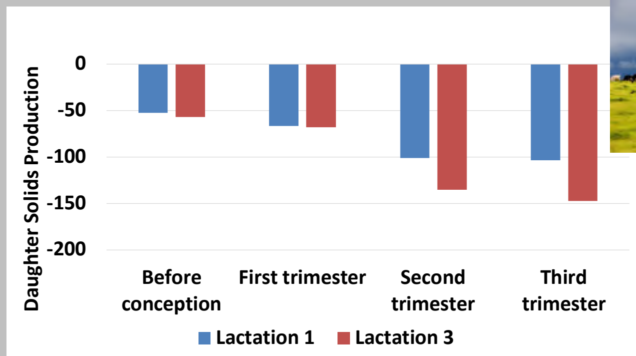


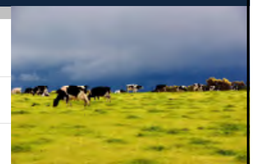


Carvalho et al., JDS 103:823

20

Dam milk yield during gestation

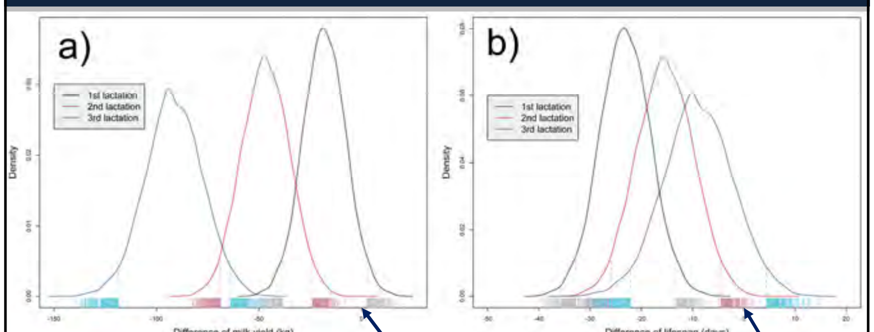




Abegglen

21

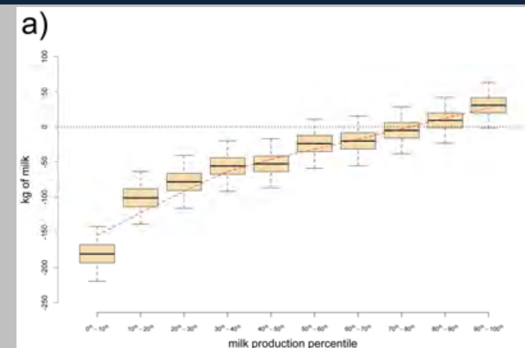
Relative to heifer



González-Recio O, Ugarte E, Bach A (2012) PLOS ONE 7(12): e51816.

22

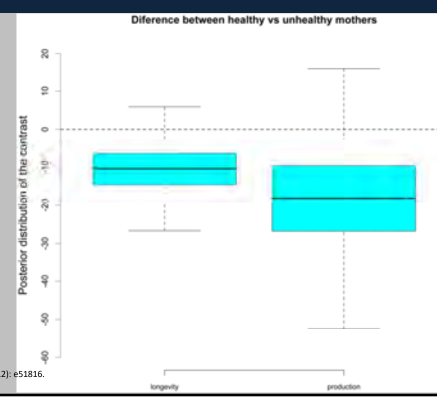
Milk production percentile



González-Recio O, Ugarte E, Bach A (2012) PLOS ONE 7(12): e51816.

23

Healthy dams favored



González-Recio O, Ugarte E, Bach A (2012) PLOS ONE 7(12): e51816.

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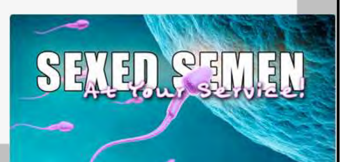
Replacement management

- **Generating replacements from heifers**
  - Programmed for success?
  - Sexed semen for heifers, beef for mature cows
- **Maternal yield and health have effects on daughter performance**
  - Statistically significant, but small and inconsistent effects
- **Accelerated heifer growth**
  - Programming = faster growth & higher yield?
  - Or … accelerated growth directly facilitate favorable programming?

25

Other management considerations

- **Strong evidence**
  - Heat stress, diet, energy balance
- **Some evidence**
  - Beef on dairy, transportation stress
- **Uncertain / unlikely**
  - Sexing sperm, fetal sex



The bullvine

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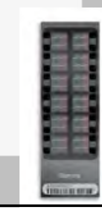
Challenges to strategy development

- **How well do observations from other countries translate to the US?**
- **Often *assuming* an epigenetic/fetal programming effect**
  - Other possibilities: germline mutations, telomere dynamics, incomplete accounting for genetic effects, etc.
- **Which tissue, and how do you access?**



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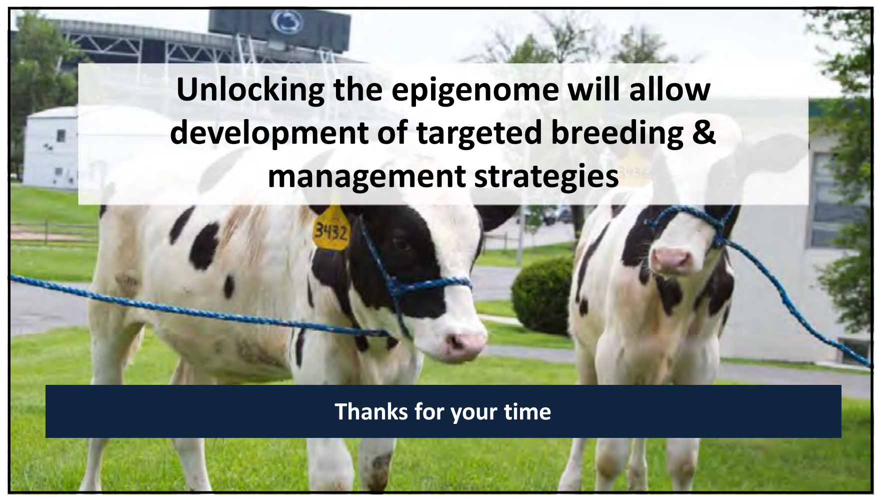


Unlocking the epigenome will allow development of targeted breeding & management strategies

Thanks for your time

28

Phenotypic & molecular signatures of fetal hyperthermia

Jimena Laporta
Assistant Professor
Mammary Gland Physiology

ANIMAL & DAIRY SCIENCES
University of Wisconsin-Madison

Mini-Symposium:
Exploring In Utero Influences on Transgenerational Performance

balchem

May 9, 2022

1

Presentation outline

1. **Heat stress, fetal programming & the epigenome**
2. **Phenotypic Signature**
   Short & long-lasting effects of *in utero* heat stress
   - Growth, productive life, survival, milk production
   - Financial implications?
3. **Molecular Signature**
   Mammary gland & liver methylation
   - Unique mammary gland methylation pattern?
   - Commonality of DMG across tissues orchestrated by *in utero* heat stress?

2

Heat stress is the largest challenge affecting the dairy industry

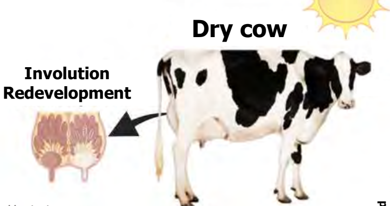
- Global temperature rise caused by climate change
- Modern dairy cows increased productivity and metabolic heat production are more sensitive to changes in temperature
- Focus of research and mitigating technologies
  → immediate drop feed intake and milk production: $ pit

Heat stress does not discriminate!

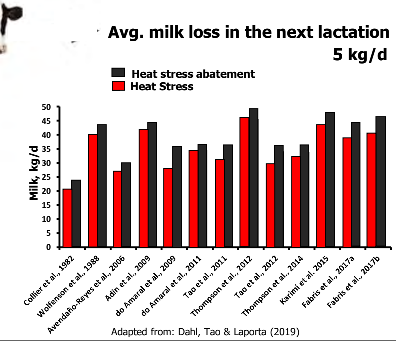
- Impact physiology, productivity, and welfare independent of age or physiological status

3

Impact of heat stress on dry cows?



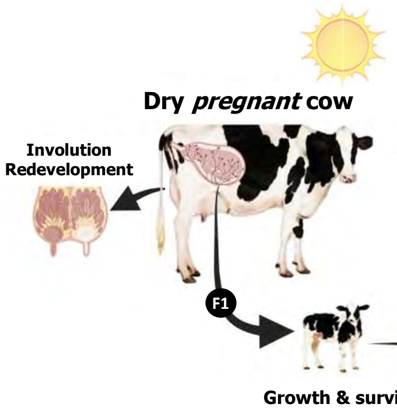
→ Heat stress exposure derails these cellular processes leading to milk yield reductions in the next lactation



Adapted from: Dahl, Tao & Laporta (2019)

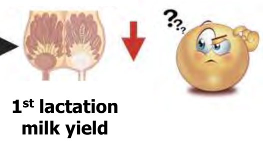
Tao et al. (2012), Dado-Senn et al (2018), Fabris et al., (2020)

4



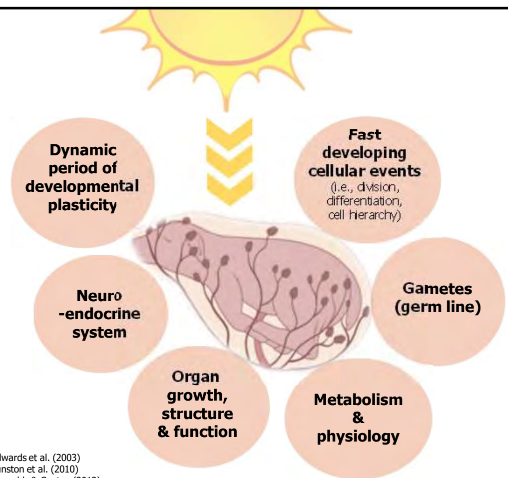
- Coincides with the last trimester of gestation, a period of exponential fetal growth
- Maternal HS leads to intrauterine hyperthermia resulting in smaller daughters less likely to survive and less productive

→ **What is the mechanistic link?**
→ **What happens beyond the first lactation?**
→ **What happens beyond the first generation?**



Monteiro et al. (2014 & 2016)

5



Fetal programming of *in utero* hyperthermia & the resulting life-long implications

The fetus is extremely sensitive to maternal influences at all stages of gestation

In utero hyperthermia can derail the developmental trajectory of organs and systems… triggering disease susceptibility and reducing future productivity

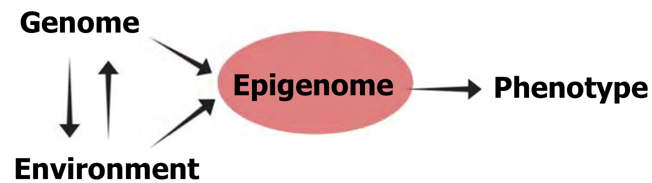
…altered epigenome?

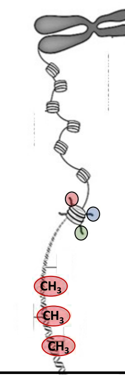
Edwards et al. (2003)
Funston et al. (2010)
Reynolds & Canton (2012)

6

Mechanistic link between intrauterine conditions and postnatal performance?

"above" or "in addition to" traditional genetic basis of inheritance





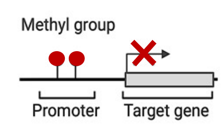
→ Epigenome orchestrates the complex interplay between E & G to determine P
→ Chemical changes that alter DNA's physical structure w/o changing its sequence
→ Regulate when & which genes are turned **on** or **off** in a specific cell type or organ

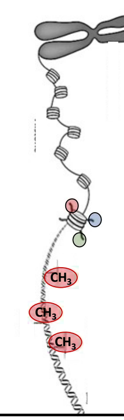
→ **Alternations in the epigenome triggered *in utero* can be stable and sustained within individuals and across generations**

7

Three main mechanisms cause epigenetic changes

- Histone modifications
- Non-coding microRNAs
- DNA methylation
  → most stable epigenetic mark





Environmental signals are…

- **R**eceived by the genome
- **R**ecorded by the genome
- **R**emembered across successive cell generations
- **R**evealed in altered gene expression and cell function
- **R**eversible
  → Key mechanism allowing for phenotypic plasticity of a fixed genotype!

Mathers & McKay (2009); Angers et al. (2010)

8

Presentation outline

1 Heat stress, fetal programming & the epigenome

2 Phenotypic Signature

Short & long-lasting effects of in-utero heat stress
- Growth, productive life, survival, milk production
- Financial implications?

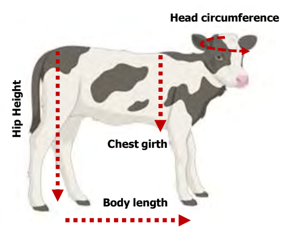
3 Molecular Signature

Mammary gland & liver methylation
- Unique mammary gland methylation pattern?
- Commonality of DMG across tissues orchestrated by intrauterine hyperthermia?

9

Postnatal hallmarks of *in utero* heat stress
"Short-term effects"

- **Gestation length**
  → ~5 d, less time for fetal and MG development
- **Birth and weaning weight**
  → 10% reduction (10 & 17 lbs.)
- **Body size**
- **Organ weights**
  → Altered immune organs
  → Larger adrenal glands
  → Smaller ovaries
  → Smaller mammary glands
    - less fat pad and parenchyma
    - fewer & smaller epithelial ductal structures



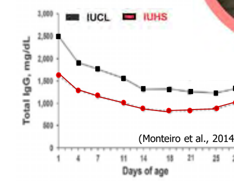
IUHS limits normal early MG developmental trajectory

Dado-Senn et al. (2021a, 2021b)

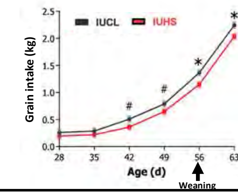
10

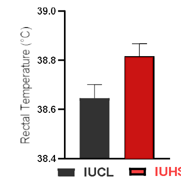
Postnatal hallmarks of *in utero* heat stress
"Short-term effects"

- **Innate & adaptive immunity**
  → Lower circulating IgG
  → Reduced apparent efficiency of IgG absorption
  → Lower peripheral blood mononuclear cell proliferation
  → Less robust immune response…
    - greater odds of a health event
    - require more assistance and treatments



- **Grain intake**
  → total dry matter, metabolizable energy, crude protein, and crude fat intake
- **Thermoregulation**
  → Elevated core body temperature
  → Elevated sweating
  → Alterations in sweat gland density and distribution!
    - less and smaller SG located further to the skim surface





Dado-Senn et al. (2021)

11

Postnatal hallmarks of *in utero* heat stress
"Long-term effects"

University of Florida 10-year data set
AFI records and lifetime events

400 Dams either HS or CL the entire dry period **(F_0)**
└ 160 Daughters **(F_1)**
  └ 50 Granddaughters **(F_2)**

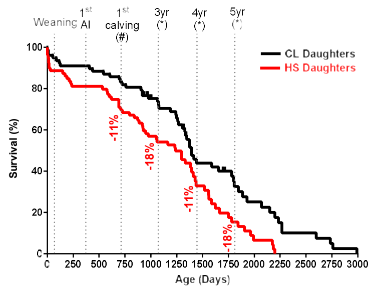
a **Productive life and lifespan?**
b **Milk loss: transient or multi lactational?**
c **Multi generational?**
d **Financial implications?**

Laporta et al. (2020)

12

a Productive life & lifespan?

Survival F1



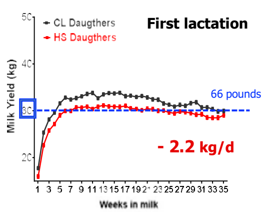
- ***In utero*** **heat stress reduces**
  - survival to 1st calving
  - survival through 1, 2, 3 lactations
  - productive life by 5 months
  - lifespan by ~ 12 months

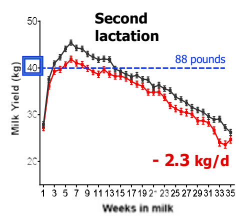
Laporta et al. (2020)

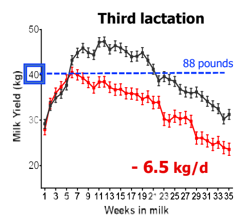
13

b Is it transient or multi lactational?

- **Milk Production**







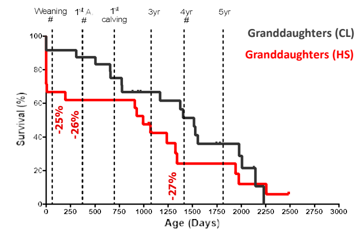
- Reduction in ECM, fat and protein yields for at least 3 lactations (despite being cooled during lactation)
- Long-lasting *in utero* programming effect of a less productive phenotype!

Laporta et al., 2020

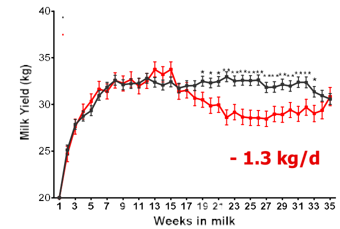
14

c Is it multigenerational?

Survival F2



First lactation milk yield F2



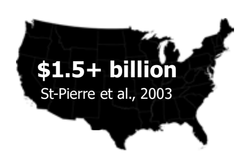
- **Late-gestation HS impacts *at least* 3 generations** manifesting 3+ years after the initial insult (F_0, grandma | F_1 in utero)

Laporta et al. (2020)

15

d Economic cost of heat stress

- Financial loss in lactating and dry cows
- Financial loss associated to *in utero* heat stress?
  - → underestimated if the subsequent loss in the progeny is not considered!



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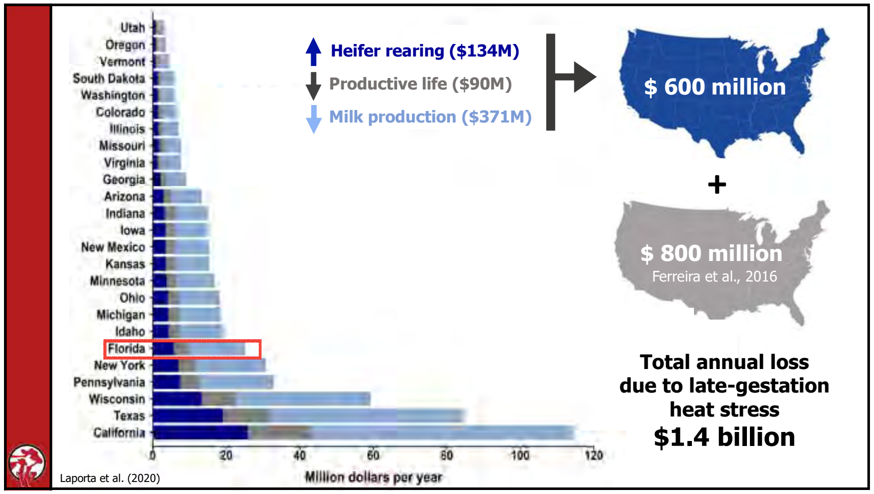
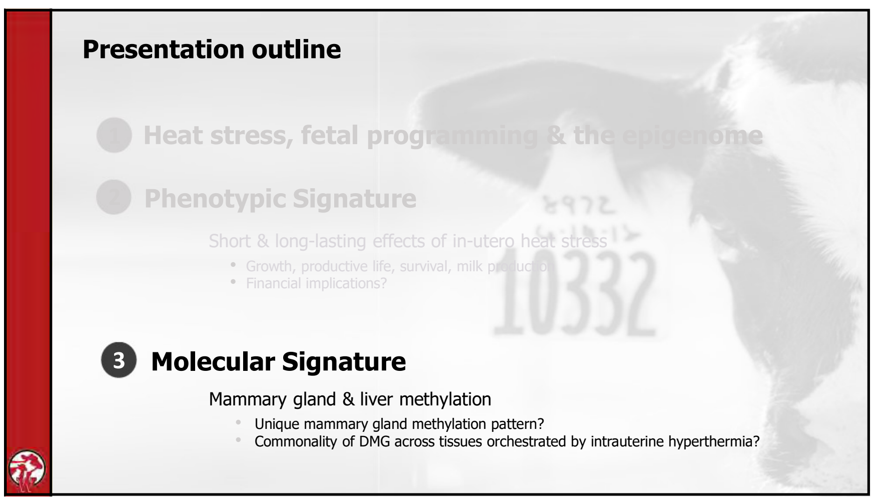
Assumptions

- **Milk yield & survival**: UF data set
- **No cows were cooled during the dry period**
- **No seasonality of calving:** 15% dry cows
- **Heat stress days per state**: avg. THI>68, NOAA
- **Number of cows per state**: USDA-NASS, 2018
- **Milk price**: $0.44/kg (avg. US prices 2010-16)
- **Feed price**: $0.11/kg of milk | **IOFC**: $0.33/kg of milk
- **Herd composition**: 35% (1st), 20% (2nd), 14% (3rd) lactations

16


Utah
Oregon
Vermont
South Dakota
Washington
Colorado
Illinois
Missouri
Virginia
Georgia
Arizona
Indiana
Iowa
New Mexico
Kansas
Minnesota
Ohio
Michigan
Idaho
Florida
New York
Pennsylvania
Wisconsin
Texas
California
0 20 40 60 80 100 120
Million dollars per year
Laporta et al. (2020)
Heifer rearing ($134M)
Productive life ($90M)
Milk production ($371M)
$ 600 million
+
$ 800 million
Ferreira et al., 2016
Total annual loss due to late-gestation heat stress
$1.4 billion

17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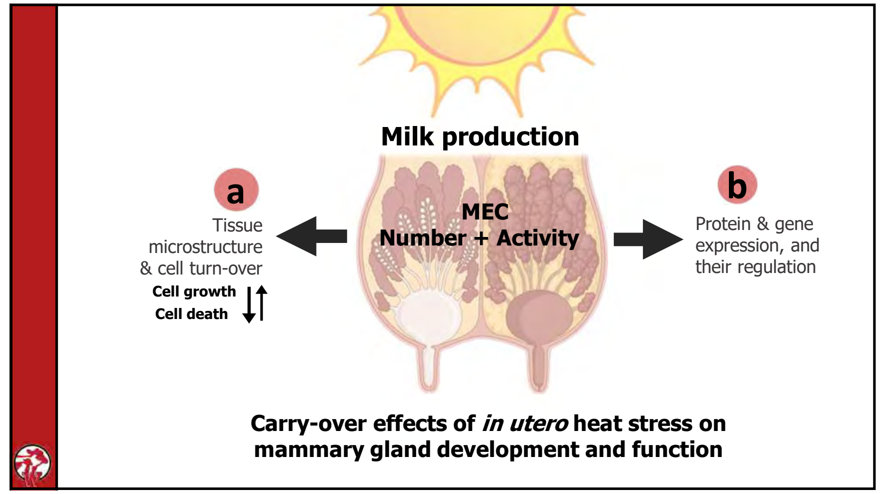
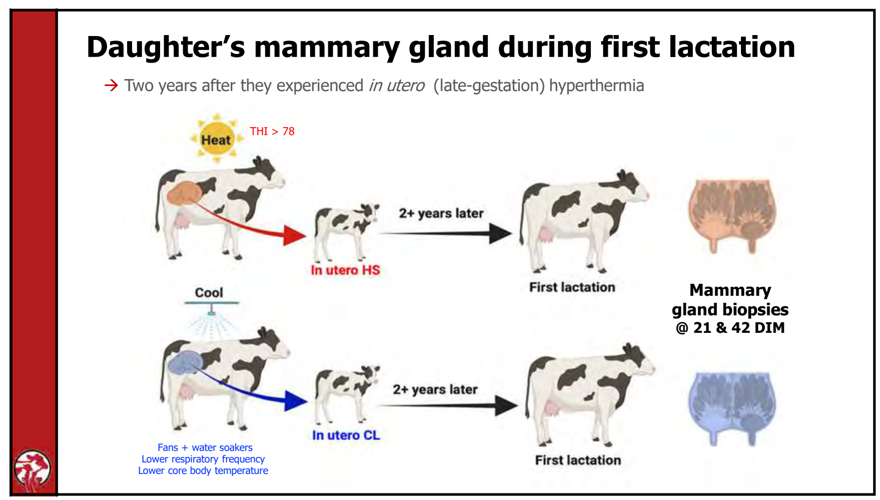
Commonality of DMG across tissues orchestrated by intrauterine hyperthermia?

18

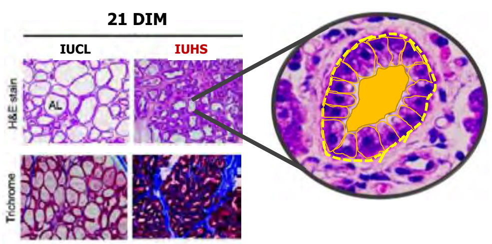

Milk production
a
Tissue microstructure & cell turn-over
Cell growth
Cell death
MEC
Number + Activity
b
Protein & gene expression, and their regulation
Carry-over effects of in utero heat stress on mammary gland development and function

19

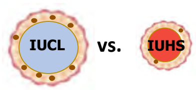

Daughter's mammary gland during first lactation
→ Two years after they experienced in utero (late-gestation) hyperthermia
Heat
THI > 78
In utero HS
2+ years later
First lactation
Mammary gland biopsies @ 21 & 42 DIM
Cool
Fans + water soakers
Lower respiratory frequency
Lower core body temperature
In utero CL
2+ years later
First lactation

20

a Daughter's mammary gland microstructure



- → 46% smaller luminal-alveolar area
- → Fewer (#) epithelial (secretory) cells
- → Lower % of proliferating cells
- → More connective (stroma) tissue



- → **Compromised synthetic & milk storage capacity**
- → **Undesirable characteristics during lactation!**
- → **Contributing to reduced lactation performance?**

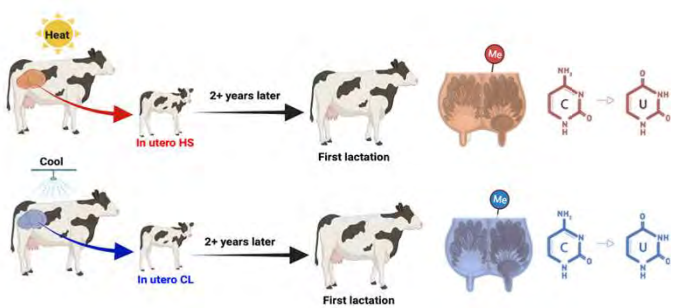
Skibiel et al. (2018)

21

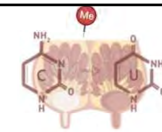
b Daughter's mammary gland DNA methylation

Are there alternations in DNA methylation triggered by *in utero* heat stress that might explain the observed phenotypic outcomes?

→ Reduced Representation Bisulfate Sequence (RRBS)



22



- 523 DM Cytosines
- **135 DM Genes**

Pathway analysis
DM Genes associated with fundamental cellular processes:
- genomic stability
- cell survival
- transcription, translation

...involved in stress response, mammary development & milk synthesis

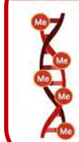
Hypomethylated genes IUHS

PTK2: Protein Tyrosine Kinase
- cell migration/attachment to ECM
- cell survival

PRKG1: Ser/threonine Protein Kinase
- apoptotic induction/growth inhibition
- circadian rhythms

WRN: DNA helicase
- DNA repair
- oxidative stress

Hypermethylated genes IUHS



ASAP1: GTPase
- regulator of stem & progenitor cells

LAP: Aminopeptidase
- antimicrobial peptide

DTX2: Ubiquitin-protein ligase
- cell-cell communication
- Notch signaling pathway

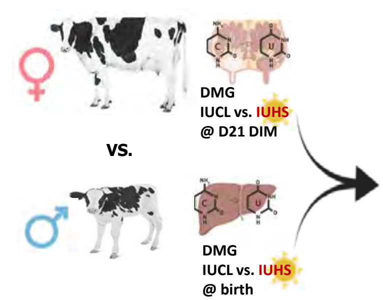
Skibiel et al. (2018b)

23

Are these DM Genes unique to the mammary gland?

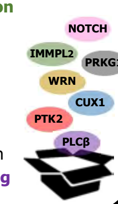
→ Common patterns of DNA methylation induced by *in utero* heat stress regardless of sex, age, or tissue type?

→ DMG associated with biological functions commonly regulated by *in utero* heat stress



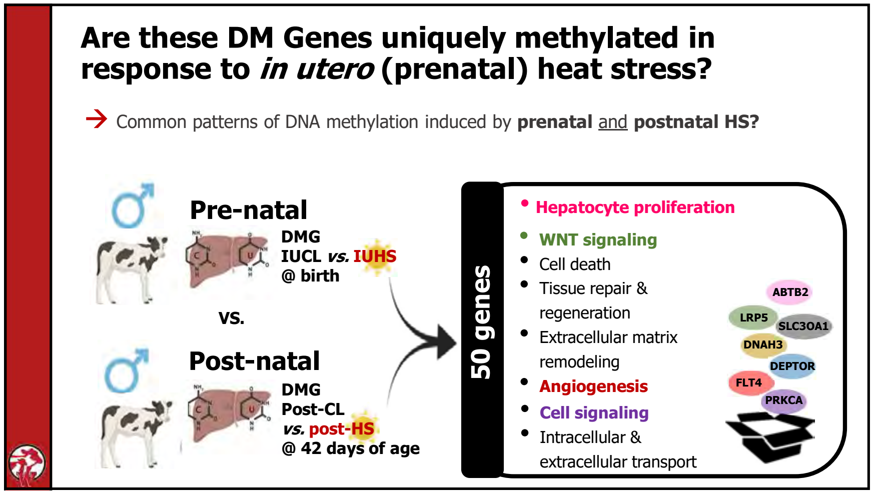
50 genes

- (20) code for ribosomal RNA
  Protein synthesis machinery?
- **Mitochondrial function**
- DNA repair
- Genomic integrity
- **Cell adhesion & migration**
- Cell viability
- Transcription/translation
- **Intracellular signaling**

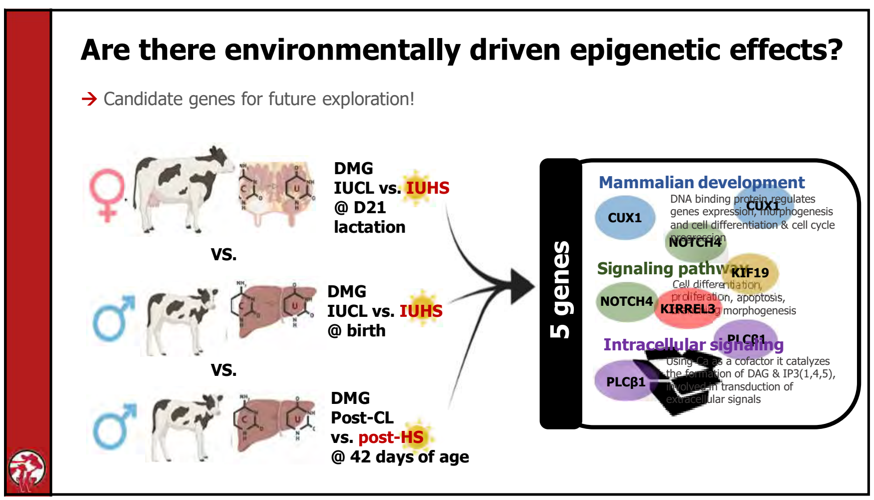


Skibiel et al. (2018b)

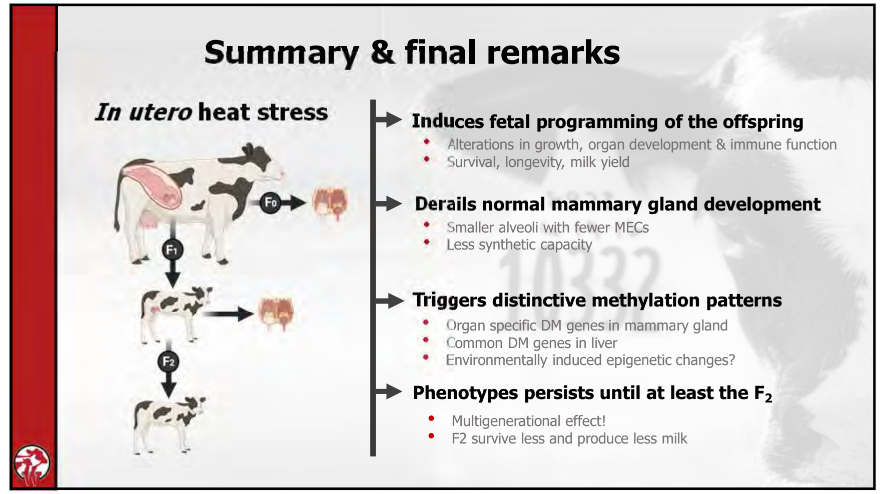
24


Are these DM Genes uniquely methylated in response to *in utero* (prenatal) heat stress?
→ Common patterns of DNA methylation induced by **prenatal** and **postnatal HS?**
Pre-natal
DMG
IUCL vs. IUHS
@ birth
vs.
Post-natal
DMG
Post-CL
vs. post-HS
@ 42 days of age
50 genes
Hepatocyte proliferation
WNT signaling
Cell death
Tissue repair & regeneration
Extracellular matrix remodeling
Angiogenesis
Cell signaling
Intracellular & extracellular transport
ABTB2
LRP5
SLC30A1
DNAH3
DEPTOR
FLT4
PRKCA

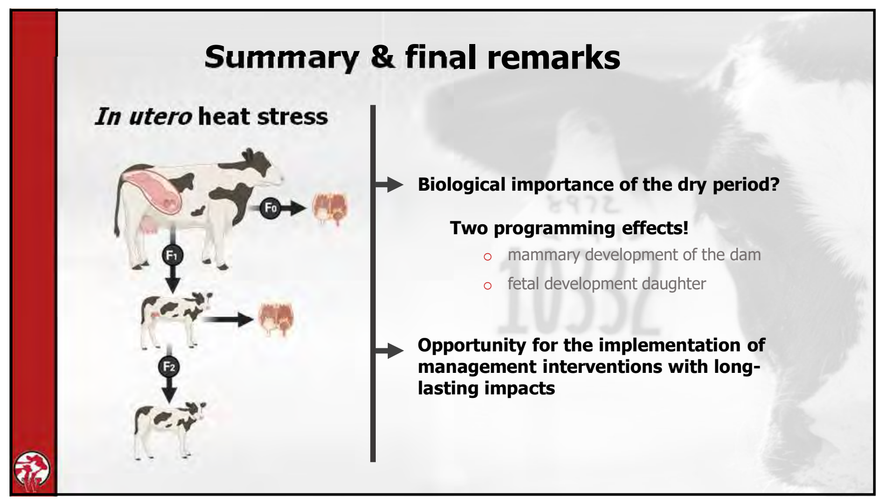
25


Are there environmentally driven epigenetic effects?
→ Candidate genes for future exploration!
DMG
IUCL vs. IUHS
@ D21
lactation
VS.
DMG
IUCL vs. IUHS
@ birth
VS.
DMG
Post-CL
vs. post-HS
@ 42 days of age
5 genes
Mammalian development
CUX1
DNA binding protein regulates genes expression, morphogenesis and cell differentiation & cell cycle progression
NOTCH4
Signaling pathway
KIF19
NOTCH4
KIRREL3
Cell differentiation, proliferation, apoptosis, morphogenesis
PLCβ1
Intracellular signaling
PLCβ1
Using Ca as a cofactor it catalyzes the formation of DAG & IP3(1,4,5), involved in transduction of extracellular signals

26


Summary & final remarks
In utero heat stress
F0
F1
F2
Induces fetal programming of the offspring
Alterations in growth, organ development & immune function
Survival, longevity, milk yield
Derails normal mammary gland development
Smaller alveoli with fewer MECs
Less synthetic capacity
Triggers distinctive methylation patterns
Organ specific DM genes in mammary gland
Common DM genes in liver
Environmentally induced epigenetic changes?
Phenotypes persists until at least the F2
Multigenerational effect!
F2 survive less and produce less milk

27


Summary & final remarks
In utero heat stress
F0
F1
F2
Biological importance of the dry period?
Two programming effects!
mammary development of the dam
fetal development daughter
Opportunity for the implementation of management interventions with long-lasting impacts

28

Collaborator @ UF

Geoffrey E. Dahl

Students & postdocs

Bethany Dado-Senn
Brittney Davidson
Sena Field
Marcela Marrero
Leticia Casarotto Trevisan
Thiago Fabris
Veronique Ouellet
Amy Skibiel

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USDA/NIFAAFRI # 2019-67015-29445
USDA/NIFAAFRI # 2015-67015-23409

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Thank you

Jimena Laporta

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@JimenaLaporta
https://lactationbiology.webhosting.cals.wisc .edu/laporta/

30

Methyl donors and epigenetic regulation of the early embryo

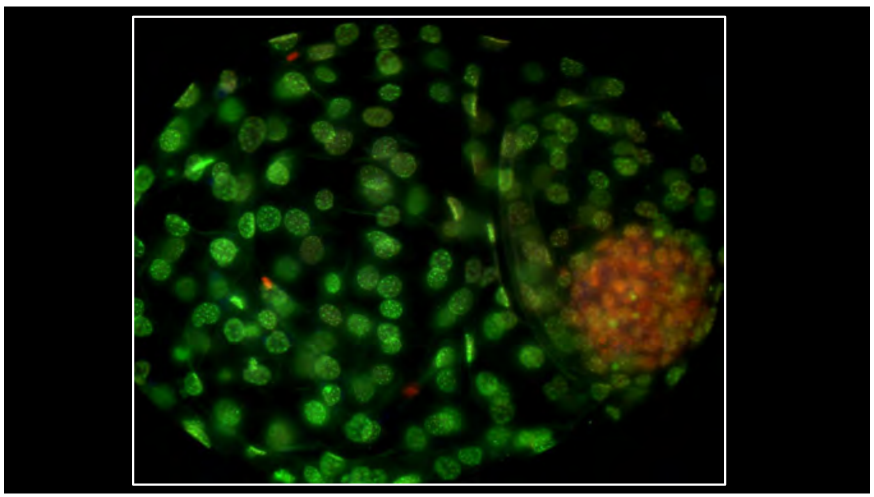
balchem
Solve Today. Shape Tomorrow.

Balchem Mini-Symposium

P.J. Hansen
Dept. of Animal Sciences
University of Florida

UF IFAS UNIVERSITY of FLORIDA | UF | ANIMAL SCIENCES | University of Florida Brahman Cattle

1



2

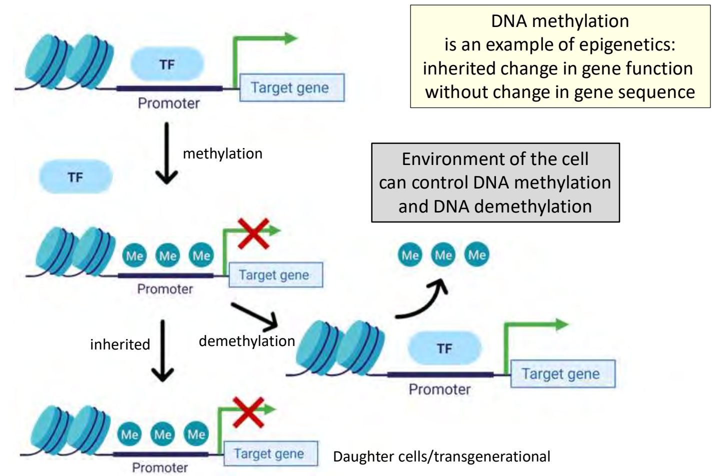
Take-home messages

- Changing DNA methylation during the earliest stages of life, when the embryo is developing from the one-cell stage to the blastocyst stage (day 7 in the cow), can change the program of development to affect postnatal phenotype
- Providing methyl donors is one way to change DNA methylation
- There is the opportunity to improve growth, reproduction or lactation by altering DNA methylation at critical times in development

Example of this idea

- Effects of choline treatment of embryos produced in vitro on birthweight and growth of the resultant calf

3



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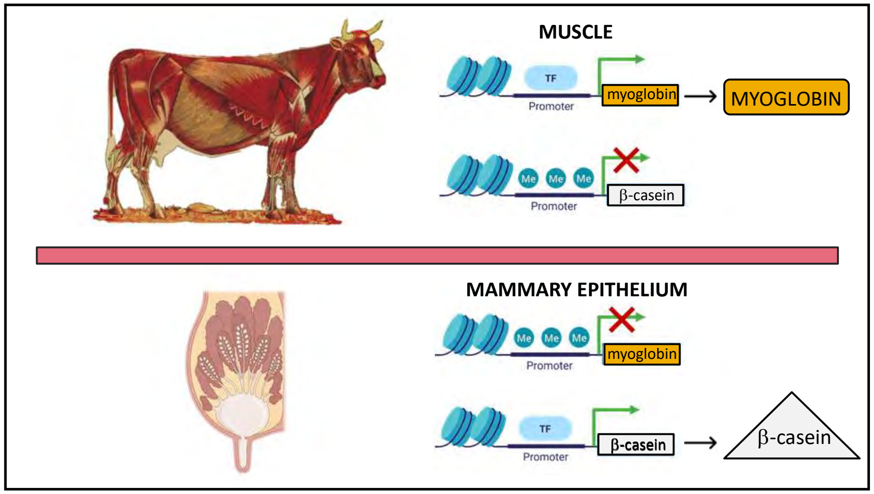

5

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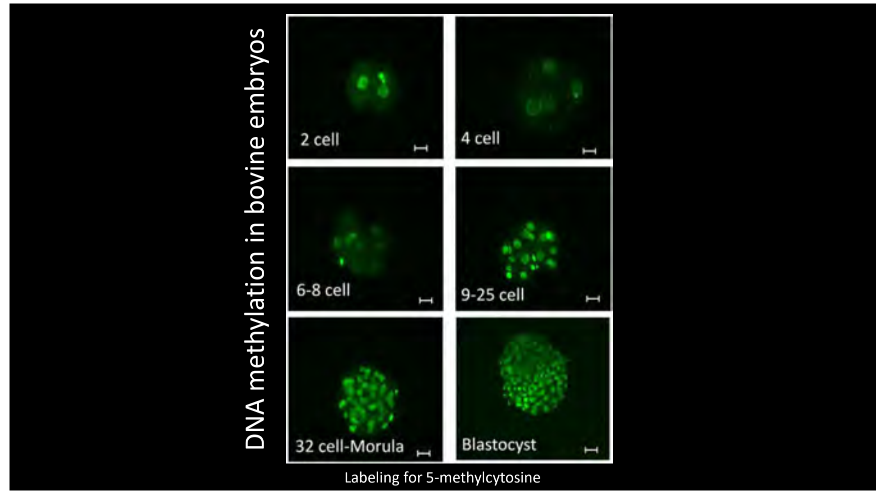
6



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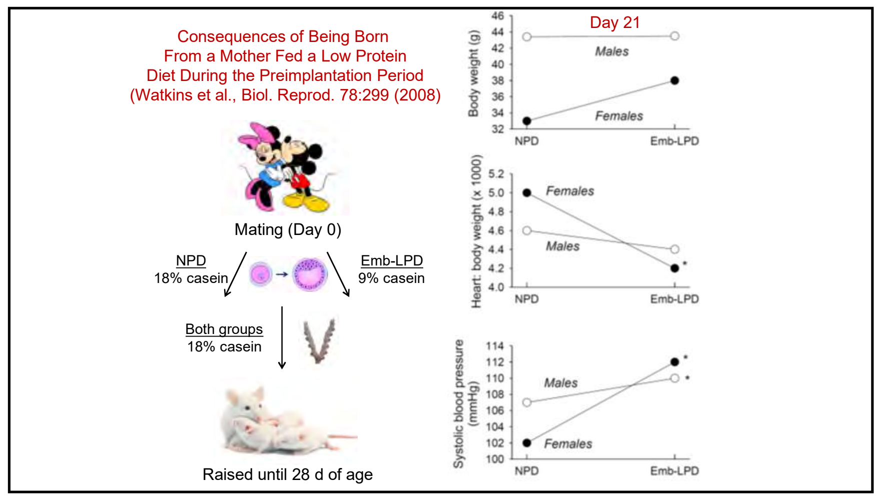
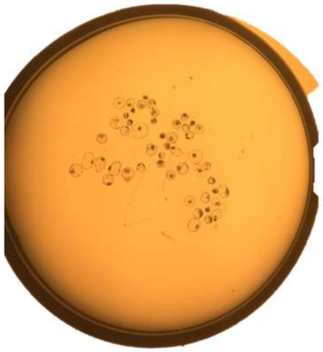


8

EXAMPLE OF ABERRANT PROGRAMMING

LARGE OFFSPRING SYNDROME
FOLLOWING EMBRYO PRODUCTION IN VITRO

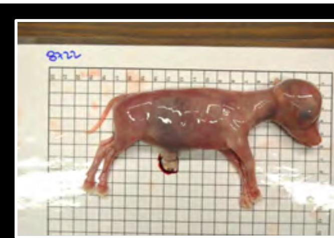
Salts
Sugars
Amino acids
Fatty acids
Proteins
miRNA
Exosomes
Surface tension
Motion
Cell-cell
interactions



9

98 kg at birth
picture at 2 days of age

10



8722 – IVF
Dried placenta weight – 9.1 g
Cotyledon diameter – 3.5 cm
Fetal weight – 152 g
Liver weight – 6.6 g
Heart weight - 1.4 g

7348 – IVF + CSF2
Dried placenta weight – 34.1 g
Cotyledon diameter – 5.6 cm
Fetal weight – 354.3 g
Liver weight – 18.6 g
Heart weight - 4.5 g

11

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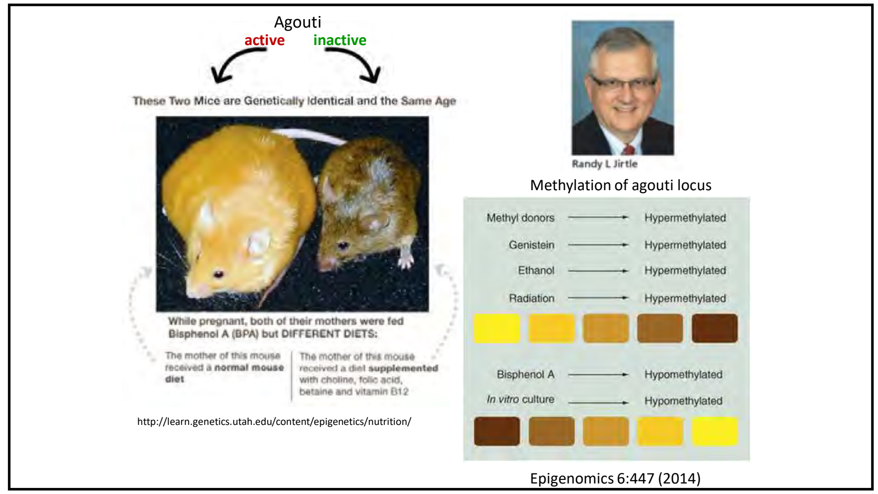
12

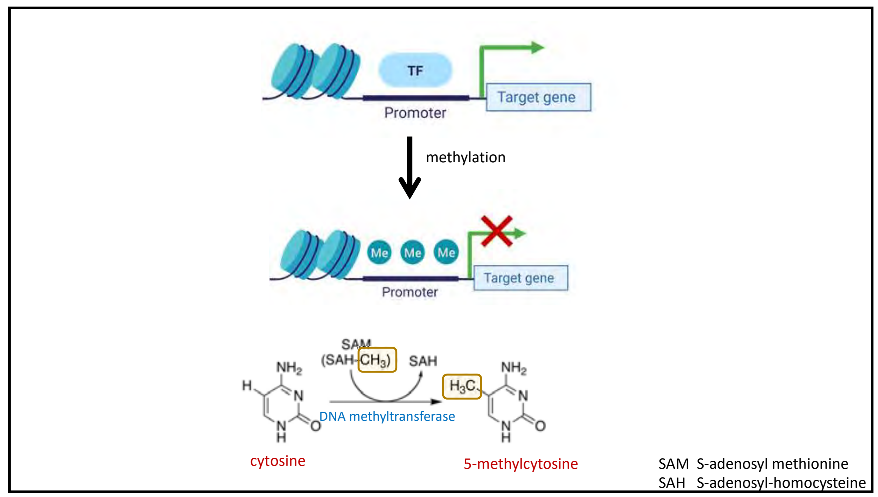

mepron
balchem
ReaShure
Precision Release Choline
Smartamine M

13

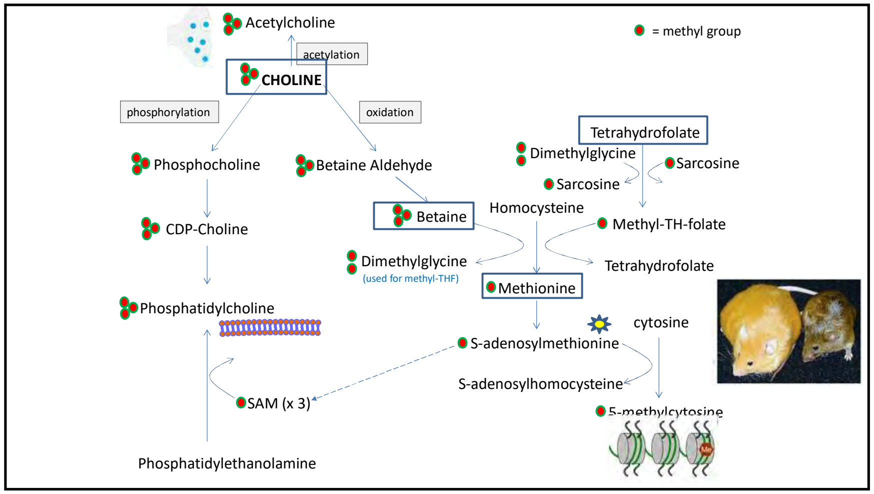
14


Agouti
active
inactive
These Two Mice are Genetically Identical and the Same Age
While pregnant, both of their mothers were fed Bisphenol A (BPA) but DIFFERENT DIETS:
The mother of this mouse received a normal mouse diet
The mother of this mouse received a diet supplemented with choline, folic acid, betaine and vitamin B12
http://learn.genetics.utah.edu/content/epigenetics/nutrition/
Randy L Jirtle
Methylation of agouti locus
Methyl donors → Hypermethylated
Genistein → Hypermethylated
Ethanol → Hypermethylated
Radiation → Hypermethylated
Bisphenol A → Hypomethylated
In vitro culture → Hypomethylated
Epigenomics 6:447 (2014)

15


TF
Promoter
Target gene
methylation
Me Me Me
Promoter
Target gene
SAM
(SAH-CH3)
SAH
DNA methyltransferase
cytosine
5-methylcytosine
SAM S-adenosyl methionine
SAH S-adenosyl-homocysteine

16



17

Take-home messages

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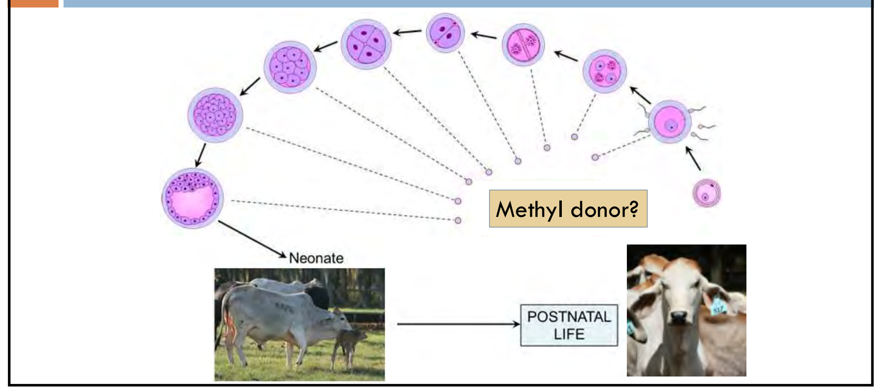
Example of this idea

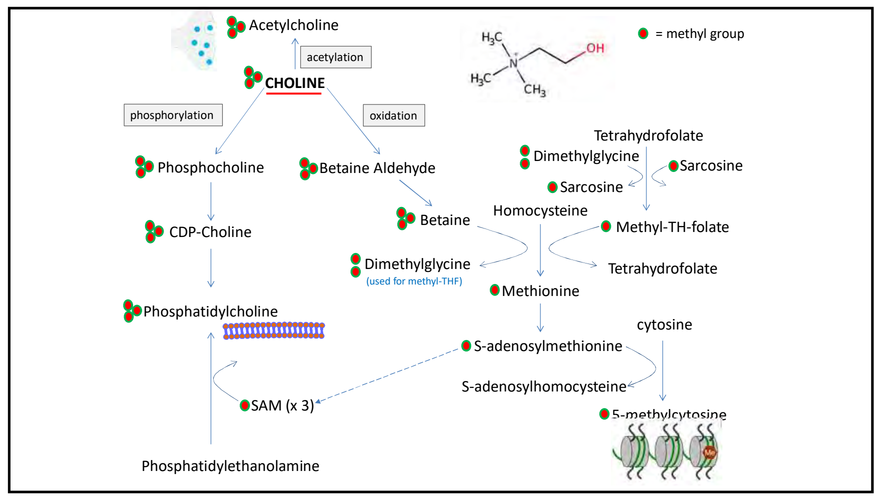
- Effects of choline treatment of embryos produced in vitro on birthweight and growth of the resultant calf

18

Does feeding of choline program the embryo to affect the calf?



19



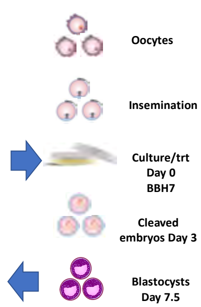
20

21

Choline effects on embryo development

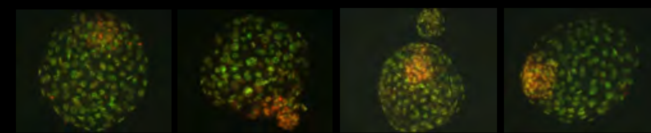
Criteria for concentration selection	[Choline chloride]	Sodium Chloride
Control	**0.00 mM**	6.37 mM
Total concentration of free choline in plasma of lactating dairy cows at week 1 postpartum	**0.004 mM**	6.37 mM
Total concentrations of choline in plasma of lactating dairy cows at week 1 postpartum	**1.30 mM**	5.07 mM
Total concentrations of choline in plasma of lactating dairy cows at week 1 postpartum assuming feeding RPC increased [choline] by 0.5 mM	**1.80 mM**	4.57 mM

Artegoitia *et al.,* 2014; Zenobi and Staples, unpublished



22

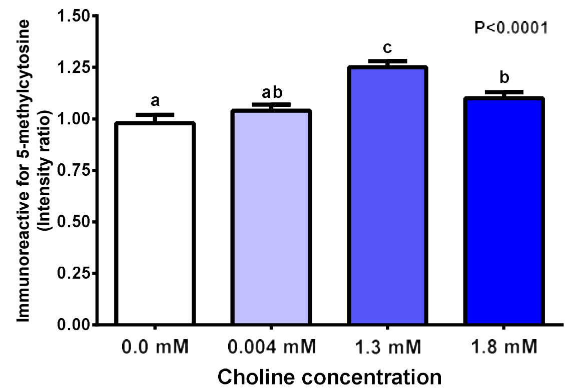
Immunofluorescence intensity for 5-methyl cytosine in blastocysts



0.0 mM	0.004 mM	1.3 mM	1.8 mM
n=44	n=62	n=50	n=50

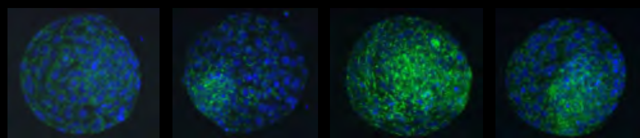
23

Global DNA methylation in blastocysts



24

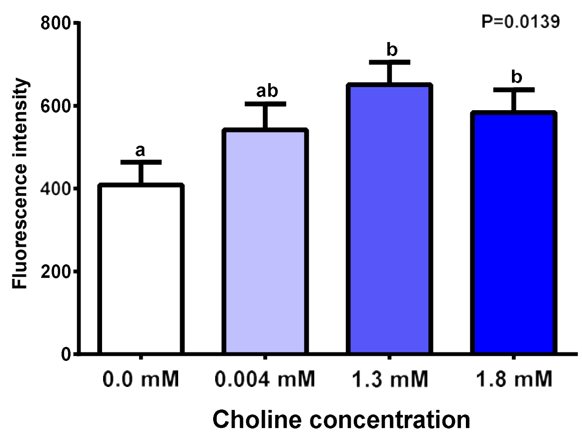
Fluorescence for lipid droplets in blastocysts



0.0 mM	0.004 mM	1.3 mM	1.8 mM
n=28	n=20	n=26	n=25

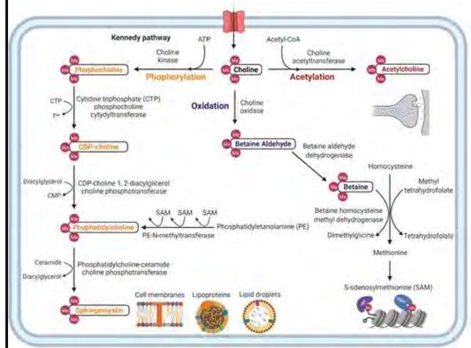
25

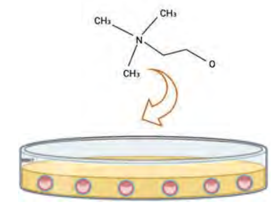
Fluorescence for lipids in blastocysts



26

How can choline modify the characteristics of the embryo?



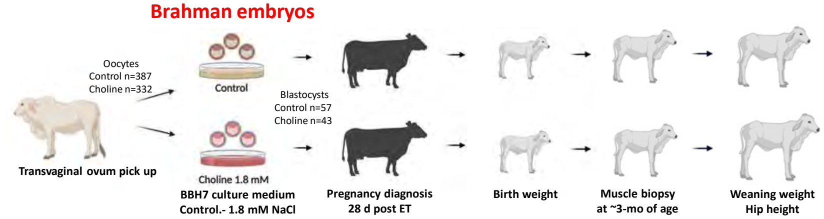


↑ Triglyceride accumulation
↑ DNA methylation

27

Effects of choline on the establishment of pregnancy and phenotype of resultant calves

Brahman embryos



Statistical analysis

- Variables with binomial distribution were analyzed with logistic regression fit to a binary distribution, using proc GLIMMIX of SAS

- Variables with a normal distribution were analyzed with ANOVA, using pro GLM of SAS

Variables

- Pregnancy outcomes
- DNA methylation in *L. dorsi*
- Gestation length
- Body size and growth

28

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Effects of choline treatment during culture on pregnancy outcomes after embryo transfer

Trait	Treatment		
	Control	Choline	P-value, treatment
Recipients pregnant at d 28, % [a]	53.6±6.0 (30/56; 54%)	43.5±7.0 (20/46; 44%)	0.3136
Recipients that calved, % [a]	42.9±7.0 (24/56; 43%)	39.1±7.2 (18/46; 39%)	0.7044
Pregnancy loss, % [a]	20.0±7.3 (6/30; 20%)	10.0±6.7 (2/20; 10%)	0.3581
Gestation length, days[b]	290.0±1.0	294.2±1.1	0.0118

[a] Data are least-squares means ± SEM and, in parentheses, the fraction and percent of cows.
[b] Data are least-squares means ± SEM. One animal in the choline group with premature calving (277 days) was removed from the data set before analysis. There was no effect of sex or sex x treatment.

30

Postnatal phenotypic traits of calves derived from in vitro produced embryos exposed to choline chloride

Postnatal traits	Treatment combination				Statistical effects, p-value		
	Control-Female	Choline-Female	Control-Male	Choline-Male	Treatment	Sex	Interaction
Number of calves at birth and weaning[b]	13/13	11/11	11/10	6/3	-	-	-
Birth weight, kg	35.1±2.2	42.9±2.3	35.0±2.5	42.6±3.0	**0.0081**	0.9284	0.9603
Adjusted birth weight, kg[c]	36.9±2.0	40.8±2.1	35.9±2.2	41.0±2.7	0.0857	0.8724	0.7667
Weaning weight, kg	233.3±10.3	246.9±11.3	202.9±13.0	239.7±18.7	0.085	0.2256	0.3435
205-d adjusted weaning weight, kg [d]	221.5±7.1	238.2±7.9	209.9±9.8	234.2±12.7	**0.0477**	0.2606	0.4176
Hip height at weaning, cm	114.4±1.8	110.0±2.3	110.1±2.5	114.1±3.3	0.3458	0.3160	0.4136
Weight:hip ratio at weaning, kg/cm	1.99±0.06	2.04±0.06	1.84±0.08	2.10±0.11	**0.0378**	0.1840	0.3314
Average daily gain, birth to weaning, kg/day[d]	0.93±0.04	0.81±0.05	0.84±0.05	0.93±0.08	0.1386	0.1687	0.4813

[a] Unless otherwise stated, data are presented as least-squares means ± SEM.
[b] Differences in number of calves at birth and weaning represent deaths in the first few weeks of life.
[c] Adjusted for gestation length in the statistical model. Final model included treatment, mating, and gestation length.
[d] Weaning weight adjusted for age at weaning using a standard equation for beef cattle.

31

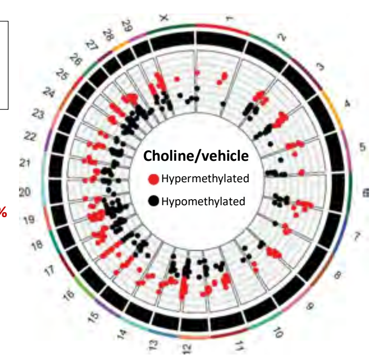
Chromosomal distribution of differentially methylated cytosines (DMC) in *L. dorsi* muscle

- MethylKit of R package (samples with at least 10X)
- Methylation analyzed by logistic regression
- P values adjusted for multiple testing
- Model: treatment, sex, and age of calf as a covariate

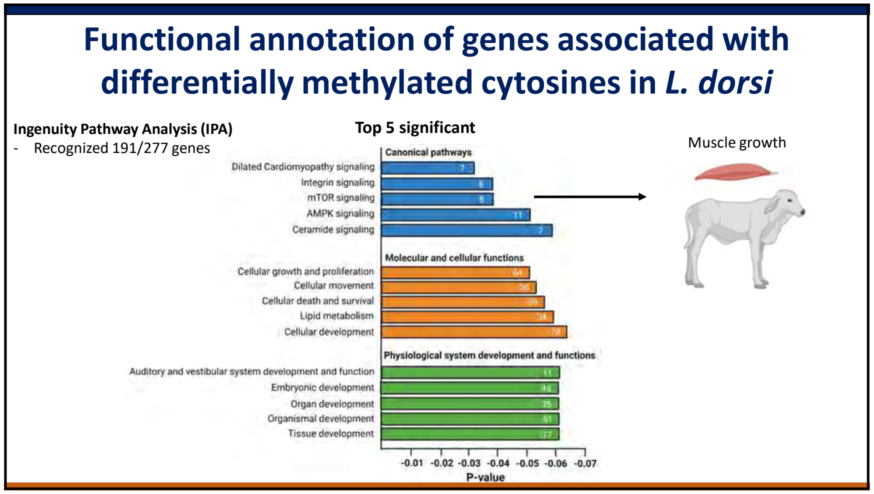
Reduce Representation Bisulfite Sequencing

DMC	Number
Total detected	8149
Total significant	670
Hypermethylated	239
Hypomethylated	431
Associated genes	277

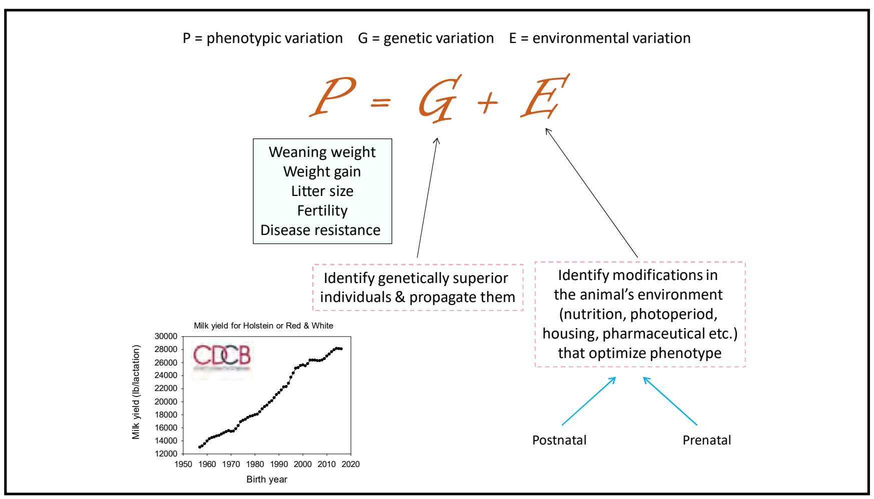
8.2%



32


Functional annotation of genes associated with differentially methylated cytosines in L. dorsi
Ingenuity Pathway Analysis (IPA)
- Recognized 191/277 genes
Top 5 significant
Canonical pathways
Dilated Cardiomyopathy signaling
Integrin signaling
mTOR signaling
AMPK signaling
Ceramide signaling
Molecular and cellular functions
Cellular growth and proliferation
Cellular movement
Cellular death and survival
Lipid metabolism
Cellular development
Physiological system development and functions
Auditory and vestibular system development and function
Embryonic development
Organ development
Organismal development
Tissue development
-0.01 -0.02 -0.03 -0.04 -0.05 -0.06 -0.07
P-value
Muscle growth

33


P = phenotypic variation G = genetic variation E = environmental variation
P = G + E
Weaning weight
Weight gain
Litter size
Fertility
Disease resistance
Identify genetically superior individuals & propagate them
Identify modifications in the animal's environment (nutrition, photoperiod, housing, pharmaceutical etc.) that optimize phenotype
Postnatal
Prenatal
Milk yield for Holstein or Red & White
CDCB
Milk yield (lb/lactation)
30000
28000
26000
24000
22000
20000
18000
16000
14000
12000
1950 1960 1970 1980 1990 2000 2010 2020
Birth year

34


Acknowledgements
Eliab Estrada
Jeremy Block
Liz Jannaman
William Ortiz
Florida Cattlemen's Association
Beef Enhancement Fund
CONACYT
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Solve Today. Shape Tomorrow
L.E. "Red" Larson Endowment
Eunice Kennedy Shriver NICHD
National Institute of Child Health & Human Development
R03 HD080855
R01 HD088352
Danny Driver
Bert Faircloth
Audy Spell
Michelle Driver

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MORE TO COME!!!
Programming Caused by Treatment of Embryos and Inseminated Cows with Choline
Lané Haimon (w/ Jesus Plascencia, mgr Sacramento Farms)
also collaborating – Joao Bittar and Thiago Amaral, UF
cultured embryos – 1.8 mM and 0.004 mM
Brahman, Brangus and Senepol cattle
Masroor Sagheer (w/ Angela Gonella Diaza and Thiago Amaral)
also collaborating – Nicolas Di Lorenzo, UF
Feeding rumen-protected choline
from day -1 to 7 relative to estrus

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Prenatal Choline Supplementation's Role in Calf Performance

2022 Florida Ruminant Nutrition Symposium
Clay Zimmerman, Ph.D.

2022
Florida Ruminant
Nutrition Symposium

1

Current Practices of Raising Preweaned Dairy Heifers are Good

Measurement	
Time to first feeding of colostrum	2.8 hours
Amount of colostrum fed in 24 hours	4.8 quarts
Calves with greater than 10 g/L serum IgG	87%
Milk fed	5.9 quarts/day
Average daily gain to weaning	1.61 lb/day
Mortality	5%

2,545 heifers; 104 Dairies; 13 states

JDS 2018; 2014 National Animal Health Monitoring System

2

Why Do We Focus on Calf Performance?

Preweaned heifer calf morbidity rate is ~34%

Effects of poor calf performance due to disease during the preweaning period can have long-term negative effects

http://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf

3

Nutritional Programming – Intake of Choline by Gestating Rats and Impact on Their Offspring

Pregnant Rat

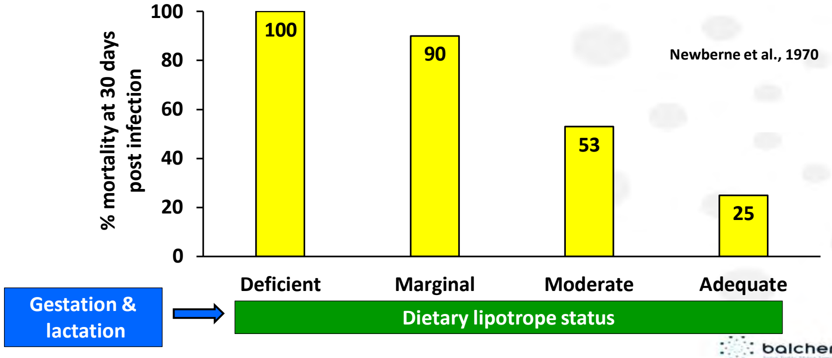
Lactating Rat

Intake of Lipotropic Compounds: Deficient, Marginal, Moderate, Adequate

4

Effect of deficiencies of lipotropes (Choline+Methionine+B_{12}) in diets of rat dams on survival of offspring post infection

Rat offspring were injected with *Salmonella typhimurium* at 100 days of age



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5

Choline Deficiency Symptoms in Baby Calves

Labored or rapid breathing, weakness, inability to rise, and anorexia within ~4 days in newly-born dairy calves fed a choline-free synthetic milk. Johnson et al., 1951. Journal of Nutrition 43:37.

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6

Two Cow-Calf Experiments at the University of Florida

Ruminally-Protected Choline (*ReaShure®*, 60 g/d) fed during transition period only

- 2018; 93 Holstein cows
  - Zenobi et al., JDS 101:1088 (cows & calves)
- 2020; 99 Holstein cows
  - Bollatti et al., JDS 103:4174 (cows)
  - Zenobi et al., 2022 – submitted for publication (calves)


J. Dairy Sci. 101:1088–1110
https://doi.org/10.3168/jds.2017-13327
Effects of supplementation with ruminally protected choline on performance of multiparous Holstein cows did not depend upon prepartum caloric intake
M. G. Zenobi, R. Gardinal, J. E. Zuniga, A. L. G. Dias, C. D. Nelson, J. P. Driver, B. A. Barton, J. E. P. Santos, and C. R. Staples
J. Dairy Sci. 103:4174–4191
https://doi.org/10.3168/jds.2019-17293
© American Dairy Science Association®, 2020
Timing of initiation and duration of feeding rumen-protected choline affects performance of lactating Holstein cows
J. M. Bollatti, M. G. Zenobi, N. A. Artusso, G. F. Alfaro, A. M. Lopez, B. A. Barton, C. D. Nelson, C. R. Staples, and J. E. P. Santos

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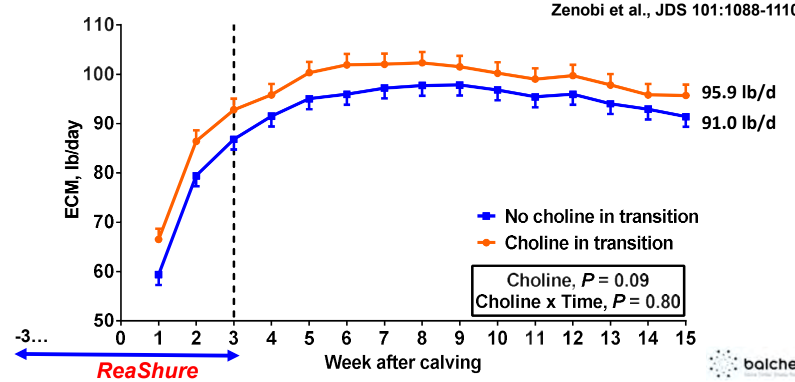
2018, 93 cows; Positive Milk Benefits From *ReaShure* Continued After Supplementation Ceased



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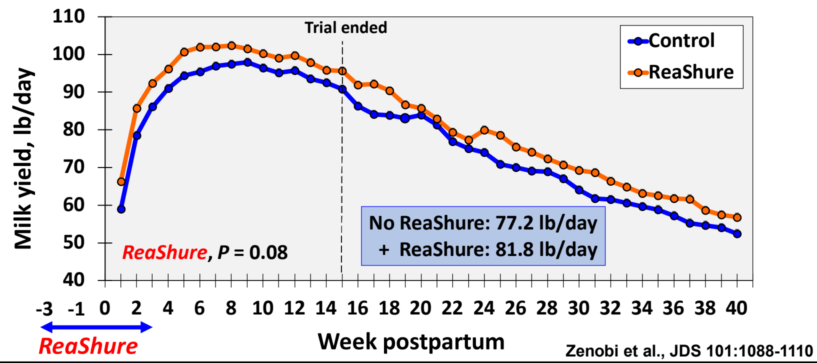
Positive Benefits of *ReaShure* Continued After Supplementation Ceased – 40 Weeks



9

Effect of Prepartum Feeding of *ReaShure* on Growth of Replacement Heifers (*in utero* effect only)

Age	No Choline	+ Choline	SEM
	n = 17	n = 18	--
Birth, lb	89	84*	2
2 months (weaning), lb	169	171	4
12 months, lb	710	738**	11
Post-calving, lb	1177	1256**	35

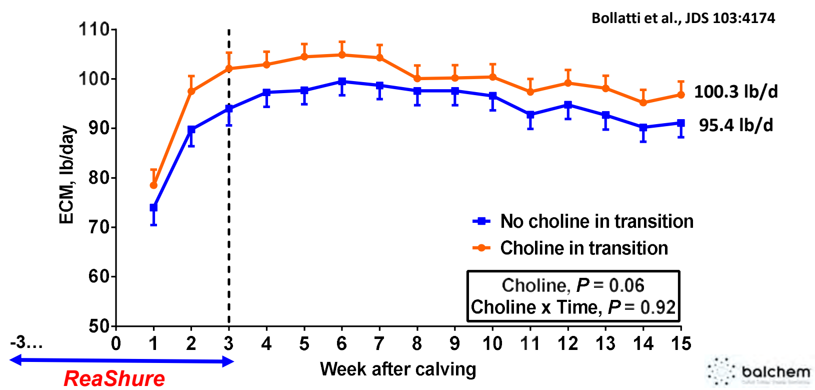
*Effect of choline, $P < 0.10$.
**Effect of choline, $P \leq 0.05$.

Average daily gain from birth to yearlings:
No choline: 1.77 lb per day
Choline: 1.86 lb per day*

Zenobi et al., 2018. J. Dairy Sci. 101:1088.

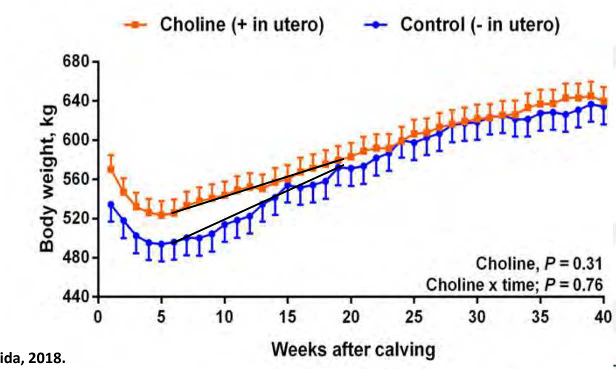
11

2020, 99 cows; Positive Milk Benefits From *ReaShure* Continued After Supplementation Ceased,



10

Body Weight of Primiparous Cows Exposed to *ReaShure* Prenatally



University of Florida, 2018.
Unpublished results

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Lifelong Impacts of Receiving Choline Biomolecules *In Utero*?

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Prenatal Choline Supplementation Improved Health and Growth of Neonatal Holstein Calves

M.G. Zenobi*, J.M. Bollatti, N.A. Artusso, A.M. Lopez, B.A. Barton, F.P. Maunsell, J.E.P. Santos, and C.R. Staples

UF UNIVERSITY of FLORIDA

2022 – submitted for publication

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Experimental Design

4 dietary treatments arranged in a 2 × 2 factorial design

- Utero effect (CTRL)

+ Utero effect (ReaShure)

- Colostrum source (CTRL)	+ Colostrum source (ReaShure)	- Colostrum source (CTRL)	+ Colostrum source (ReaShure)
NN = 16	NC = 16	CN = 15	CC = 12

Zenobi et al., 2022 – submitted for publication

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Heifer Calf Results – *In Utero* Effects

Zenobi et al., 2022 – submitted for publication

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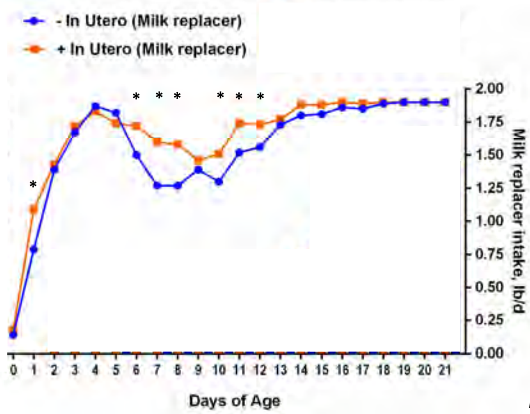
Late Gestation Exposure to Choline Biomolecules Increased DMI of Milk Replacer and Starter During the First 21 d of Age of Heifers

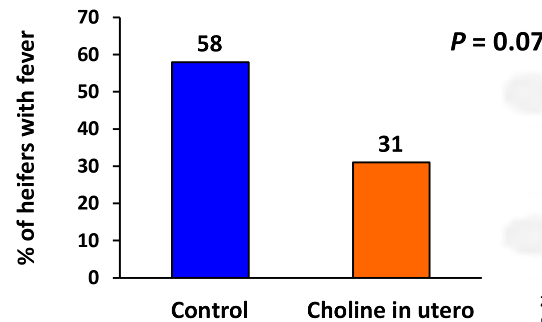


Zenobi et al., 2022 – submitted for publication

Starter
In utero, $P = 0.08$

Milk replacer
In utero, $P < 0.01$
In utero × age, $P < 0.01$

In Utero Effects - Incidence of Fever



Zenobi et al., 2022 – submitted for publication

Rectal temperatures measured daily.

Fever: >103.1°F.

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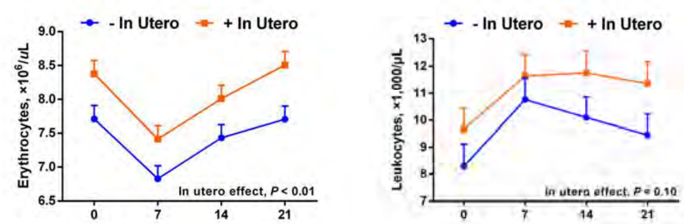
Heifers Born from *ReaShure* Supplemented Dams Had Increased Concentrations of Red and White Blood Cells



Zenobi et al., 2022 – submitted for publication

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Effect of Transition Feeding of *ReaShure* on Growth of Replacement Heifers (*in utero* effect only)

Age	No Choline	+ Choline	SEM
	n = 23	n = 23	--
Birth, lb	92	89	3
56 d of age, lb	161	162	4
300 d of age, lb	604	630	12

*Effect of choline, $P < 0.10$.

Average daily gain from weaning to yearlings:
No choline: 1.70 lb per day
Choline: 1.80 lb per day *

Zenobi et al., 2022 – submitted for publication

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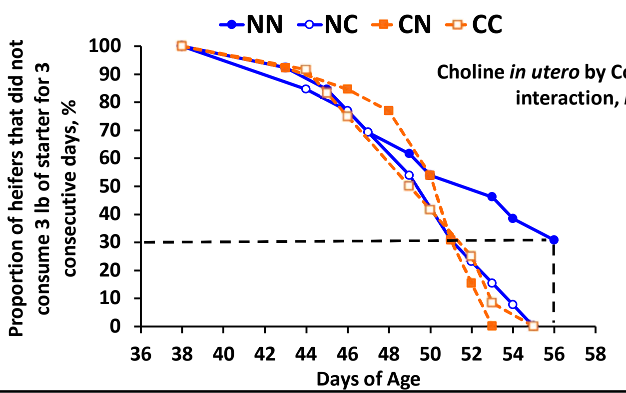
Time to event curves for intake of starter by female Holstein calves exposed to choline biomolecules *in utero* or not (*in utero* effect) and receiving colostrum harvested from dams fed with or without *ReaShure* (colostrum source effect).



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Prenatal choline supplementation modulated LPS-induced inflammatory responses of neonatal Holstein calves

M.G. Zenobi*, J.M. Bollatti, N.A. Artusso, A.M. Lopez, F.P. Maunsell, B.A. Barton, J.E.P. Santos, and C.R. Staples

Zenobi et al., 2022 – submitted for publication

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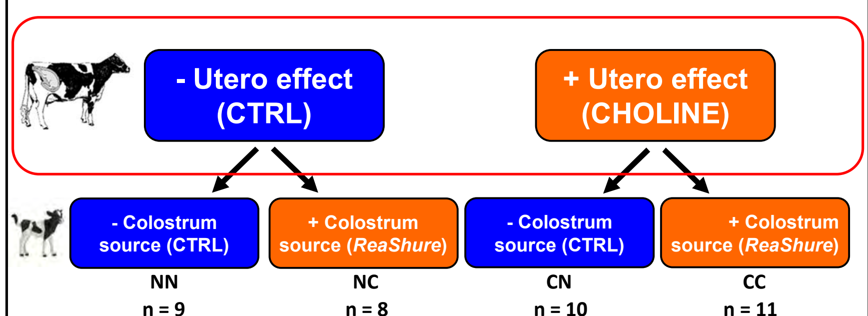
Experimental Design

4 dietary treatments arranged in a 2 × 2 factorial design



Zenobi et al., 2022 – submitted for publication

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Bull Calf Results – in Utero Effects
LPS challenge at 21 days of age
Zenobi et al., 2022 – submitted for publication
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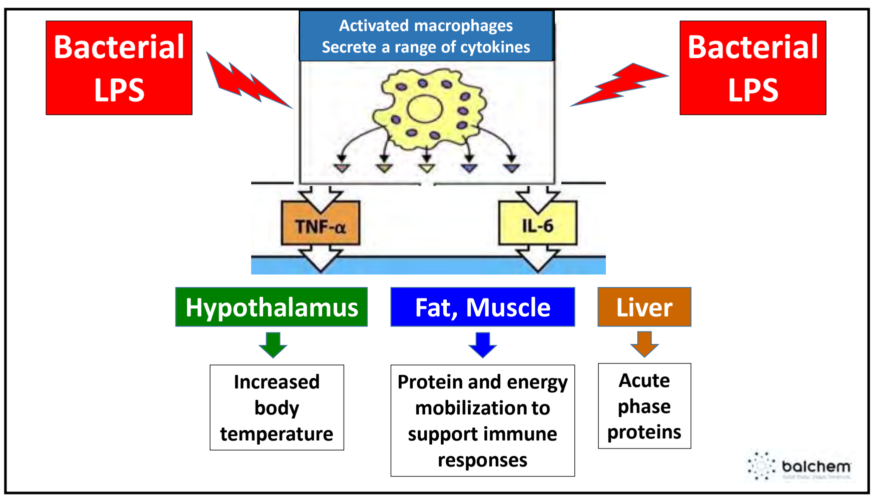
Bacterial LPS
Activated macrophages
Secrete a range of cytokines
Bacterial LPS
TNF-α
IL-6
Hypothalamus
Fat, Muscle
Liver
Increased body temperature
Protein and energy mobilization to support immune responses
Acute phase proteins
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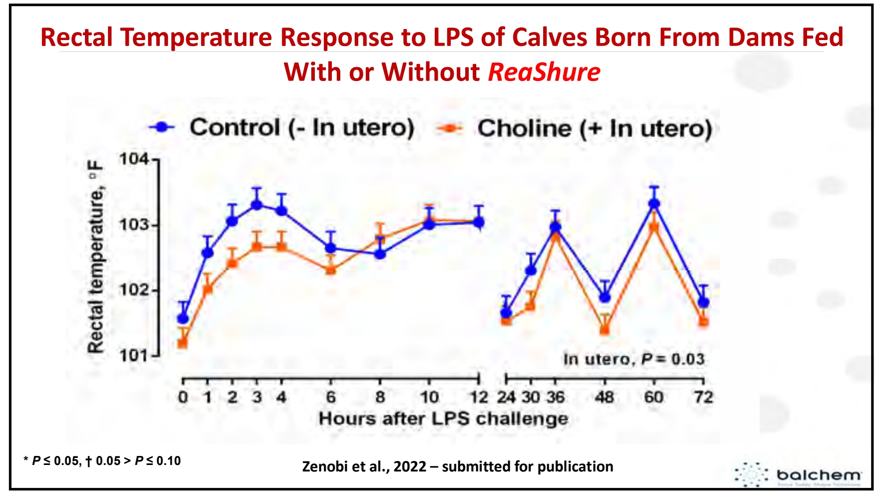
Rectal Temperature Response to LPS of Calves Born From Dams Fed With or Without ReaShure
Control (- In utero)
Choline (+ In utero)
Rectal temperature, °F
104
103
102
101
In utero, P = 0.03
0 1 2 3 4 6 8 10 12 24 30 36 48 60 72
Hours after LPS challenge
* P ≤ 0.05, † 0.05 > P ≤ 0.10
Zenobi et al., 2022 – submitted for publication
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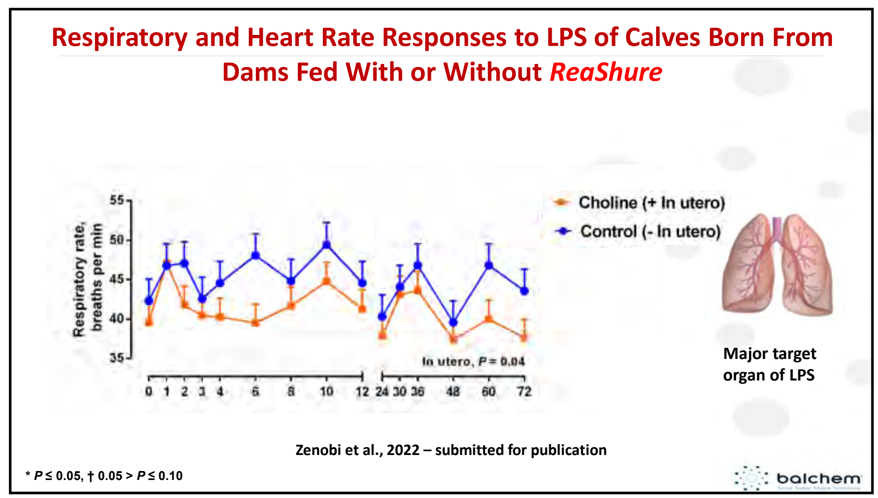
Respiratory and Heart Rate Responses to LPS of Calves Born From Dams Fed With or Without ReaShure
Choline (+ In utero)
Control (- In utero)
Respiratory rate, breaths per min
55
50
45
40
35
In utero, P = 0.04
0 1 2 3 4 6 8 10 12 24 30 36 48 60 72
Major target organ of LPS
Zenobi et al., 2022 – submitted for publication
* P ≤ 0.05, † 0.05 > P ≤ 0.10
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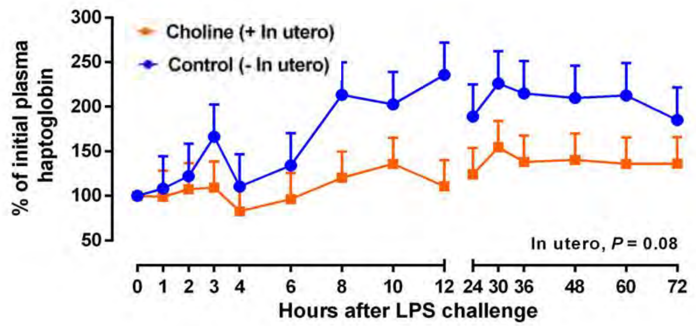
Plasma Haptoglobin Response to LPS of Calves Born From Dams Fed With or Without *ReaShure*



Zenobi et al., 2022 – submitted for publication

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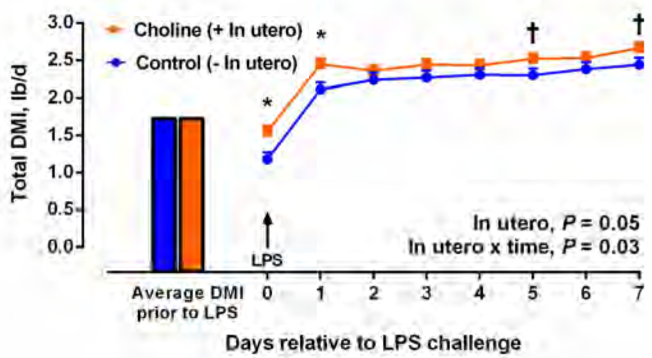
Greater DMI after LPS administration by bulls born from dams fed *ReaShure*



* $P \leq 0.05$, † $0.05 > P \leq 0.10$

Zenobi et al., 2022 – submitted for publication

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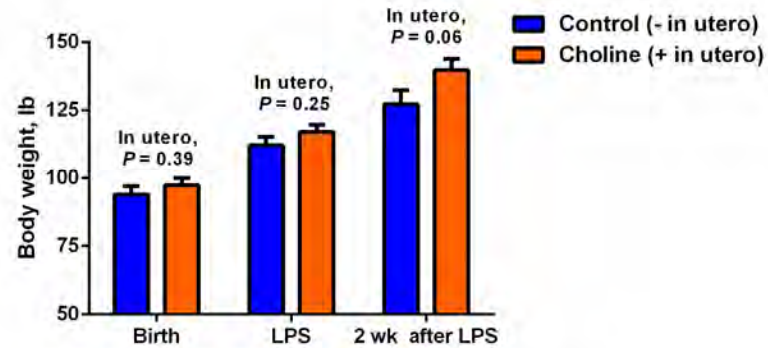
Body Weight After LPS Administration of Bulls Born From Dams Supplemented With or Without *ReaShure*



Zenobi et al., 2022 – submitted for publication

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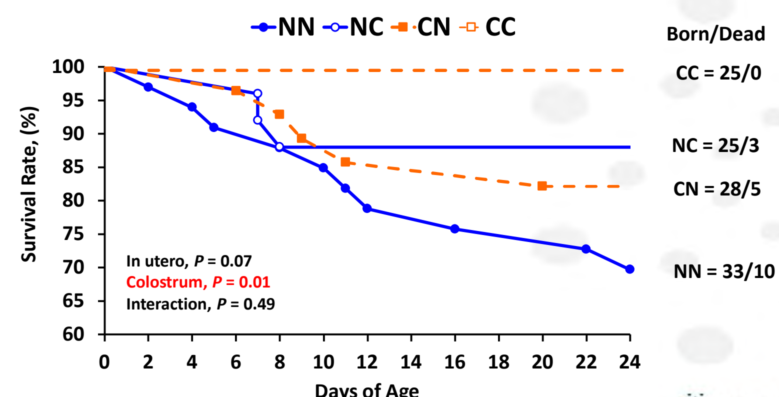
Survival curves using males and females (n = 111)



Zenobi et al., 2022 – submitted for publication

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Replication of Improved ADG of Holstein Calves Born From Dams Fed *ReaShure*

Birth to ~50 weeks of age by <u>heifers</u>		Birth to 5 weeks of age by <u>bulls</u> given LPS
2018	**2022**	**2022**
1.77 vs. 1.86 lb/d; *P* = 0.06 n = 35	1.70 vs. 1.80 lb/day *P* = 0.09 n = 46	0.96 vs. 1.23 lb/day *P* = 0.06 n = 38

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Lifelong Impacts of Receiving Choline Biomolecules *In Utero*?

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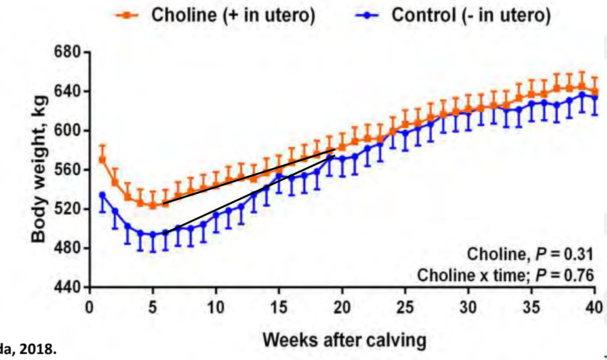
Body Weight of Primiparous Cows Exposed to *ReaShure* Prenatally



University of Florida, 2018.
Unpublished results

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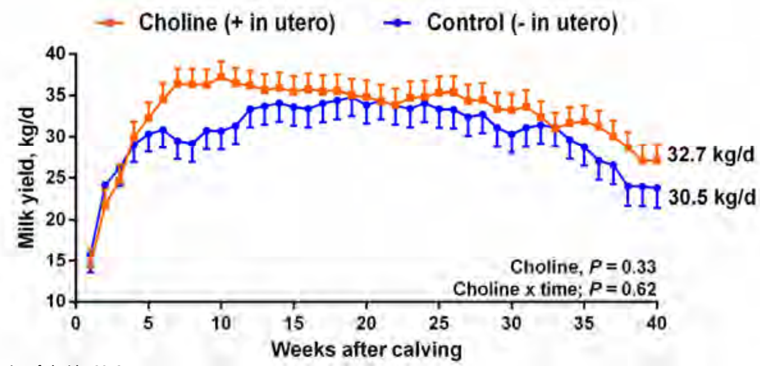
Milk Yield of Primiparous Cows Exposed to *ReaShure* Biomolecules *in Utero*



University of Florida, 2018.
Unpublished results

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Choline – Modes of Action?

1. Meeting a choline requirement for organ development and maturation

Adequate Intake | **Requirements**

425 mg/day

450 mg/day

550 mg/day

??? g/day

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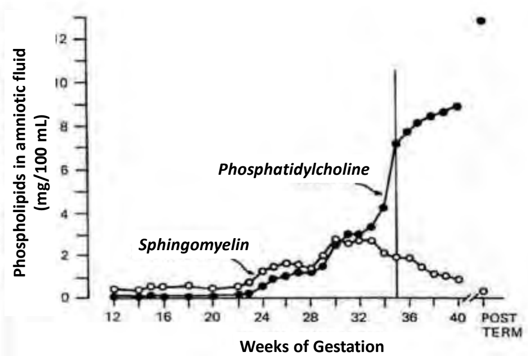
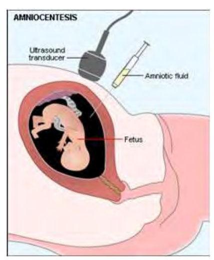
Phosphatidylcholine (PC) is key to maturation of lungs *in utero*





Amniocentesis to measure PC in the amniotic fluid during pregnancy to assess lung maturity.

Gluck and Kulovich, 1972

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Composition of Surfactant from Lungs/Intestine

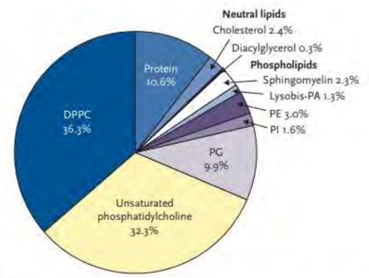
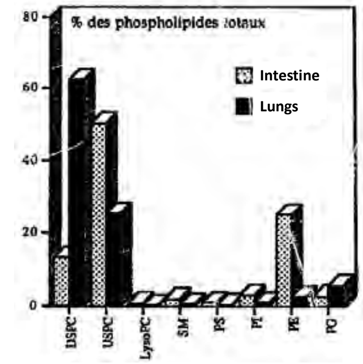


Figure 1
Composition of surfactant. Representative composition of bovine surfactant from lung lavage fluid is shown. Components are expressed as a percentage of weight. DPPC: dipalmitoylphosphatidylcholine; PA; phosphatidic acid; PE: phosphatidylethanolamine; PG: phosphatidylglycerol; PI: phosphatidylinositol. Reproduced from [15] with permission from the publisher.



Chakraborty et al., 2003 | PC = Phosphatidylcholine | Rubio et al., 1995

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How Might Choline be Improving Growth and Immune Function in Dairy Replacements?

1. Meeting a choline requirement for organ development and maturation.
2. Improved expression of key genes responsible for growth, health, and immunity due to greater methylation of DNA *in utero*

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Recent Human Research Study from Cornell University

DOI: 10.1096/fj.202101217R

FASEB JOURNAL

RESEARCH ARTICLE

Prenatal choline supplementation improves child sustained attention: A 7-year follow-up of a randomized controlled feeding trial

Charlotte L. Bahnfleth[1] | Barbara J. Strupp[1,2] | Marie A. Caudill[1] | Richard L. Canfield[1]

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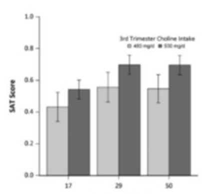
Recent Human Research Study from Cornell University

3rd Trimester Pregnant Women

- **480 mg choline/day (Adequate Intake) vs. 930 mg choline/day**
- Their offspring were assessed for cognitive ability utilizing an SAT (Sustained Attention Task) testing protocol

Results:

- Children 7 years of age showed **improved sustained attention in 12 minute sessions if exposed to 930 mg/d *in utero***



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Questions?

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Improving beef progeny performance through developmental programming

2022 Florida Ruminant Nutrition Symposium

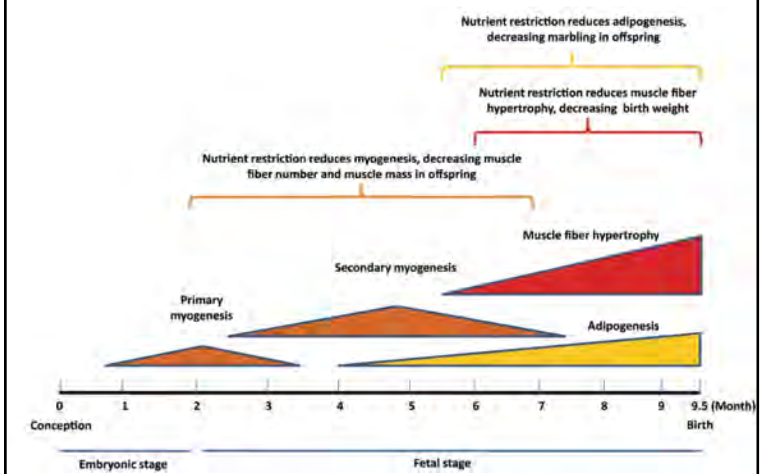
UF UNIVERSITY of FLORIDA

Philipe Moriel - Associate Professor

Range Cattle Research & Education Center - University of Florida, Ona, FL

1

Fetal growth



Adapted from Du et al. (2010) JAS 88:E51–E6

2

Study	Gestation trimester	Birth body weight	Preweaning growth	Post-weaning growth
Corah et al., 1975 (Exp. 1)	Third	+	+	Not reported
Corah et al., 1975 (Exp. 2)	Third	+	+	Not reported
Hough et al., 1990	Third	ND	ND	Not reported
Greenwood et al., 2005	Second + third	+	+	+
Banta et al., 2006	Third	ND	ND	ND
Stalker et al., 2006	Third	ND	+	ND
Stalker et al., 2007	Third	+	+	+
Martin et al., 2007	Third	ND	+	+
Larson et al., 2009	Third	+	+	+
Micke et al., 2010	First and/or second	+	Not reported	Not reported
Long et al., 2010	Early	ND	ND	-
Funston et al., 2010	Third	ND	+	ND
Underwood et al., 2010	Second	ND	+	+
Long et al., 2012	Early	ND	ND	ND
Mulliniks et al., 2012	Third	Not reported	ND	ND
Winterholler et al., 2012	Third	+	+	Not reported
Radunz et al., 2012	Second + third	+	+	ND
Bohnert et al., 2013	Third	+	+	ND
Shoup et al., 2015a	Third	ND	+	Not reported
Shoup et al., 2015b	Third	Not reported	Not reported	ND
Wilson et al., 2015	Third	ND	ND	ND
Summers et al. 2015a	Third	ND	Not reported	Not reported
Summers et al. 2015b	Third	Not reported	ND	+
Wilson et al., 2016a	Third	ND	ND	ND
Wilson et al., 2016b	Third	+	ND	ND
Kennedy et al., 2016	Third	+	Not reported	Not reported
Moriel et al., 2016	Third	ND	ND	ND
Marquez et al., 2017	Second or third	ND	ND	Not reported
Nepomuceno et al., 2017	Third	ND	ND	ND
McLean et al., 2018	First	ND	+	+/-
Maresca et al., 2018	Second + third	Not reported	Not reported	Not detected
Kennedy et al., 2019	Third	+	+	Not reported
Maresca et al., 2019	Second + third	+	ND	Not reported
Tanner et al., 2020	Second + third	ND	+	Not reported
Moriel et al., 2020	Third	ND	ND	+
Palmer et al., 2020	Third	ND	+	Not reported
Rodrigues et al., 2021	Second + third	+	ND	Not reported
ND = no statistical difference		14 of 33 studies	17 of 32 studies	8 of 22 studies

3

Impacts of maternal precalving nutrition (*No Supp.* vs. *Supp.*) on body condition score (BCS) and reproduction of cows and growth and immune response of their calves (studies[1] at the Range Cattle REC; Ona, FL)

UF UNIVERSITY of FLORIDA

	Study 1		Study 2		Study 3		Study 4		Study 5	
	No Supp.	Supp.	No Supp.	Supp.	No Supp.	Supp.	No Supp.	Supp.	No Supp.	Supp.
Initial BCS	5.7	5.7	5.5	5.5	5.3	5.4	5.0	5.0	5.5	5.5
Calving BCS	5.8^{a}	6.1^{b}	5.0^{a}	5.4^{b}	5.2^{a}	5.8^{b}	4.7^{a}	5.6^{b}	5.0^{a}	5.5^{b}
Pregnancy, %	91.7	94.4	78.5	75.8	96.2	96.3	82^{a}	95^{b}	93.3	86.8
Calf weaning weight, lb	275^{a}	295^{b}	579^{a}	597^{b}	561^{a}	591^{b}	535^{a}	563^{b}	557^{a}	581^{b}
Response to vaccination, %	56.1^{a}	81.5^{b}	-	-	21^{a}	54^{b}	-	-	-	-

^{ab} Means without a common superscript differed ($P < 0.05$).

[1] Study 1 = 0 or 2.2 lb/day of molasses + urea for 57 days before calving (*Moriel et al., 2020*). doi:10.1093/jas/skaa123
Study 2 = 0 or 2.2 lb/day of molasses + urea for 47 days before calving (*Palmer et al., 2020*). doi:10.1016/j.livsci.2020.104176
Study 3 = 0 or 2.2 lb/day of dried distillers grains for 90 days before calving (*Palmer et al., 2022*). doi:10.1093/jas/skac022
Study 4 = 0 or 2.2 lb/day dried distillers grains for 70 days before calving (*Izquierdo et al., 2022*). In review
Study 5 = 0 or 2.2 lb/day dried distillers grains for 77 days before calving (*Vedovatto et al., 2022*). In review

In all studies, cows and their calves were managed similarly from calving until calf weaning. Calves were early weaned at 2 to 3 months of age in Study 1 and normally weaned at 8 to 9 months of age in Studies 2, 3, 4, and 5.

4

Opportunities

- Nutrient restriction
  - *Long- vs. short-term?*
- Nutrient excess?
- Diet composition?
- Energy source?
- Protein source and amount?
- Minerals and fatty acids?
- Timing of supplementation?
- Frequency of supplementation?
- Feed additives
  - *Monensin*

5

Beef Enhancement Funds
Florida Cattlemen's Association

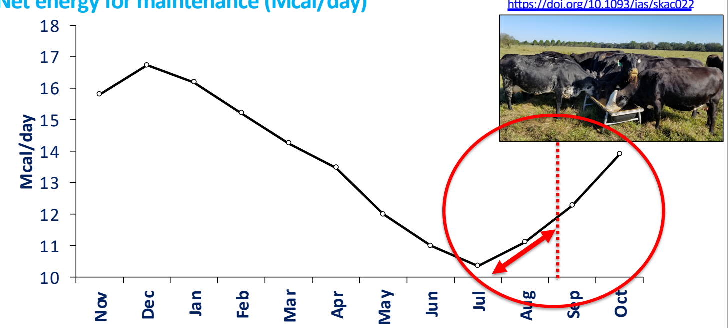
Fetal Programming

Timing of supplementation

6

Net energy for maintenance (Mcal/day)

Palmer et al. (2022) J. Anim. Sci. **100**:1–17
https://doi.org/10.1093/jas/skac022



Supplementation offered:
Day 0 to 84 = dried distillers grains (DDG)
Day 84 end of breeding season = Molasses + urea

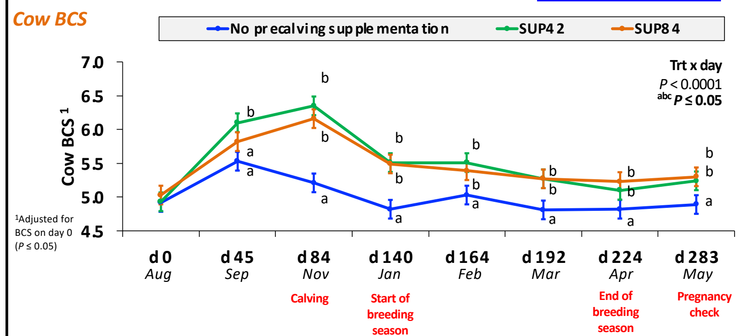
Day 0 = Start of study
Day 84 = start of calving
Day 140 to 224 = Breeding season

Treatments	Day of the study: Day 0 to 42	Day 42 to 84 (*Calving*)	Day 84 until end of breeding season
NO precalving supplementation	0	0	4 lb/day
Supplement day 0 to 84 – SUP84	2.2 lb/day	2.2 lb/day	4 lb/day
Supplement day 0 to 42 – SUP45	4.4 lb/day	0	4 lb/day

7

Results
Cow BCS

Palmer et al. (2022) J. Anim. Sci. **100**:1–17
https://doi.org/10.1093/jas/skac022

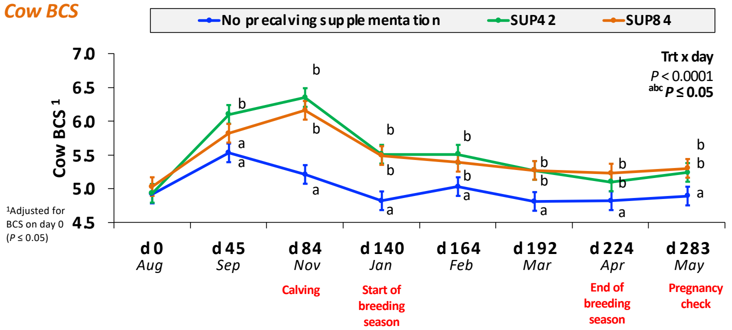


Treatments	Day of the study: Day 0 to 42	Day 42 to 84 (*Calving*)	Day 84 until end of breeding season
NO precalving supplementation	0	0	4 lb/day
Supplement day 0 to 84 – SUP84	2.2 lb/day	2.2 lb/day	4 lb/day
Supplement day 0 to 42 – SUP45	4.4 lb/day	0	4 lb/day

8

Results
Cow BCS

Palmer et al. (2022) J. Anim. Sci. 100:1–17
https://doi.org/10.1093/jas/skac022

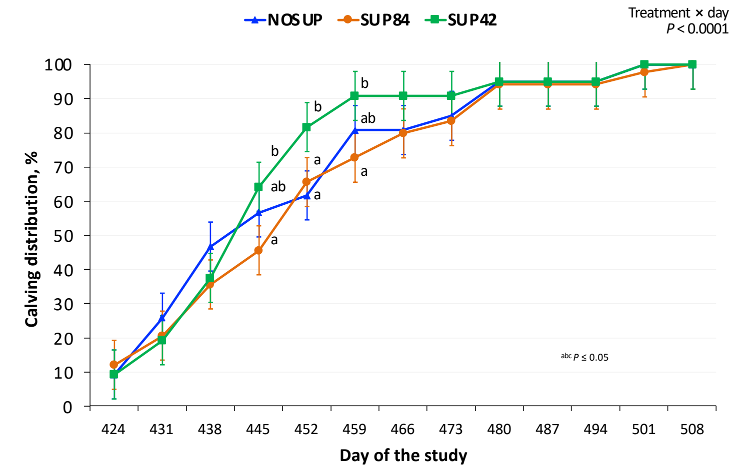


	Treatment				P - value
	NO Sup	SUP84	SUP42	SEM	Treatment
Calving date, day of the study	82	83	86	4.7	0.76
Pregnancy rate (day 283), % of total	96.3	96.2	88.0	4.81	0.39

9

Calving distribution

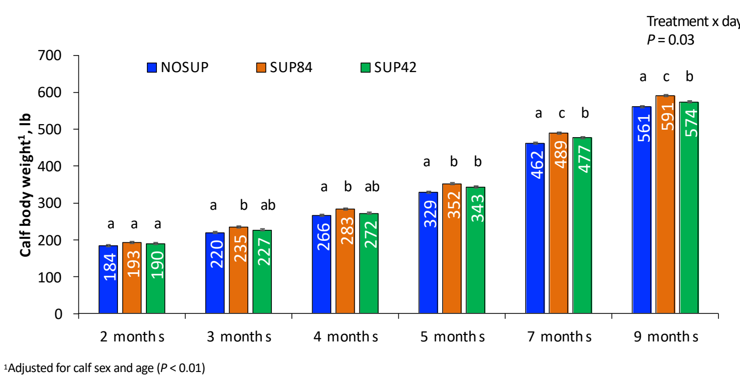
Palmer et al. (2022) J. Anim. Sci. 100:1–17
https://doi.org/10.1093/jas/skac022



10

Results – Preweaning calf body weight

Palmer et al. (2022) J. Anim. Sci. 100:1–17
https://doi.org/10.1093/jas/skac022



Treatments	Day of the study		
	Day 0 to 42	Day 42 to 84 (Calving)	Day 84 until end of breeding season
NO precalving supplementation	0	0	4 lb/day
Supplement day 0 to 84 – SUP84	2.2 lb/day	2.2 lb/day	4 lb/day
Supplement day 0 to 42 – SUP45	4.4 lb/day	0	4 lb/day

11

Results – Post-weaning immune response of steers

Palmer et al. (2022) J. Anim. Sci. 100:1–17
https://doi.org/10.1093/jas/skac022

Steer innate and humoral immune response

	Treatment				P - value	
Item	CON	SUP42	SUP84	SEM	Trt	Trt × Day
Plasma cortisol, µg/dL	2.13	2.29	2.15	0.16	0.76	0.79
Plasma haptoglobin, mg/mL	0.25	0.30	0.28	0.02	0.40	0.78
Serum BVDV-1						
Titers, $\log_2$	3.46	4.41	3.91	0.38	0.21	0.87
Seroconversion, % total	78	85	88	7.2	0.64	0.27
Serum PI3						
Titers, log2	2.53[a]	4.30[b]	3.73[ab]	0.44	0.07	0.51
Seroconversion, % total						
day 347	21[a]	63[b]	54[b]	11	0.32	0.01
day 389	80	82	83			

[ab] $P \leq 0.05$

12

Results – Steer carcass characteristics

Palmer et al. (2022) J. Anim. Sci. 100:1–17
https://doi.org/10.1093/jas/skac022

	Treatment				
Item	CON	SUP42	SUP84	SEM	*P* - value
Hot Carcass Weight, kg	337	338	338	5.5	0.98
Dressing Percent, %	59.7	60.5	59.8	0.30	0.12
12th rib fat thickness, cm	1.77	1.69	1.62	0.089	0.49
Longissimus muscle area, cm²	79.2	80.8	80.7	1.58	0.74
KPH, %	2.92	2.62	2.67	0.13	0.20
Yield Grade	3.8	3.6	3.5	0.14	0.33
Marbling	521[a]	570[b]	545[ab]	15	0.07
Average choice, %	5[a]	36[b]	17[ab]	9.3	0.10
Low choice, %	72	46	58	10	0.17
Select, %	23	19	25	8	0.87

[ab] $P \leq 0.05$

13

Beef Enhancement Funds
Florida Cattlemen's Association

Fetal Programming

Frequency of supplementation

14

Frequency of precalving supplementation

80 days before calving:
120 Brangus cows (20 bahiagrass pastures; 6 cows/pasture)

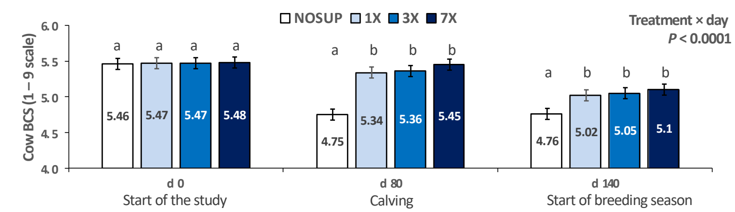
NOSUP *= no precalving supplementation*
1X *= 14 lb of DDG offered on Monday (14 lb of DDG/cow/week)*
3X *= 4.66 lb of DDG offered on Monday, Wednesday and Friday (14 lb of DDG/cow/week)*
7X *= 2 lb of DDG offered daily (14 lb of DDG/cow/week)*

Calving to weaning:
All cows and calves managed similarly

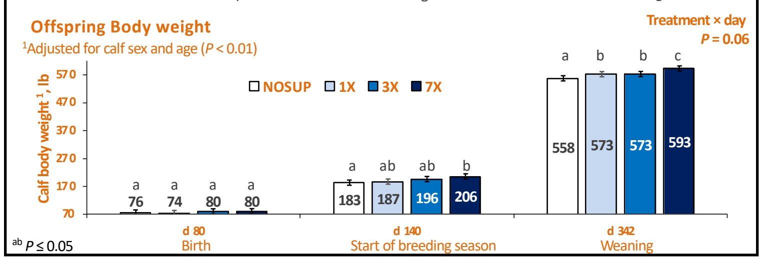
15

NOSUP *= no precalving supplementation*
1X *= 14 lb of DDG offered on Monday (14 lb of DDG/cow/week)*
3X *= 4.66 lb of DDG offered on Monday, Wednesday and Friday (14 lb of DDG/cow/week)*
7X *= 2 lb of DDG offered daily (14 lb of DDG/cow/week)*

Cow BCS



Offspring Body weight
[1]Adjusted for calf sex and age ($P < 0.01$)



[ab] $P \leq 0.05$

16

FETAL PROGRAMMING

Monensin

17

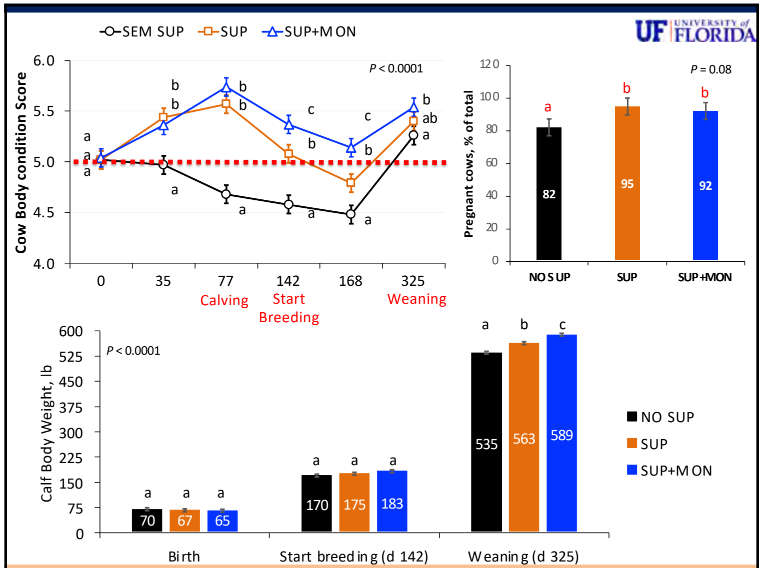
Inclusion of monensin into precalving supplementation

70 days before calving:
160 Brangus cows (16 bahiagrass pastures; 10 cows/pasture)

Treatments :
NO SUP = No precalving supplementation
SUP = 2 lb of DDG daily
SUP + MON = 2 lb of DDG daily + 200 mg de monensin daily

Calving to weaning:
All cows and calves managed similarly!


18



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FETAL PROGRAMMING

Methionine

20

Early-gestation

Brangus cows offered methionine-rich diets

30 days prepartum until end of the breeding season

Item	Control	Fishmeal	Methionine	SEM	P-value
Birth weight, kg	32.6	32.6	32.1	1.2	0.96
Weaning weight adjusted for 205 days of age, kg	201.3	213.8	213.5	8.4	0.54

Item	Control	Fishmeal	Methionine	SEM	P-value
ADG, kg/d	0.83[a]	1.00[b]	1.01[b]	0.01	0.04
Final BW, kg	248.1[a]	255.3[b]	255.7[b]	2.2	0.04
DMI, % of BW	2.27	2.28	2.25	0.02	0.60
G:F	0.16[a]	0.19[b]	0.19[b]	0.01	0.02

Silva et al. (2021) ANIMAL

Semitendinosus muscle of male calves at 30 days of age:

- Perturbed coexpression patterns in the offspring's muscle.
- Nearly 2% of all evaluated cytosines were differentially methylated between maternal diets.
- Unpreserved modules implicated in **myogenesis, adipogenesis, fibrogenesis**, canonical Wnt/β-catenin pathway, ribosome structure, mitochondrial activities, ATP synthesis and other functions.

Liu et al. (2020) BMC Genomics

21

Late-gestation

Brangus heifers (n = 36/yr; 4 astures/treatment; 2 yr)

Moriel et al. (2021) J. Anim. Sci. doi:10.1093/jas/skaa123

- **NOSUP =** No Molasses + urea supplementation
- **MOL** = 2.2 lb/d of Molasses + urea (DM)
- **MOLMET =** 2.2 lb/d of **MOL** + **18 g/d of methionine hydroxy analog** (Alimet, Novus)
  - **Sugarcane Molasses + Urea**
    - 20% CP and 70% TDN (DM)
    - Offered 2x/week (Tuesdays and Fridays)
  - **Supplementation period**
    - 56 d prepartum = ***d 0 of the study***
    - Ended when all cows within each pasture have calved = ***d 74 of the study***
- **d 75 until the end of the breeding season (d 164)**
  - 3.5 lb DM/d of Molasses + urea

22

Calf Early-weaning

- **d 147…**
  - Start of the breeding season
  - Early-weaning
- **d 154 until 201**
  - Individual drylot pens
    - High concentrate-based TMR (3% of BW; DM)
      - 75% TDN and 22% CP (DM)
    - 2.2 lb/d of ground stargrass (*Cynodon nlemfuensis*) hay
- **d 160 and 188**
  - Vaccination against bovine respiratory disease
    - Bovi Shield Gold 5 + One Shot

23

Late-gestation

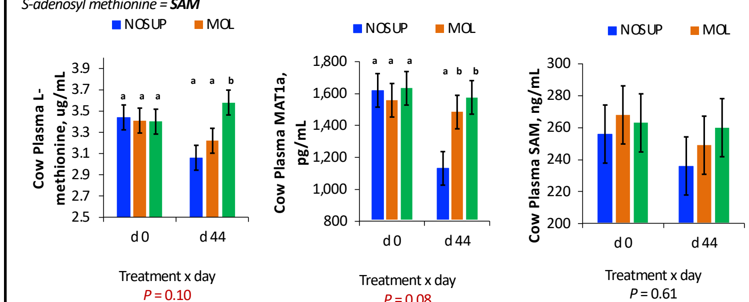
Brangus heifers (n = 36/yr; 4 astures/treatment; 2 yr)

Moriel et al. (2021) J. Anim. Sci. doi:10.1093/jas/skaa123

Cow Plasma L-methionine, MAT1a, and SAM (Years 1 and 2)

*Methionine Adenosyltransferase 1A = **MAT1a***

*S-adenosyl methionine = **SAM***



[ab] P ≤ 0.05

24

Late-gestation

Brangus heifers (n = 36/yr; 4 astures/treatment; 2 yr)

Moriel et al. (2021) J. Anim. Sci. doi:10.1093/jas/skaa123

Maternal performance	Treatments			SEM	*P*	
	NOSUP	MOL	MOLMET		Trt.	Trt. x day
Cow BCS (1-9 scale)						
d 0	5.67	5.65	5.69	0.084	0.04	0.10
d 44 (near calving)	5.77 [a]	6.10 [b]	6.17 [b]			
d 147 (early weaning)	4.85	4.95	5.01			
BCS change						
d 0 to 44	0.09 [a]	0.42 [b]	0.49 [b]	0.081	0.002	
d 44 to 147	-0.93 [b]	-1.16 [b]	-1.17 [b]	0.099	0.10	
Pregnant cows d 288, %	83.3	90.0	90.9	10.1	0.82	
Calving date 2nd calf, day of the study	453	452	445	7.4	0.68	

[ab] $P \leq 0.05$

25

Late-gestation

Brangus heifers (n = 36/yr; 4 astures/treatment; 2 yr)

Moriel et al. (2021) J. Anim. Sci. doi:10.1093/jas/skaa123

Item	Treatment			SEM	*P*	
	NOSUP	MOL	MOLMET		Trt.	Trt. x day
Calf birth Body Weight [1], lb	55.5	61.6	58.2	2.2	0.13	
Body Weight [1], lb						
d 147 – Early weaning	174 [a]	185 [b]	189 [b]	7.0	0.54	0.10
d 154 – Drylot entry	178 [a]	194 [b]	196 [b]	7.0		
d 201 – Drylot exit	275 [a]	293 [b]	293 [b]	7.0		
ADG, lb/day						
Birth to weaning (d 147)	1.28	1.26	1.37	0.064	0.48	
Drylot (d 154 to 201)	1.85 [a]	2.00 [b]	2.18 [b]	0.068	0.02	
Birth to d 201	1.41 [a]	1.59 [b]	1.65 [b]	0.081	0.05	
Drylot (d 154 to 201)						
Total DM intake, lb/d	8.22	8.63	8.63	0.249	0.41	
G:F, d 154 to 201 [1]	0.246	0.243	0.236	0.006	0.51	

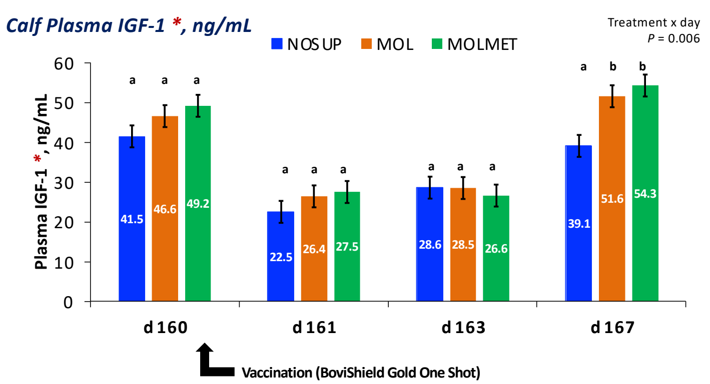
[1]Adjusted for calf sex ($P \leq 0.05$)
[ab] $P \leq 0.05$

26

Late-gestation

Brangus heifers (n = 36/yr; 4 astures/treatment; 2 yr)

Moriel et al. (2021) J. Anim. Sci. doi:10.1093/jas/skaa123



*Adjusted for plasma concentrations of IGF-1 on d 154
[ab] $P \leq 0.05$

27

Late-gestation

Brangus multiparous cows (70 days before calving)

Palmer et al. (2020) Livestock Sci. doi:10.1016/j.livsci.2020.104176

- **160 multiparous Brangus cows (4 pastures/treatment; 10 cows/pasture)**
  - Stratified by:
    - BW = 548 ± 54
    - BCS = 5.45 ± 0.75

Treatments

2 × 2 factorial

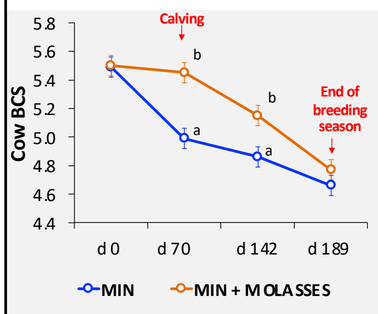
	Methionine (MET)	No Methionine (NoMET)
Trace Mineral (MIN)	**MIN × MET** 56 g/d Min + 15 g/d Met	**MIN × NoMET** 56 g/d Min + 0 g/d Met
Molasses + MIN (MOL)	**MOL × MET** 1.4 kg/d Mol + 56 g/d Min + 15 g/d Met	**MOL × NoMET** 1.4 kg/d Mol + 56 g/d Min + 0 g/d Met

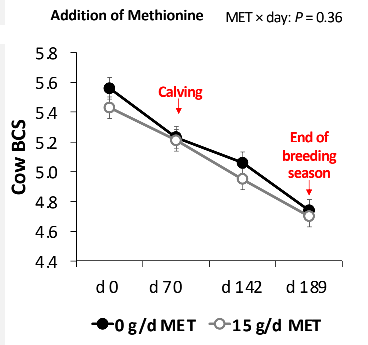
28

Late-gestation

Brangus multiparous cows (70 days before calving)

Palmer et al. (2020) Livestock Sci. doi:10.1016/j.livsci.2020.104176





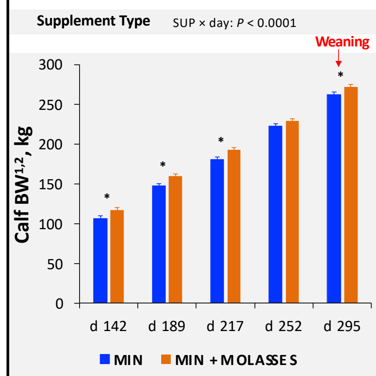
$^{ab}P \leq 0.05$

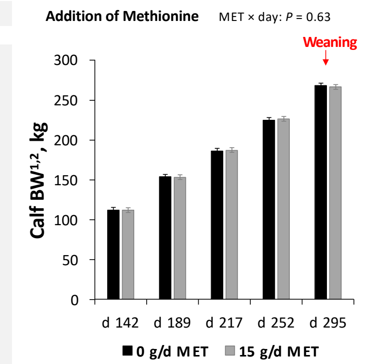
29

Late-gestation

Brangus multiparous cows (70 days before calving)

Palmer et al. (2020) Livestock Sci. doi:10.1016/j.livsci.2020.104176





$^{*}P \leq 0.05$

[1]Adjusted for calf sex ($P < 0.05$)

[2]Adjusted for calving date ($P < 0.05$)

30

Current challenges

Inconsistent results

Multiple possible explanations

- Cow milk production
- Epigenetics
- Pre- vs. postnatal nutrition
- Breed
- Sex-specific responses
- Immunological challenges
- Longer periods of evaluation
- Multigeneration studies

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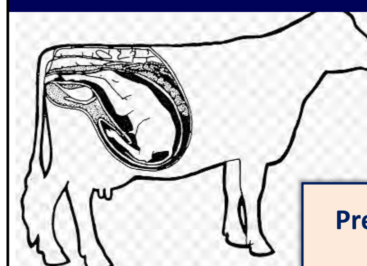

Prenatal vs. Postnatal Nutrition

32

Immunological challenge in the feedlot

Effects of maternal supplementation of protein and energy during late gestation were detected for calf ADG immediately after a vaccination challenge against BRD pathogens but not during pre-vaccination period.

Treatments (starting 56 days precalving):

- **NOSUP =** No Molasses + urea supplementation
- **MOL** = 2.2 lb/d of Molasses + urea (DM)
- **MOLMET =** 2.2 lb/d of **MOL** + **18 g/d of methionine hydroxy analog** (Alimet, Novus)

Item	Treatment: No Supplement	Treatment: Molasses	Treatment: Molasses Methionine	SEM	P-value
ADG[1], lb/day					
Birth to early weaning	1.28	1.26	1.37	0.064	0.48
Postweaning drylot	1.85 [a]	2.00 [b]	2.18 [b]	0.068	0.02
Birth to day 201	1.41 [a]	1.59 [b]	1.65 [b]	0.081	0.10

[1]Adjusted for calf sex ($P \leq 0.05$)

[ab] $P \leq 0.05$

Moriel et al. (2020) J. Anim. Sci. doi:10.1093/jas/skaa123

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Bos indicus

34

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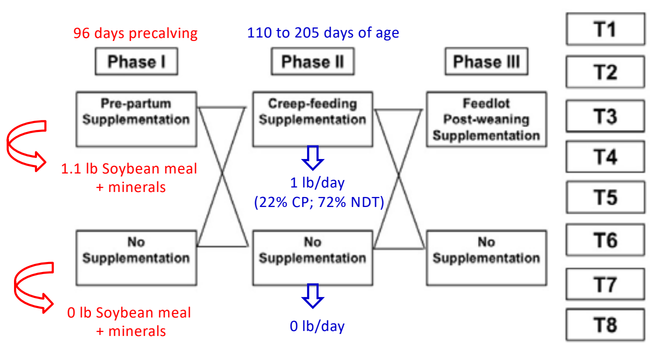
498 multiparous Nelore cows (950 lb; BCS = 5.5) Factorial = 2 x 2 x 2



Nepomuceno et al. (2017) Livestock Sci. 195:58–62

35

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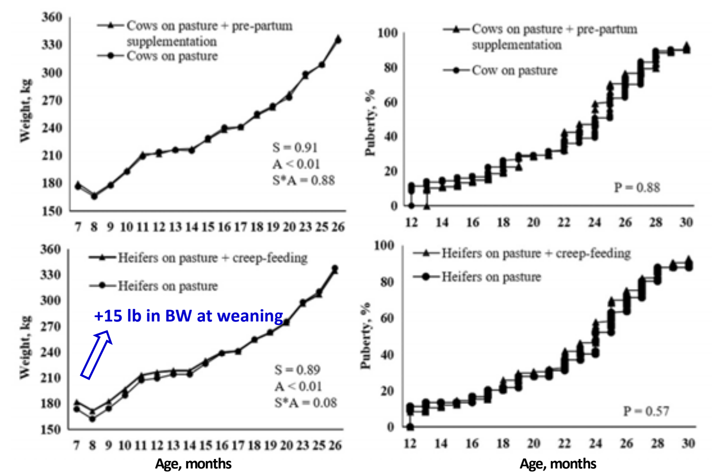
498 multiparous Nelore cows (950 lb; BCS = 5.5)



Nepomuceno et al. (2017) Livestock Sci. 195:58–62

36

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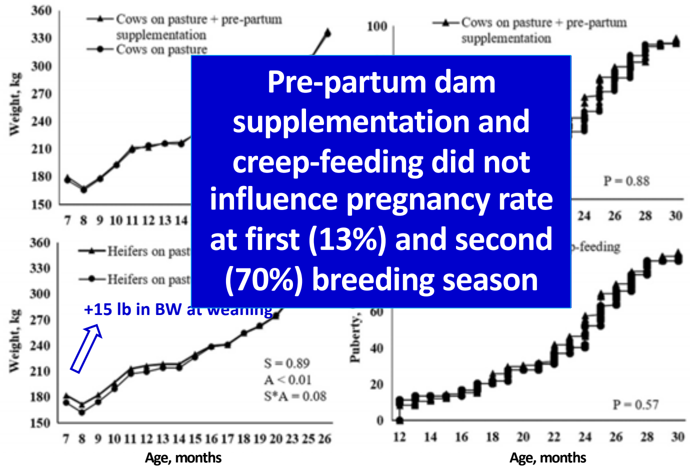
498 multiparous Nelore cows (950 lb; BCS = 5.5)



Pre-partum dam supplementation and creep-feeding did not influence pregnancy rate at first (13%) and second (70%) breeding season

Nepomuceno et al. (2017) Livestock Sci. 195:58–62

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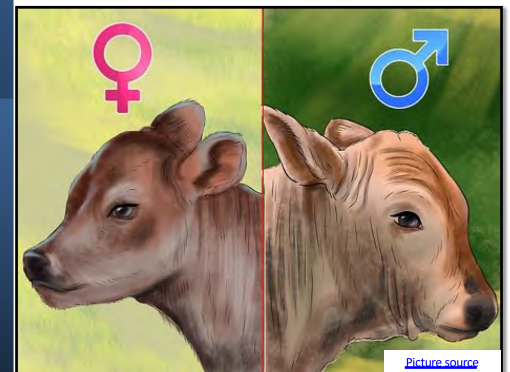
Sex-specific responses

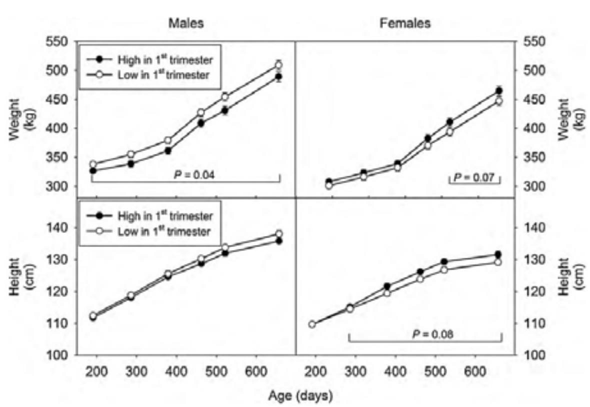


38

Sex-specific responses

Protein restriction during the first trimester of gestation increased post-weaning BW of male calves but decreased post-weaning BW of females compared to meeting protein requirements during first trimester of gestation

G.C. Micke et al. / Animal Reproduction Science 121 (2010) 208–217



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Longer evaluations periods

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Longer evaluation periods

- Opposite outcomes to offspring performance during shorter vs. longer periods of evaluation.
- Low precipitation vs. high precipitation during gestation
  - Decreased birth and weaning BW of calves
  - Increased longevity and percentage of females calving after 8 years of age (Beard et al., 2019)
- Multiple generations (F1 daughter and F2 granddaughters)
  - Laporta et al. (2020)
    - 10 years of consecutive data collection
    - Maternal heat stress during late gestation decreased milk production
    - Daughters during first, second and third lactations,
    - Granddaughters during their first lactation

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Final messages

- Opportunity for beef producers to enhance offspring growth, immune function and reproduction
- Offspring outcomes can be variable
- Enhanced offspring growth more consistent during preweaning vs. post-weaning phase
- Current research challenges and opportunities:
  - Less data on *Bos indicus*
  - Pre- vs. postnatal calf nutrition, sex-specific outcomes, and multiple generations beyond F1 offspring.

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https://doi.org/10.3389/fanim.2021.728635


frontiers in Animal Science

OPEN ACCESS

Improving Beef Progeny Performance Through Developmental Programming

Philipe Moriel[1], Elizabeth A. Palmer[1], Kelsey M. Harvey[1] and R. F. Cooke[1]*

Maternal nutritional management during gestation appears to modulate fetal development and imprint offspring postnatal health and performance, via altered organ and tissue development and tissue-specific epigenetics. This review highlighted the studies demonstrating how developmental programming could be explored by beef producers to enhance offspring performance (growth, immune function, and reproduction), including altering cow body condition score (BCS) during pregnancy and maternal supplementation of protein and energy, polyunsaturated fatty acids (PUFA), trace minerals, frequency of supplementation, specific amino acids, and vitamins. However, this review also highlighted that programming effects on offspring performance reported in the literature were highly variable and depended on level, duration, timing, and type of nutrient restriction during gestation. It is suggested that maternal BCS gain during gestation, rather than BCS per se, enhances offspring preweaning growth. Opportunities for boosting offspring productive responses through maternal supplementation of protein and energy were identified more consistently for pre- vs. post-weaning phases. Maternal supplementation of specific nutrients (i.e.,

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Thank you

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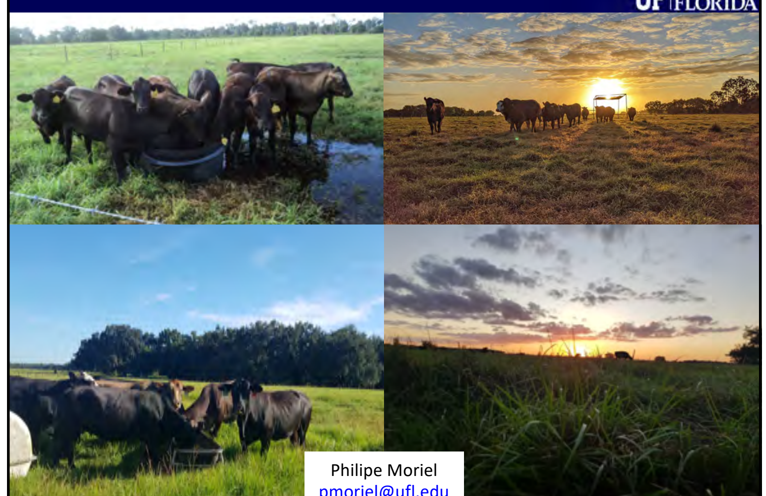

Philipe Moriel
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Methyl Donors and Fetal and Neonatal Development

Fernanda Batistel

1

Developmental Programming & Epigenetics

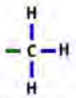
Conrad Waddington
University of Cambridge

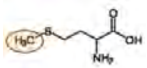
- **Epigenetics**: is the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail change in DNA sequence."

Wu and Morris 2001, *Science*

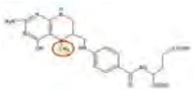
2

Methyl Donors

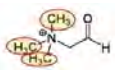
- Methyl group/s: 
- Examples:



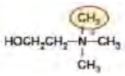
Methionine
(1 methyl group)



Folic acid
(1 methyl group)



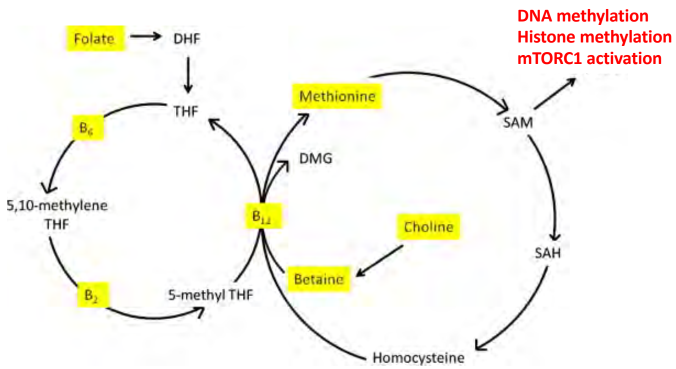
Betaine
(3 methyl groups)



Choline
(3 methyl groups)

3

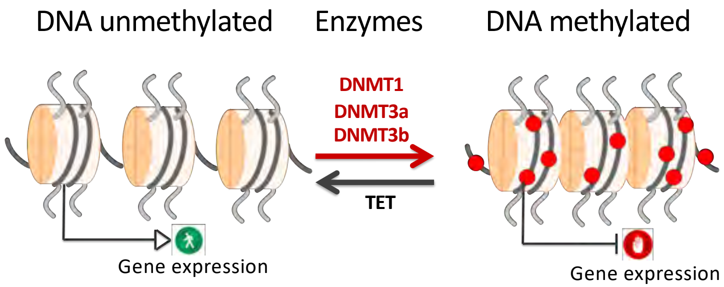
Methyl Donors and One-carbon Metabolism



Mentch, 2016

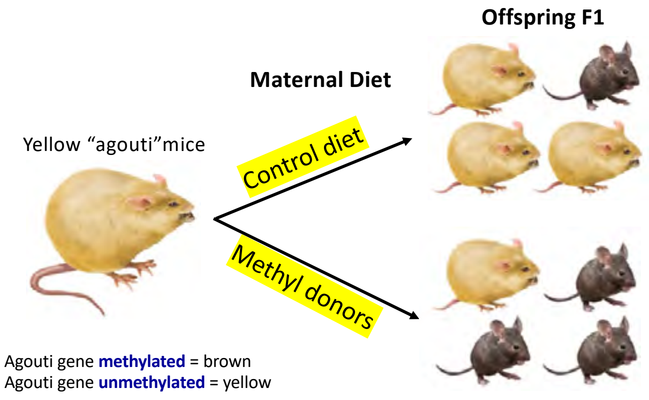
4

Methyl Donors and DNA Methylation



DNMT = DNA methyltransferase
TET =

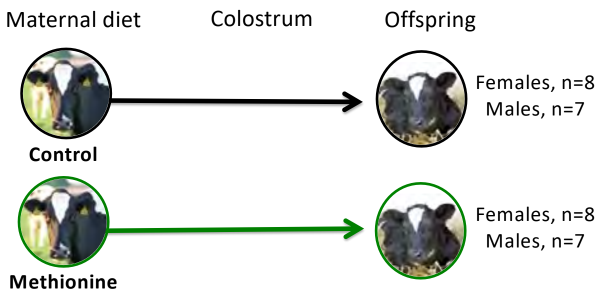
Dietary Methyl Donors and DNA Methylation



Agouti gene **methylated** = brown
Agouti gene **unmethylated** = yellow

Waterland et al. 2007 FASEB Journal; Gu et al. 2017, Science

Methionine Supply During the End of Gestation

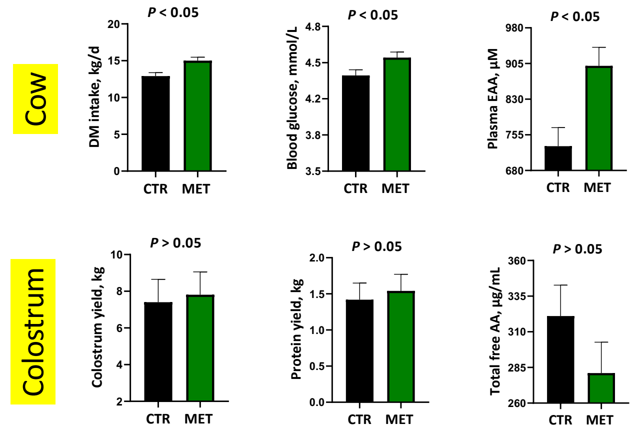


- Last 21 d pregnancy
- Lys:Met 2.9:1

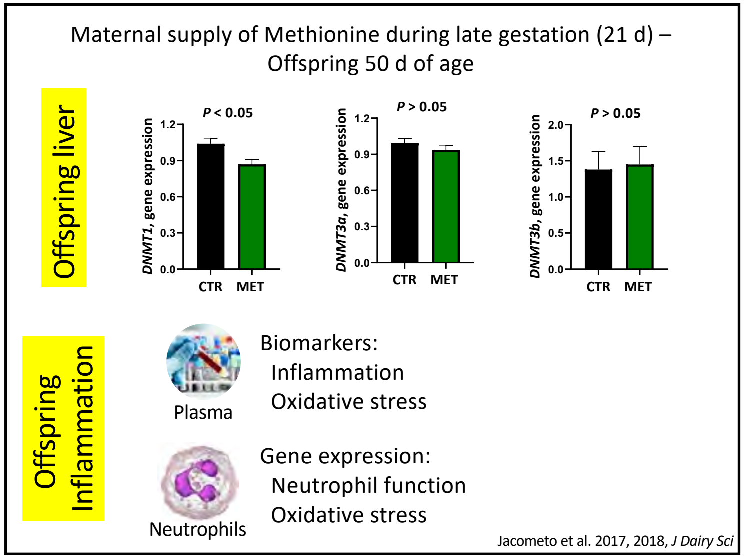
- First 50 days of age:
  - Calf performance
  - Liver biopsy

Zhou et al. 2016; Jacometo et al. 2016, 2018

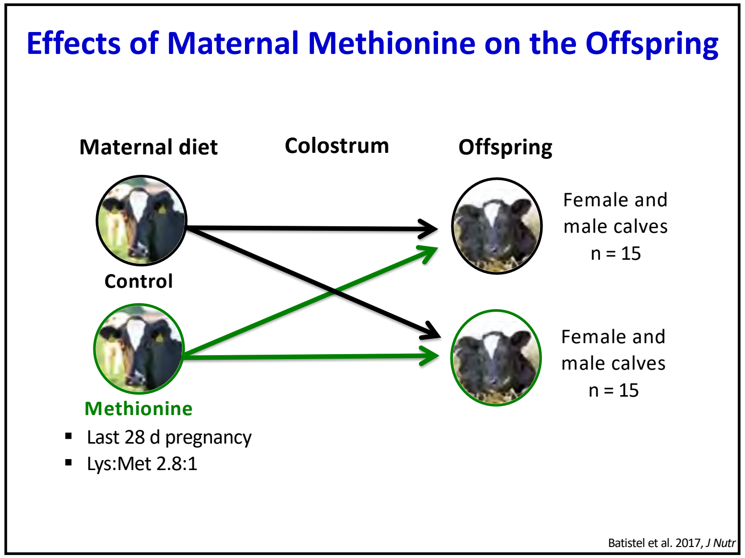
Maternal supply of Methionine during late gestation (21 d)



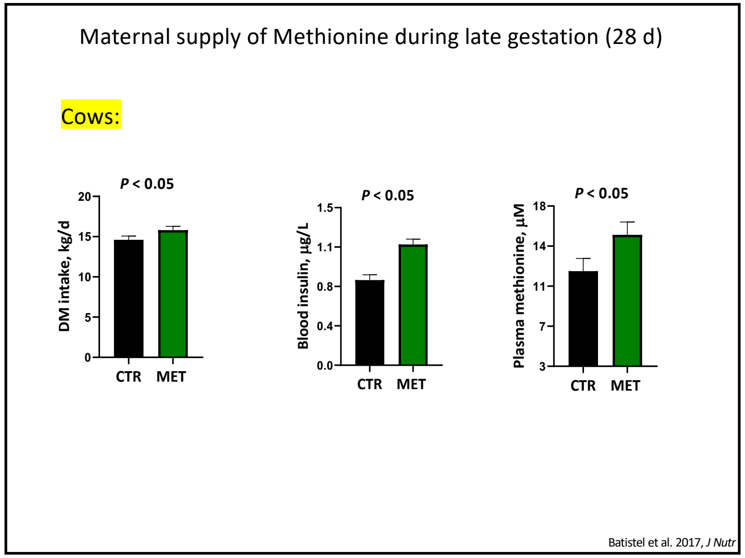
Jacometo et al. 2017, *J Dairy Sci*


Maternal supply of Methionine during late gestation (21 d) – Offspring 50 d of age
Offspring liver
P < 0.05
DNMT1, gene expression
CTR MET
P > 0.05
DNMT3a, gene expression
CTR MET
P > 0.05
DNMT3b, gene expression
CTR MET
Offspring Inflammation
Plasma
Biomarkers:
Inflammation
Oxidative stress
Neutrophils
Gene expression:
Neutrophil function
Oxidative stress
Jacometo et al. 2017, 2018, J Dairy Sci

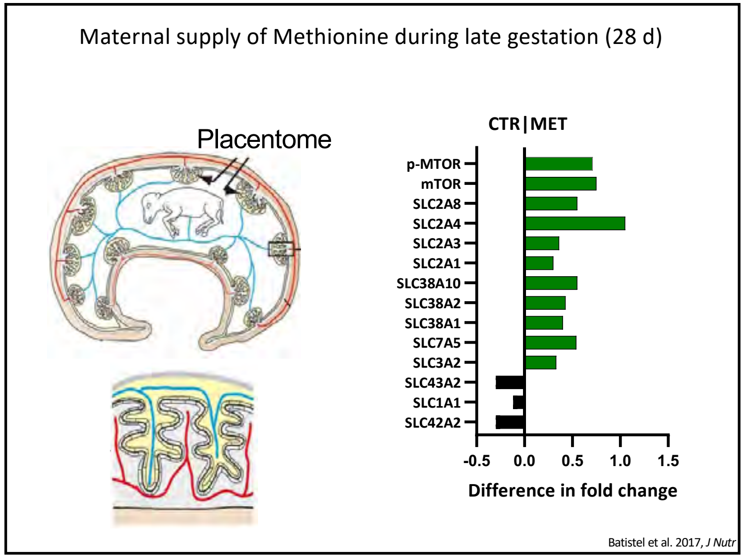
9


Effects of Maternal Methionine on the Offspring
Maternal diet
Colostrum
Offspring
Control
Methionine
Last 28 d pregnancy
Lys:Met 2.8:1
Female and male calves n = 15
Female and male calves n = 15
Batistel et al. 2017, J Nutr

10


Maternal supply of Methionine during late gestation (28 d)
Cows:
P < 0.05
DM intake, kg/d
CTR MET
P < 0.05
Blood insulin, μg/L
CTR MET
P < 0.05
Plasma methionine, μM
CTR MET
Batistel et al. 2017, J Nutr

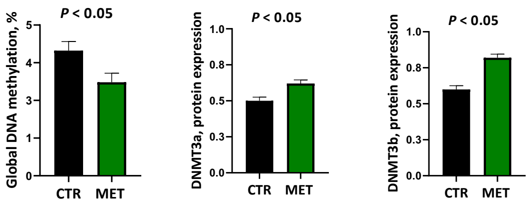
11


Maternal supply of Methionine during late gestation (28 d)
Placentome
CTR|MET
p-MTOR
mTOR
SLC2A8
SLC2A4
SLC2A3
SLC2A1
SLC38A10
SLC38A2
SLC38A1
SLC7A5
SLC3A2
SLC43A2
SLC1A1
SLC42A2
-0.5 0.0 0.5 1.0 1.5
Difference in fold change
Batistel et al. 2017, J Nutr

12

Maternal supply of Methionine during late gestation (28 d)

Placenta:

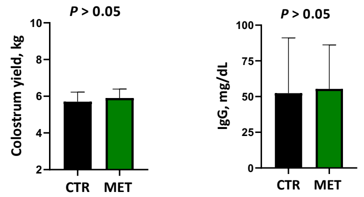


Batistel et al. 2019; *J Nutr*

13

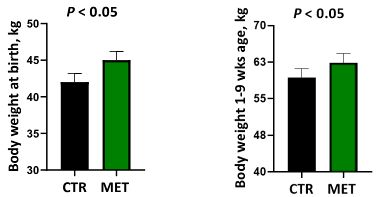
Maternal supply of Methionine during late gestation (28 d)

Colostrum



- No difference:
  - Amino acids
  - Fatty acids

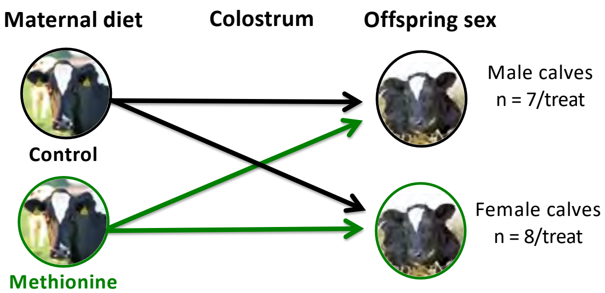
Offspring



Batistel et al. 2017, *J Nutr*
Alharthi et al. 2018, *J Anim Sci Biotech*

14

Effects of Maternal Methionine on the Offspring

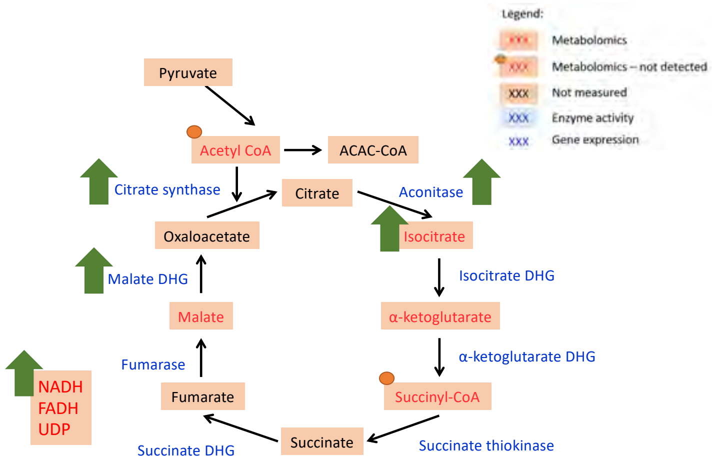


- Last 28 d pregnancy
- Lys:Met 2.8:1

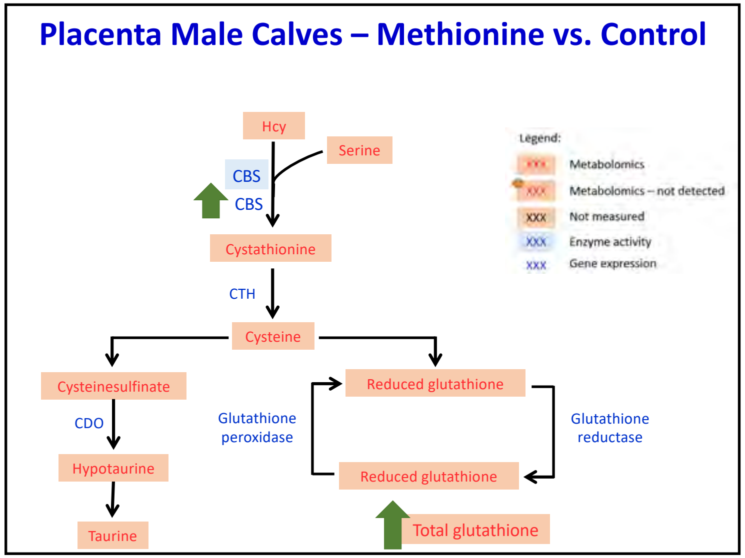
Batistel et al. 2019, *J Nutr*

15

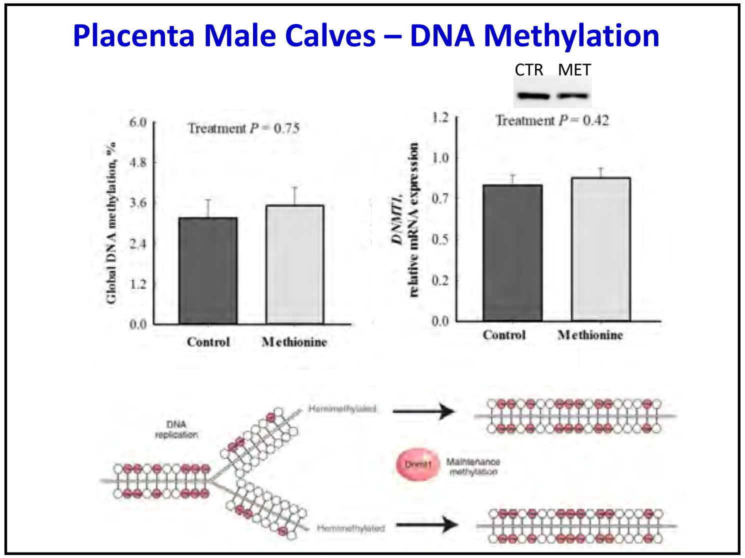
Placenta Male Calves – Methionine vs. Control



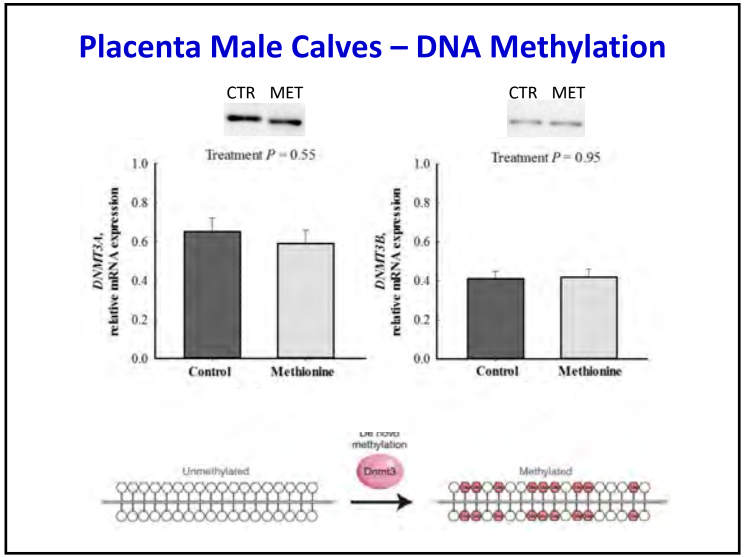
16


Placenta Male Calves – Methionine vs. Control
Hcy
Serine
CBS
CBS
Cystathionine
CTH
Cysteine
Cysteinesulfinate
CDO
Hypotaurine
Taurine
Reduced glutathione
Glutathione peroxidase
Glutathione reductase
Reduced glutathione
Total glutathione
Legend:
Metabolomics
Metabolomics – not detected
Not measured
Enzyme activity
Gene expression

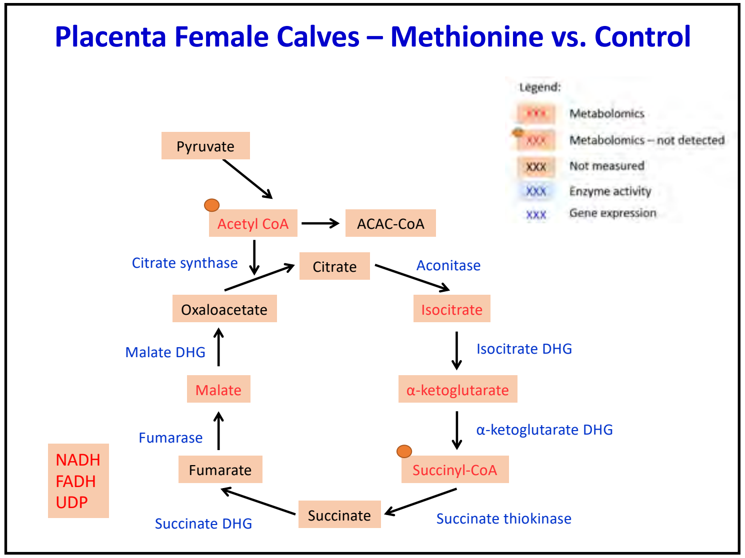
17


Placenta Male Calves – DNA Methylation
CTR MET
Treatment P = 0.75
Global DNA methylation, %
6.0
4.8
3.6
2.4
1.2
0.0
Control
Methionine
Treatment P = 0.42
DNMT1, relative mRNA expression
1.2
1.0
0.7
0.5
0.2
0.0
Control
Methionine
DNA replication
Hemimethylated
Maintenance methylation
Hemimethylated

18

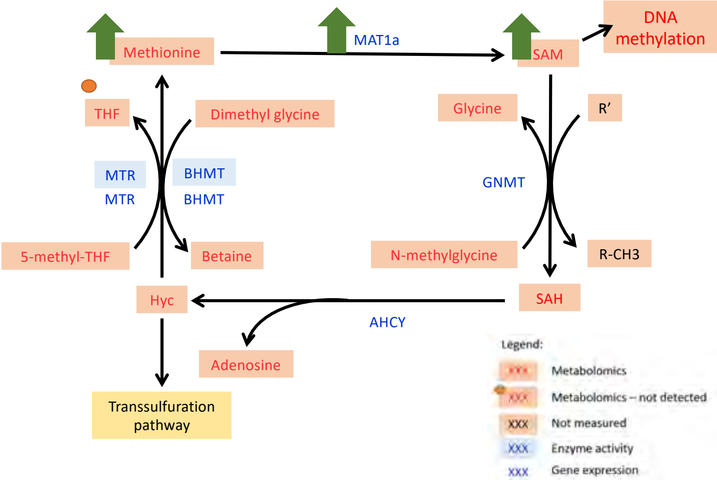

Placenta Male Calves – DNA Methylation
CTR MET
Treatment P = 0.55
DNMT3A, relative mRNA expression
1.0
0.8
0.6
0.4
0.2
0.0
Control
Methionine
CTR MET
Treatment P = 0.95
DNMT3B, relative mRNA expression
1.0
0.8
0.6
0.4
0.2
0.0
Control
Methionine
Unmethylated
De novo methylation
Dnmt3
Methylated

19


Placenta Female Calves – Methionine vs. Control
Legend:
Metabolomics
Metabolomics – not detected
Not measured
Enzyme activity
Gene expression
Pyruvate
Acetyl CoA
ACAC-CoA
Citrate synthase
Citrate
Aconitase
Oxaloacetate
Isocitrate
Malate DHG
Isocitrate DHG
Malate
α-ketoglutarate
Fumarase
α-ketoglutarate DHG
NADH
FADH
UDP
Fumarate
Succinyl-CoA
Succinate DHG
Succinate
Succinate thiokinase

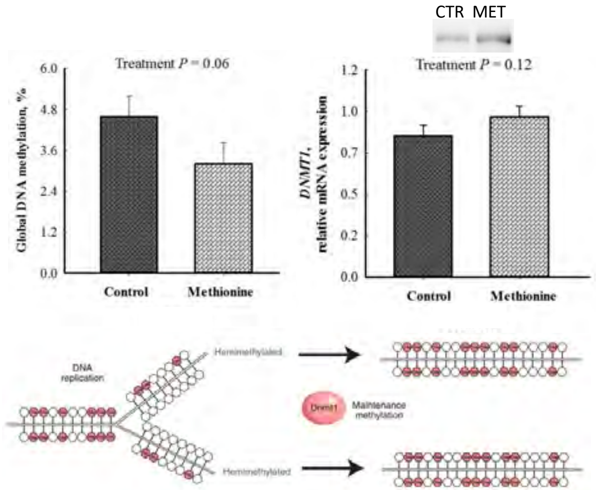
20

Placenta Female Calves – Methionine vs. Control



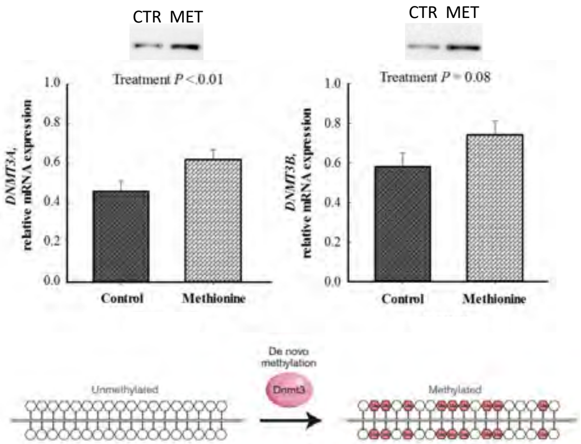
21

Placenta Female Calves – DNA Methylation



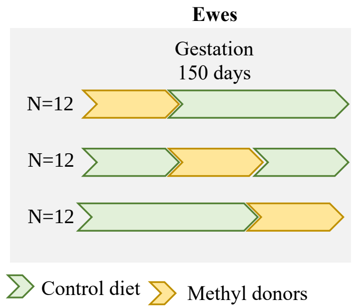
22

Placenta Female Calves – DNA Methylation



23

Methionine Supply During Different Stages of Pregnancy



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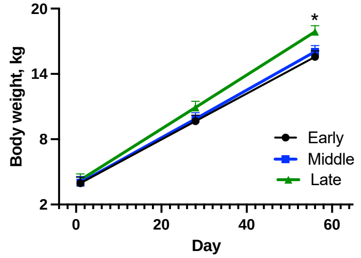
Methionine Supply During Different Stages of Pregnancy



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Take Home Message

- Maternal supply of Met during late pregnancy enhances the rate of calf development in utero and postnatal growth.
  - Mediated by placenta metabolism.
  - Offspring sex-specific metabolic changes.
- The gestational phase of intervention affects lamb growth.

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Thank you!

fernandabatistel@ufl.edu

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Update on Vitamin D Nutrition for Dairy Cows

Corwin D. Nelson
Associate Professor of Physiology
Department of Animal Sciences
University of Florida, Gainesville, FL, USA

Florida Ruminant Nutrition Symposium
May 9-11, 2022

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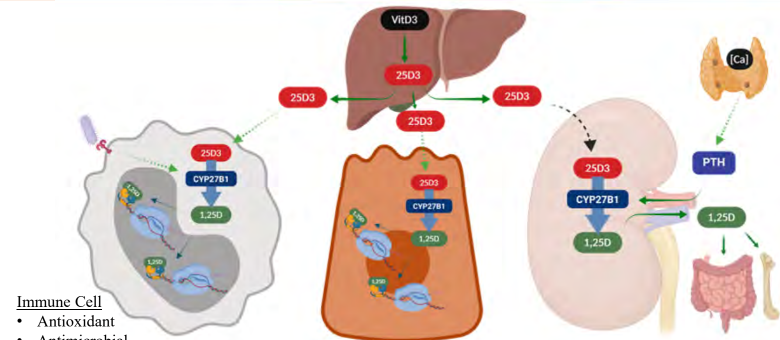
1

Overview of Vitamin D Physiology



Immune Cell
- Antioxidant
- Antimicrobial

Mammary Cell
- Development
- Glucose & Ca transport

Endocrine
- Bone mineral content
- Ca & P transport

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2

Summary: Vitamin D Physiology

Vitamin D Metabolism
- Requires enzymatic activation
- 1,25D (Calcitriol) is active form – tightly regulated
- 25D (Calcidiol) is major form in blood
  - Precursor to 1,25D
  - Indicative of vitamin D intake

Modes of Action
- Most physiological responses to 1,25D are accomplished through the VDR.
- The VDR is expressed in nearly every cell of the body
  - Calcium & phosphorus homeostasis
  - Skeletal development
  - Immune
  - Mammary
  - Reproduction

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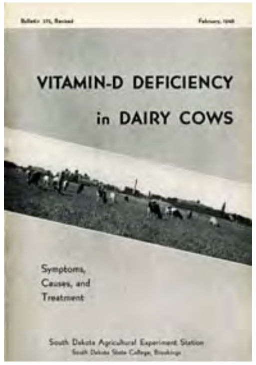
3

Vitamin D Nutrition & Status of Cattle

- **Dairy cow diets typically include supplemental vitamin D_3**
  - Support Ca and P homeostasis
  - Lack of sun-exposure
- **Vitamin D status is indicated by serum 25(OH)D concentrations**
  - Stable half-life
  - Reliable indicator of intake (diet or sun)

4

Lack of Vitamin D Causes Skeletal Deformities

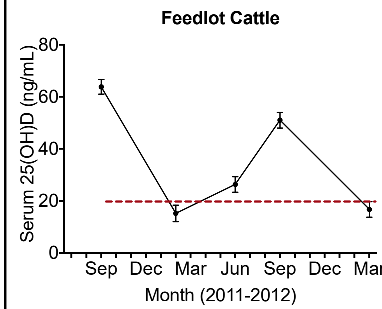


Symptoms of severe vitamin-D Deficiency are shown in this cow, which collapsed about a week after this picture was taken. Note rigid position, swollen joints, knees springing forward, and elevation of back.

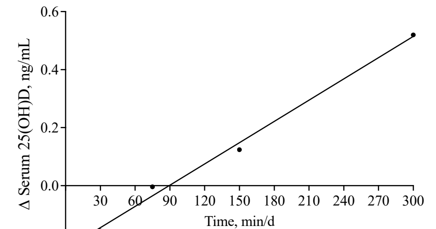
Calves dropped by vitamin-D-deficient cows were usually weak, and the legs of many were crooked because of lack of minerals in the bones. The calf shown above died within a week after it was born.

5

Effect of Time and Season on Vitamin D Status



Adapted from Casas et al., 2015, Domest. Anim. Endo.



Change in $25(OH)D_3$ concentration of dairy cows versus daily time spent at pasture in June 2010 at 56°N in Denmark for 28 d.

Hymoller 2012, Brit. J. Nutr. 108:666-71

6

Vitamin D Nutritional Recommendations

1 mg = 40,000 IU
1 μg = 40 IU

Stage	IU/kg BW	μg/kg BW	IU/kg DM	μg/kg DM	IU/d
Lactating	**40**	**1.0**	**900 to 1,400**	**17 to 25**	**28,000**
Dry	30	0.75	1,600	40	22,500
Close-up	30	0.75	2,270	57	22,700
Fresh	**40**	**1.0**	**1,750 to 2,000**	**44 to 52**	**28,000**
Calves	**32**	**0.8**	**3,200**	**80**	**3,200**
Heifers (450-650kg)	30	0.75	1,500	37.5	13,500 - 19,500
Beef	6.6	0.165	275	6.8	2,000 – 5,000

Mature BW = 680 kg

NASEM, 2021, *Nutrient Requirements of Dairy Cattle*, 8th Edition

7

CAUTION: Vitamin D Toxicity!!!

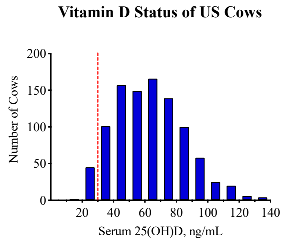
Vitamin D has greater risk of toxicity compared with other vitamins
- Does not have hepatic storage capacity like vitamin A
- Endogenous synthesis upon sun exposure is regulated

Symptoms of overfeeding vitamin D (>500 KIU cholecalciferol)
- Decreased appetite and ADG in feedlot animals
- Decreased milk yield in dairy cows
- Lethargic

Clinical vitamin D toxicity
- Calcification of soft tissues
- Results from massive overdosing of vitamin D, millions of IUs

8

Vitamin D Status of Dairy Cows



Nelson 2016, J. Dairy Science, 99:10150-10160

- < 5 ng/mL serum 25(OH)D will result in rickets
- 10-20 ng/mL serum 25OH)D insufficient for Ca and P homeostasis, bone health
- >30 ng/mL sufficient for immunity in theory
- > 100 ng/mL does not occur naturally, potential for beneficial or negative effects
- Toxicity occurs with massive (>10⁶ IU) vitamin D_3 doses

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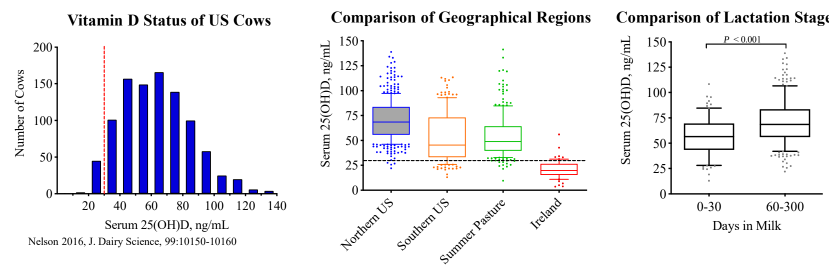
Vitamin D Status of Dairy Cows



Nelson 2016, J. Dairy Science, 99:10150-10160

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Vitamin D Status and Health Outcomes

Condition	Y/N	N	Serum 25(OH)D, ng/mL Mean	SE	25th%	Median	75th%	*P*-value
Hyperketonemia	N	257	89.2	1.0	69.7	88.5	106.8	0.01
	Y	22	102.0	4.7	75.4	100.2	124.6	
Lameness	N	260	91.1	1.1	71.2	90.0	108.6	<0.01
	Y	19	78.4	2.7	65.2	77.1	90.0	
Mastitis	N	264	90.1	1.0	70.6	88.8	107.7	0.68
	Y	15	92.1	4.7	69.1	91.7	107.1	
Metritis	**N**	**259**	**91.4**	**1.0**	**71.8**	**89.8**	**108.1**	**<0.01**
	Y	**20**	**75.3**	**4.3**	**49.9**	**66.4**	**92.3**	
Ret. Placenta	**N**	**261**	**90.9**	**1.0**	**71.1**	**89.3**	**108.4**	**<0.01**
	Y	**18**	**79.6**	**3.9**	**60.3**	**73.8**	**105.7**	
Uterine Disease	**N**	**187**	**91.3**	**1.1**	**71.2**	**89.6**	**108.1**	**<0.01**
	Y	**92**	**81.2**	**3.2**	**66.1**	**75**	**106.1**	

Wisnieski, 2020, J Dairy Sci. 103(2):1795-1806.

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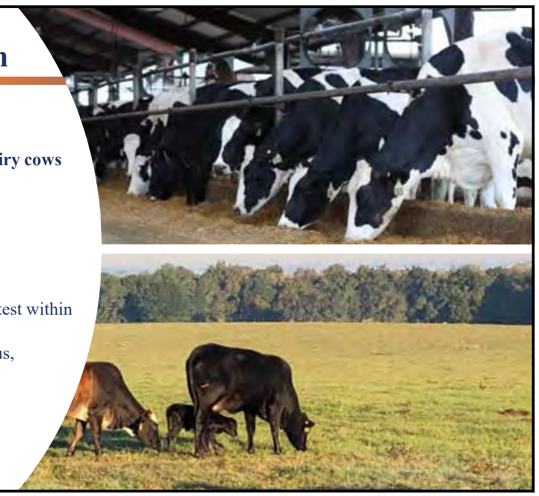
Opportunities for Vitamin D Intervention

- **Vitamin D nutrition for peripartum dairy cows**
  - Post-partum hypocalcemia
  - Pluripotent effects of vitamin
    - Immunity
    - Mammary function
- **Vitamin D nutrition for calves**
  - Risk of morbidity and mortality greatest within first few weeks of life
  - Calves born with low vitamin D status, i.e., 10 to 20 ng/mL



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Vitamin D and Postpartum Ca

1. Hypocalcemia results from failure of Ca homeostatic mechanisms, almost never vitamin D deficiency
2. Effect of increasing vitamin D from 20 to 50 KIU/d on transition Ca *__unknown__*
3. Increasing dietary vitamin D_3 > 50 KIU/d (1.5 mg/d) does not prevent hypocalcemia
4. Properly managed negative DCAD improves postpartum calcium

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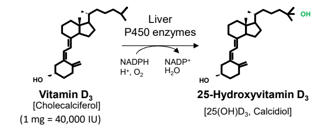
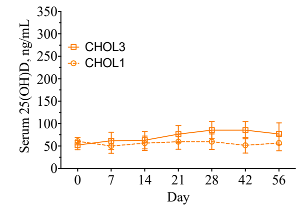
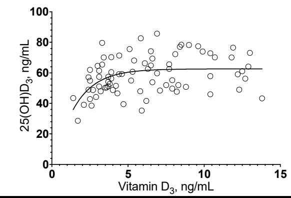
Calcidiol: An Alternative and Effective Vitamin D Source



1 vs. 3 mg/d   1 vs. 3 mg/d





Poindexter, 2020. J. Dairy Sci. 103:805-822.

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Effect of Calcidiol on Transition Cows

Summary of two University of Florida transition cow experiments

Feeding 3 mg calcidiol compared with cholecalciferol:

- **Increased Ca digestibility**
- **Increased serum Ca and P**
- **Increased milk yield by 4 kg/d in first 42 DIM**
- **Increased colostrum yield and net energy yield**

Martinez et al. 2018. J. Dairy Sci.
Poindexter 2021, Ph D Dissertation, Univ. Florida
Vieira-Neto, 2021, J. Dairy Sci. 104:107796-10811

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Effect of Source of Vitamin D and DCAD on Transition Cows

Hypothesis: 25-hydroxyvitamin D_3 combined with negative DCAD would improve postpartum Ca homeostasis

Experimental design

- 80 Holstein cows (52 parous, 28 nulliparous)
- Blocked by parity and previous lactation 305 d MY
- Enrolled in treatments at 252 d of gestation
- Factorial arrangement of treatments:
  - 2 levels of DCAD (+130 mEq/kg or -130 mEq/kg)
  - 2 sources of vitamin D_3 (cholecalciferol or calcidiol)

Rodney et al. 2018. J. Dairy Sci. 101:2519-2543.
Martinez et al. 2018a. J. Dairy Sci. 101:2544-2562.
Martinez et al. 2018b. J. Dairy Sci. 101:2563-2578.

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Effect of Source and Amount of Vitamin D on Transition Cows

Hypothesis: Increasing amount of calcidiol will increase mineral balance and productive performance of cows compared with equivalent of cholecalciferol

Experimental Design:

- 177 Holstein cows
- Enrolled at 242 d gestation
- Fed prepartum TMR with -77 mEq/kg DM DCAD, 0.53% Ca
- Factorial arrangement of treatments:
  - ***CAL1:*** supplement containing 1 mg calcidiol
  - ***CAL3:*** supplement containing 3 mg calcidiol
  - ***CHOL1:*** supplement containing 1 mg (40,000 IU) cholecalciferol
  - ***CHOL3:*** supplement containing 3 mg (120,000 IU) cholecalciferol

Poindexter 2021, Ph D Dissertation, Univ. Florida
Vieira-Neto, 2021, J. Dairy Sci. 104:107796-10811

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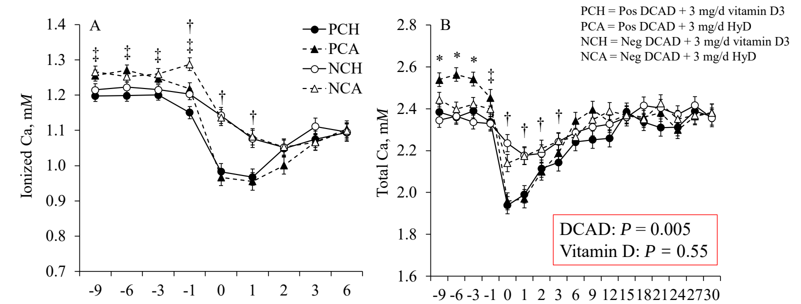
Prepartum DCAD is More Effective Than Vitamin D source



Rodney et al. 2018. J. Dairy Sci.

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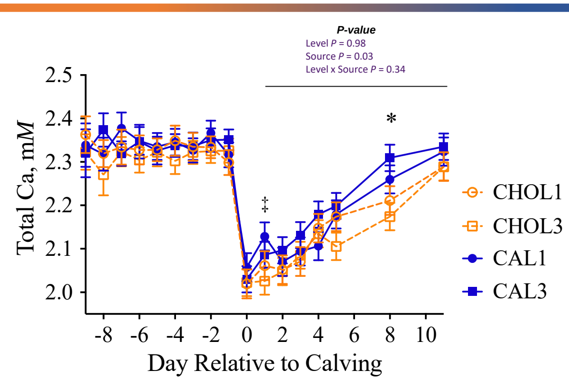
Prepartum Calcidiol Restored Postpartum Ca Faster



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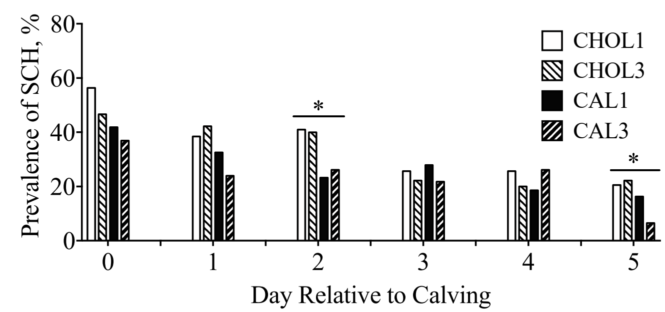
Prepartum Calcidiol Decreased Subclinical Hypocalcemia



Poindexter, unpublished

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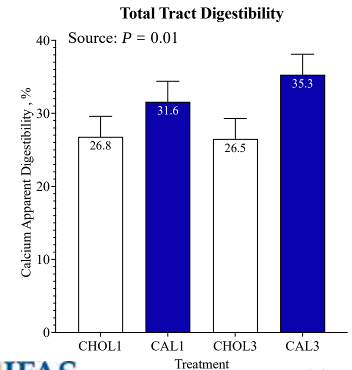
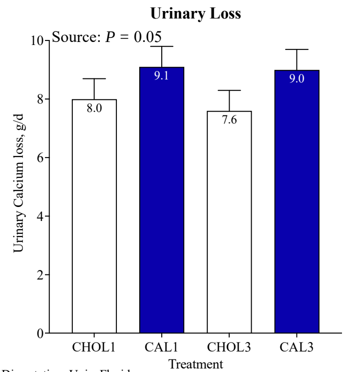
Prepartum Calcidiol Increased Prepartum Ca Flux





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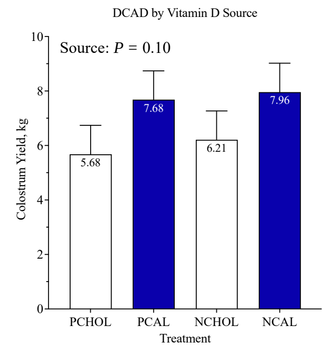
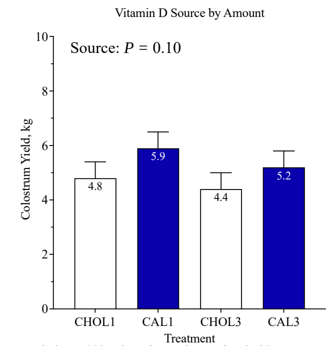
Prepartum Calcidiol Increased Colostrum Yield



Martinez et al. 2018. J. Dairy Sci. 101:2544-2562.



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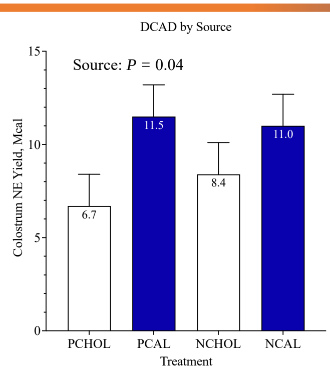
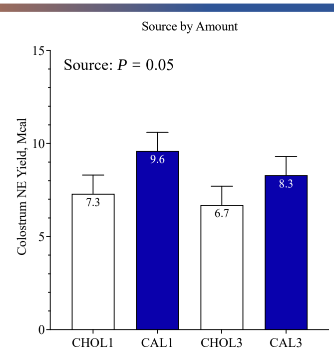
Prepartum Calcidiol Increased Colostrum Net Energy Yield



Martinez et al. 2018. J. Dairy Sci. 101:2544-2562.



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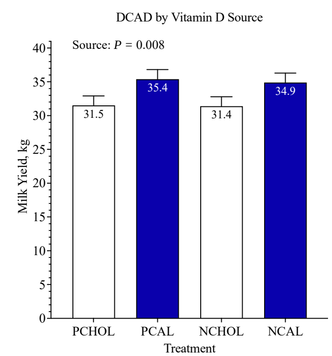
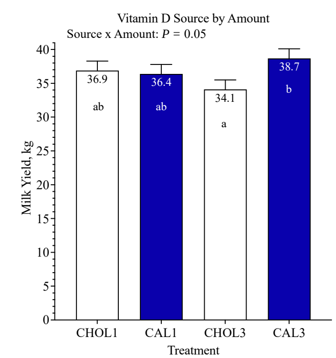
Prepartum Calcidiol Increased Milk Yield



Martinez et al. 2018. J. Dairy Sci. 101:2544-2562.



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Opportunities for Vitamin D Intervention

- **Vitamin D nutrition for peripartum dairy cows**
  - Post-partum hypocalcemia
  - Pluripotent effects of vitamin
    - Immunity
    - Mammary function
- **Vitamin D nutrition for calves**
  - Risk of morbidity and mortality greatest within first few weeks of life
  - Calves born with low vitamin D status, i.e., 10 to 20 ng/mL

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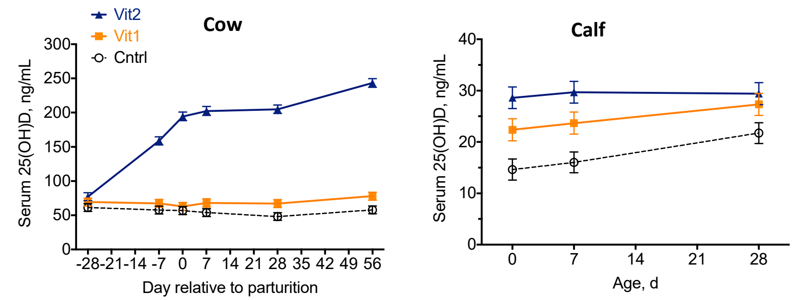
Maternal Vitamin D Determines Neonatal Vitamin D



Effect	Cow	Calf
Trt	<0.001	<0.001
Trt x Time	<0.001	0.167

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Effect of Supplemental Vitamin D Source and Amount for Calves

Treatment[1]	Cholecalciferol, µg/kg BW	Calcidiol, µg/kg BW	Total Vitamin D, µg/kg BW	Total Vitamin D, IU/kg BW[2]
CON	0.25	-	0.25	10
CHOL1.5	1.75	-	1.75	70
CHOL3	3.25	-	3.25	130
CAL1.5	0.25	1.5	1.75	-
CAL3	0.25	3.0	3.25	-

[1] Holstein male calves (n = 45; 9/treatment) fed treatment from 5 to 131 d of age
[2] NASEM 2021 recommendation = 0.8 µg/kg BW (32 IU/kg BW)

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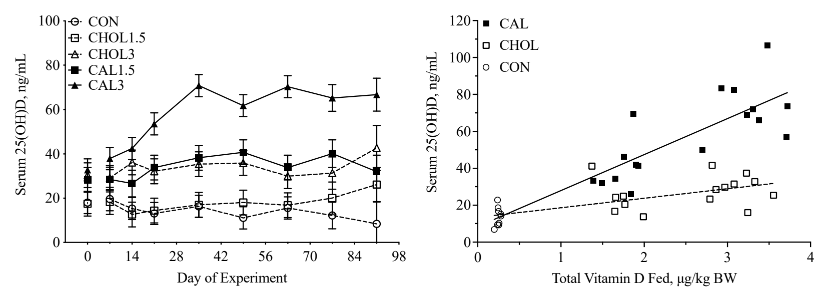
Effect of Supplemental Vitamin D Source and Amount for Calves



Blakely 2018 MS Thesis, Univ. Florida

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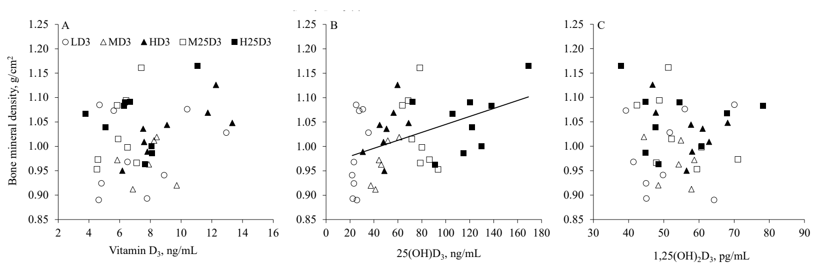
Effect of Supplemental Vitamin D Source and Amount for Calves



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Calcidiol Increased Pre-weaning Growth of Heifers

Effect of calcidiol supplementation on growth and dry matter intake of calves

Measure	Treatment: Control	Treatment: Calcidiol	SE	*P*-value
No. calves	71	74	-	-
Cholecalciferol, µg/kg BW	2.3	2.3	-	-
Calcidiol, µg/kg BW	**0**	**1.7**	**-**	**-**
Serum 25(OH)D, ng/mL	**42.5**	**70.1**	**2.2**	**<0.001**
Growth and DMI				
BW d 56, kg	**81.0**	**83.3**	**1.6**	**0.07**
BW gain, kg/d	**0.811**	**0.874**	**0.035**	**0.02**
Hip height d 56, cm	92.1	92.8	0.6	0.09
MR DMI, kg/d	1.14	1.20	0.05	0.09
Post-wean				
BW d 98, kg	**111.5**	**115.0**	**1.4**	**0.02**
BW gain, kg/d	766	787	31	0.44
Hip Height d 98, cm	100.5	101.6	0.4	0.01
Starter DMI, kg/d	2.33	2.28	0.07	0.62

Wells, 2022, PhD Dissertation, Univ. Florida

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Summary & Conclusions

Transition cows - Feeding 3 mg calcidiol compared with cholecalciferol:

- **Increased Ca digestibility**
- **Increased serum Ca and P**
- **Increased milk yield by 4 kg/d in first 42 DIM**
- **Increased colostrum yield and net energy yield**

Calves - feeding calcidiol compared with cholecalciferol:

- **More effectively improved vitamin D status**
- **Increased bone mineral density**
- **Increased pre-weaning BW gain in dairy heifers**

Funding:
Southeast Milk Checkoff
DSM Nutritional Products
Church & Dwight
Landus Cooperative

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Supplemental Data Slides

The following slides are data tables from transition cow experiments with supplemental calcidiol:

- Effect of level and source of vitamin D, Poindexter, M.B. 2021 University of Florida, PhD Dissertation
- Effect of vitamin D source and level of DCAD, Martinez et al. 2018. J. Dairy Sci. 101:2544-2562

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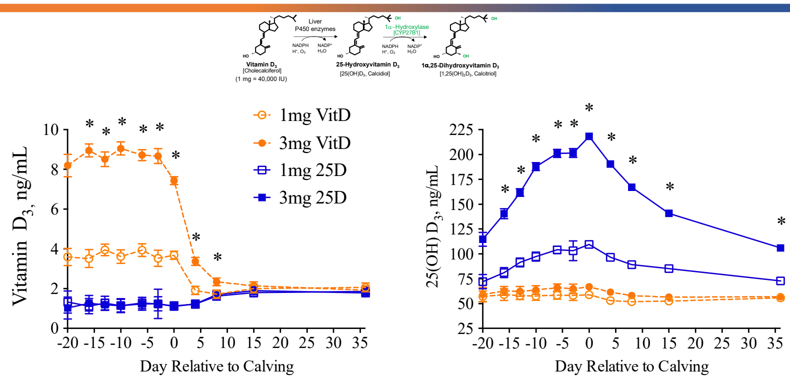
Effect of Calcidiol on Vitamin D metabolites



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Effect of Calcidiol on Serum Vitamin D

Table 3. Effect of source and amount of vitamin D fed prepartum on concentrations of vitamin D metabolites in serum of Holstein cows

	Treatment[1]					Parity			P-value[2]			
	CHOL		CAL									
Item	1 mg	3 mg	1 mg	3 mg	SEM	Nulliparous	Parous	SEM	Source	Amt	Source × Amt	Parity
Prepartum[3]												
Vitamin D_3, ng/mL	3.7	8.6	1.2	1.2	0.2	4.1	3.3	0.2	<0.001	<0.001	<0.001	<0.01
25(OH)D_3, ng/mL*	**58.3**	**63.5**	**93.8**	**173.6**	**3.34**	**99.01**	**95.56**	**2.61**	**<0.001**	**<0.001**	**<0.001**	**0.40**
24,25(OH)$_2D_3$, ng/mL	2.1	2.2	2.7	5.7	0.3	3.5	3.0	0.2	<0.001	<0.001	<0.001	0.17
Ratio 24,25D to 25D*	0.030	0.030	0.023	0.030	0.003	0.021	0.035	0.003	0.28	0.35	0.34	<0.001
1,25(OH)$_2$D, pg/mL	**49.0**	**47.0**	**47.2**	**45.9**	**3.9**	**38.1**	**56.4**	**3.7**	**0.7**	**0.63**	**0.92**	**0.002**
Postpartum[4]												
Vitamin D_3, ng/mL	2.3	3.4	1.5	1.5	0.1	2.1	2.2	0.1	<0.001	<0.001	<0.001	0.50
25(OH)D_3, ng/mL*	**54.4**	**60.0**	**90.7**	**185**	**2.4**	**97.0**	**87.8**	**1.9**	**<0.001**	**<0.001**	**<0.001**	**0.002**
24,25(OH)$_2D_3$, ng/mL	2.1	2.4	3.8	9.4	0.3	5.0	3.8	0.3	<.0001	<0.001	<0.001	0.008
Ratio 24,25D to 25D*	0.033	0.037	0.037	0.058	0.004	0.033	0.05	0.003	0.01	<0.001	0.02	<0.001
1,25-(OH)$_2$D, pg/mL	**82.1**	**87.8**	**78.3**	**81.3**	**4.8**	**59.2**	**105.5**	**5.5**	**0.12**	**0.19**	**0.69**	**<0.001**

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Prepartum Calcidiol Increased Serum Ca and P

Table 4. Effect of source and amount of vitamin D on serum mineral concentrations.

	Treatment[1]					Parity			P-value[2]			
	CHOL		CAL									
Item	1 mg	3 mg	1 mg	3 mg	SEM	Nulliparous	Parous	SEM	Source	Amt	Source × Amt	Parity
Prepartum[3]												
Ca, mM	2.33	2.31	2.34	2.34	0.02	2.34	2.32	0.02	0.27	0.56	0.59	0.48
Mg, mM	0.84	0.85	0.83	0.82	0.02	0.83	0.81	0.01	0.25	0.74	0.73	0.01
P, mM	**1.89**	**1.86**	**1.97**	**2.05**	**0.03**	**2.01**	**1.88**	**0.03**	**<0.001**	**0.49**	**0.08**	**<0.001**
Postpartum[4]												
Ca, mM	**2.13[ab]**	**2.11[b]**	**2.15[ab]**	**2.17[a]**	**0.02**	**2.17**	**2.12**	**0.01**	**0.03**	**0.98**	**0.34**	**0.02**
Mg, mM	0.88	0.86	0.87	0.85	0.02	0.88	0.85	0.01	0.53	0.07	0.93	0.26
P, mM	**1.72**	**1.68**	**1.75**	**1.80**	**0.04**	**1.79**	**1.69**	**0.24**	**0.03**	**0.84**	**0.20**	**0.004**
Milk fever, %[5]	5.1	2.2	2.3	4.4	-	0.0	4.6	-	0.94	0.90	0.40	-
SCH, %[6]	**71.1**	**80.0**	**72.1**	**60.8**	**-**	**44.2**	**79.2**	**-**	**0.05**	**0.98**	**0.12**	**<0.001**

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Prepartum Calcidiol Increased Prepartum Ca Digestibility

Table 5. Effect of source and amount of vitamin D fed prepartum on mineral digestibility and retention prepartum.

	Treatment[1]					Parity			P-value[2]			
	CHOL		CAL									
Item	1 mg	3 mg	1 mg	3 mg	SEM	Nulliparous	Parous	SEM	Source	Amt	Source × Amt	Parity
Ca intake, g/d	43.4	45.4	47.6	44.9	2.5	53.33	37.26	1.88	0.44	0.88	0.34	<0.001
Ca fecal excretion, g/d	30.9	31.8	31.5	28.3	1.7	29.0	32.3	1.1	0.39	0.50	0.23	0.05
Ca absorption, g/d	14.1	13.1	16.0	16.6	2.0	8.7	21.2	1.8	0.12	0.92	0.65	<0.001
Ca digestibility, %	**26.8**	**26.5**	**31.6**	**35.3**	**2.8**	**22.0**	**38.1**	**2.2**	**0.01**	**0.51**	**0.46**	**<0.001**
Urine pH	5.77	5.87	5.75	5.72	0.08	6.03	5.53	0.09	0.23	0.65	0.34	<0.001
Urine Ca, mg/L	**307**	**319**	**330**	**370**	**17**	**318**	**345**	**13**	**0.03**	**0.11**	**0.38**	**0.15**
Urine Ca, g/d	**8.0**	**7.6**	**9.1**	**9.0**	**0.7**	**6.1**	**10.7**	**0.5**	**0.05**	**0.70**	**0.84**	**<0.001**
Retention, g/d	5.5	4.1	5.9	6.9	2.2	2.2	9.0	1.9	0.37	0.89	0.52	0.02

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Prepartum Calcidiol Increased Colostrum Net Energy Yield

Effect of DCAD and source of vitamin D fed prepartum on colostrum yield and composition in Holstein cows[1]

	Positive DCAD		Negative DCAD			P-value[2]		
Item	Cholec	Calcidiol	Cholec	Calcidiol	SEM	DCAD	Vitamin D	DCAD x Vitamin D
Colostrum yield, kg	5.86	7.68	6.21	7.96	1.06	0.77	0.10	0.97
Fat, %	4.02	5.37	5.40	4.24	0.54	0.83	0.87	0.02
Fat, kg	0.25	0.43	0.30	0.39	0.08	0.93	0.12	0.58
Protein, %	11.9	15.8	14.9	14.9	0.89	0.23	0.03	0.04
Protein, kg	0.66	1.20	0.88	1.17	0.17	0.57	0.02	0.47
Lactose, %	2.94	2.47	2.49	2.41	0.14	0.07	0.05	0.16
Lactose, kg	0.18	0.19	0.17	0.19	0.03	0.87	0.50	0.82
SNF, %	16.8	20.6	19.6	19.6	0.86	0.27	0.03	0.03
5.6	0.95	1.57	1.19	1.55	0.22	0.62	0.03	0.56
TS, %	20.9	26.0	25.1	23.9	1.00	0.31	0.05	0.002
TS, kg	1.21	2.00	1.49	1.94	0.29	0.70	0.04	0.55
Net Energy, Mcal/kg	1.16	1.48	1.44	1.33	0.07	0.35	0.10	0.001
Net Energy, Mcal	**6.7**	**11.5**	**8.4**	**11.0**	**1.7**	**0.73**	**0.04**	0.53
Urea N, mg/dL	35.2	39.5	35.4	38.7	2.3	0.90	0.10	0.82
IgG, g/L	45.3	57.7	50.6	60.1	3.8	0.31	0.005	0.70
Somatic cell score	6.45	6.96	6.38	7.18	0.44	0.87	0.14	0.74

Martinez et al. 2018a. J. Dairy Sci. 101:2544-2562.

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Prepartum Calcidiol Increased Colostrum Net Energy Yield

Effect of source and amount of vitamin D fed prepartum on colostrum

	Treatment[1]								P-value[2]			
	CHOL		CAL			Parity						
Item	1 mg	3 mg	1 mg	3 mg	SEM	Nulliparous	Parous	SEM	Source	Amt	Source× Amt	Parity
Yield, kg	4.8	4.4	5.9	5.2	0.6	4.5	5.7	0.4	0.10	0.35	0.80	0.04
Fat, %	5.7	6.3	6.2	6.8	0.4	7.6	4.9	0.3	0.20	0.14	0.99	<0.001
Fat, kg	**0.29**	**0.28**	**0.41**	**0.38**	**0.05**	**0.37**	**0.31**	**0.04**	**0.04**	**0.68**	**0.78**	**0.24**
Protein, %	14.4	14.0	14.7	13.7	0.4	13.8	14.6	0.3	0.89	0.06	0.50	0.07
Protein, kg	0.69	0.60	0.86	0.71	0.08	0.62	0.81	0.06	0.08	0.15	0.68	0.03
Lactose, %	3.33	3.24	3.24	3.40	0.08	3.35	3.26	0.06	0.72	0.64	0.11	0.30
Lactose, kg	0.16	0.14	0.20	0.18	0.02	0.15	0.19	0.02	0.10	0.38	0.85	0.06
SNF, %	19.11	18.64	19.32	18.57	0.04	18.62	19.21	0.03	0.85	0.10	0.70	0.15
SNF, kg	0.88	0.81	1.14	0.96	0.11	0.83	1.07	0.08	0.06	0.25	0.64	0.04
TS, %	24.8	25.1	25.6	25.6	0.6	26.2	24.3	0.5	0.27	0.78	0.75	0.006
TS, kg	**1.20**	**1.07**	**1.58**	**1.31**	**0.16**	**1.21**	**1.37**	**0.12**	**0.05**	**0.20**	**0.64**	**0.38**
Net energy, Mcal/kg	1.50	1.53	1.56	1.57	0.05	1.65	1.44	0.03	0.28	0.71	0.83	<0.001
Net energy, Mcal	**7.3**	**6.7**	**9.6**	**8.3**	**1.0**	**7.7**	**8.3**	**0.7**	**0.05**	**0.34**	**0.73**	**0.53**
SCS	7.2	7.2	7.2	7.1	0.2	7.5	6.9	0.2	0.83	0.89	0.90	0.03
IgG, g/L	108	110	114	103	6	97	120	5	0.94	0.48	0.33	<0.001
IgG, g	494	477	634	505	65	426	629	55	0.18	0.25	0.38	0.003

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Prepartum Calcidiol Increased Milk Yield

Effect of DCAD and source of vitamin D fed prepartum on performance in the first 49 d postpartum in Holstein cows

	Positive DCAD		Negative DCAD			P-value		
Item	Cholec	Calcidiol	Cholec	Calcidiol	SEM	DCAD	Vitamin D	DCAD x Vitamin D
Yield, kg/d								
Milk	**31.5**	**35.4**	**31.4**	**34.9**	1.4	0.79	**0.008**	0.90
3.5% FCM	**37.0**	**40.1**	**37.5**	**41.9**	1.8	0.50	**0.04**	0.72
ECM	**35.6**	**38.6**	**36.0**	**40.4**	1.7	0.53	**0.03**	0.68
Fat								
%	4.56	4.37	4.62	4.77	0.12	0.05	0.89	0.15
Yield, kg	1.43	1.53	1.46	1.66	0.81	0.33	0.07	0.54
True protein								
%	3.16	3.10	3.14	3.25	0.09	0.48	0.73	0.36
Yield, kg	0.98	1.07	0.97	1.11	0.06	0.82	0.06	0.70
Lactose								
%	4.70	4.74	4.77	4.76	0.04	0.24	0.73	0.59
Yield, kg	1.49	1.67	1.54	1.67	0.07	0.78	0.03	0.73
Somatic cell score	2.30	2.51	2.25	2.77	0.36	0.77	0.31	0.67

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Martinez et al. 2018a. J. Dairy Sci. 101:2544-2562.

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Prepartum Calcidiol Increased Milk Yield

Effect of source and amount of vitamin D fed prepartum on lactation performance.

	Treatment[1]								P-value[2]			
	CHOL		CAL			Parity						
Item	1 mg	3 mg	1 mg	3 mg	SEM	Nulliparous	Parous	SEM	Source	Amt	Source × Amt	Parity
Milk, kg/d[3]	**36.9[ab]**	**34.1[a]**	**36.4[ab]**	**38.7[b]**	**1.39**	**31.2**	**41.8**	**1.3**	**0.12**	**0.85**	**0.05**	**<0.001**
ECM, kg/d[4]	**37.5**	**35.1**	**38.1**	**39.9**	**1.47**	**32.4**	**42.8**	**1.4**	**0.06**	**0.84**	**0.14**	**<0.001**
Fat, %	4.33	4.53	4.68	4.58	0.11	4.66	4.40	0.09	0.08	0.69	0.18	0.06
Fat, kg/d	1.45	1.33	1.49	1.54	0.07	1.29	1.62	0.06	0.07	0.63	0.19	<0.001
Protein, %	3.24	3.26	3.42	3.27	0.06	3.31	3.29	0.04	0.12	0.29	0.14	0.74
Protein, kg/d	1.08	0.97	1.10	1.12	0.05	0.92	1.21	0.04	0.12	0.41	0.26	<0.001
Lactose, %	4.73	4.72	4.61	4.67	0.04	4.73	4.64	0.03	0.06	0.48	0.32	0.06
Lactose, kg/d	1.61	1.46	1.53	1.65	0.08	1.34	1.78	0.06	0.50	0.83	0.07	<0.001
SCS	2.79	3.57	3.39	3.09	0.31	3.55	2.87	0.28	0.82	0.39	0.05	0.10

Poindexter 2021 PhD Dissertation, Univ. Florida

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Department of Animal Sciences

Choline: An Essential Nutrient for Dairy Cows

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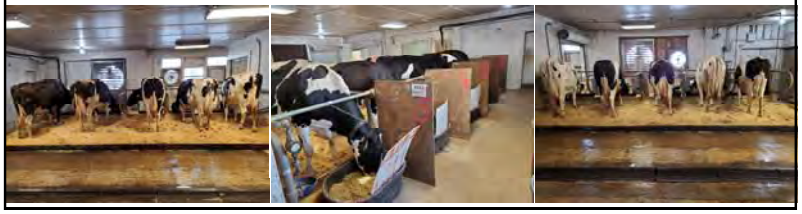
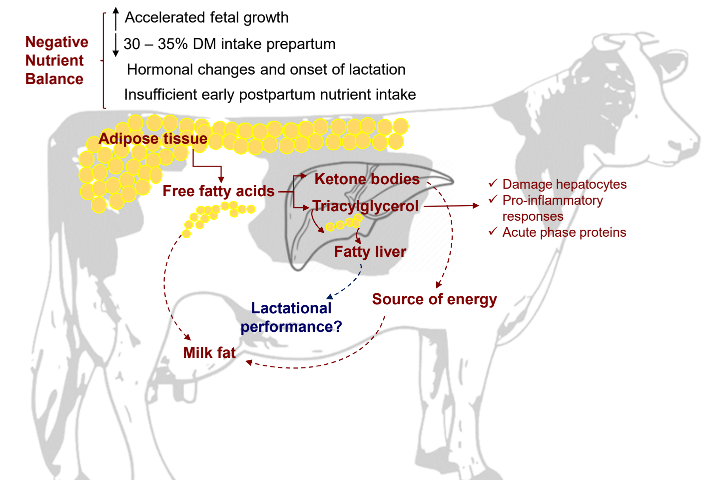


1

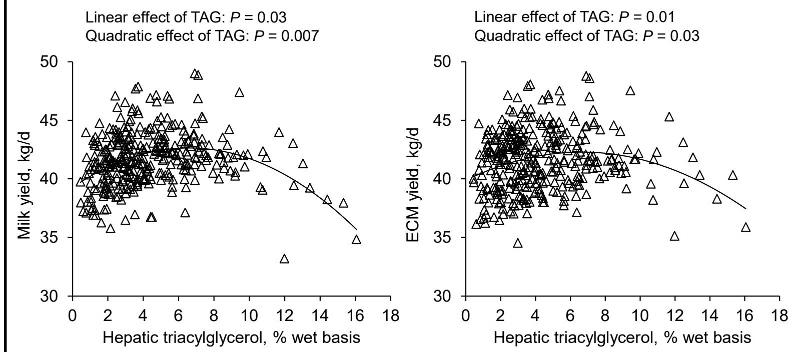
Biology of Transition Cow



Adapted from Drackley (1999) J. Dairy Sci. 82:2259-2273

2

Hepatic Triacylglycerol and Yields of Milk and ECM



Arshad and Santos (2022) J. Dairy Sci. 104:1-17

3

Hepatic Triacylglycerol and Incidence of Diseases and Survival

	Hepatic triacylglycerol, % wet-basis			*P*-value	
Item	2.5	5.0	7.5	Linear	Quadratic
Clinical diseases, %					
Retained placenta	9.3 ± 2.7	11.9 ± 3.2	15.1 ± 4.7	0.12	---
Metritis	12.5 ± 3.4	18.2 ± 4.5	25.7 ± 6.8	0.01	---
Puerperal metritis	6.7 ± 2.3	10.2 ± 3.4	13.5 ± 5.4	0.07	---
Mastitis	14.2 ± 3.2	16.9 ± 3.3	19.9 ± 4.5	0.15	---
Morbidity	36.2 ± 5.0	41.3 ± 5.4	46.7 ± 6.9	0.10	---
Multiple diseases	8.7 ± 2.9	13.7 ± 4.1	21.1 ± 6.6	0.01	---
Left the herd by 300 DIM, %	8.9 ± 1.9	11.0 ± 1.8	13.7 ± 2.8	0.10	---
Subclinical diseases, %					
Hyperketonemia	15.2 ± 3.1	24.7 ± 3.8	37.5 ± 5.6	< 0.01	---
Hypocalcemia	30.3 ± 6.5	40.8 ± 7.1	52.4 ± 8.1	< 0.01	---

Arshad and Santos (2022) J. Dairy Sci. 104:1-17

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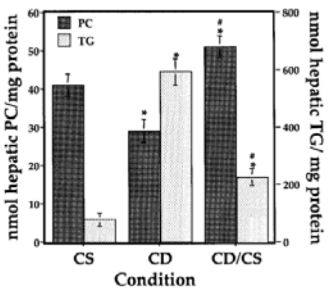
Choline: An Essential Nutrient

- ✓ Choline is an essential nutrient for all mammals; required for the normal function of cells
- ✓ In early 90's, it was well established that dietary choline is completely degraded by rumen microbes and, that's why, choline must be fed in a rumen-protected form to dairy cows
- ✓ Choline deficiency results in the development of fatty liver in most species

Taylor et al. (2018) Nut. Today 53:240-253
Sharma and Erdman (1989) J. Dairy Sci. 72:2772-2776



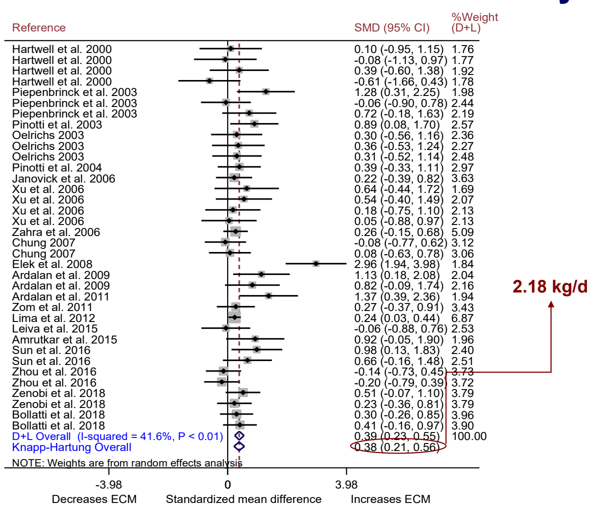
Waite et al. (2002). J. Nutr. 132:68-71

Nutrient Requirements of Dairy Cattle (NRC, 2021)

- ✓ NRC (2021) currently recommends a minimum 1,000 mg of choline per kilogram dry matter of milk replacer for calves
- ✓ However, the recommended amount of choline, either for the dry or lactating dairy cow, has not been established

5

Effect of RPC on ECM Yield: A Meta-Analysis



Arshad et al. (2020) J. Dairy Sci. 103:282–300

6

Effect of RPC on Health: A Meta-Analysis

Least squares means and respective SEM for incidence of clinical diseases according to supplementation of choline ion when fed during transition period in dairy cows

Item	Means (Exp.),[2] n	Treatment[1] Control	Choline	*P*-value
Retained placenta	38 (11)	10.6 ± 2.9	7.5 ± 2.2	0.06
Metritis	28 (09)	11.7 ± 2.2	8.7 ± 1.8	0.19
Mastitis	34 (11)	14.8 ± 3.0	11.7 ± 2.5	0.09
Milk fever	38 (11)	2.5 ± 1.5	1.5 ± 0.9	0.23
Displaced abomasum	38 (11)	6.0 ± 1.7	5.2 ± 1.5	0.67
Ketosis	36 (10)	12.0 ± 3.0	12.1 ± 3.0	0.96
Disease cases/cow	40 (12)	0.55 ± 0.1	0.48 ± 0.1	0.23

[1]Treatment as a categorical parameter in the statistical models (not supplemented vs. supplemented) because the majority of experiments that reported diseases supplemented choline ion at 12.9 g/d. The mean (± SD) amounts of supplemental choline for experiments reporting data on health was 13.3 ± 2.6.

[2]Number of treatment means (experiments) that contributed data for statistical analyses.

Arshad et al. (2020) J. Dairy Sci. 103:282–300

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Hypotheses

- ✓ Rumen-protected choline (RPC) reduces hepatic triacylglycerol and increases glycogen contents during induction of fatty liver in Holstein dairy cows
- ✓ The reduction in hepatic triacylglycerol content is mediated by increasing triglyceride-rich lipoprotein secretion rate

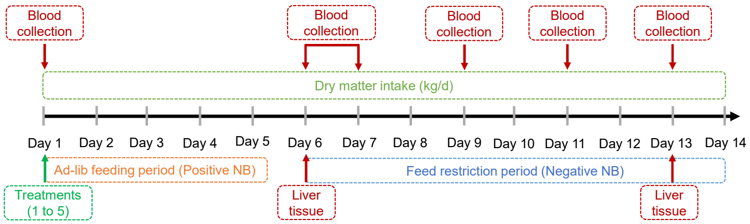
Objectives

- ✓ Determine the effect of sources of RPC with a low (28.8%) or high concentration (60.0%) of choline chloride when fed as 0, 12.9 or 25.8 g/d of choline ion
  - ✓ Hepatic composition and mRNA
  - ✓ Blood metabolites
  - ✓ Secretion rate of triglyceride-rich lipoprotein

8

Experimental Design

- ✓ Randomized complete block design
- ✓ Dry pregnant Holstein cows (n = 110)
- ✓ 232 ± 3.9 days of gestation
- ✓ Blocked by BCS (4.02 ± 0.50)



- ✓ **1) CON** = 0 g of choline ion
- ✓ **2) L12.9** = 12.9 g/d of choline ion as RPC form with a low concentration (28.8% choline chloride)
- ✓ **3) L25.8** = 25.8 g/d of choline ion as RPC form with a low concentration (28.8% choline chloride)
- ✓ **4) H12.9** = 12.9 g/d of choline ion as RPC form with a high concentration (60% choline chloride)
- ✓ **5) H25.8** = 25.8 g/d of choline ion as RPC form with a high concentration (60% choline chloride)

9

Statistical Analyses

✓ Data were analyzed by ANOVA using mixed-effects models in SAS

$$Y = \mu + TRT + Day + TRT \times Day + Cov + BCS_0 + BW_0 + Twin + \mathit{Cow}\,(\mathit{TRT}) + \mathit{BLK} + e$$

- ✓ μ = Overall mean
- ✓ TRT = Fixed effect of treatments
- ✓ Day = Fixed effect of day
- ✓ TRT x Day = Fixed effect of the interaction between TRT and day
- ✓ COV = Fixed effect of linear covariate value for blood metabolites
- ✓ BCS, BW, Twin = fixed effects of the respective values start of experiment
- ✓ *Cow (TRT)* = Random effect of cow nested within treatment
- ✓ *BLK* = Random effect of block
- ✓ e = random residual

✓ Ad libitum and feed-restricted periods analyzed separately

✓ Orthogonal contrasts
- ✓ Effect of RPC [CON vs. ¼·(L12.9 + L25.8 + H12.9 + H25.8)]
- ✓ Effect of amount [½·(L12.9 + H12.9) vs. ½·(L25.8 + H25.8)]
- ✓ Effect of source [½·(L12.9 + L25.8) vs. ½·(H12.9 + H25.8)]
- ✓ Effect of interaction [½·(L12.9 + H25.8) vs. ½·(H12.9 + L25.8)]

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Energy Measures, and Hepatic Metabolism

	Treatment						*P*-value			
Item	CON	L12.9	L25.8	H12.9	H25.8	SEM	RPC	Source	Amount	S x A
NE intake, Mcal/d	7.44	7.42	7.19	7.69	7.69	0.21	0.72	0.02	0.48	0.47
Blood metabolites										
Fatty acids, m*M*	0.94	0.94	0.92	0.93	0.86	0.06	0.64	0.36	0.35	0.56
β-hydroxybutyrate, m*M*	0.96	0.90	0.87	0.90	0.89	0.06	0.20	0.90	0.63	0.85
Hepatic composition, as-is										
Triacylglycerol, %	9.32	6.59	5.05	6.61	6.00	0.55	< 0.01	0.28	0.02	0.29
Glycogen, %	1.83	2.59	3.55	3.13	4.07	0.18	< 0.01	< 0.01	< 0.01	0.91
Hepatic mRNA, fold change										
BHMT	1.00	1.19	1.04	1.30	1.19	---	0.05	0.10	0.12	0.74
MTTP	1.00	1.13	1.16	1.03	1.42	---	0.09	0.42	0.05	0.07
ATG3	1.00	1.18	1.12	1.09	1.11	---	0.01	0.31	0.64	0.37
ERN1	1.00	0.85	0.88	0.82	0.83	---	0.02	0.47	0.67	0.85

Arshad et al. (2020) J. Dairy Sci. (Suppl. 1) Abstr. 103

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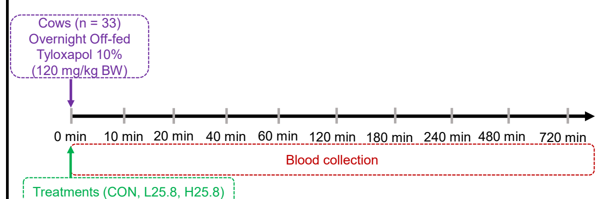
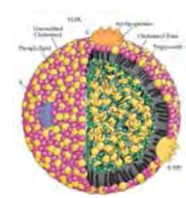
Experimental Design Day 14 – Secretion Rate of Triglyceride-rich Lipoprotein

✓ Rumen-protected choline reduces hepatic triacylglycerol content by increasing triglyceride-rich lipoprotein secretion rate

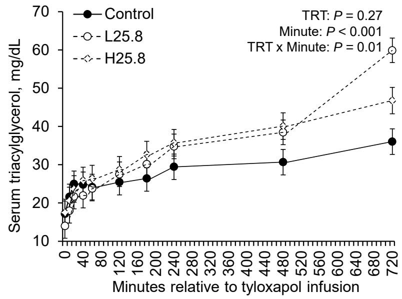




✓ Blood was assayed to determine concentrations of serum triacylglycerol after tyloxapol infusion

12

Effect of RPC on Hepatic Secretion of Triglyceride-Rich Lipoprotein



Item	CON	L25.8	H25.8	SE	RPC	Source
AUC triacylglycerol, mg/dL/min	21,747	32,323	28,699	3,706	0.03	0.43
Hepatic mRNA, fold change						
MTTP	1.00	0.98	1.58	---	0.10	0.01
ATG3	1.00	1.10	1.12	---	0.08	0.79
PLIN2	1.00	0.66	0.62	---	0.03	0.80

Arshad et al. (2022) J. Dairy Sci. Abstr. (Accepted)

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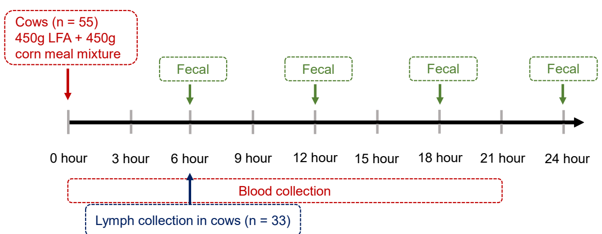
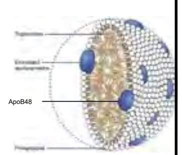
Effect of RPC on Serum and Lymph Metabolites After Fat Feeding





- ✓ Blood was assayed to assess serum triacylglycerol
- ✓ Lymphatic fluid was collected to quantify
  - ✓Free fatty acids; β-hydroxybutyrate; glucose; and triacylglycerol

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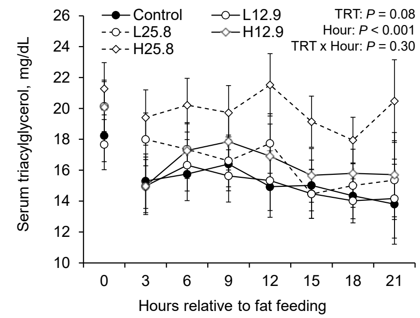
Effect of RPC on Serum and Lymph Metabolites After Fat Feeding



Item	CON	L25.8	H25.8	SE	RPC	Source
Lymph metabolites						
Free fatty acids, m*M*	0.71	0.65	0.69	0.07	0.69	0.68
β-hydroxybutyrate, m*M*	0.77	0.79	0.83	0.06	0.57	0.61
Glucose, m*M*	2.66	2.69	3.13	0.16	0.23	0.07
Triacylglycerol, mg/dL	11.4	15.7	15.9	3.4	0.08	0.98

Arshad et al. (2022) J. Dairy Sci. Abstr. (Accepted)

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Conclusions

- ✓Hepatic lipidosis beyond 4 to 7% hepatic triacylglycerol was associated with impaired production, health, and survival
- ✓Supplementing choline ion as RPC during the transition period improved productive performance
- ✓Supplementation of RPC
  - ✓Reduced hepatic triacylglycerol, and increased hepatic glycogen
  - ✓Enhanced the rate of secretion of triglyceride-rich lipoprotein to facilitate the export of triacylglycerol
  - ✓Increased concentrations of serum or lymphatic triacylglycerol after fat feeding
- ✓Feeding RPC during negative nutrient balance promotes lipotropic effects independent of source that reduces the risk of fatty liver in dairy cows

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Acknowledgements

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- Dairy Research Unit, UF

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- Karla Ferreira
- Iago Leão
- Kleves Almeida
- Vitória Camargo
- Félix Welter

Pictures by Bonnie Mohr http://www.bonniemohr.com/

17

Florida Ruminant Nutrition Symposium – 2022

Nitrogen efficiency as predictor of production performance in commercial dairy herds

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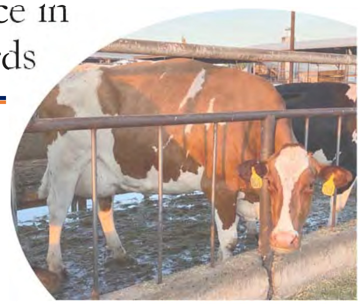
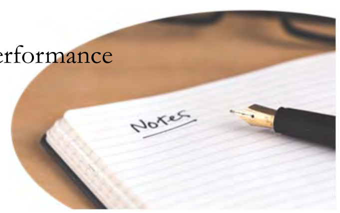
Department of Animal Sciences, University of Florida, Gainesville, FL, USA



1

Outline

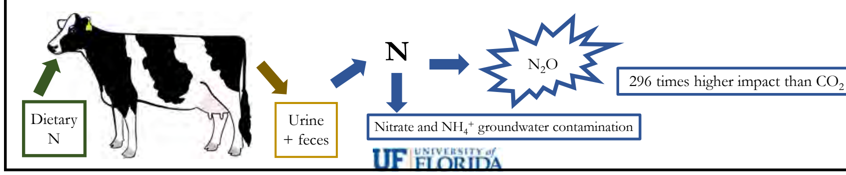
- Introduction
  - Nitrogen Utilization Efficiency: Challenges
  - Research Rationale
  - Hypothesis and Objectives
- Material and Methods
  - Data Collection from Commercial Dairies
  - Statistical Modeling
- Results
  - Optimal NUE for Maximizing Lactation Performance
  - Diet Composition and NUE
- Conclusion
- Future Directions



2

Introduction

- Nitrogen utilization efficiency (**NUE**; milk N/N intake) is typically low in dairy cattle
  - 20 to 35% (Chase et al., 2009)
- Excreted dietary N is lost in the form of NH_3, N_2O and NO_3
  - Contributes to acidification, eutrophication, and climate change and may negatively affect human health (Groenestein et al., 2019)



3

Introduction

- Increased N efficiency may have production and economic benefits to dairy farming
  - Milk yield and income over feed cost (**IOFC**) of Canadian dairy farms

Item	Cluster				SE	*P*-value
	NE22	NE27	NE30	NE36		
Milk yield, kg/d	28.7^{b}	31.1^{a}	31.9^{a}	32.5^{a}	0.82	<0.01
IOFC, $/cow per day	14.3^{c}	16.4^{bc}	17.2^{ab}	18.2^{a}	0.55	<0.001

NE22; nitrogen efficiency 22%, NE27; nitrogen efficiency 27%, NE30; nitrogen efficiency 30%, NE36; nitrogen efficiency 36%,

Adapted from Fadul-Pacheco et al., 2017

Research Rationale

Determining optimal nitrogen utilization efficiency is crucial to enhance the profitability and environmental performance indicators of commercial dairy farms

4

Introduction

Hypothesis:

Nitrogen utilization efficiency (**NUE**) can be used as a production performance indicator for dairy herds

Objectives:

To evaluate the association between milk production and NUE

To estimate the NUE that maximizes milk production

To evaluate dietary factors affecting NUE in dairy cows

5

Materials and Methods

California – Jun to Aug 2020
- 13 dairy farms
- 143 pens
- Over 30,000 cows
- Over 200 feed samples

Texas – Nov to Feb 2020/2021
- 10 dairy farms
- 139 pens
- Over 30,000 cows
- Over 180 feed samples

Florida – Oct to to date 2020/2021
- 5 dairy farms
- 25 pens
- About 6,000 cows
- Over 80 feed samples

Farm selection criteria

- Individual milk yield was measured
- Individual milk components were analyzed
- Daily feed offered and orts were recorded (average pen DMI – EZFeed, FeedWatch)
- Ingredient composition of total mixed ration was available
- Cow information was available (DIM, parity, pen at the day of milk test, pregnancy status – DC305, DHIplus, PCDart)

6

Sampling Schedule

Milk test day

-4d | 0d | 4d

1st visit – information on:
-Feeding times
-Pen/diet allocation
-Diet composition
-Survey

Feed sample collection
- 4 to 4 d relative to test

-Backup
-Milk test results

Feed analysis

- Diet ingredients were dried, ground (1 mm) and pen diets were reconstituted
- Reconstituted pen diets were analyzed for CP, aNDF, ADF, Starch, EE and WSC
- Nitrogen utilization efficiency was calculated based on pen average N intake and cow milk composition

7

Statistical Analysis

The MIXED procedure of SAS was used (v. 9.4, SAS Institute Inc., Cary, NC)

The model included;

Y = NEL + NEQ + Par + LS + (NEL × Par) + (NEQ × Par) + (NEL × LS) + (NEQ × LS)

Y= dependent variable;
NEL = linear covariate of NUE;
NEQ = quadratic covariate of NUE;
Par = cow parity (primiparous *vs.* multiparous);
LS = lactation stage (early-, mid-, late-lactation);
Interactions

Pen nested within herd and location was used as random effect

A stepwise backward elimination was used to remove non-significant interactions one predictor at a time based on the largest *P*-value.

Full model: MY = NEL + NEQ + Par + LS + (NEL × Par) + (NEQ × Par)+ (NEL × LS) + (NEQ × LS)

After analyzing the model once :
MY = NEL + NEQ + Par + LS + (NEL × Par) + (NEQ × Par) + (NEL × LS) + (NEQ × LS)

Remove interaction with largest *P*-value

8

Statistical Analysis

The MIXED procedure of SAS was used (v. 9.4, SAS Institute Inc., Cary, NC)

The model included;

Y = NEL + NEQ + Par + LS + (NEL × Par) + (NEQ × Par) + (NEL × LS) + (NEQ × LS)

Y= dependent variable;
NEL = linear covariate of NUE;
NEQ = quadratic covariate of NUE;
Par = cow parity (primiparous *vs.* multiparous);
LS = lactation stage (early-, mid-, late-lactation);
Interactions

Pen nested within herd was used as random effect

A stepwise backward elimination was used to remove non-significant interactions one predictor at a time based on the largest *P*-value.

Full model: MY = NEL + NEQ + Par + LS + (NEL × Par) + (NEQ × Par) + (NEL × LS) + (NEQ × LS)

Rerun the model: MY = NEL + NEQ + Par + LS + (NEQ × Par) + (NEL × LS) + (NEQ × LS)

Final model: MY = NEL + NEQ + Par + LS

9

Results

Table 1. Mean, minimum, maximum and standard deviation of 285 pens (total # cows 70461)

Item	N	Mean	Minimum	Maximum	SD
Herd	23	3063	320	5462	1614
Pen size	285	247	24	597	110
Parity	285	2.15	1	4.41	0.88
DIM	285	164	7	393	86
DMI, kg/d	285	24.96	10.0	33.8	3.6
Milk yield, kg/d	285	36.9	16.2	62.5	8.1
3.5% FCM, kg/d	285	40.2	18.2	65.5	7.6
ECM, 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	12.45	6.7	28.74	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
NUE, %	285	27.95	14.2	51.47	4.94

Production variables are averages of each pen included in the model
$Nitrogen utilization efficiency calculated as milk N (from true protein) divided by N intake

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Results

Table 2. Mean, minimum, maximum and standard deviation of 61 lactating cow diets

Item, % of DM	N	Mean	Minimum	Maximum	SD
CP	61	17.14	11.48	20.69	1.41
Starch	61	22.38	10.72	28.71	3.99
aNDFom	61	28.14	23.25	34.15	2.72
ADF	61	19.66	14.76	24.87	2.19
Lignin	61	3.58	1.68	5.14	0.92
WSC	61	7.73	3.10	13.71	2.39
EE	61	5.35	2.93	7.11	0.86
Ash	61	7.97	5.50	11.44	1.32

Diets were reconstituted with individual ingredients collected in each farm and analyzed with wet chemistry method

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Association Between NUE and Production Parameters

Table 3. Association between production parameters and nitrogen utilization efficiency in dairy cows

Item	*P*-values							
	NE	Parity	DIM	NE × NE	NE×P	NE×NE×P	NE×D	NE×NE×D
Milk yield, kg/d	<0.01	<0.01	<0.01	<0.01	NS	NS	NS	NS
ECM, kg/d	<0.01	<0.01	<0.01	NS	NS	NS	<0.01	NS
3.5%FCM, kg/d	<0.01	<0.01	<0.01	NS	<0.01	<0.01	NS	NS
Protein, %	<0.01	0.9074	<0.01	NS	NS	NS	NS	NS
Fat, %	<0.01	0.4926	<0.01	NS	NS	NS	NS	NS
Protein yield, kg/d	0.1992	<0.01	<0.01	NS	<0.01	<0.01	NS	<0.01
Fat Yield, kg/d	0.4719	<0.01	<0.01	NS	<0.01	<0.01	NS	<0.01

[1, 2, 3] NE, P and LS defined as nitrogen utilization efficiency, parity and lactation state, respectively.

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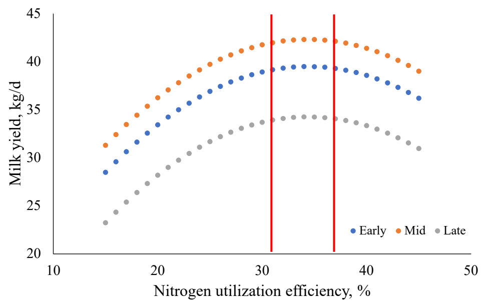
Milk Yield



NE ($P < 0.01$)
NE × NE ($P < 0.01$)
Parity ($P < 0.01$)
Lactation stage ($P < 0.01$)

Figure 1. Daily milk yield in early, mid and late lactation dairy cows according to nitrogen utilization efficiency (NUE).

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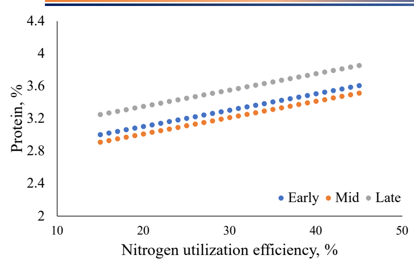
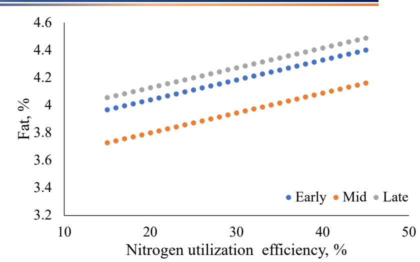
Milk Components



NE covariate ($P < 0.01$)
Parity ($P = 0.91$)
Lactation stage ($P < 0.01$)

Figure 2. Daily milk protein percentage in early, mid and late lactation dairy cows according to nitrogen utilization efficiency (NUE).



NE covariate ($P < 0.01$)
Parity ($P = 0.49$)
Lactation stage ($P < 0.01$)

Figure 3. Daily milk fat percent in early, mid and late lactation dairy cows according to nitrogen utilization efficiency (NUE).

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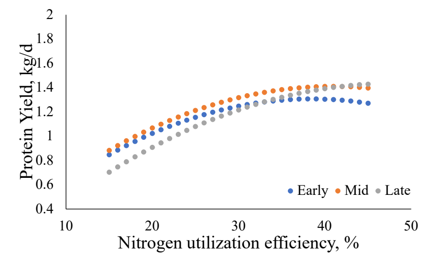
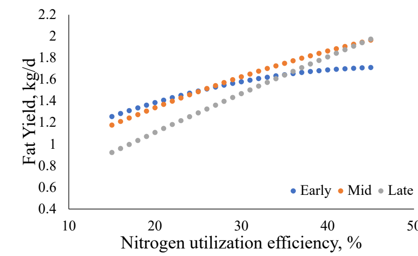
Milk Components Yield



NE covariate ($P < 0.20$)
Parity ($P < 0.01$)
Lactation stage ($P < 0.01$)
NE × P ($P < 0.01$)
NE × NE × P ($P < 0.01$)
NE × NE × LS ($P < 0.01$)

Figure 4. Daily milk protein yield in early, mid and late lactation dairy cows according to nitrogen utilization efficiency (NUE).



NE covariate ($P = 0.47$)
Parity ($P < 0.01$)
Lactation stage ($P < 0.01$)
NE × P ($P < 0.01$)
NE × NE × P ($P < 0.01$)
NE × NE × LS ($P < 0.01$)

Figure 5. Daily milk fat yield in early, mid and late lactation dairy cows according to nitrogen utilization efficiency (NUE).

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Correlation of NUE and diet components

Table 4. Pearson correlation coefficients for N utilization efficiency and diet composition

Parameter	Nitrogen efficiency, %	Crude protein, %	Starch, %	aNDF, %	WSC, %
Nitrogen efficiency, %	1	-0.43	0.18	0.18	0.06 *
Crude protein, %		1	-0.26	-0.30	-0.14
Starch, %			1	-0.49	-0.50
aNDF, %				1	0.18
WSC, %					1

*P-value > 0.10
[1]WSC – water soluble carbohydrates

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Future Directions

- Evaluate income over feed costs of commercial pens used for data collection
- Estimate manure N excretion in pens
- Compare NRC and CNCPS models for N requirements using our dataset

17

Conclusions

Nitrogen utilization efficiency can be used as an indicator for milk yield and milk composition in dairy cows although some of the responses are dependent on lactation stage or parity of cows
Based on our association equations, NUE from 31 to 37% will yield greatest daily milk production
Dietary factors affecting NUE still warrant further evaluation

18

Acknowledgements

Dr. Diwakar Vyas
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Milk Check-Off
All dairy farmers who allowed us to sample their herds

Thank you!

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19

Assessing Feed Efficiency and its Association with Production, Health and Reproduction in Dairy Cows

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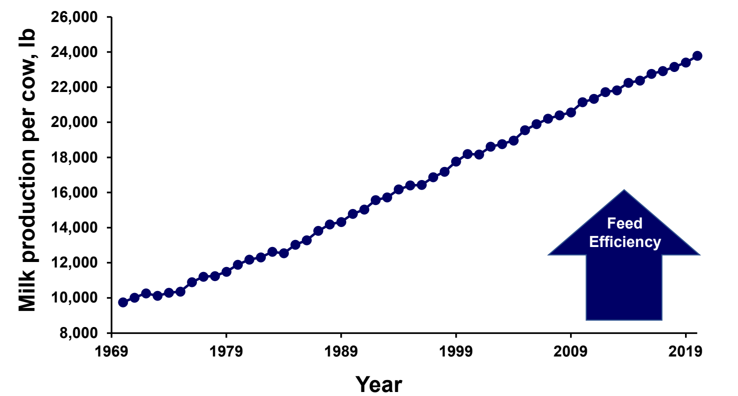
Department of Animal Sciences
University of Florida

1

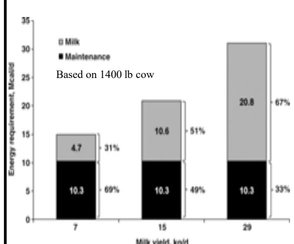
Milk Production Over the Years

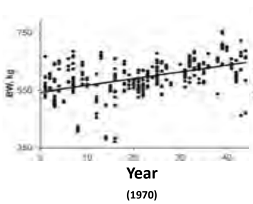


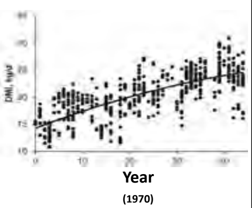
USDA-ERS, 2020

2

Feed Efficiency Over the Years

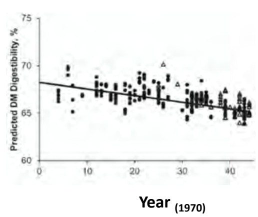






Capper et al. (2009) J. Animal Sci. 87:2160

- ✓ **Maintenance requirements increased over the years**
  - ✓ NRC (2001) and NASEM (2021)
    - ✓ 2001: Metabolic BW x 0.08 Mcal/d
    - ✓ 2021: Metabolic BW x 0.10 Mca/d

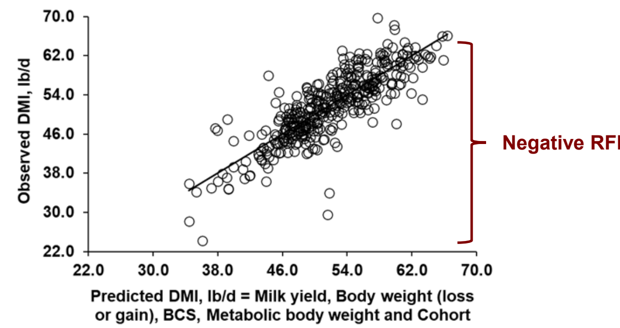


Potts et al. (2017) J. Dairy Sci. 100:5400–5410

3

Residual Feed Intake: A Better Trait to Measure Feed Efficiency

- ✓ **Residual feed intake (RFI) is a trait that directly measures feed conversion efficiency**
- ✓ **Differs from Gross Feed Efficiency (ECM/DMI):**
  - ✓ Energy required for production, maintenance, tissue accretion/loss, and adjusted for cohort

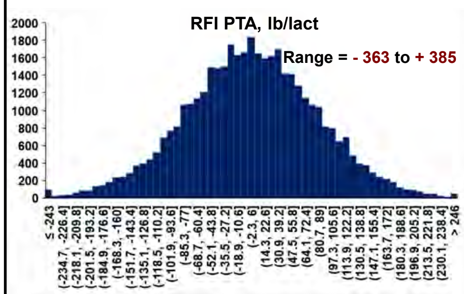


Nehme Marinho et al. (2021) J. Dairy Sci. 104: 5493-5507

4

Why Residual Feed Intake?

- ✓ **Increases profitability**
  - ✓ Feed represents more than 50% of total cost in dairy farms
- ✓ **Less land utilization for the same amount of production**
- ✓ **Candidate for selection:**
  - ✓ Genetic variability
  - ✓ Heritability = **0.14**



Trait	Heritability
Milk	0.20
Fat	0.20
Productive life	0.08
SCS	0.12
Udder composite	0.27
BW composite	0.40
Cow conception rate	0.02
Daughter pregnancy rate	0.04
Mastitis	0.031
Metritis	0.014

CDCB, 2022

VanRaden et al.,2021, USDA AIP report

5

Superior Cows?

- ✓ Is RFI repeatable across lactational stages?
  - ✓ Are the most efficient cows in early lactation also the most efficient cows in mid-lactation?
- ✓ Does selecting for RFI impact tissue mobilization, health and reproductive performance of dairy cows?
  - ✓ Decreasing intake will also reduce **energy balance**!
- ✓ **Main objectives:**
  - ✓ Determine the associations between ranking of RFI in mid-lactation with measures of body tissue mobilization, productive performance, incidence of diseases, and survival
  - ✓ Determine the associations between ranking of RFI and reproductive performance in lactating Holstein cows

6

Materials and Methods

✓ Study 1

- ✓ Retrospective cohort study
- ✓ Data from **399** cows, 154 primiparous and 245 multiparous cows
- ✓ Experimental freestall barn with individual feeding gates

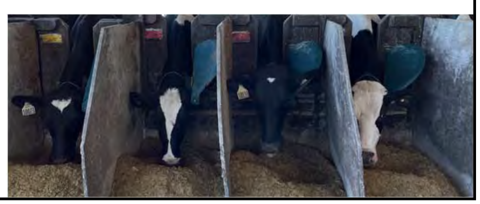
✓ Study 2

- ✓ Retrospective cohort study
- ✓ Data from **851** cows, 342 primiparous and 509 multiparous cows
- ✓ Experimental freestall barn with individual feeding gates

Linear model to predict DMI:

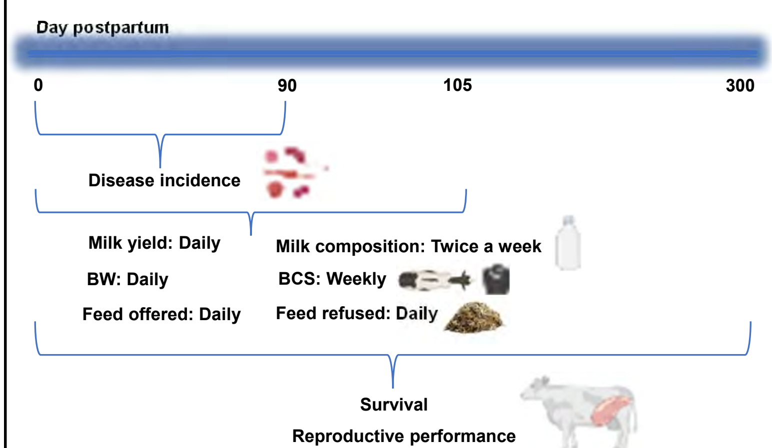
$$\text{DMI} = \mu + \text{milk energy} + \text{BW}^{0.75} + \text{body E change} + Trt(experiment) + e$$

$$\text{RFI} = \text{Observed} - \text{Predicted}$$



7

Measurements



8

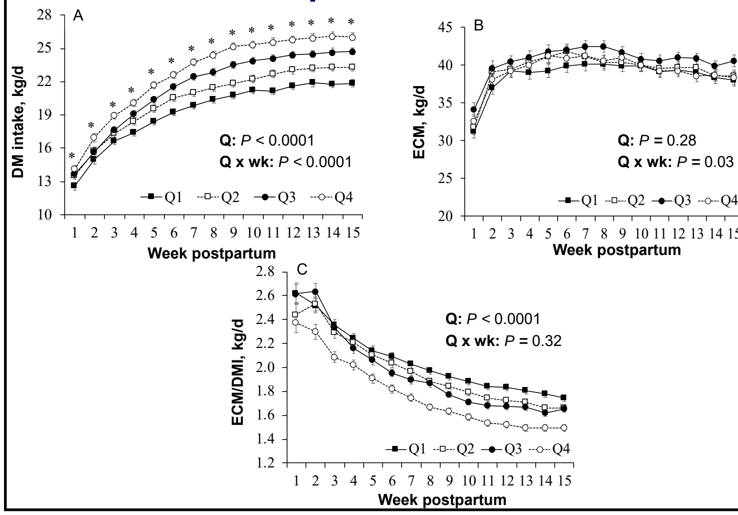
Association Between RFI and Performance in Early Lactation

	RFI in mid-lactation, quartiles					
Item, early lactation	Q1	Q2	Q3	Q4	SEM	P-value
Cows, n	98	98	99	98	---	---
DMI, kg/d	16.0	16.9	17.3	18.4	0.3	< 0.001
Energy-corrected milk, kg/d	37.2	38.5	39.4	38.3	0.8	0.32
Energy balance, Mcal/d	-9.0	-8.1	-8.2	-5.5	0.5	< 0.001
BW change, kg/d	-1.38	-1.38	-1.20	-1.20	0.11	0.41
Body condition, 1 to 5	3.35	3.31	3.30	3.21	0.03	0.001
Milk N, % N intake	39.6	38.8	39.7	36.0	0.7	< 0.001

Nehme Marinho et al. (2021) J. Dairy Sci. 104: 5493-5507

9

Association Between RFI and Performance up to 105 DIM



10

Association Between RFI with Incidence of Diseases and Survival

	RFI in mid-lactation, quartiles					
Item	Q1	Q2	Q3	Q4	SEM	P-value
Cows, n	98	98	99	98	---	---
Somatic cell score	2.38	2.66	2.83	2.66	0.19	0.41
Retained placenta, %	12.2	13.3	11.1	14.3	3.3	0.92
Metritis, %	13.3	19.4	17.2	22.5	4.0	0.40
Mastitis, %	15.3	13.3	12.1	15.3	3.5	0.89
Displaced abomasum, %	1.0	2.0	3.0	4.1	1.5	0.60
Lameness, %	10.2	5.1	2.0	8.2	2.4	0.14
Respiratory, %	2.0	3.1	1.0	2.0	1.4	0.81
Left herd by 300d, %	10.2	13.3	5.1	9.2	2.9	0.29

Nehme Marinho et al. (2021) J. Dairy Sci. 104: 5493-5507

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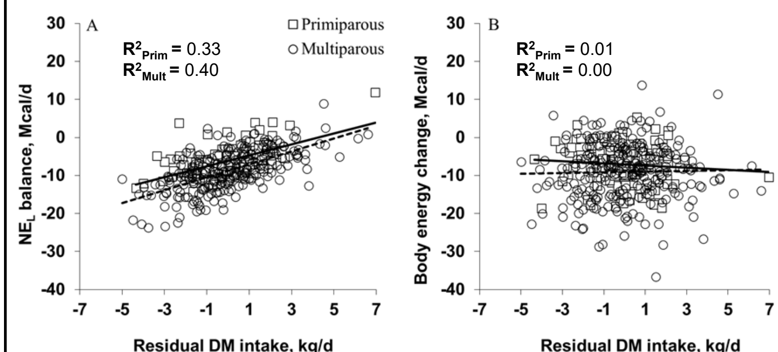
RFI in Early Lactation and Predictors of Energy Status



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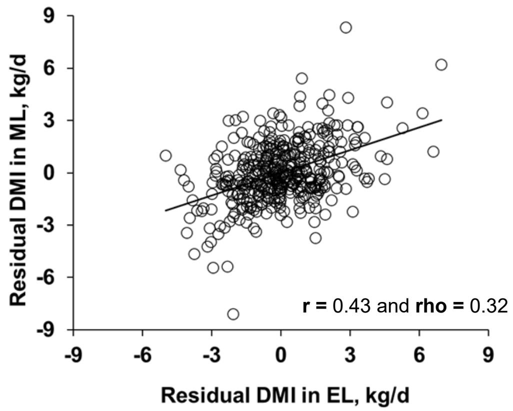
12

RFI is Repeatable Across Lactational Stages



Nehme Marinho et al. (2021) J. Dairy Sci. 104: 5493-5507

13

Association Between RFI and Reproductive Performance

Item	RFI in mid-lactation, quartiles Q1	Q2	Q3	Q4	SEM	P-value
Cows, n	212	213	213	213	---	---
Inseminated, %	98.4	99.1	97.7	99.1	0.8	0.7
First AI						
Pregnant d 74, %	31.0	30.9	30.5	26.5	3.5	0.72
Second AI						
Pregnant d 74, %	38.5	29.0	27.4	17.6	4.2	<0.001
Pregnancy per AI all AI, %	31.4	30.6	31.2	24.5	2.2	0.03
Pregnant by 300 d, %	79.0	80.7	82.4	71.5	3.3	0.05
21-d cycle pregnancy rate	21.2	21.1	22.0	16.6	1.9	0.02

Nehme Marinho and Santos (2022) Front. Anim. Sci. 3:847574

14

Can we Select for RFI?

- ✓ **Feed Saved (FSAV)**
  - ✓ Includes the economic values of cow body weight composite (BWC) with residual feed intake (RFI)
  - ✓ FSAV PTA represents the expected pounds of feed saved per lactation
- ✓ **Formulas:**

$PTA\ FSAV = -1(PTA\ RFI) - 151.8\ (PTA\ \text{BWC})$

$\text{BWC} = (0.23\ x\ stature) + (0.72\ x\ strength) + (0.08\ x\ body\ depth) + (0.17\ x\ rump\ width) - (0.47\ x\ dairy\ form)$: each unit represents 35 lb of mature BW

- ✓ **Example**

	Cow A	Cow B	Cow C
Weight (lb)	1500	1570	1430
BWC	0	+1.5	-1.5
Milk yield (lb/lact)	25,000	25,000	25,000
Expected DMI (lb/lact)	18,000	18,300	17,500
Actual DMI (lb/lact)	18,000	18,500	17,300
RFI (lb/lact)	0	+200	-200
Feed saved (lb/lact)	0	-428	+428

$PTA\ FSAV = -1(\mathbf{-200}) - 151.8\ (\mathbf{-1.5}) = \mathbf{+428}$ lb of feed saved per lactation

15

Acknowledgements

- ✓Dr. Adeoye Oyebade
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- ✓Richard Lobo
- ✓Dr. Roney Zimpel

FFAR Foundation for Food and Agriculture Research

CDCB COUNCIL ON DAIRY CATTLE BREEDING

UF IFAS Research UNIVERSITY of FLORIDA Department of Animal Sciences

The Foundation for The Gator Nation

16

2022 Florida Ruminant Nutrition Symposium
Gainesville, FL
May 9-11, 2022

Nutrient partitioning during immunological challenge

C. R. Krehbiel
Department of Animal Science
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Nebraska Lincoln

1

Pre and post weaning factors affecting immunity and subsequent feedlot health, performance and carcass quality

Preweaning	Postweaning
Prenatal nutrition	Transportation
Colostrum intake	Commingling
BVDV-PI	Receiving management
Preweaning health	Receiving diet
Preshipment management	Metaphylactic treatments

Galyean, Duff, and Rivera (2022)

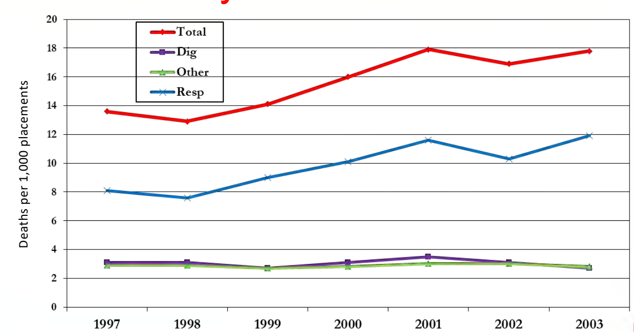
2

Stress (NASEM, 2016)

- Non-specific response of the body to any demand placed on it
- An abnormal or extreme adjustment in the physiology of an animal to cope with adverse effects of its environment and management
- The purpose of proper animal husbandry is to reduce the risk to the animal by stressors; weaning stress, commingling, environmental, handling, nutritional, and people stress
- Reduce the risk of clinical disease and enhance performance and carcass merit

3

Mortalities by cause



Loneragan (2004)

4

Etiology of Respiratory Disease

- Environmental stressors (Lillie, 1974; Duff and Galyean, 2007)
  - Exhaustion
  - Commingling
  - Other sick cattle
  - Climate
  - Feeding changes
  - Handling
  - Processing

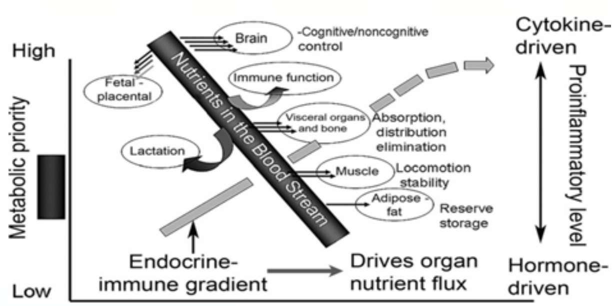
5

Pathogenesis

- Compromised immune system
- Viral infections precede bacterial
- Bacteria
  - Commensal
  - Spread from URT to lung
- Bronchopneumonia

6

Mechanistic effects of inflammation



(Hammond, 1952; Elsasser et al., 2008)

7

Feed intake

Dry matter feed intake of newly arrived calves (% of body weight)

Days from receiving	***Healthy (SD)***	***Sick (SD)***
0 to 7	1.55 (0.51)	0.90 (0.75)
0 to 14	1.90 (0.50)	1.43 (0.70)
0 to 28	2.71 (0.50)	1.84 (0.66)
0 to 56	3.03 (0.43)	2.68 (0.68)

Hutcheson and Cole (1986)

8

Energy

- Cattle requirements for energy
  - Net energy for maintenance (NE_m)
    - Metabolism (normal body functions)
  - Net energy for gain (NE_g)
    - Anabolism (muscle and fat deposition)
  - Negative energy balance
    - Catabolism (body wasting)
    - Low feed intake during receiving period

9

Metabolic Costs of the Immune Response

- Decreased ME intake has been associated with decreases in FHP in pigs (Labussière et al., 2011; Campos et al., 2014) and ruminants (Ferrell et al., 1986; Ferrell and Koong, 1987; McCurdy et al., 2010).
  - Decreased maintenance requirements
- Decreased DM intake associated with immune challenge
- During an immune challenge, an increase in ME_m was observed in pigs (Campos et al., 2014).
  - Resulted in decreased ME_g (3.3 to 1.7 Mcal/d) and subsequent protein and fat deposition.

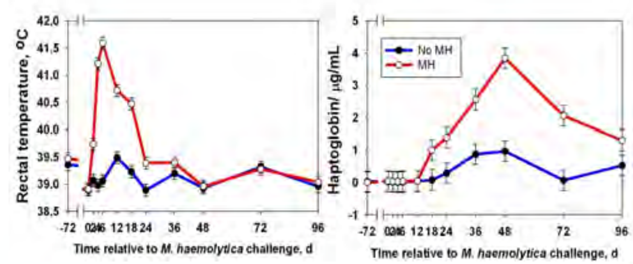
10

Metabolic Costs of the Immune Response

- Fever increases caloric demand 7 to 15% per degree Celsius increase in body temperature (Rauw, 2012).
  - Calories come from body stores.
- Increased fractional rate of protein synthesis in liver (141%) and plasma (161%) in chickens.
- Certain types of proteins are synthesized at accelerated rates, whereas many individual amino acids may be wasted for processes such as gluconeogenesis.
- Under stress animals allocate their limited resources between combating the stressor and maintaining other functions.

11

Effects of exposure to BVDV-PI type 1b and MH challenge on rectal temp and serum haptoglobin concentration



(Burciaga-Robles et al., 2010)

12

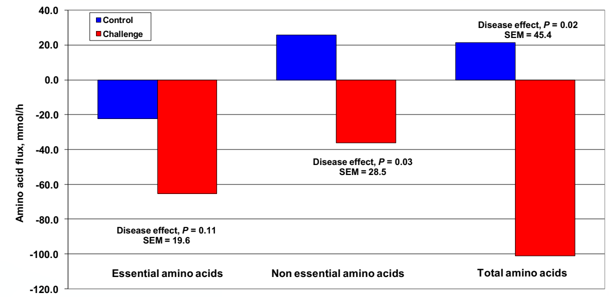
Arterial concentration of amino acids in steers fed or fasted with or without a *M. haemolytica* intratracheal challenge

	Disease			*P*-Value	
	Control	Challenge	SEM	Disease	Diet
Essential amino acids, µM	793	722	38.7	0.22	0.68
Non essential amino acids, µM	1173	924	46.2	<0.001	0.67
Total amino acids, µM	1966	1645	81.4	0.11	0.67

(Burciaga-Robles et al., 2011)

13

Amino acid hepatic flux in steers fed or fasted with or without a *M. haemolytica* intractracheal challenge



(Burciaga-Robles et al., 2011)

14

Amino acid hepatic flux in steers with or without a *M. haemolytica* intractracheal challenge

	Control	Challenge	*P*-value	SEM
Ornithine, mmol/h	-2.03	-19.01	0.08	7.03
Tryptophan, mmol/h	0.48	-2.01	0.04	0.87
Lysine, mmol/h	-2.03	-19.02	0.08	7.04
Tyrosine, mmol/h	-0.74	7.12	0.004	1.47
Phenylalanine, mmol/h	-2.44	-6.84	0.06	1.55
Histidine, mmol/h	-5.72	-13.94	0.03	2.57
Aspartic acid, mmol/h	-0.04	-0.91	0.04	0.31
Leucine, mmol/h	-2.44	-6.84	0.06	1.55
Glutamine, mmol/h	45.87	16.64	0.06	10.32

(Burciaga-Robles et al., 2011)

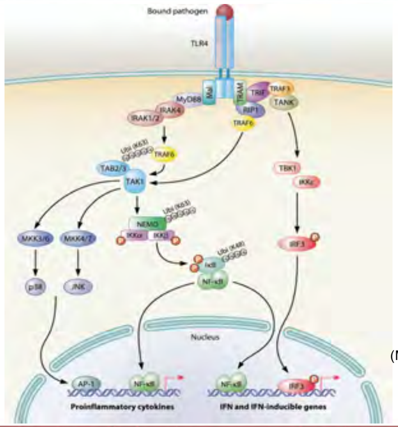
15

Signals that link pathogen sensing and growth biology

- Bacterial infection stimulates the production of a number of the proinflammatory cytokines, including interleukin-1 (IL-1), IL-6, and TNFα, by macrophages/monocytes and neutrophils (Matsumura et al., 2000).
- Subsequently, a wide variety of pathologic and host defense reactions are induced, such as fever, pain and synthesis of acute-phase proteins (Matsumura et al., 2000).

16

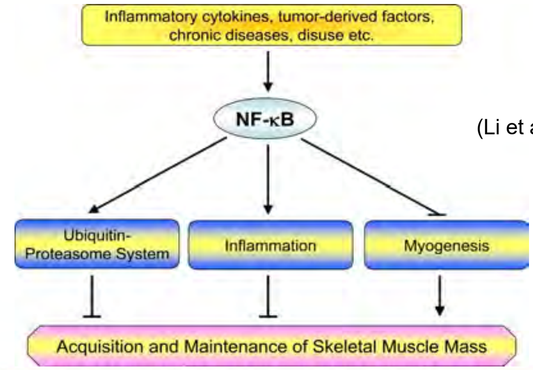
Principles in TLR signaling



(Mogensen, 2009)

17

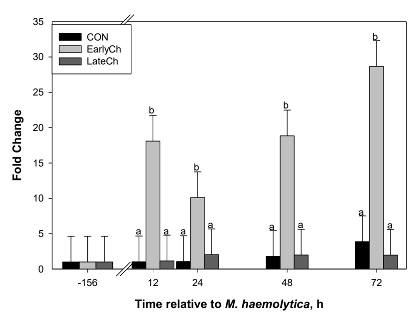
NF-κB and Muscle Catabolism



(Li et al., 2008)

18

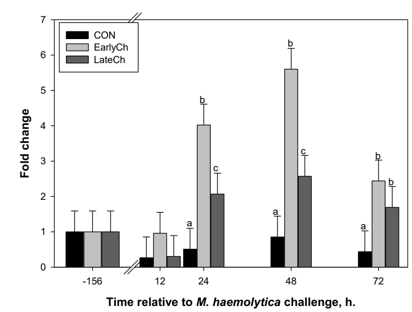
Gene expression of TLR4 in LDM



(Carlos-Valdez et al., 2011)

19

Gene expression of TLR4 in SCF



(Carlos-Valdez et al., 2011)

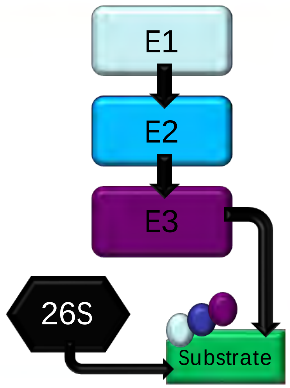
20

Ubiquitin-Proteasome Pathway

Genes that were up-regulated

- RWD domain containing 1
- SUMO1 activating enzyme subunit 1
- F-box and WD containing repeat domain 12
- Ubiquitin specific peptidase 15
- Ankyrin repeat and SOCS box-containing 2
- 26S proteasome subunit 1

(Johnson, 2009)



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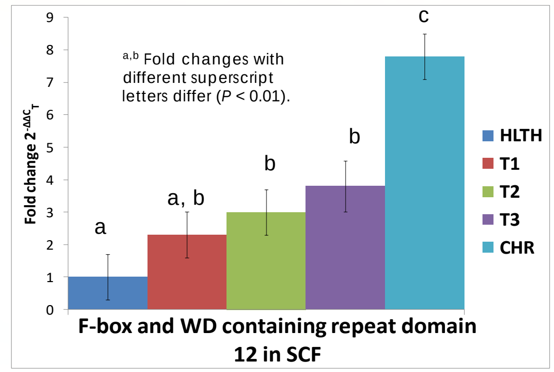
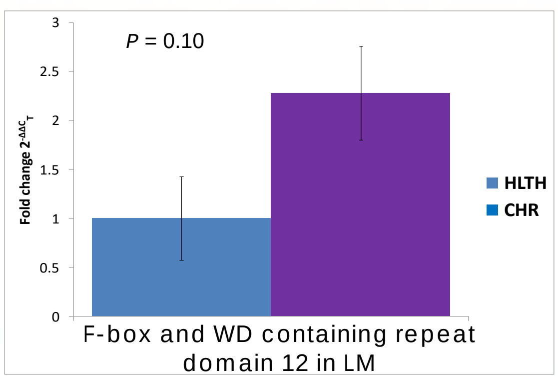


Johnson, 2009

22



Johnson, 2009

23

Summary

- Acute BRD
  - Increased liver removal of amino acids
  - Decrease arterial [AA] and increased [haptoglobin]
  - Increased expression of cytokines (TLR4, TNFα, IL-6)
  - Increased expression of genes associated with the ubiquitin-proteasome pathway (FBXW12 and 26S)
- These could explain a part of the muscle wasting phenomenon observed in chronic calves, and potentially HCW in calves treated for BRD.

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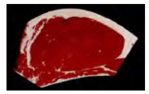
Significance of BRD

- Morbid cattle ⇒
  - – ↓ carcass weight
  - – ↓ ribeye area
  - – ↓ marbling (↓ quality)



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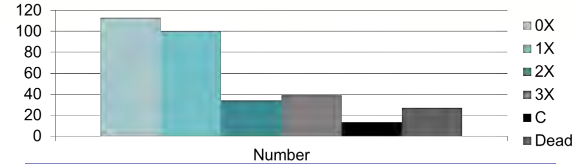
Morbidity

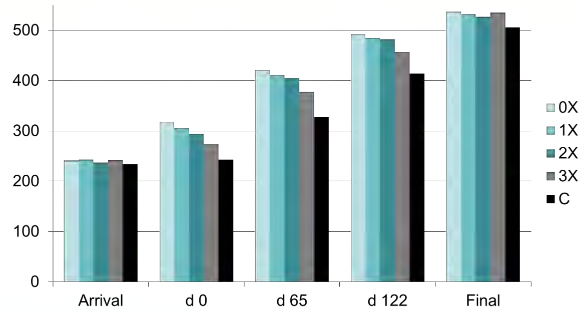


Item	0X	1X	2X	3X	C	Dead
Count[1]	113	100	34	39	13	27
%	33.5	29.7	10.1	11.6	3.9	8.0
Finishing Phase Allocation						
n	54	54	34	39	12	-
Pens	9	9	6	6	2	-
DOF	163	163	163	182	189	-

[1]Does not include calves removed due to lameness (n=6) or not included in the finishing phase due to protocol non-compliance (n=5).

26

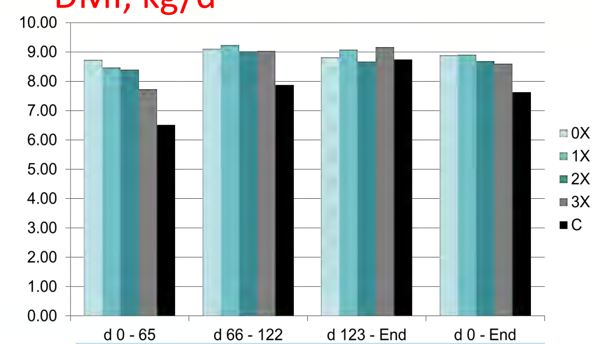
BW, kg



Arrival	d 0	d 65	d 122	Final
SEM = 9.15	SEM = 18.38	SEM = 19.90	SEM = 17.23	SEM = 10.59
L, $P = 0.84$	L, $P < 0.001$	L, $P < 0.001$	L, $P < 0.001$	L, $P = 0.58$
Q, $P = 0.51$	Q, $P = 0.01$	Q, $P = 0.03$	Q, $P = 0.51$	Q, $P = 0.18$
3 vs. C, $P = 0.11$	3 vs. C, $P < 0.001$	3 vs. C, $P < 0.001$	3 vs. C, $P = 0.11$	3 vs. C, $P = 0.01$

27

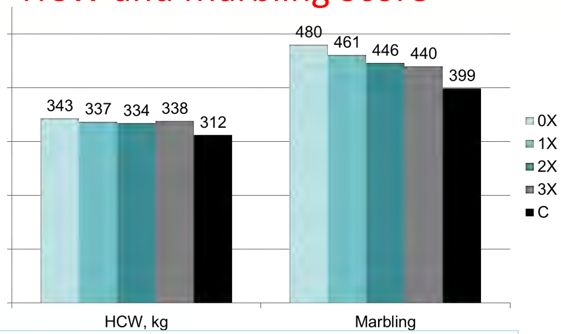
DMI, kg/d



d 0 - 65	d 66 - 122	d 123 - End	d 0 - End
SEM = 0.54	SEM = 0.43	SEM = 0.42	SEM = 0.40
L, $P < 0.001$	L, $P = 0.64$	L, $P = 0.50$	L, $P = 0.13$
Q, $P = 0.18$	Q, $P = 0.79$	Q, $P = 0.62$	Q, $P = 0.75$
3 vs. C, $P < 0.001$	3 vs. C, $P = 0.02$	3 vs. C, $P = 0.37$	3 vs. C, $P = 0.007$

28

HCW and Marbling Score



HCW, kg	Marbling
SEM = 7.85	SEM = 28.7
L, *P* = 0.03	L, *P* = 0.06
Q, *P* = 0.21	*Q*, *P* = 0.68
3 vs. C, *P* = 0.007	3 vs. C, *P* = 0.22

29

Effects of BRD on HCW, kg

Study	Untreated	Treated	Change	*P*-value
Holland et al. (2010) n = 193 heifers	343	336	-7	> 0.10
Garcia et al. (2010) n = 642	371	366	-5	= 0.06
Schneider et al. (2009) n = 5,976	323	315	-8	< 0.10
Gardner et al. (1999) n = 204 steers	332	319	-13	< 0.01

Average decrease = 8.3 ± 3.4 kg

30

Effects of BRD on 12th rib fat-thickness, cm

Study	Untreated	Treated	Change	*P*-value
Holland et al. (2010) n = 193 heifers	1.48	1.36	-0.12	> 0.05
Garcia et al. (2010) n = 642	1.17	1.02	-0.15	< 0.01
Schneider et al. (2009) n = 5,976	1.17	1.09	-0.08	< 0.01
Gardner et al. (1999) n = 204 steers	1.17	0.93	-0.24	< 0.01

Average decrease = 0.15 ± 0.07 cm

31

Effects of BRD on marbling score 400 = Small 00

Study	Untreated	Treated	Change	*P*-value
Holland et al. (2010) n = 193 heifers	480	449	-31	< 0.10
Garcia et al. (2010) n = 642	538	534	-4	= 0.22
Schneider et al. (2009) n = 5,976	538	525	-13	< 0.01
Gardner et al. (1999) n = 204 steers	338	327	-11	= 0.16

Average decrease = 15 ± 12 units

32

Results: BRDX

Effect of number of times treated for BRD during the receiving period on ultrasound estimates, lung consolidation and adhesion scores, and carcass characteristics of crossbred steers

Variable	Antimicrobials administered[1]				Pooled SEM	*P*-value[2]		
	0X	1X	2X	3/4X		**Overall *P*-value**	**Linear Contrast**	Quadratic Contrast
Ultrasound Estimates[3]								
d 91 REA, sq cm**	81.3	84.1	77.0	73.7	1.49	**<0.01**	**<0.01**	0.05
d 91 12^{th}-rib fat, cm	0.82	0.82	0.81	0.72	0.04	**0.21**	**0.08**	0.25
d 91 IMF*	4.55	4.29	4.42	4.04	0.15	**0.10**	**0.04**	0.70
d 138 REA, sq cm**	88.9	89.6	84.6	83.8	1.71	**0.05**	**0.01**	0.65
d 138 12^{th}-rib fat, cm	0.94	0.93	0.91	0.91	0.05	**0.95**	**0.60**	0.99
d 138 IMF	4.60	4.28	4.49	4.24	0.15	**0.32**	**0.22**	0.83
Lung Score[4]								
Consolidation**	0.38	0.54	1.06	0.97	0.16	**0.01**	**<0.01**	0.42
Adhesion	0.73	0.96	0.67	0.83	0.14	**0.47**	**0.99**	0.81
HCW, kg**	372	369	360	353	3.66	**<0.01**	**<0.01**	0.63
Dressing percentage**	65.5	64.6	64.2	64.0	0.27	**<0.01**	**<0.01**	0.23
REA, sq cm**	91.8	93.9	90.8	87.3	1.56	**0.05**	**0.03**	0.09
12th-rib fat, cm	1.33	1.28	1.36	1.40	0.09	**0.83**	**0.49**	0.63
KPH fat, %	2.17	2.01	2.08	2.00	0.06	**0.26**	**0.16**	0.55
Marbling number[5]	451	428	426	406	16.7	**0.29**	**0.10**	0.91
Prime and Choice[6], %*	70.3	56.5	60.2	36.2	9.15	**0.06**	**0.03**	0.54
Yield grade	2.81	2.60	2.75	2.91	0.16	**0.59**	**0.53**	0.26
Liver Score[7]	0.67	0.23	0.63	0.46	0.27	**0.65**	**0.86**	0.61

(Wilson et al., 2016)

33

How does the proinflammatory response decrease cattle growth and carcass merit?

- Surviving the immune insult becomes priority
- Initial processes catabolic in nature to retrieve needed substrate from storage depots
  - Increased demand of calories due to febrile response
  - Decrease in calorie consumption
- During infection, cytokines not only regulate the immune response, but also modify growth by redirecting nutrients in support of immune function

(Spurlock, 1997)

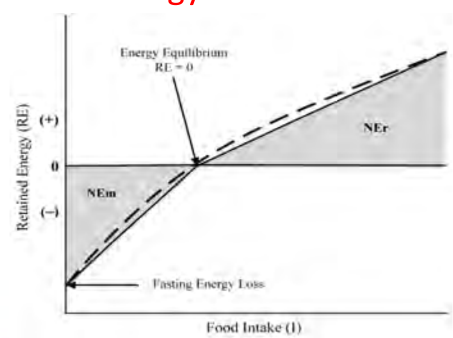
34

How do we incorporate disease effects in the Net Energy System?

- Determine magnitude and duration of the increase in ME_m due to an immune challenge.
  - Account for associated changes in DMI.
- Implications for ME_g
- Determine severity of insult impacts on energy retained as fat and protein.
- Model changes in nutrient flux and metabolism and formulate diets to enhance tolerance of infections.

35

Energy Concepts: Retained Energy and Feed Intake



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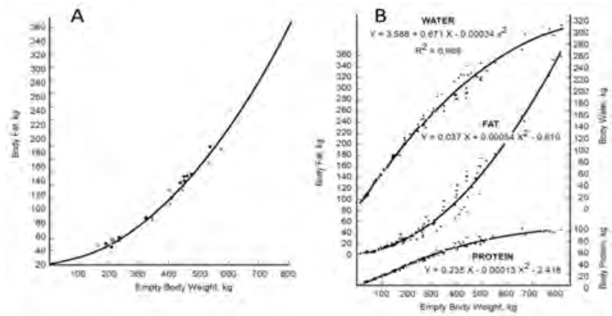
Relationship between empty BW and body composition



(Simpfendorfer, 1974; NRC, 1996; 2000; 2016)

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Implications

- During inflammation, nutrient use is shifted towards survival rather than growth
- To diminish alteration in growth
  - Mediate proinflammatory response
  - Maintain calorie and nutrient intake
  - Continue to assess and develop nutritional and health strategies to alleviate disease
- Work toward management systems that prepare cattle for transition (vaccination, weaning, nutrition programs).

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Questions?

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Mechanisms of hypophagia during disease

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1

Outline

- Nutrient demand during disease challenges
- Implications of reduced intake
- Why the drop in feed intake?
- Opportunities to intervene?

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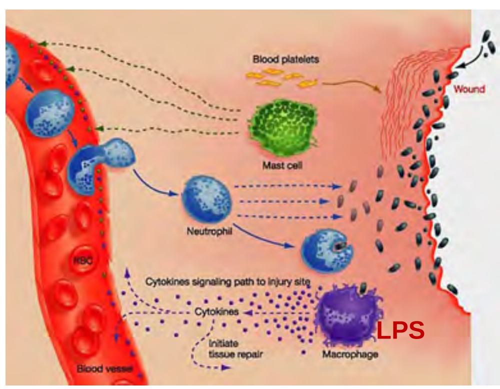
Innate immune response

•LPS = lipopolysaccharide from cell wall of Gram negative bacteria

•Cytokines = hormones produced mostly by immune cells



www.uic.edu/classes/bios/bios100/lecturesf04am/lect23.htm

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Metabolic costs of disease

1. Increased metabolic activity
   10 – 40% increase over basal maintenance energy
2. Reduced nutrient availability
3. Altered priorities for nutrient utilization
4. Increased turnover rates in the immune system
5. Damage to host tissues
6. "Genetic cost" - to offspring

Colditz et al., 2008

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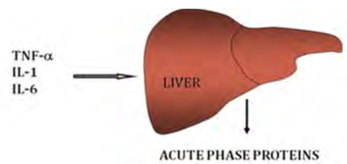
Estimated essential amino acid costs of immune response

2% for proliferation of immune cells
1% for increased immune activity
9% for liver acute phase response



(Relative to baseline requirements)

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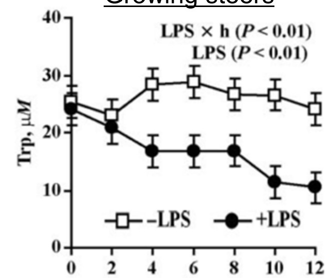
Klasing, 2007

5

Serum depletion of amino acids after LPS

Growing steers



Similar effects for:
- Leucine
- Isoleucine
- Phenylalanine
- Threonine
- Serine
- Alanine
- Glycine
- Asparagine
- Ornithine
- Glutamate

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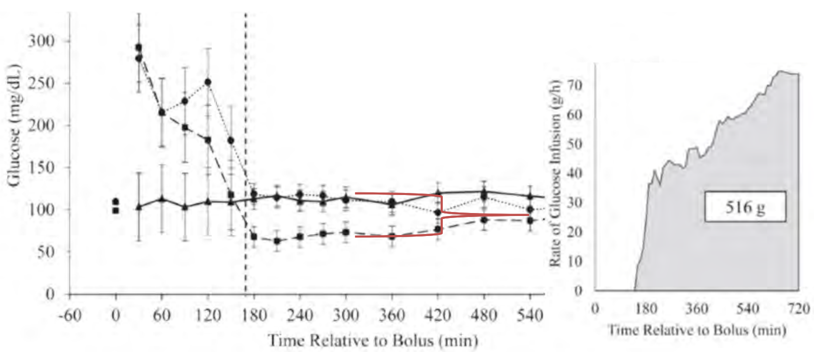
Waggoner et al., 2009

6

What is the glucose drain?



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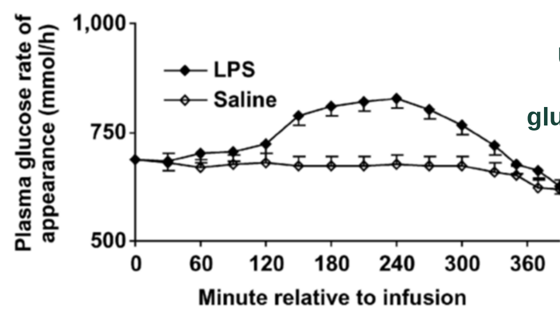
Kvidera et al., 2016

7

What is the glucose drain?



Up to a 33% increase in glucose turnover rate

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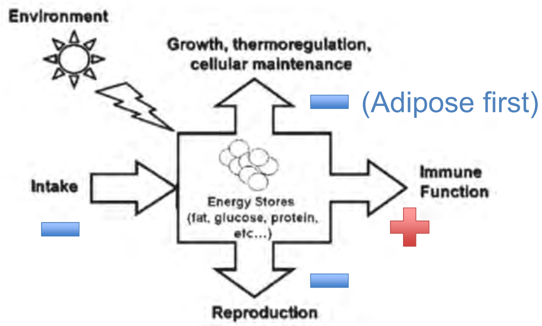
Waldron et al., 2006

8

Resource allocation during disease



French et al., 2009

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Outline

- Nutrient demand during disease challenges
- Implications of reduced intake
- **Why the drop in feed intake?**
- Opportunities to intervene?

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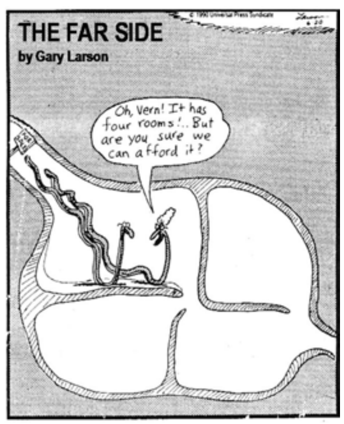
Nematode infection model

Does infection or immune response cause more problems in this disease scenario?



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Nematode infection model

- 36 ewe lambs, 5 months old (naïve)
- 4 treatments:
  - *T. colubriformis* larvae (80/kg BW/dose)
  - *T. colubriformis* larvae + weekly IM injection of Depredone (glucocorticoid)
  - Depredone (uninfected, immune-suppressed)
  - Control (uninfected)
- Duration: 3 doses per week until d 72

Greer et al., 2005

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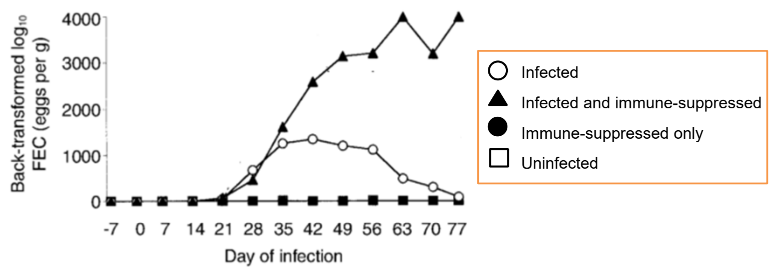
Fecal Egg Counts



Infected immune-suppressed vs. infected ($P < 0.001$)

Greer et al., 2005

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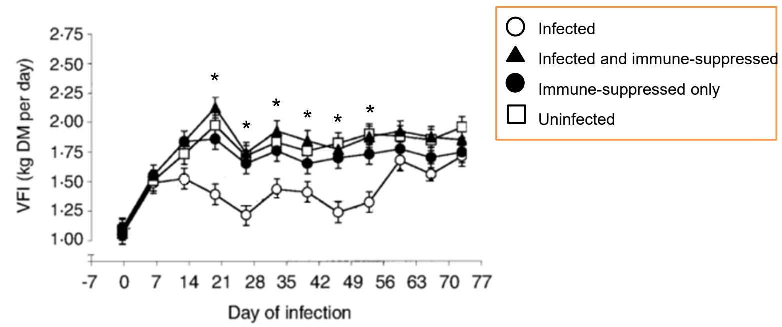
Dry matter intake: immune system is to blame



Greer et al., 2005

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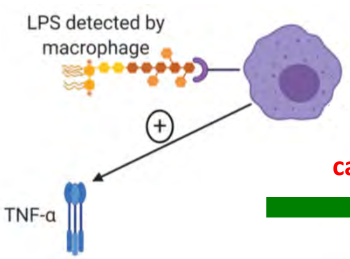
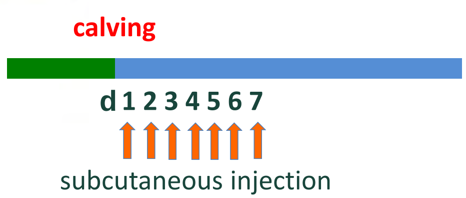
Do repeated abiotic inflammatory signals alter intake?



- 33 Holstein cows were assigned to 1 of 3 treatments (n = 11) at calving.
- 3 treatments: 0, 1.5, or 3.0 µg tumor necrosis factor/kg BW.



Yuan et al., 2013

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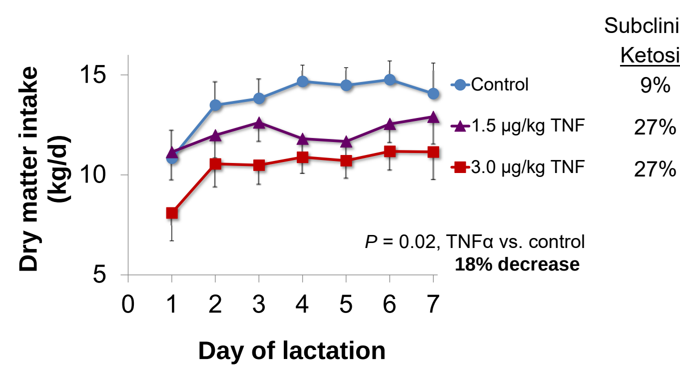
Immune signal suppresses intake



Yuan et al., 2013

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Why decrease intake during disease??

1. **Avoid consuming additional pathogens / toxins**
2. **Avoid predation**
3. **Refuse to supply key nutrients to bacteria**
   Especially trace minerals
4. **Control the innate immune response**
   Prevent the immune system from over-reacting

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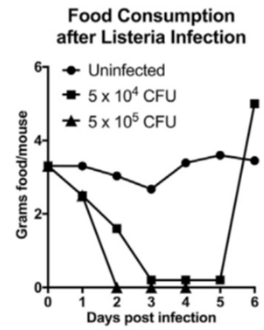
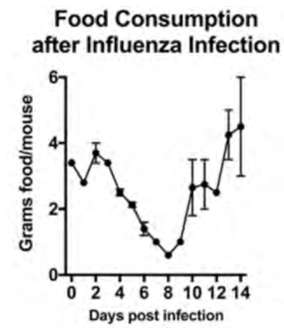
Starve a fever, drown a cold





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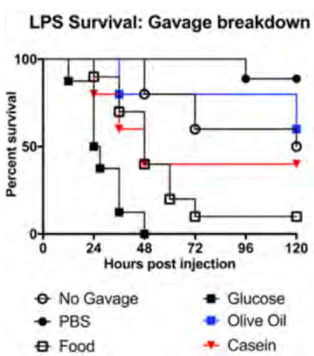
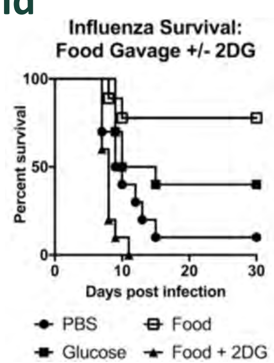
Wang et al., 2016

18

Starve a fever, drown a cold





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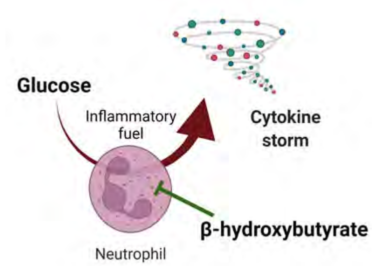
Wang et al., 2016

19

Hypophagia helps to prevent uncontrolled inflammation



- Glucose is the main fuel used by phagocytic innate immune cells, and promotes inflammation as a result
- BHB directly suppresses activation of inflammatory pathways and immune activation
- Not eating should decrease glucose and increase BHB

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Outline

- Nutrient demand during disease challenges
- Implications of reduced intake
- Why the drop in feed intake?
- **Opportunities to intervene?**

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Are nutrient interventions wise for sick cattle?

- Low blood glucose is common in some disease states, particularly postpartum dairy cows. Infuse glucose?
  - May be counter-productive if it exacerbates an inflammatory state
- What about drenching sick calves with no interest in milk?
- Should we use anti-inflammatories along with antibiotics for cattle with infections?

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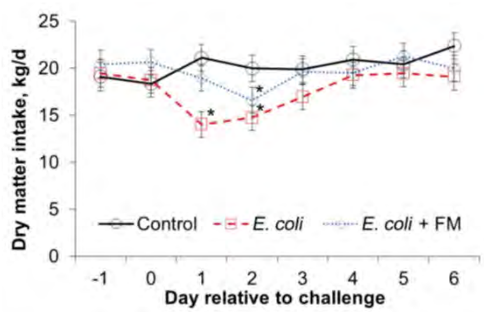
Anti-inflammatories can help in some cases

- Cows challenged with E. coli mastitis had reduced DMI for 2 d
- Treating with flunixin meglumine (Banamine) at onset of clinical symptoms delayed and lessened the reduction



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Yeiser et al., 2012

23

What about dietary formulation for the hospital pen?

Possible changes:

- Increased protein / essential amino acid supply
- Greater forage content
- Increased antioxidant supply
- Plant polyphenols

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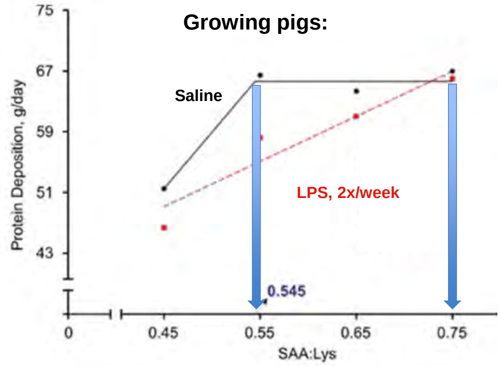
Inflammation may alter AA requirements



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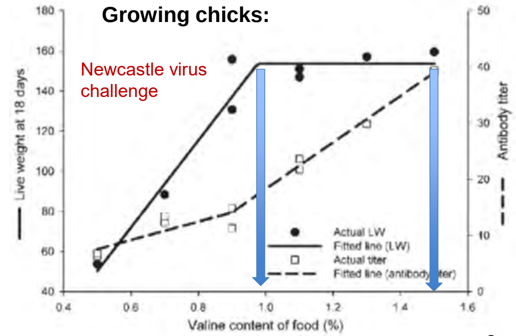
Kim et al., 2012

25

Immune response may alter AA requirements



Sandberg et al., 2007 from Bhargava et al., 1970

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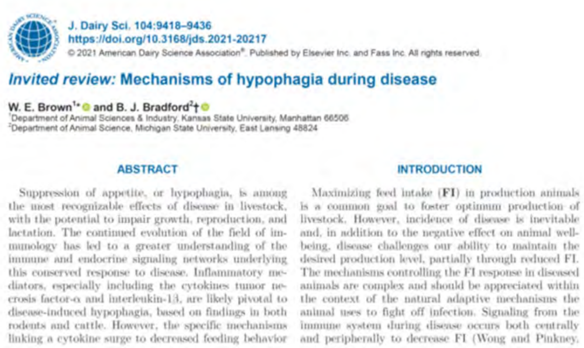
Dr. Billy Brown



J. Dairy Sci. 104:9418–9436
https://doi.org/10.3168/jds.2021-20217

Invited review: Mechanisms of hypophagia during disease

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ABSTRACT

Suppression of appetite, or hypophagia, is among the most recognizable effects of disease in livestock, with the potential to impair growth, reproduction, and lactation. The continued evolution of the field of immunology has led to a greater understanding of the immune and endocrine signaling networks underlying this conserved response to disease. Inflammatory mediators, especially including the cytokines tumor necrosis factor-α and interleukin-1β, are likely pivotal to disease-induced hypophagia, based on findings in both rodents and cattle. However, the specific mechanisms linking a cytokine surge to decreased feeding behavior

INTRODUCTION

Maximizing feed intake (**FI**) in production animals is a common goal to foster optimum production of livestock. However, incidence of disease is inevitable and, in addition to the negative effect on animal well-being, disease challenges our ability to maintain the desired production level, partially through reduced FI. The mechanisms controlling the FI response in diseased animals are complex and should be appreciated within the context of the natural adaptive mechanisms the animal uses to fight off infection. Signaling from the immune system during disease occurs both centrally and peripherally to decrease FI (Wong and Pinkney,

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Want to talk cows?

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Improving nitrogen efficiency through diet formulation

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1

CFAES

Why improving N efficiency in dairy cows?

- How N use efficiency evaluated
  - Increasing milk N ÷ N intake
- Reducing feed protein supply
  - Economic impact
- Reducing N excretion and NH_3 emission from manure
  - Environmental impact

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CFAES

Two different views on improving N use efficiency

1) Improving milk N ÷ N intake
   - N utilization within cows
   - Diet manipulation
2) Improving N use efficiency on a farm basis
   - N utilization at farm level
   - Diet manipulation

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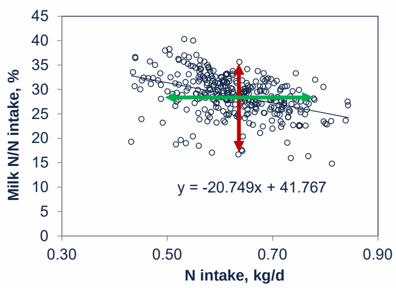
CFAES

Increasing milk N ÷ N intake



- Energy supply
- Reducing dietary protein
- Formulating a diet for AA

(Digestion studies at OSU by Dr. Weiss over 30 years)

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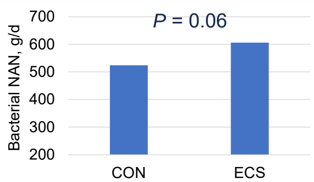
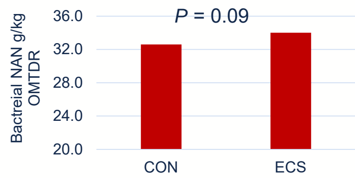
CFAES

Increasing rumen starch digestibility

- Feeding α-amylase-enhanced corn and corn silage (Enogen®)

	Design	Positive responses
Cueva et al., 2021	Randomized block design	Milk yield, ECM, feed efficiency, milk protein yield
Rebelo et al, 2021	Latin square design	Milk yield, feed efficiency, protein yield, microbial protein synthesis
Krogstad and Bradford, 2022	Randomized block design	Digestibility of DM, starch, NDF, and CP





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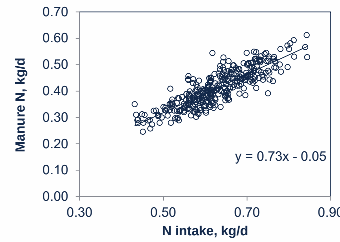
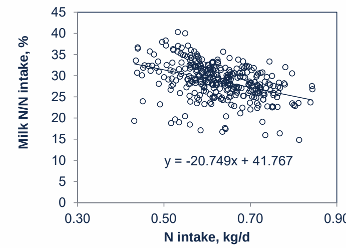
CFAES

Lowering dietary protein to improve N use efficiency





(Digestion studies at OSU by Dr. Weiss over 30 years)

Deficient protein supply decreases milk yield and milk protein yield

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CFAES

Lowering Dietary Protein Supply

References	CP, % of DM	Milk N efficiency, %	Urinary N excretion	Milk yield
Colmenero and Broderick, 2006	**16.5 vs. 15.0**	**31 → 34**	**30% ↓**	**-**
Chen et al., 2011	**16.8 vs. 15.6**	**30 → 32**	**15% ↓**	**-**
Cabrita et al., 2011	**16.0 vs. 14.8**	**29 → 33**	**35% ↓**	**2 kg ↓**
Lee et al., 2011	**16.5 vs. 14.8**	**28 → 32**	**30% ↓**	**3 kg ↓**
Lee et al., 2012	**15.7 vs. 13.6**	**29 → 34**	**28% ↓**	**4 kg ↓**
Arriola Apelo et al., 2014	**16.9 vs. 15.0**	**33 → 35***		**2 kg ↓***

* Not statistically different

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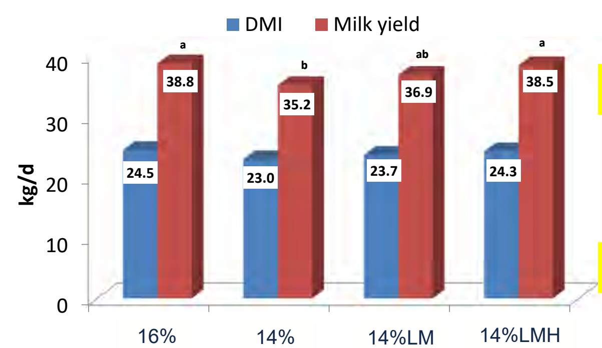
Reduced protein with AA supplementation



SEM = 0.74
***P* = 0.004**

SEM = 0.46
***P* = 0.06**

Lee et al., 2012

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Reduced protein with AA supplementation

	16%	14%	14%LM	14%LMH	SEM	*P*-value
Milk protein, %	2.98	2.94	2.99	3.03	0.030	0.23
Yield, kg/d	1.13[a]	1.01[b]	1.10[a]	1.14[a]	0.025	<0.01
Urine N, g/d	143[a]	92[b]	87[b]	97[b]	5.7	<0.01
Milk N ÷ N intake	29[b]	34[a]	35[a]	35[a]	0.99	<0.01

Lee et al., 2012

No responses to RP-AA
- RP-Met and Lys; Lee et al., 2012, 2015, 2019
- RP-Met decreased milk protein %; Potts et al., 2020
- RP-Lys; Malacco et al., 2022
- N-acetyl-L-Methionine; Amaro et al., 2022

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Expectation when a diet is formulated for AA

- Increasing dietary N use efficiency
- Optimizing production, i.e., milk protein
- Lowering N excretion

- Feeding RP-AA may become more popular
- More variety of RP-AA will be available

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Feeding RP-AA to lactating cows

- What we must know when RP-AA are used is
  - Bioavailability
  - Rumen bypass and intestinal digestibility
- What if wrong bioavailability is used?
  - Creating an imbalance of metabolizable AA
  - Inefficient use of dietary AA or AA provided from RP-AA
    - No production response, an increase in N excretion
  - Increasing a feed cost but no returns

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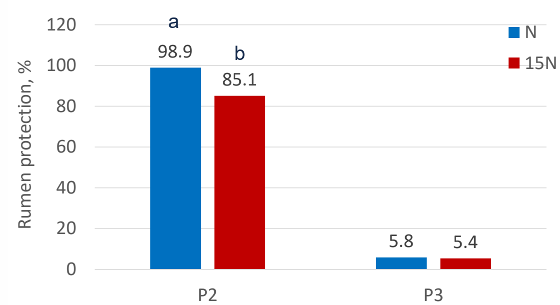
Rumen protection of 2 prototypes



Rebelo et al. unpublished

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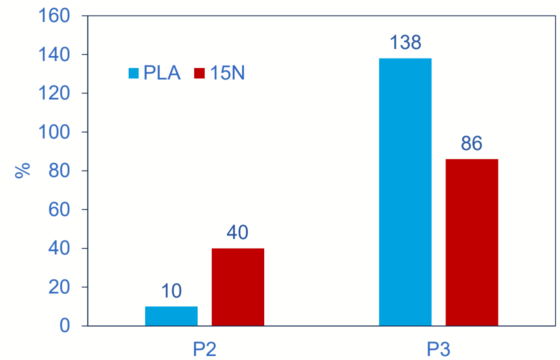
Intestinal digestibility



PLA, plasma Lys appearance; Rebelo et al. unpublished

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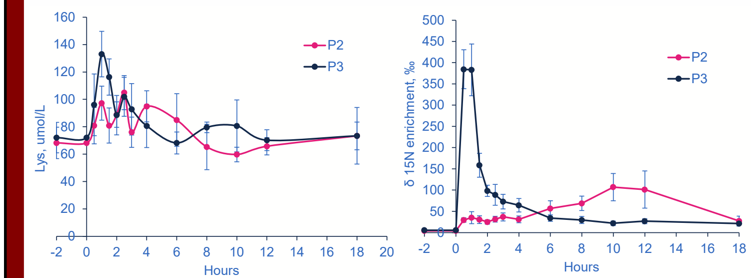
Underestimation of intestinal digestibility with PLA for P2



PLA was not able to reflect the slow absorption of Lys from P2

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Summary

- Increasing milk N ÷ N intake
  - Reducing N excretion
  - Reducing feed costs
- Strategies from diet formulation
  - Energy supply to the rumen and mammary glands
  - Feed adequate protein, not deficient and excessive
  - More studies are needed for better consistent responses to feeding AA
  - A gold standard of an in vivo method is needed to determine bioavailability of RP-AA

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Is reducing N excretion the best approach to increase N efficiency?

- Manure values as fertilizer
- Risk of performance
  - How low can dietary protein go?
  - What AA should be added?
  - Do we know bioavailability of RP-AA?
  - Does stage of lactation affect the responses?
  - Does it work for group-feeding?

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Different approach to increase N use efficiency

- Increasing N use efficiency by lowering ammonia emissions from manure
- Why reducing ammonia emission?
  - Increasing farm-based N use efficiency
  - Manure value
  - Local environmental issues
    - Odor
    - Air quality
    - P runoff

https://www.dtnpf.com/agriculture/web/ag/crops/article/2022/03/23/nitrogen-fertilizer-prices-keep-new

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Manure N can affect P utilization by crops

The ratio of N to P required = 6:1

The ratio of N to P required = 6:1 – 7:1

Application based on N or P??

Ammonia volatilization

Manure N and P ratio = 2:1 → 4:1 or 5:1

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Environmental Issues from Dairy Production

- Greenhouse gas emissions
  - Methane from enteric fermentation and manure
  - Nitrous oxide
- Ammonia emission

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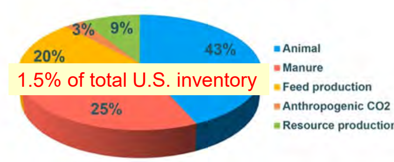
Environmental Footprints of Dairy Production

- Greenhouse gas emissions
- Fossil energy use
- Non-precipitation water use
- Reactive nitrogen (ammonia) loss

Greenhouse gas emissions



1.5% of total U.S. inventory

Fossil energy use:
0.3% of total U.S. Consumption
Non-precipitation water use:
3% of total freshwater withdraw

(LCA; Rotz et al., 2021 ADSA virtual meeting)

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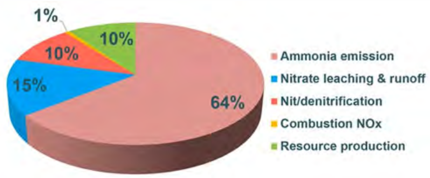
Environmental Footprints of Dairy Production

- Reactive nitrogen (ammonia) loss



24% of total U.S. inventory
5 to 76% of regional inventories

- Conclusions
  - Dairy contribution to GHG: SMALL
  - Dairy contribution to fossil energy consumption: SMALL
  - Dairy contribution to reactive nitrogen: Considerable

(LCA; Rotz et al., 2021 ADSA virtual meeting)

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How can we lower ammonia emissions from manure

- Direct manure treatment
- Indirect manure treatment

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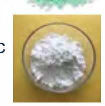
Direct manure treatment

Urease inhibitors
- Cyclohexylphosphoric triamide
- Phenylphosphorodiamide
- N-(n-butyl) thiophosphoric triamide



Extra costs!

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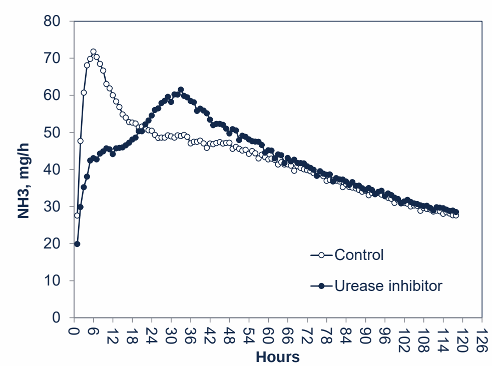
Effects of urease inhibitor on ammonia emission from manure



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Indirect manure treatment:
Diet manipulation to lower ammonia emissions

- Altering manure characteristics
  - Not easy and not as effective as direct manure treatment
  - No or minimal cost
  - No negative effects on production

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Feeding corn distillers grains with solubles replacing soybean meal

	SBM	DG
Ingredients, DM %		
Corn silage	42	42
Alfalfa silage	10	10
Corn grain	13	13
SBM	15	0
DDGS	0	29
Chemical composition, % DM		
CP	17.6	17.6
NDF	30.5	30.0
Starch	20.4	21.5

(Morris et. al., 2018; Lee et al., 2020)

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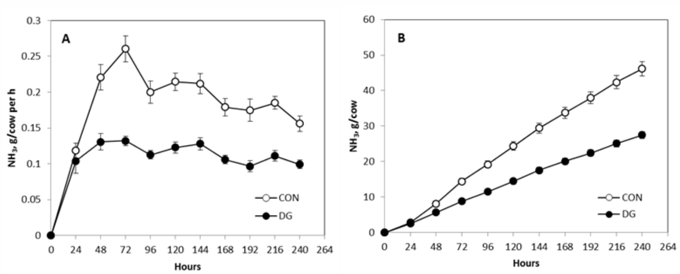
CFAES

Feeding corn distillers grains with solubles replacing soybean meal



A 35% reduction in ammonia emission with 30% DG in the ration

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CFAES

Feeding corn distillers grain with solubles replacing soybean meal

Fresh manure	SBM	DG
Feces : urine	1.6	1.9
Manure, kg/d	82	79
Manure N, g/d	478	419
Fecal N contribution, %	40	45
Urinary N contribution, %	60	55
Urine pH	8.5	7.5
DCAD (mg/kg)	**192**	**65**

	SBM	DG
Milk yield, lbs/d	41.0	41.3
Milk protein, %	3.26[a]	3.11[b]
Milk fat, %	3.81[a]	3.00[b]

(Morris et. al., 2018; Lee et al., 2020)

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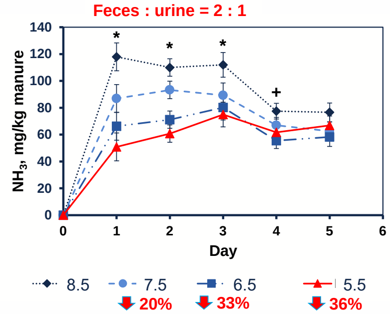
Preliminary study

- Effects of reduced urine pH on manure pH and ammonia emissions from manure

Observations per treatment = 4

	Treatment			
Urine pH	8.5	7.5	6.5	5.5
Fecal pH	6.3	6.3	6.3	6.3
Manure pH	7.7	7.5	7.1	6.9



(Zynda et al., 2021; in press)

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Animal study

	CON	MID	LOW
N	9	9	9
Ingredient, % DM			
Forage	59.7	59.7	59.7
Concentrate	40.3	40.3	40.3
Chemical Composition			
CP	16.3	16.2	16.2
NDF	32.0	32.8	31.8
Starch	26.9	26.2	26.2
DCAD, mEq/kg DM	**192.8**	**101.3**	**1.2**

	Diets			P-value	
	CON	MID	LOW	Linear	Quad
Fecal pH	6.38	6.29	6.28	0.43	0.72
Urine pH	**8.58**	**8.33**	**6.72**	<0.01	<0.01
Manure pH	**7.57**	**7.40**	**6.96**	<0.01	0.46

(Zynda et al., 2021; in press)

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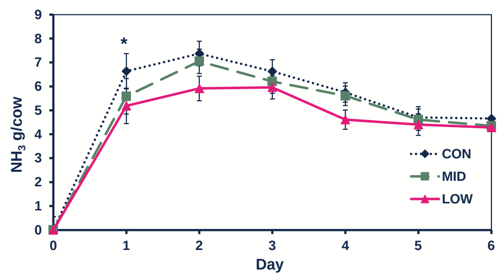
Animal study



	Diet			P-Values	
	CON	MID	LOW	Linear	Quad
Cumulative NH_3 g/cow	35.8	33.6	30.8	**0.16**	**0.89**

15%

(Zynda et al., 2021; in press)

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Summary

- Lowering ammonia emissions from manure
  - Increasing farm-based N utilization
  - Reducing environmental impacts
  - Increasing the value of manure as fertilizer
- Direct manure treatment is most effective
  - Not practical without federal or state-level support
- Indirect manure treatment: diet manipulation
  - Difficult but potential
  - Less effective compared with direct treatment
  - More research on practical dietary manipulation
  - More research on long-term ammonia emission and manure values

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OARDC

Thank you!

Chanhee (Chan) Lee
Lee.7502@osu.edu

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CFAES

33

Pre- and postnatal muscle and adipose tissue growth in beef cattle

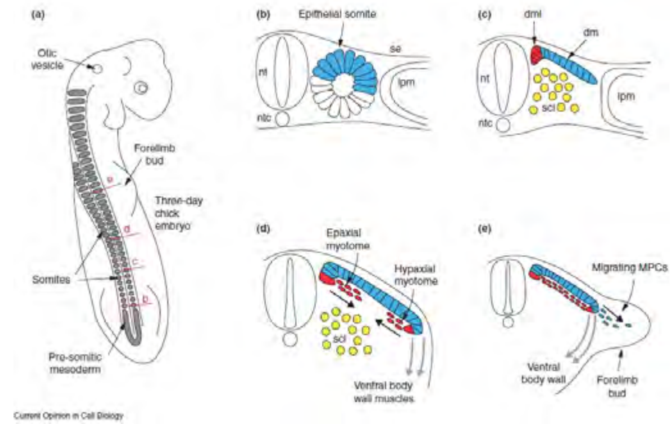
WASHINGTON STATE UNIVERSITY
Department of Animal Sciences

Min Du
Professor and Endowed Chair
Department of Animal Sciences
Washington State University

1

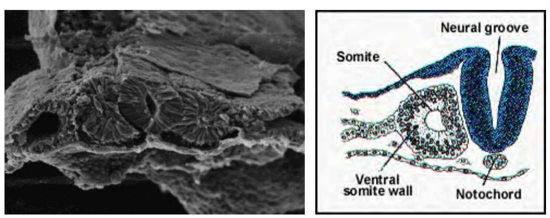
Embryonic muscle and adipose development

WASHINGTON STATE UNIVERSITY
Department of Animal Sciences

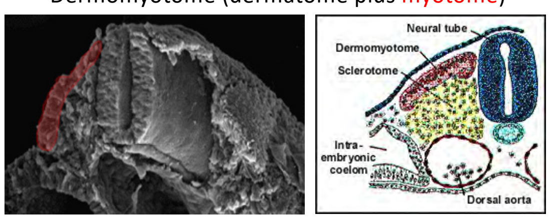


Sclerotome (SCL), neural tube (nt), notochord (ntc), surface ectoderm (se), and lateral plate mesoderm (lpm)

2

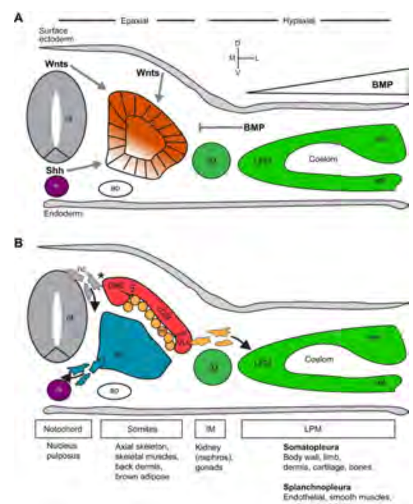


Dermomyotome (dermatome plus myotome)



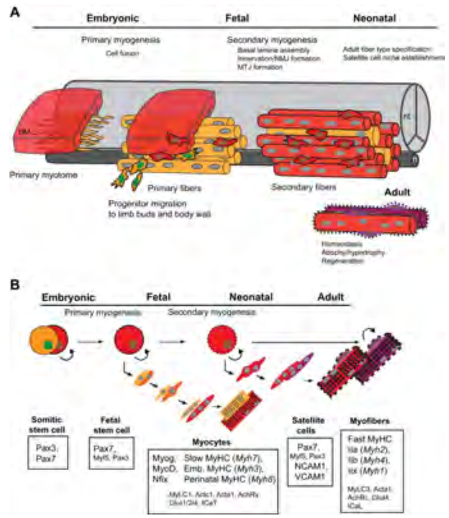
Mouse: E9, Human: E23-24

3



Chal and Pourquie, *Development*, 2017, 144: 2104.

4



Chal and Pourquie, *Development*, 2017, 144: 2104.

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Skeletal muscle development

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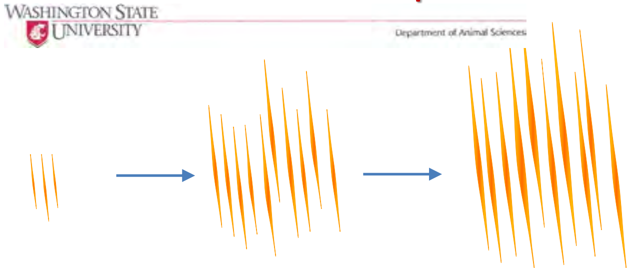

First 3 months

- Formation of new muscle fibers

3 to 7 months of pregnancy,

- Formation of new muscle fibers
- Growth of muscle fibers

7 months and after,

- Growth of muscle fibers

- Increase of muscle fiber formation during the fetal stage will increase later lean growth.

6

Secondary/primary muscle ratio is reduced in sheep fetus with nutrient restriction

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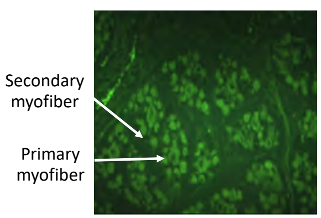
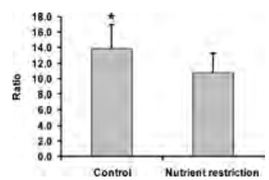
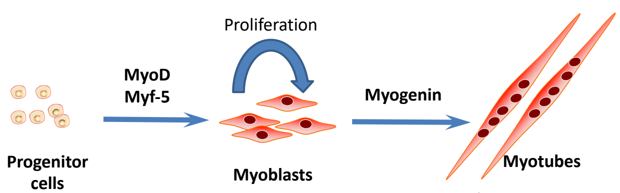




50% nutrient restriction during G28 to 78, when secondary to primary fiber ratio was examined.



Zhu et al, *Biology of Reproduction*, 2004, 71: 1968.

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Fetal muscle development

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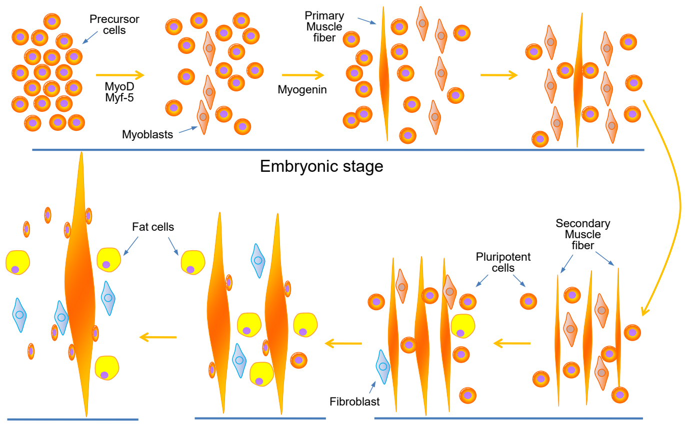
- Besides formation of muscle fibers, fetal muscle development also involves formation of adipocytes (adipose tissue) and formation of fibrogenic cells (connective tissue).
- Adipocytes formed during the fetal stage and neonatal stage accumulate lipids during fattening stage, forming marbling.
- Excessive formation of connective tissue makes the meat tough.

8

Fetal muscle development



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Skeletal muscle and adipose tissue are mainly developed during the fetal and neonatal stages

Washington State University — Department of Animal Sciences

- Skeletal muscle development (increase in muscle fiber number) occurs during the fetal stage.
- Adipose tissue development mainly occurs during the fetal and neonatal stages, but extends lifelong.
- Maternal nutrition has dramatic effects on the development of fetal skeletal muscle and adipose tissue, programming long-term performance of offspring.

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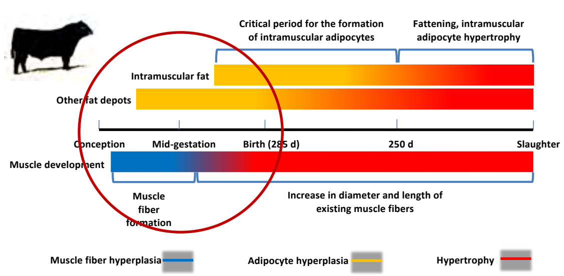
Fetal skeletal muscle and adipose development

Washington State University — Department of Animal Sciences

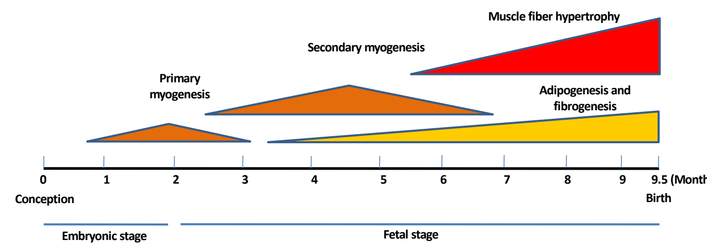


Du et al., *Animal Frontiers*, 2017, 7: 5.

11

Timeline of fetal skeletal muscle development of beef cattle

Washington State University — Department of Animal Sciences



- Timelines for muscle and fat development.
- Myogenesis (muscle formation), adipogenesis (fat formation) and fibrogenesis (connective tissue formation).

12

Maternal nutrient restriction and fetal muscle development

WASHINGTON STATE UNIVERSITY Department of Animal Sciences

- Maternal nutrition during different stages of pregnancy has specific effects on fetal development, especially muscle and adipose tissue development.
- Maternal undernutrition at mid-gestation leads to less muscle development; the excessive energy is deposited in adipose tissue in later life, reducing lean/fat ratio.
- Maternal undernutrition at late-gestation reduces the density of satellite cells, reducing postnatal muscle growth but largely recoverable.

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Muscle growth and lean:fat ratio

WASHINGTON STATE UNIVERSITY Department of Animal Sciences

- On the other hand, late gestation and neonatal stage are important for intramuscular adipogenesis.
- better maternal nutrition at late gestation and neonatal stage promote intramuscular adipocyte formation and increase intramuscular fat (marbling) development of progeny.

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Maternal nutrient and marbling

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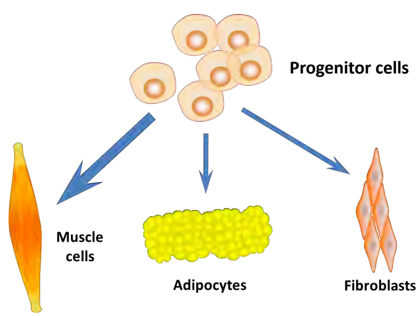

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Maternal nutrient restriction and fetal muscle development

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- Maternal physiological and nutritional status affects progenitor cell proliferation and development into muscle, fat and fibrogenic cells, affecting the lean/fat ratio, production efficiency and beef quality.
- Examples:
  - Nutrient deficiency during mid-gestation decreases the number of progenitor cells, forming less muscle fibers, decreasing muscle mass and lean/fat ratio.
  - Example: Runt piglets always have a lower lean:fat ratio compared to their littermates.

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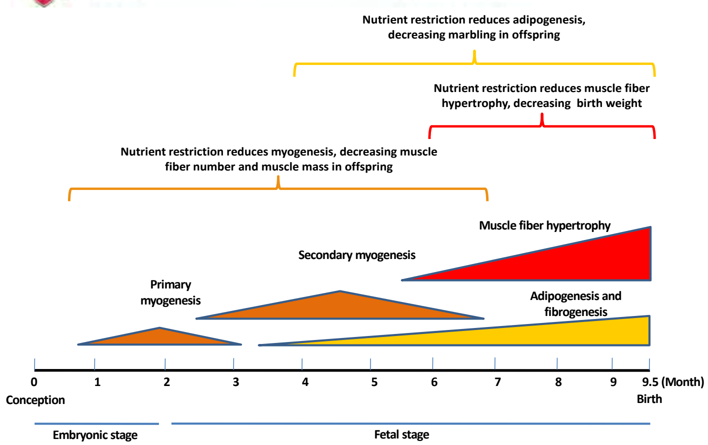
Timeline of fetal muscle development in beef cattle



Du et al., *JAS*, 2010, 88: E51.

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Nutrition during mid-gestation affects progeny performance

Animals

- At 120 to 150 d of gestation, cows were allotted randomly to one of two dietary treatment, either native range (**NR,** n = 12) or improved pasture (**IP,** n = 14) with increased forage production, for 60 days.
- Esophageal extrusa samples:
  - IP varied from 11.1% crude protein of organic matter early in the test period to 6.0% at the end of the grazing period.
  - NR ranged from 6.5% crude protein of organic matter during early grazing to 5.4 % at the end.

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Effects of cows grazing either native range or improved pasture from 120 to 180 days of gestation on growth of steer progeny

	Treatment		
Item	Native range[1]	Improved pasture[2]	*P*-value
Birth weight, kg	38.7 ± 2.0	36.6 ± 1.9	0.46
Weaning weight, kg	242.1 ± 3.7	256.2 ± 3.5	0.02
Final body weight, kg	538.0 ± 8.3	560.2 ± 7.7	0.07
Average daily gain, kg/d	1.489 ± 0.067	1.656 ± 0.062	0.05
Total body weight gain, kg	180.2 ± 8.0	200.37 ± 7.5	0.05
Live weight at slaughter, kg	520.6 ± 7.7	543.9 ± 7.1	0.04

Underwood et al., *Meat Science,* 86:588-593.

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Effects of cows grazing either native range or improved pasture from 120 to 180 days of gestation on carcass characteristics of steer progeny

	Treatment		
Item	Native range[1]	Improved pasture[2]	*P*-value
Kidney, Pelvic and Heart fat, % of HCW	3.96 ± 0.25	3.59 ± 0.24	0.32
HCW, kg	329.5 ± 4.8	348.2 ± 4.5	0.01
Yield grade	3.54 ± 0.18	3.84 ± 0.17	0.23
Marbling score[3]	420 ± 16	455 ± 15	0.12

Underwood et al., *Meat Science,* 86:588-593.

20

Muscle characteristics of steers from cows grazing either native range or improved pasture from 120 to 180 days of gestation

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Item	Treatment: Native range[1]	Treatment: Improved pasture[2]	*P*-value
Longissimus muscle area, cm^2	75.4 ± 2.2	78.7 ± 2.0	0.26
Semitendinosus, % of HCW	1.16 ± 0.07	1.20 ± 0.07	0.19
Longissimus muscle WBSF, N	37.29 ± 1.28	31.00 ± 1.19	0.004
Collagen content, µg/mg of *Ld* muscle	19.2 ± 1.9	15.7 ± 1.9	0.08
Ether extract (fat, %)	4.82 ± 0.53	6.00 ± 0.49	0.06

Likely, the difference in tenderness is due to the reduction in collagen content and increase in lipid content ---- production and quality problems having a fetal origin.

Underwood et al., *Meat Science,* 86:588-593.

21

Summary

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- Maternal nutrition alters fetal development which has long-term effect on the growth performance of offspring.
- Grazing on improved pasture appears to enhance intramuscular adipogenesis and marbling, while reduces collagen content, resulting in tenderer meat.
- Poor maternal nutrition reduces growth potential and muscle development in offspring.
- How could we solve this production problem?
  - If we supplement cows with proteins, would that increase muscle growth?

22

Maternal protein supplementation diverts adipogenesis to myogenesis in beef steers

Washington State University — Department of Animal Sciences

- Mid-gestation is an important period for muscle and adipose tissue development.
- Thirty six crossbred beef cows were randomly placed on a control diet (100% NRC requirements, n = 12, **C**), nutrient restricted (70% of requirements, n = 12, **NR**), or a nutrient restricted diet with protein supplement (**NRP**, n = 12) designed to equal flow of amino acids to the small intestine of C diet from d 45 to 185 of gestation.
- Then, all groups of cows were placed together, managed to meet requirements and allowed to calve.
- Steers were slaughtered at 405 days of age.

23

Maternal protein supplementation diverts adipogenesis to myogenesis in beef steers

Washington State University — Department of Animal Sciences

Item	Treatment: C[1]	Treatment: NR[2]	Treatment: NRP[3]	*P*-value: Treatment
Live BW, kg	567 ± 22[a]	588 ± 15[a]	615 ± 18[a]	0.240
HCW, kg	375.8 ± 13.8[a]	377.4 ± 9.6[a]	398.2 ± 11.2[a]	0.313
LM area, cm^2	86.4 ± 4.2[a]	88.0 ± 3.0[a]	90.3 ± 3.4[a]	0.762
St muscle (kg)	2.44 ± 0.15[b]	2.55 ±0.10[ab]	2.87±0.12[a]	0.067
St muscle % HCW	1.25±0.05[b]	1.35±0.03[ab]	1.44±0.04[ad]	0.02
KPH, % HCW	3.05 ± 0.25[a]	2.88 ± 0.17[a]	2.30 ± 0.20[b]	0.050

Underwood et al., *Unpublished data*.

24

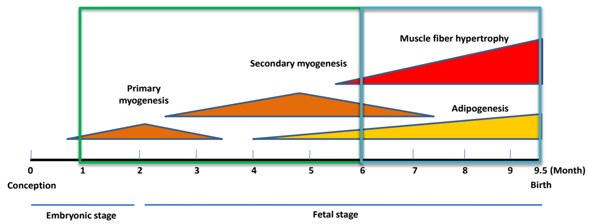
Studies were done in Brazilian cows…

Washington State University — Department of Animal Sciences

Control: No protein/energy supplementation

Treatment 1: Protein/energy supplementation (1 kg/d 28% CP) from 1-6 months of gestation

Treatment 2: Protein/energy supplementation (1.5 kg/d 28% CP) from 6 - 9 months of gestation

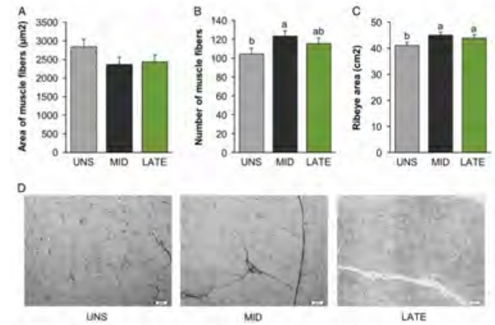


Marquez et al. *Animal*, 2017, 2184

25

Similar studies were done in Brazil…

Washington State University — Department of Animal Sciences



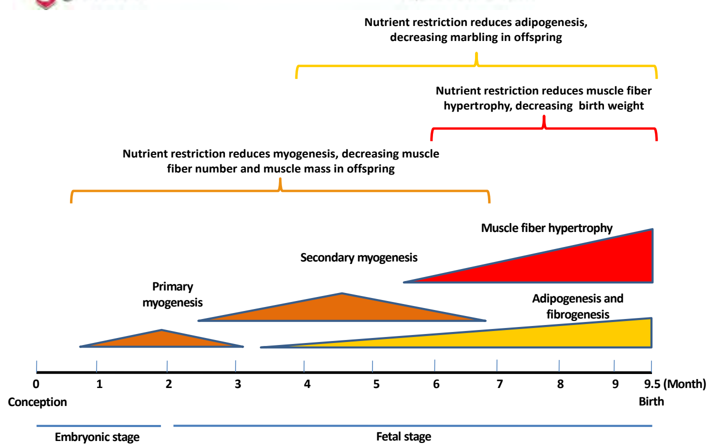
- Protein supplementation during early to mid-gestation increased muscle fiber number and muscle mass of offspring cattle.

Marquez et al. *Animal*, 2017, 2184

26

Timeline of fetal muscle development in beef cattle

Washington State University — Department of Animal Sciences



Du et al., *JAS*, 2010, 88: E51.

27

Conclusion so far…

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- Fetal programming has a major role in determining the production efficiency of beef cattle, as well as beef quality.
- Nutrition during pregnancy affects lean/fat ratio, feed efficiency and beef quality.
- Through manipulation of maternal nutrition, we will be able to maximize the growth potential and meat quality of offspring.

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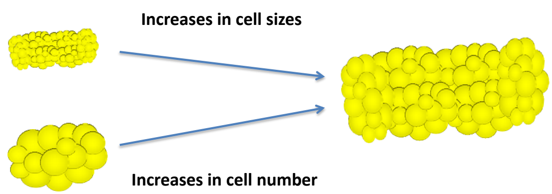
Formation of adipose tissue and marbling fat

WASHINGTON STATE UNIVERSITY — Department of Animal Sciences

- Marbling fat is critical for the eating quality of beef.
- Increase in marbling fat is due to: increase in number (hyperplasia) and/or size (hypertrophy) of intramuscular adipocytes.



29

Enhance marbling through nutritional management of calves

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- Can we also induce marbling through nutritional management of calves?
- There is a “marbling window”, when feeding a high grain diet to calves can effectively enhance marbling.

30

Increases adipocyte number and marbling

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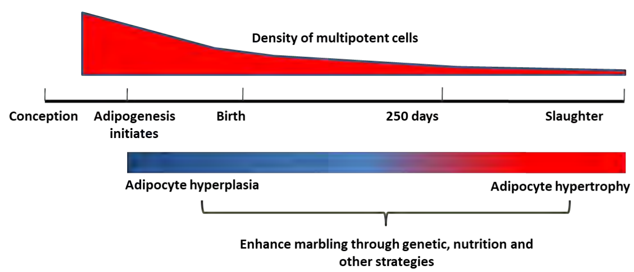
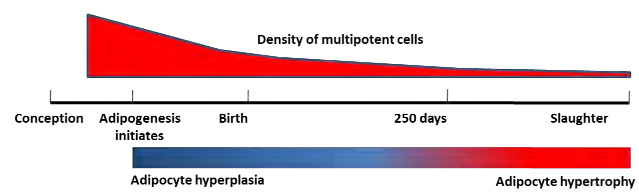


- Enhancing adipocyte formation increases marbling.
- The number of multipotent cells decreases as animals become older.
- Thus, to increase adipocyte number in beef cattle, fetal and early post-weaning stages are the most effective stages.

31



- For beef cattle, increase of adipocyte number occurs between mid-gestation to about 250 days of age.
- These adipocytes accumulate lipids during the later stage.
- “Fattening” stage in feedlots: cattle is fed a grain-based diet, increasing adipocyte size.

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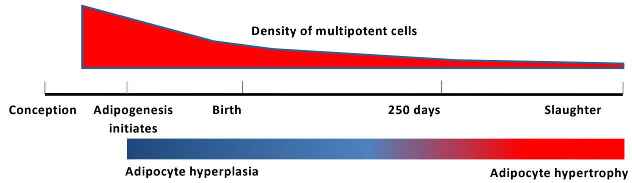

- However, the increase in adipocyte size occurs in all fat depots.
- The majority of lipids accumulates in visceral and subcutaneous fat, but only limited amount goes to intramuscular fat.
- If we can specifically increase intramuscular adipocyte number, we will be able to increase marbling without increasing overall adiposity.

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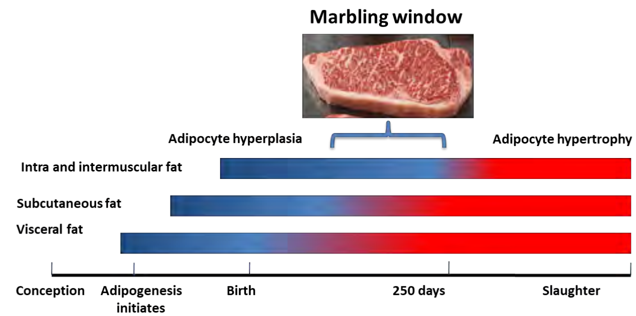
Challenges to specifically increase marbling?



- Nutrient supplementation during "marbling window" specifically increases intramuscular adipocyte number.

Du et al., *Journal of Animal Science,* 2013, 91:1419.

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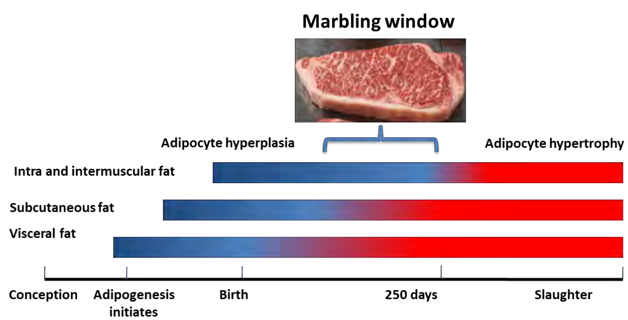
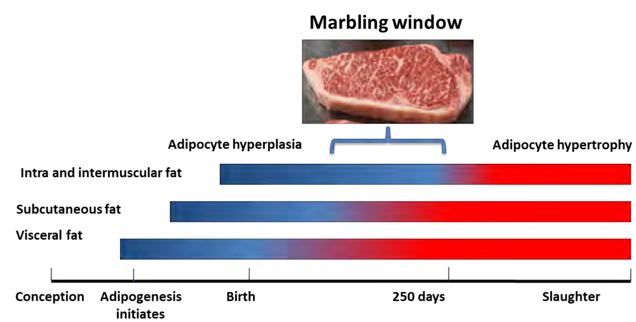


- Increases intramuscular adipocytes without overall increase in fat deposition in other depots.
- During fattening stage, lipid accumulation in intramuscular adipocytes forms abundant marbling.

Du et al., *Journal of Animal Science,* 2013, 91:1419.

35

Intramuscular adipocytes and marbling

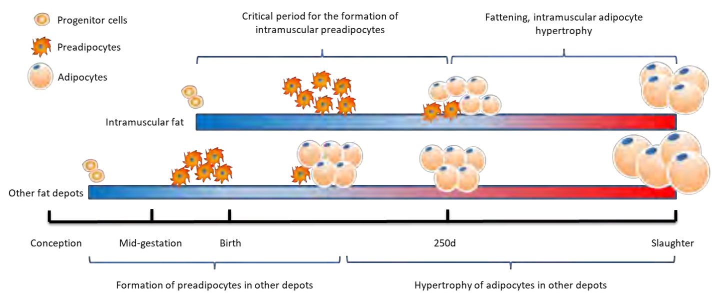


- Marbling window: Early weaning to around 250 days

Du et al., *Journal of Animal Science,* 2013, 91:1419.

36

Timing of adipocyte formation differs among different depots

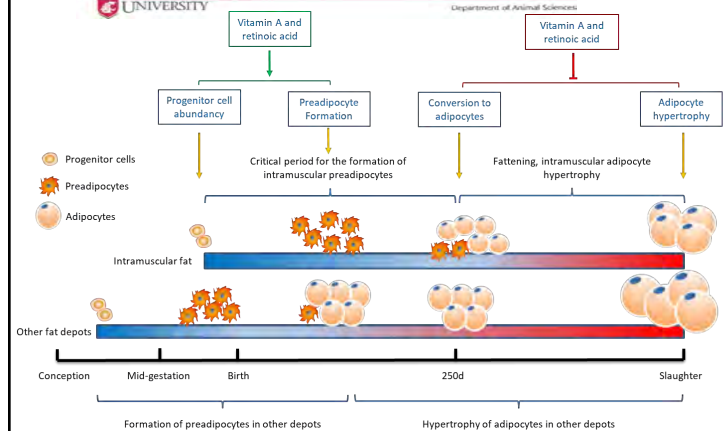


- Retinoic acid, via retinoic acid receptor, is required for adipogenic progenitor proliferation and their adipogenic commitment.

Wang et al., *Meat Science*, 2016, 120: 100.

37

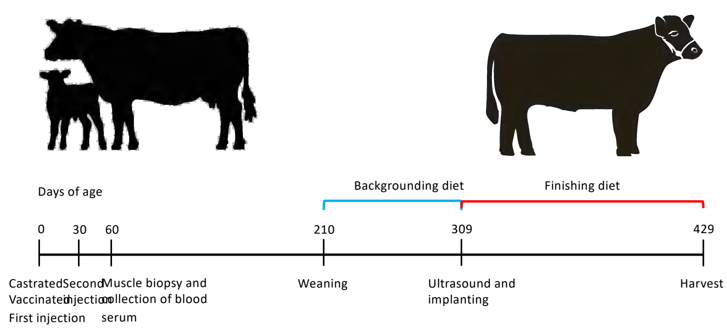
Critical control points for intramuscular adipogenesis



Wang et al., *Meat Science*, 2016, 120: 100.

38

Neonatal vitamin A administration increases marbling and growth rate



39

Neonatal vitamin A administration increases marbling and growth rate

Response	0 IU (n = 9)	150,000 IU (n = 7)	300,000 IU (n = 9)	SE	P-value
Ultrasound at transition to the finishing stage					
IMF, %	3.96^{b}	4.93^{a}	4.34^{ab}	0.26	0.036*
Rib fat, cm	0.38	0.38	0.41	0.03	0.659
Rump fat, cm	0.53	0.53	0.43	0.05	0.240
REA, cm^{2}	53.5	56.1	58.3	2.21	0.337
Carcass characteristics					
Carcass weight, kg	345.8	359.1	352.7	6.58	0.527
Dressing percent, %	59.8	58.8	59.7	0.51	0.468
Marbling score	583.3^{b}	671.7^{a}	610.0^{ab}	20.20	0.016*
KPH, %	2.34	2.18	2.15	0.14	0.661
REA, cm^{2}	82.1	85.6	84.3	5.42	0.565
Back fat, cm	1.30	1.45	1.27	0.10	0.421
Yield grade	2.99	3.07	2.95	0.18	0.921

[&] 500 = small 0; 600 = modest 0; 700 = moderate 0

Harris et al., *JASB*, 2018, 9:55.

40

Neonatal vitamin A administration increases marbling and growth rate

Response	0 IU (n = 9)	150,000 IU (n = 7)	300,000 IU (n = 9)	SE	P-value
Birth to pre-weaning					
Birth weight, kg	35.4	35.9	35.8	0.58	0.932
Average daily gain, kg	0.88^{b}	0.98^{a}	1.00^{a}	0.02	0.034
Backgrounding					
Weaning weight, kg	223.6^{b}	245.1^{ab}	246^{a}	5.98	0.018*
Dry matter intake, kg/head/d	9.09	8.89	9.25	0.39	0.320
Average daily gain, kg	1.35	1.37	1.47	0.17	0.784
Feed/gain ratio, kg	6.73	6.55	6.43	0.62	0.944
Finishing					
Weight at 309-d, kg	312.1^{b}	333^{ab}	339.7^{a}	8.65	0.040*
Dry matter intake, kg/head/d	8.95	9.45	9.09	0.85	0.829
Average daily gain, kg	2.06	1.99	1.90	0.17	0.627
Feed/gain ratio, kg	4.35	4.76	4.79	0.13	0.219

Harris et al., *JASB,* 2018, 9:55.

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Early nutrition and its impacts on overall cattle performance

- Vitamin A administration during early development promotes adipocyte formation, providing sites for later intramuscular fat accumulation.
- Thus, it is effective in enhancing marbling.
- In addition, it might also promote muscle growth, which was further studied.

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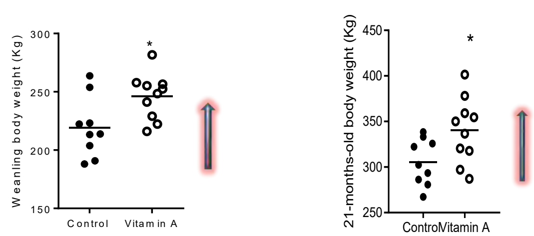
Early nutrition and its impacts on overall cattle performance

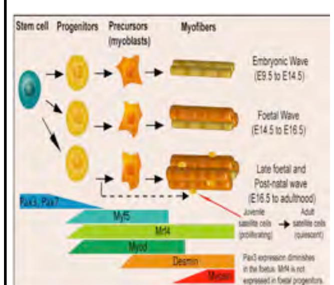


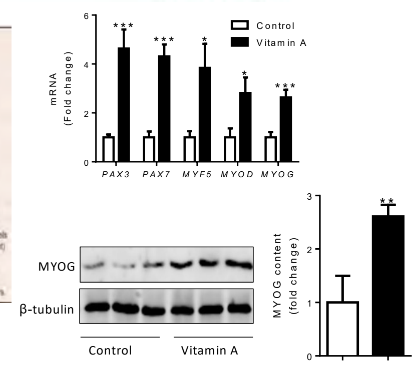
Weaned at 6 months old

Wang et al., *JASB,* 2018, 9: 82.

43

Neonatal vitamin A administration increases satellite cell density and myogenesis





Biopsy at 2 months old

Wang et al., *JASB,* 2018, 9: 82.

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Early nutrition and its impacts on overall cattle performance

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Department of Animal Sciences

- Vitamin A administration during early development promotes intramuscular adipocyte formation, providing sites for later intramuscular fat accumulation.
- In addition, it promotes calf growth, which is likely due to enhanced satellite cell activation and myogenesis.
- These changes are associated with enhanced angiogenesis.
- Mechanisms need to be further explored.

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Critical control points for intramuscular adipogenesis

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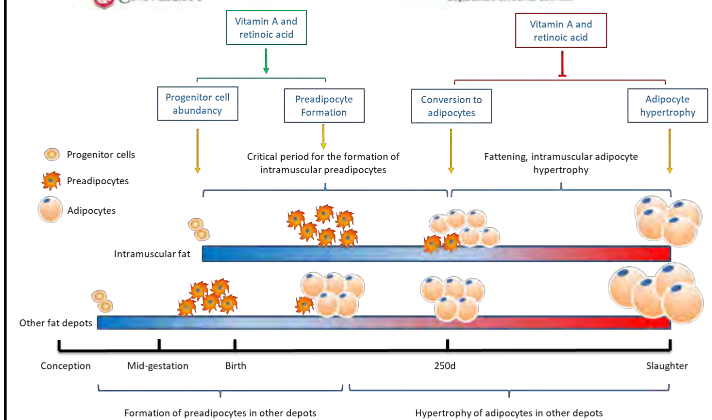

Wang et al., *Meat Science*, 2016, 120: 100.

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Nutritional management to maximize genetic potential

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- The long-gestation period of beef cattle allows stage-specific and nutrient specific (precision) management.
- Cow nutrition, in this case, vitamin A, during the fetal stage and lactation stage is also critically important.
- Proper nutrition, especially providing stage-specific nutrients, will improve livestock growth efficiency to maximize their genetic potential.

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Early nutrition and its impacts on overall cattle performance

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- Besides affecting muscle and adipose tissue development, early nutrition also impacts cattle reproduction and milk production.
- Percentage of cows return to the estrus at the time of breeding is correlated with body condition scores.
- As a result, early nutrition also has large impacts on cattle reproduction and breeding.

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Cow calf production cycle and nutritional management

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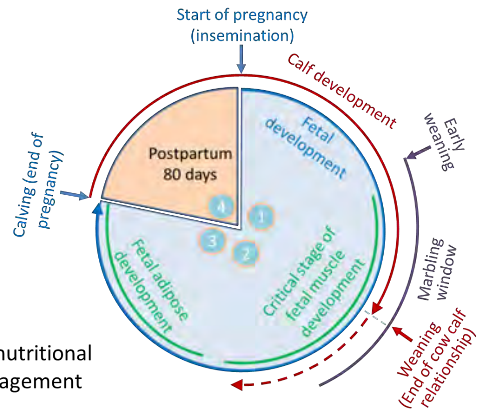

Du et al., *Animal Frontiers*, 2017, 7: 5.

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Early nutrition and its impacts on overall cattle performance

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- Provide cows with better nutrients have long-term impacts in improving the performance of cow-calf production.
- By improving:
  - Enhancing pregnancy rate.
  - Calf muscle development, more lean meat.
  - Calf marbling, better quality.

More calves with better quality

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Funding support

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National Institutes of Health
The Nation's Medical Research Agency

USDA United States Department of Agriculture
National Institute of Food and Agriculture

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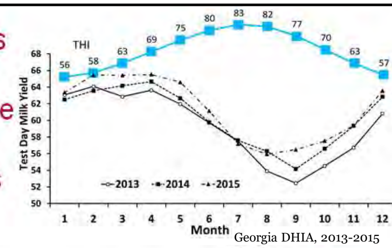
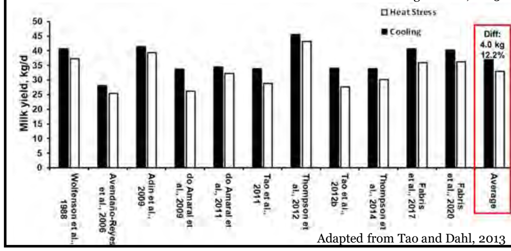
Environmental effects on calf performance and responses to different feeding programs

Sha Tao, Ruth M. Orellana Rivas
University of Georgia

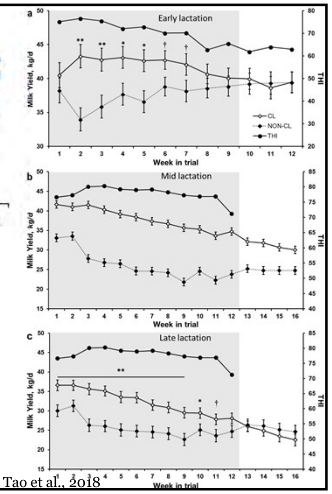
Heat stress impairs performance of mature dairy cows



Georgia DHIA, 2013-2015

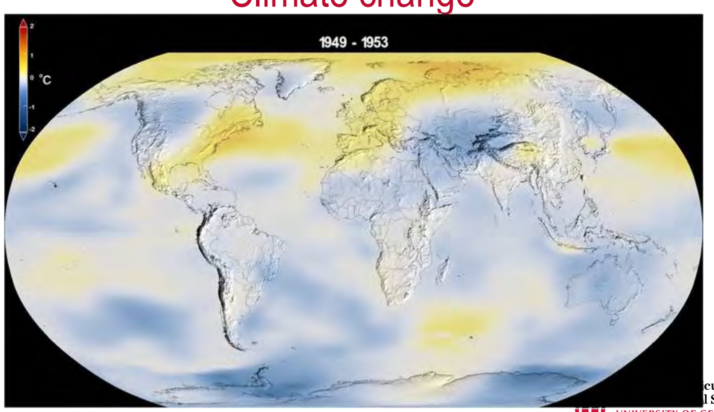


Adapted from Tao and Dahl, 2013



Tao et al., 2018

Climate change



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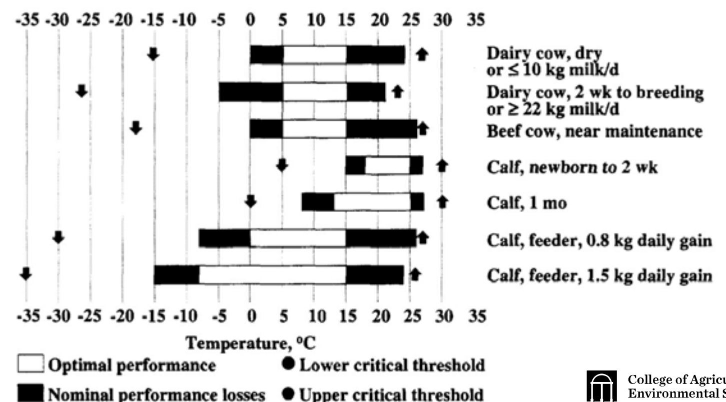
Thermoneutral zone for calves



Hahn, 1994

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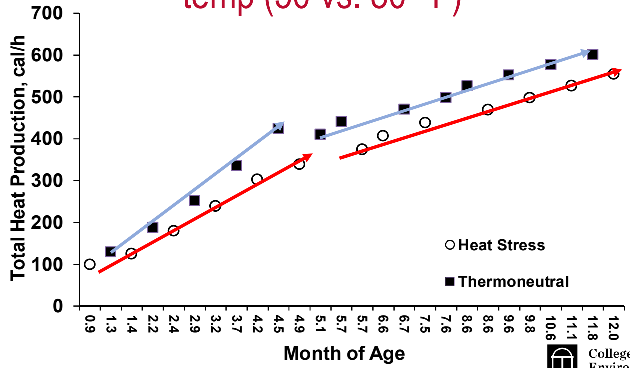
Total heat production of Holstein calves housed in constant temp (50 vs. 80 °F)



Adapted from Kibler, 1960

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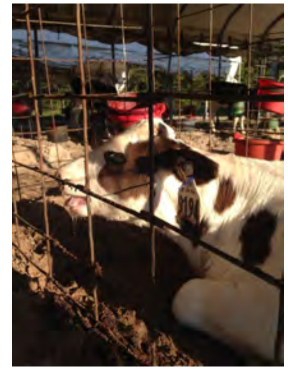
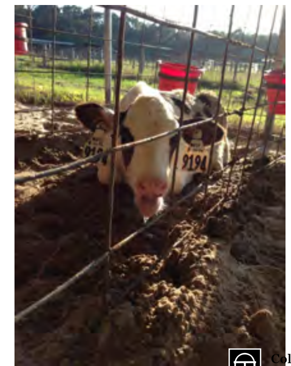
Neonatal calves also experience heat stress!





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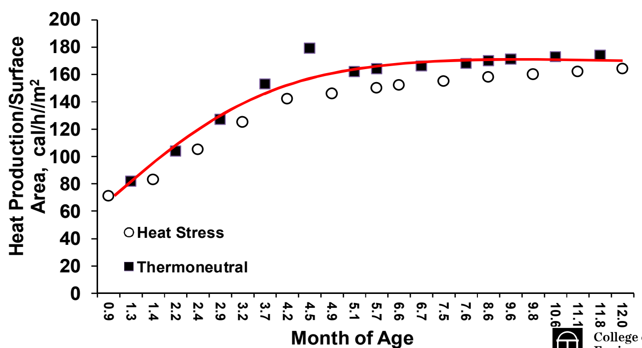
Heat production/surface area of Holstein calves housed in constant temp (50 vs. 80 °F) increases over time



Adapted from Kibler, 1960

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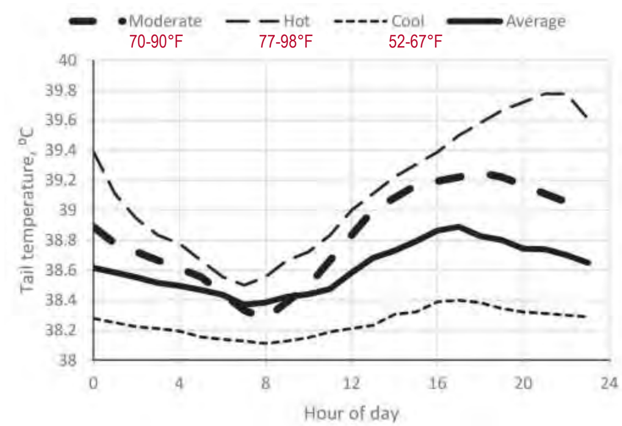
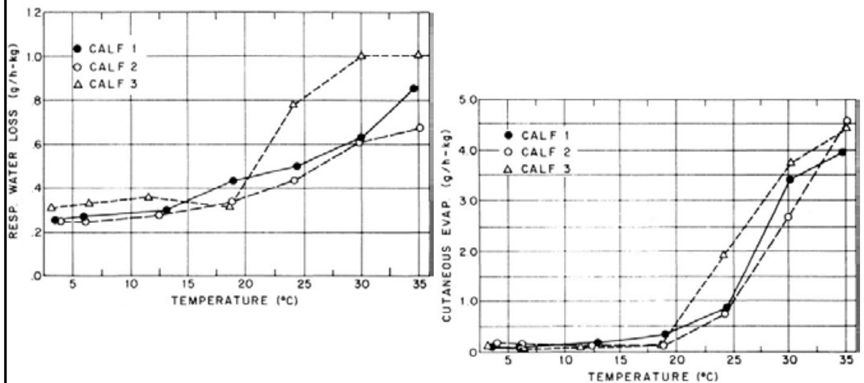
Daily variation in calf body temperatures



Hill et al, 2016

Evaporative cooling is initiated around 20 °C in calf



Gebremedhin et al., 1981

How does heat stress affect calf performance?

Very limited research in preweaned calves

Relationship between environments and body temperature and respiration rate of preweaned calves under a barn

J. Dairy Sci. 103
https://doi.org/10.3168/jds.2020-18381
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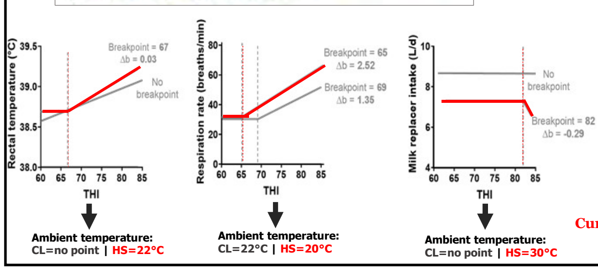
Methods for assessing heat stress in preweaned dairy calves exposed to chronic heat stress or continuous cooling

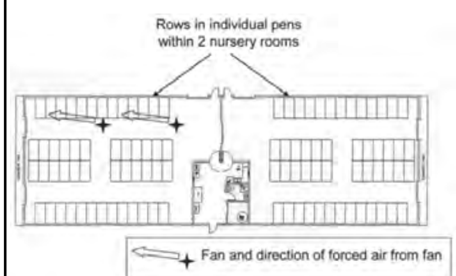
B. Dado-Senn, V. Ouellet, G. E. Dahl, and J. Laporta
Department of Animal Sciences, University of Florida, Gainesville 32611

Segmented regression models to estimate THI thresholds for significant changes in physiological responses under HS or CL



Curtesy of Dr. Jimena Laporta, UW-Madison

Cooling preweaned calves by fans in a nursey barn in Ohio summer



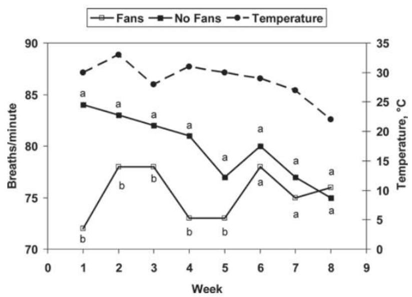
https://www.calfnotes.com/new/en/2020/10/18/calf-note-220-calf-management-in-summer-part-2/

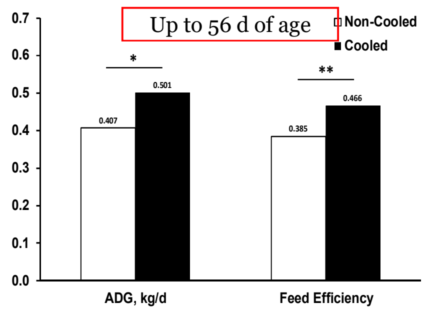
Average ambient temp: 22 °C (9-36 °C)

Calves were housed open hutches in a nursery barn and were either cooled by fans from 8AM to 5 PM or not.

Adapted from Hill, et al., 2011

Cooling preweaned calves by fans improve growth and efficiency in a nursey barn in Ohio summer

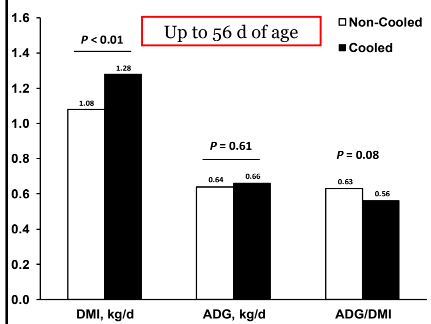


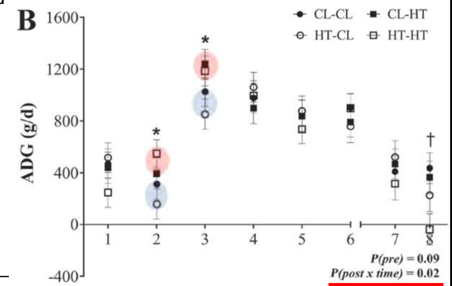


All calves were weaned at 42 d

Adapted from Hill, et al., 2011

Cooling preweaned calves by fans improves intake but not growth during summer in Florida





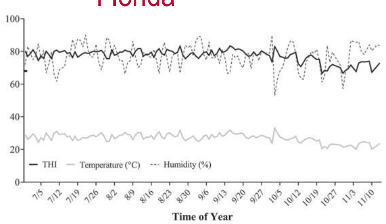
All calves were raised on autofeeder;
Weaning process from d 42-52

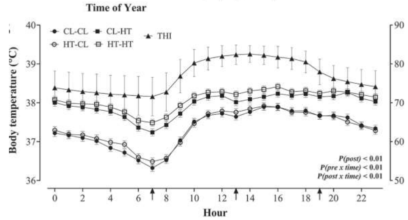
Dado-Senn, et al., 2020

Curtesy of Dr. Jimena Laporta, UW-Madison

Cooling by fans reduces preweaned calves' body temp during summer in Florida

Curtesy of Dr. Jimena Laporta, UW-Madison



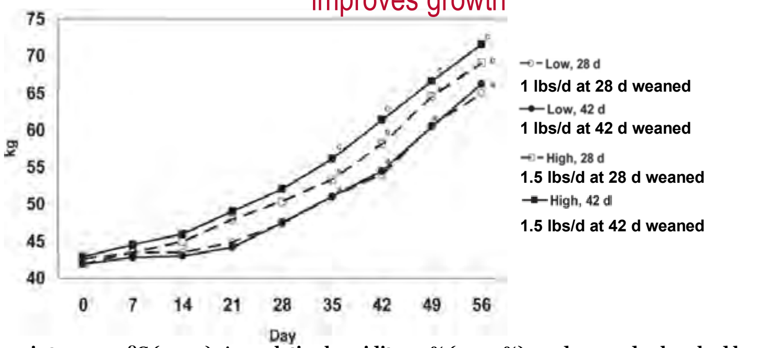


Dado-Senn, et al., 2020

Potential approaches to mitigate heat stress impacts

- Management
  - Dry and clean bedding
  - Shade
  - Improve ventilation
    - Natural wind by hutch elevation
    - Cooling by fan
- Nutrition
  - Water
  - Calf starter
  - Amount and frequency of milk replacer feeding

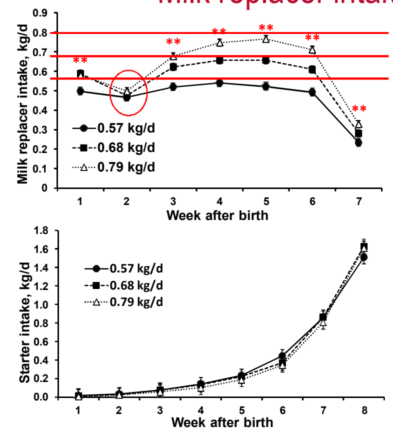
Increasing the amount of milk replacer (21:21 MR) fed in summer improves growth



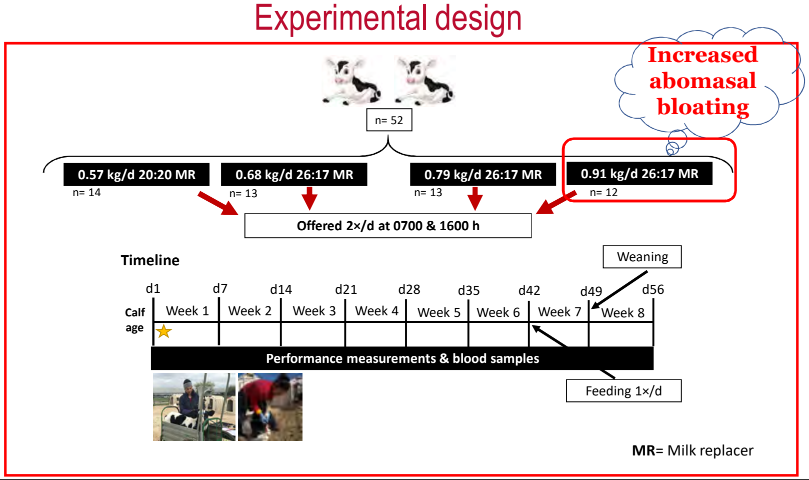
Ave air temp: 24 °C (13-34), Ave relative humidity: 72% (25-99%), 42 d weaned calves had lower starter intake than 28 d, but milk replacer rate did not affect starter intake

Adapted from Hill et al., 2012

Milk replacer intake & starter intake

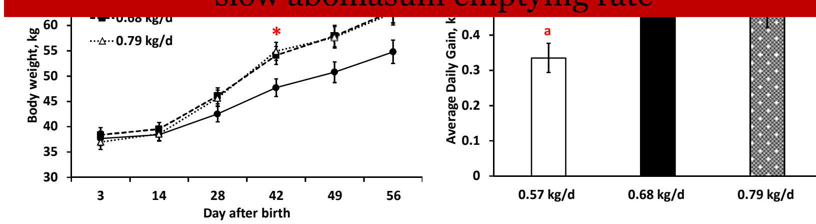


Experimental design



Calves fed 0.68 kg/d and 0.79 kg/d had similar body weight and ADG but greater than 0.57 kg/d

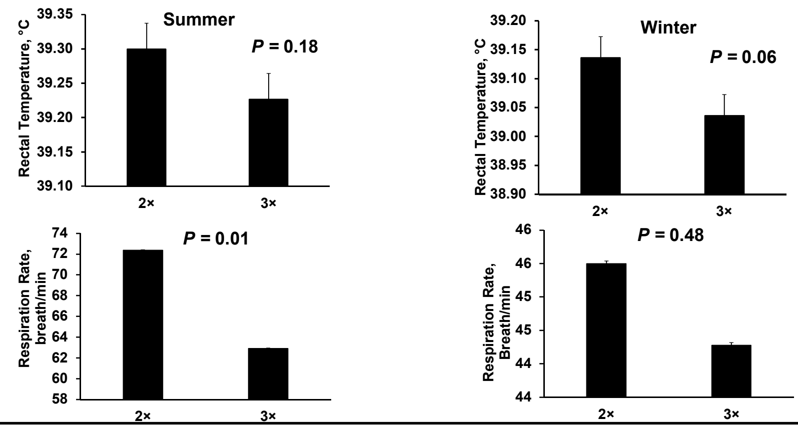
Increasing feeding rate during summer can potentially slow abomasum emptying rate



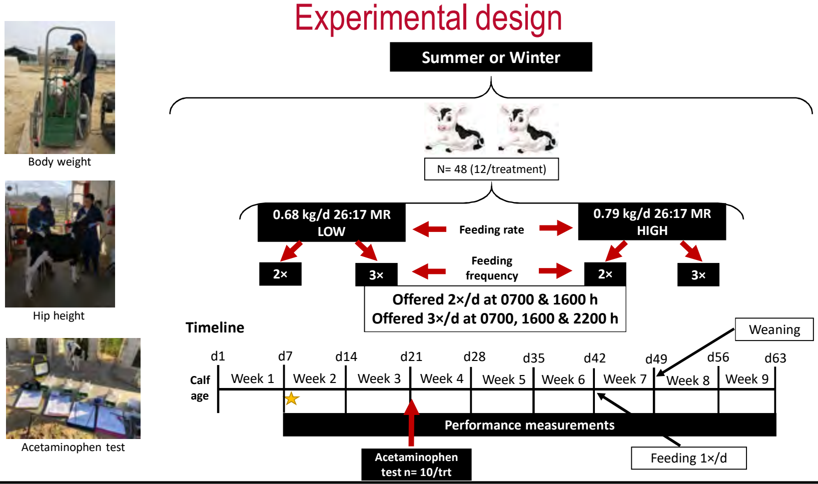
Potential solution

- Increasing feeding frequency:
  - Accelerates abomasum emptying
- Would increasing feeding frequency during summer improve calf performance when larger amount of MR is fed?

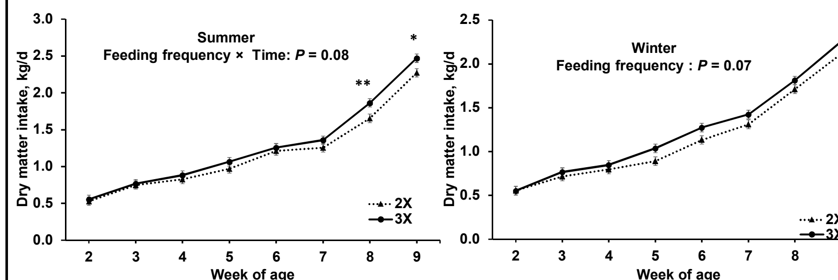
Feeding more frequently reduced heat load in the summer



Experimental design



Feeding more frequently improves intake



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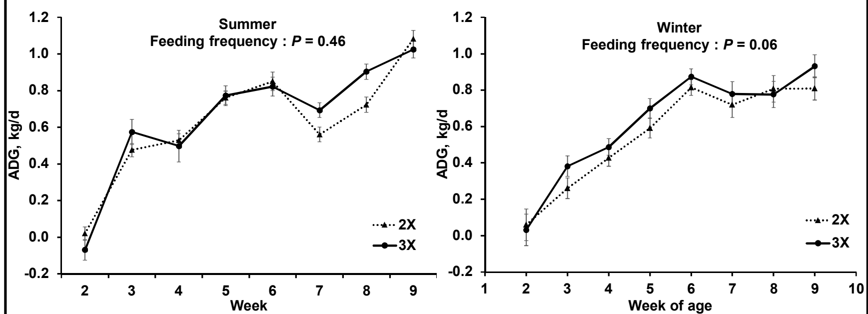
Feeding milk replacer more frequently improves growth only during winter



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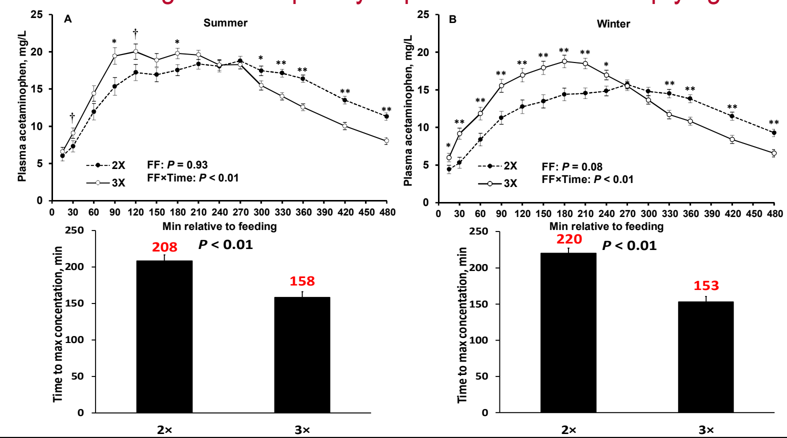
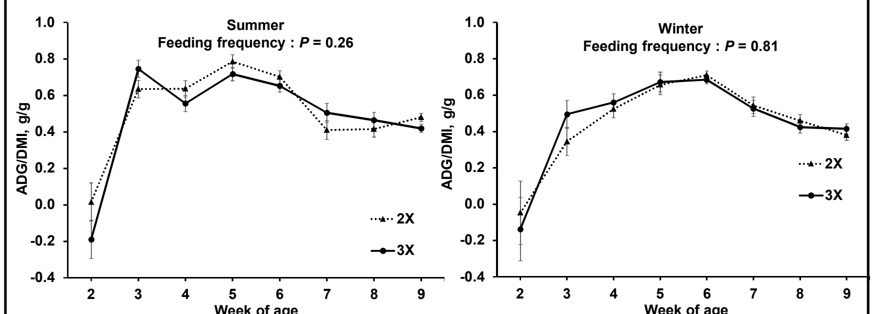
Feeding more frequently improves abomasal emptying



Feeding milk replacer more frequently doesn't affect feed efficiency



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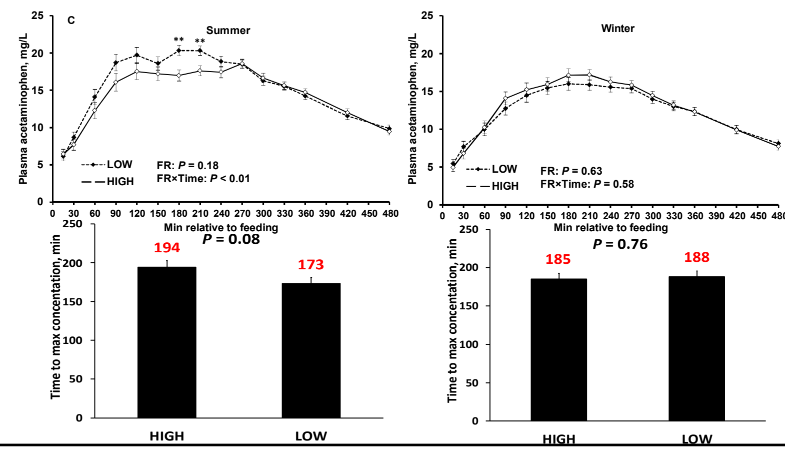
Feeding more milk replacer only delays abomasal emptying during summer



Conclusions

- Preweaned calves experience heat stress, and initiate evaporative cooling around 20 °C
- Day to day and within a day temperature variation have negative impact on calf performance
- Feeding too much milk replacer with limited frequency may not benefit calf growth.

Methods for silage conservation to improve quality

Oscar Queiroz (presenter), Ivan Eisner, Audrey Segura, Giuseppe Copani, Erik Dorr

1

Definition of Silage

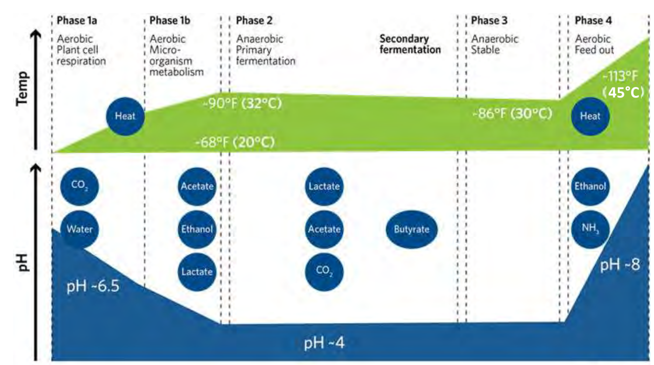
Silage is defined by the USDA as "any crop that is harvested green and preserved in a succulent condition by partial fermentation in a more-or-less airtight container such as a silo"

2

Ensiling Process and feed out

3

Phases of ensiling



Microorganisms play a key role during most of the phases! Feedout

4

5

CCP 1 – People: safety and decision making

- Have we done everything possible to ensure safety for us, our employees and our family members?
- Who is empowered to make the decision to **start**, **alter** or **stop** the harvest and ensiling process?

Every serious injury or fatality during the ensiling process could have been prevented!

Identify the decision-makers and empower them to make the 'right' decision in a timely manner.

6

CCP 2 – Maturity

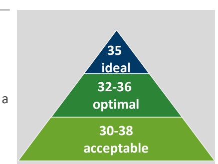
- Maturity reflects the antagonism between quantity (yield) and quality (digestibility).
- Consider the balance between total NDF and total starch versus NDF-d and Starch-d when determining optimal maturity.

Optimal harvest time point:
1/2 -2/3 milk line

7

CCP 3 – Moisture (or DM, if you prefer)

- The target moisture for whole plant maize for silage is 35% DM, with a narrow ideal range of 32-36% DM and a realistic range of 30-38% DM.



Bottom line:
Hitting target moisture is critical for significantly reducing the risk of undesirable fermentation, minimizing effluent, achieving pack density (minimizing porosity), and excluding oxygen from storage.

How to measure dry matter at the farm?
SS-AGR-178/AG181: Methods of Forage Moisture Testing (ufl.edu)

8

Impact of maturity on the composition of corn plants

Effect of maturity at harvest on yield characteristics of 111 day season corn

Item	Days from Black layer [1]								P-value[2]	
	-18	-13	-10	-6	-3	3	8	SEM	Day	Day*Day
% DM	33.5	32.5	35.7	34.4	37.9	39.7	40.7	0.27	<0.01	<0.01
Yield[3]	23.8	24.4	24.9	24.7	29.4	28.0	29.6	0.19	<0.01	0.98
Grain %	40.7	44.2	48.3	47.9	48.1	52.1	50.4	0.32	<0.01	<0.01
NDF-Digestibility (plant)[4]	51.8	51.1	47.5	45.1	41.6	42.0	43.3	0.43	<0.01	<0.01
NDF-Digestibility (cob)[4]	33.5	22.4	-	-	-	21.2	23.6	0.83	<0.01	<0.01
Crude Protein	8.7	8.5	7.9	7.3	7.3	7.3	6.4	0.08	<0.01	0.05

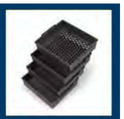
[1]Days from Black layer: -18= August 22; -13= August27; -10= August 30; -6= September 3; -3= September 6; 3= September 12; 8= September 17. Black layer approximately September 9, 2013.
[2]Lin= P- value for the linear effect response to plant maturity Quad= P-value for the quadratic effect response to plant maturity (days from Black layer)
[3]Silage Yield in DM metric ton/hectare
[4]28-h in situ digestibility as percentage of plant
[5]Protein as percentage of plant height on DM basis

Row, 2015

CCP 4 – Particle length

- Common recommendation: between 1 and 2 cm on average.
- Wetter silage needs longer particle length to avoid effluent outflow.
- Dry silage needs shorter particle length to ensure sufficient compaction.
- Check the distribution of particles using Penn State Separator

Bottom line:

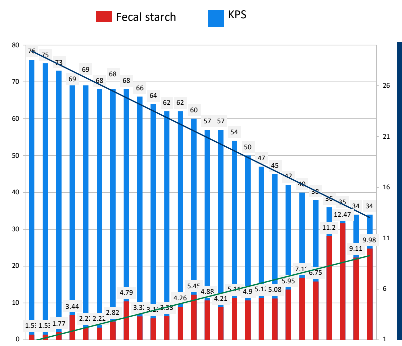


To ensure the optimal level of the effective fiber for dairy cows this part of the sample should retain:

3-8 % top screen

< 10% bottom pane

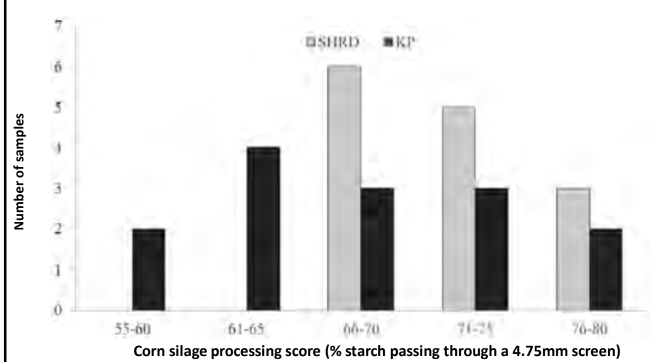
CCP 5 – Kernel Processing Score (KPS)- After the fact



- KPS≥70, optimal
- 50 ≤KPS≤ 69 adequate
-
- KPS<50 inadequate
- Every 1.0 % fecal starch ~ 0.3 kg milk (Ferguson, 2003)

Source: Braman and Kurtz, 2015

CCP 5 – Kernel Processing Score (KPS)- Shredlage (SHRD) vs. Conventional (KP)



Source: Vanderwerff et al., 2015

CCP 5 – Kernel Processing Score (KPS)- After the fact

CCP 5 – Kernel Processing Follow-up During Harvesting

32 oz cup- technique

Water separation technique

How to follow-up processing during harvesting?
https://fyi.extension.wisc.edu/forage/files/2014/01/KernelProcessing-FOF.pdf

CCP 6 - Manage packing for optimum density

Follow "the rule of 400": The total weight of the packing tractors in kg should be calculated as:

Packing weight in kg: 400 x Filling rate t/hour

Control speed:

Packing speed: as slow as possible, about 4-5 km/h

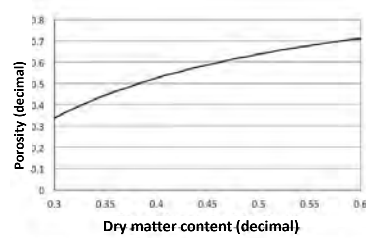
Maximum 10 cm layers of crop

Goal is to achieve 700kg/m^3

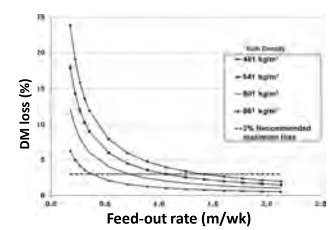
Operational weight t:
New Holland T8050 – 9.3 t
John Deere 7R – 11.4 t

If the filling speed is 100 t per hour, the total packing weight of the tractors should be above 40 t.

Bulk density will impact early fermentation establishment and feed-out



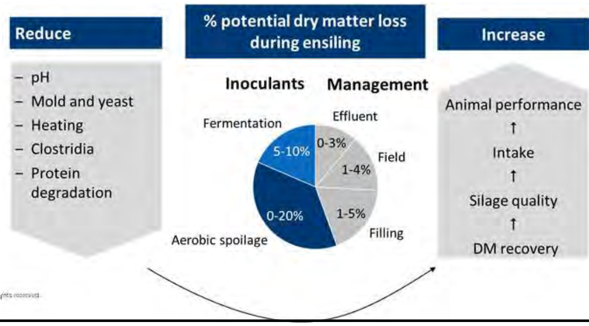
Porosity of silage packed to constant DM density (225 kg of DM/m3) as a function of DM content assuming an ash content of 5% (adapted from Holmes and Muck, 2009)



Dry matter losses at the feed-out face of a silo as influenced by bulk density and feed-out rate (adapted from Holmes and Muck, 2007)

CCP 7 – Inoculant

- Silage inoculants contain selected strains of bacteria, capable of positively impacting the fermentation.
- Usually inoculants are divided in homo or heterofermenters and their combination.



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CCP 7 – Inoculant- Homofermenters

- Focus on fast acidification of silage by the increase of lactic acid concentration
- More efficient fermentation resulting on lower DM loss
- Reduce load of undesirable bacteria (ex. Clostridium)
- No antifungal properties
- Homofermentative and facultative heterofermentative lactic acid bacteria

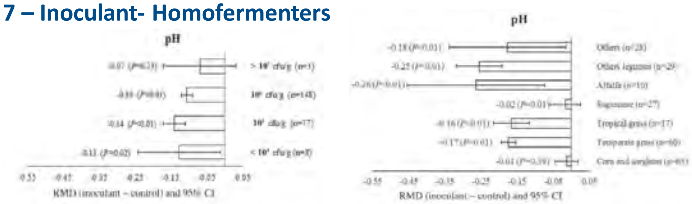
Chemical composition of Alfalfa silage inoculated with homofermentative bacteria

	Control	FA+LC	FA+LP
pH	5[b]	4.0[a]	4.2[a]
DM (%)	31.1	30.8	31.0
Lactic ac. (g/kg)	8.3[a]	21.6[b]	19.8[b]
Butiric ac (g/kg)	17.2[b]	8.6[a]	8.2[a]
Ammonia (g/kg of N)	108.5[b]	50.2[a]	45.3[a]
DM loss (%)	6.1[b]	4.1[a]	3.6[a]

FA= *Enterococcus hirae*, LC= *Lactobacillus casei*, LP= *Lactobacillus plantarum* (adapted from Cai, 1999)

18

CCP 7 – Inoculant- Homofermenters



			RMD[3] (95% CI)		Heterogeneity[4]	
Item	Control[1] (SD)	N[2]	Random effect	*P*-value	*P*-value	I^2 (%)
DMI (kg/d)	17.80 (3.72)	32	0.26 (-0.03, 0.54)	0.08	<0.01	71.5
DM digestibility (%)	69.9 (4.42)	6	-0.42 (-1.22, 0.39)	0.31	0.47	0.0
Milk yield (kg/d)	25.04 (7.13)	38	0.37 (0.09, 0.65)	<0.01	0.06	28.3
Feed efficiency	1.49 (0.26)	10	0.03 (-0.01, 0.06)	0.18	0.68	0.0
Milk fat (%)	3.77 (0.43)	40	0.04 (0.00, 0.08)	0.08	<0.01	69.2
Milk protein (%)	3.06 (0.23)	37	0.02 (0.00, 0.03)	0.06	<0.01	42.3

[1]= Uninoculated treatment. [2]N=Number of comparisons between inoculated and uninoculated treatments. [3]RMD = raw mean differences
[4]P-value to X^2 (q) test of heterogeneity; I^2= proportion of total variation of size effect is due to heterogeneity.

Oliveira et al., 2017

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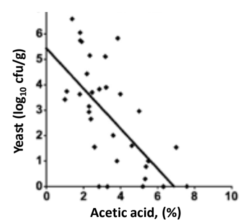
CCP 7 – Inoculant- Heterofermenters

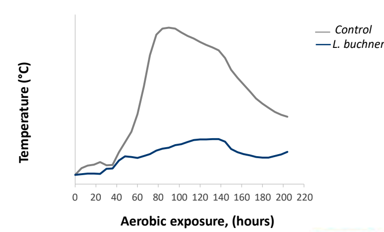
- Focus on increasing acetic acid (antifungal)
- Reduction of yeast
- Increase in aerobic stability of silage

Relationship between the concentration of acetic acid (DM) and number of yeast in corn silage



Kleinschmidt and Kung, 2006

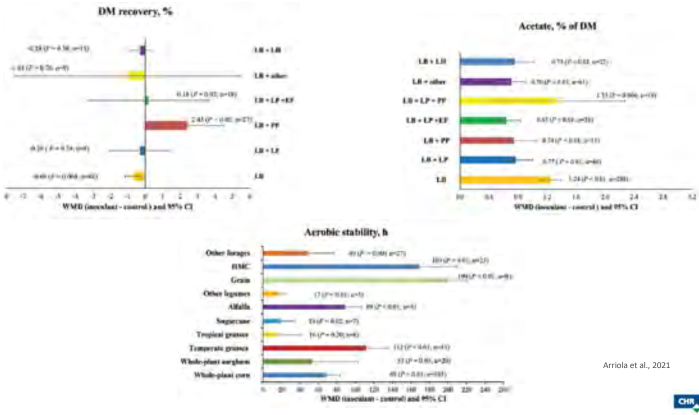
Effect of *L. buchneri* on aerobic stability on corn silage



Copani et al, 2019

20

CCP 7 – Inoculant- Heterofermenters and combinations



Arriola et al., 2021

21 LB= *L. buchneri*, LH= *L. hilgardii*, LP= *L. platarum*, PP= *P. pentosaceus*, EF= *E. faecium*, Others = (*L. lactis, L. casei, P. acidilactici*)
WMD = weighted raw mean

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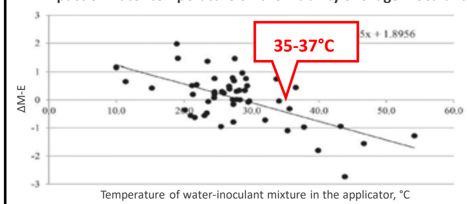
CCP 7 – Inoculant

Impact of water temperature on the viability of silage inoculants



Windle and Kung et al., 2016

In order for any inoculant to work,

- Clean the applicators before the harvest
- Avoid hot, dirty water and heating of the inoculant solution
- Dissolve the product according to the instructions

ΔM-E= Log difference between the measured and expected numbers of LAB

22

22

CCP 8 – Sealing

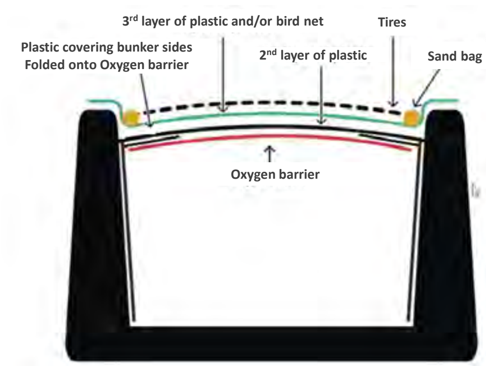
- Once packing is completed, apply top layer protection for increased nutrient preservation.
- Use oxygen barrier film.
- Cover quickly and make sure the tires are touching each other!

Bottom line:
Cover quickly, efficiently, thoroughly and safely!
Oxygen is the enemy!

23

23

Sealing management – would you leave 30,000 € in cash unprotected?



Any item left out is a step in the wrong direction

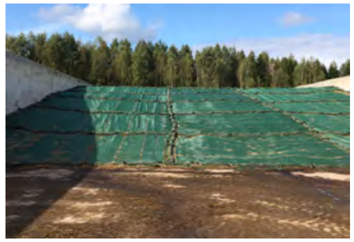
A bunker dimensioned 10x30 m and 3 m high holds 900 m³ of forage. At 35 €/m³ this equals 31,500 €

24

24

CCP 8 – Sealing

Good approach: tires mostly touch each other. But no protection against birds.



Better approach: gravel bags create a good barrier against air penetration under the plastic. Bird net prevents cover against damages by birds.

CCP 8 – Sealing – Oxygen Barrier and Top spoilage losses

Characteristics of plastic films

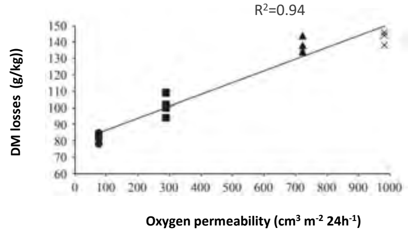
	Plastic film			
Item	OB	PE	PVC	PVOH
Nominal Thickness (µm)	125	200	300	200
Measured Thickness (µm)	121 ± 3.2	189 ± 4.2	280 ±6.1	192 ± 3.98
Oxygen permeability ($cm^3\ m^{-2}$ per day)	75 ± 1.6	722 ± 19.6	982 ±32.3	289 ± 5.1

OB, Polyethylene/polyamide; PE, Polyethylene; PVC, polyvinyl chloride; PVOH, PE/ polyvinyl alcohol.

Bernardes et al., 2011

CCP 8 – Sealing – Oxygen Barrier and Top spoilage losses (Corn silage)

Relationship between DM losses and permeability
Y= 0.0718x + 79.217
R^2=0.94



● OB, Polyethylene/polyamide; ▲ PE, Polyethylene; X PVC, polyvinyl chloride; PVOH, ■ PE/ polyvinyl alcohol. Bernardes et al., 2011

Take home message: critical control points for high quality silage

- Safety first!
- Maturity stage and dry matter content: Optimum balance between volume and quality.
- Optimal length of chop (particle size).
- Compaction. Rule of 400!
- Seal fast and completely.
- Protect what's good - avoid rodents, birds – and plastic that moves!
- Science-based, research-proven inoculant.

Extra slides

29

No go

A lot of good silage is not fed. Spoiled silage is not removed and the volume is not recorded.

Holes in the plastic (made by birds, rodents) cause a lot of spoiling due to air penetration

Protection net prevents against damages and save a lot fo losses

30

No go

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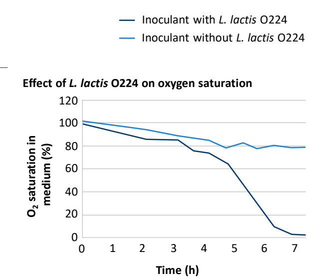
Lactococcus lactis (O-224, DSM11037)

Oxygen scavenging ability

L. lactis (O-224) works quickly to remove oxygen, which inhibits any aerobic spoilage microorganisms and improves conditions for anaerobic lactic acid bacteria

Reduces spoilage of silage

Early onset of anaerobic conditions saves crop sugars as nutrients for lactic acid bacteria. It results in fast pH reduction and accelerate the fermentation.



The goal of silage making is to preserve the harvest crop by **ANAEROBIC FERMENTATION** (Without Oxygen).

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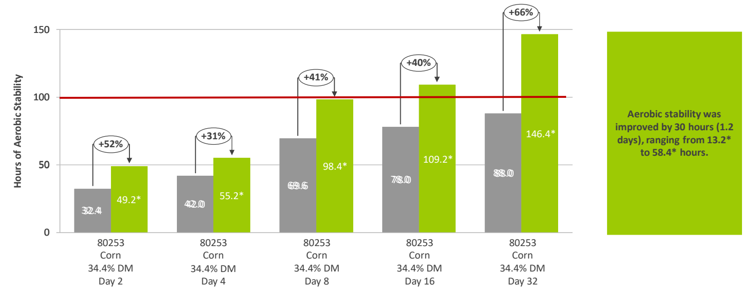
Oxygen is the enemy

Good compaction is essential to avoid aerobic deterioration

210 kg/m³	265 kg/m³
Gas filled porosity 48.9 %	Gas filled porosity 36.1 %

Even with good compaction more than 30 % of volume in the bunker is air! Lactococcus lactis O224 helps to create anaerobic conditions faster.

The combination *L. buchneri* and *L. lactics* improved aerobic stability at early feed out in corn silage



*P<0.05 significantly different from untreated.

Forage Conservation for winter cow-calf production systems

UF | IFAS UNIVERSITY of FLORIDA
Range Cattle Research & Education Center

2022 Florida Ruminant Nutrition Symposium

Joao (Joe) Vendramini

1

Introduction

Hay = Forage preserved by drying above 85% DM

Baleage = Forage preserved by fermentation in a bale with lesser DM concentration than hay (≤ 85% DM) but greater than silage (≥ 30-35% DM)

Haylage = Forage preserved by fermentation with lesser DM concentration than hay (≤ 85% DM) but greater than silage (≥ 30-35% DM)

Silage = Forage preserved by fermentation at ≤ 30-35% DM

Vendramini and Moriel (2019)

2

Introduction

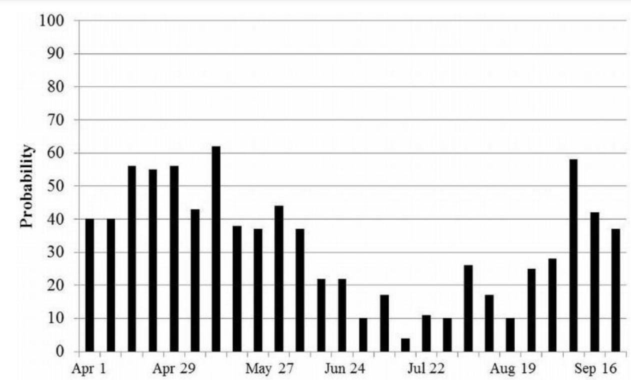
Intake and apparent digestibility of Big Bluestem hay and Baleage

Item	DM (%)	DM Intake (% BW)	DMD (%)	DDMI (%)	NDFD (%)	DNDFI (%)
Hay	89	1.5	545	0.83	590	0.66
Baleage	45	1.7	504	0.91	537	0.66
P value	--	0.07	0.03	0.67	<0.01	0.97
SE	--	0.08	46.6	0.06	43.4	0.06

Burns and Fisher (2012)

3

Introduction



Adapted from Bates et al., 1989.

4

Hay

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5

Hay

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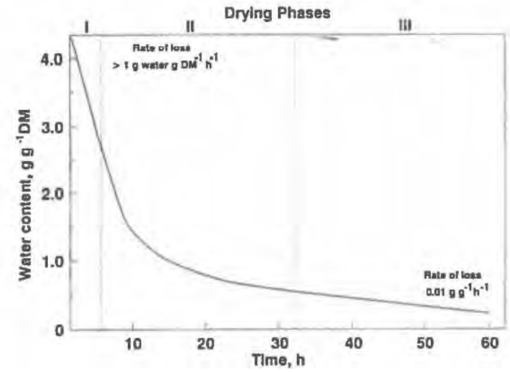

6

Hay

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https://www.youtube.com/watch?v=maZAtL5KE7w&t=267s

7

Hay

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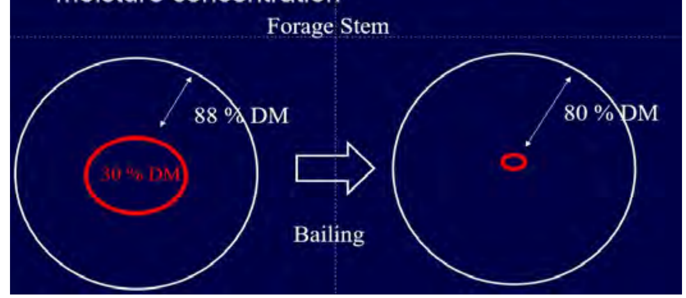
- ✓ Hay "Sweating"
- ✓ Inaccurate measurement of moisture prior to baling, which may lead to baling at higher moisture concentration



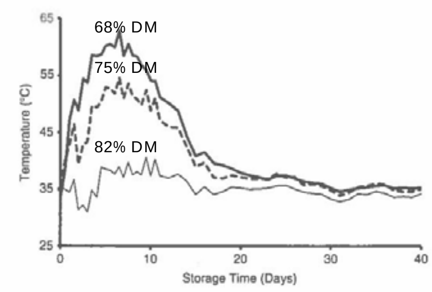
8

Hay

- Small increase in temperature is expected; however, large increases in temperature may be evidence of microbe fermentation
- Large increases in temperature results in DM and nutritive value losses in hay. In addition, it can cause spontaneous combustion

9

Hay



10

Hay

11

Hay

Storage System	Dry Matter (%)	Animal Refusal (%)	TOTAL (%)
Ground	28	22	50
Gravel	31	17	48
Tires	35	6	41
Rack	26	6	32
Rack with cover	12	2	14
Barn	2	1	3

* Hay stored for 7 months

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Haylage

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Haylage

- Warm-season grasses have undesirable characteristics for successful preservation by fermentation
  - High water concentration
  - Decreased concentration of water-soluble carbohydrates (WSC)
  - The main WSC stores is starch, and LAB do not have the ability to ferment starch directly (McDonald et al. 1991)

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Haylage

15

Haylage

- There is a misperception that the fermentation process would increase forage nutritive value
- Harvesting at the optimum regrowth interval is crucial to have baleage with acceptable quality

Weeks	Herbage Accumulation (kg/ha)	CP (%)	TDN (%)
2	1500	16	56
4	2100	13	57
6	3200	9	52
8	3600	7.5	48
10	4600	8.0	46

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Haylage - DM concentration

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17

Haylage - DM concentration

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	High DM	Low DM	*P* value
DM (%)	53	22	<0.01
pH	4.4	4.7	<0.01
Lactic acid (%)	4.3	2.8	<0.01
Acetic acid (%)	1.2	3.9	<0.01
Ammonia (%)	7.6	13.7	<0.01

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Haylage - Inoculants

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19

Haylage - Inoculants

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Item	Inoculant								*P* value	SE
	Control	B500	BPII	ESA	F20	F600	HQ	VS-3		
pH	4.6[b]	4.95[ab]	4.9[a]	4.8[ab]	4.8[ab]	4.91[ab]	4.7[ab]	4.6[b]	0.007	0.1
Lactic acid, % DM	2.23[a]	0.60[ab]	0.91[ab]	1.64[ab]	1.59[ab]	0.47[b]	1.64[ab]	1.97[a]	0.01	0.68
Acetic acid, % DM	2.45[ab]	3.32[a]	2.35[ab]	2.46[ab]	2.41[ab]	1.84[ab]	2.03[ab]	0.32[b]	0.04	0.32
Propionic acid, % DM	0.35	0.39	0.29	0.40	0.35	0.57	0.31	0.27	0.40	0.15
Butyric acid, % DM	3.24	3.90	3.19	4.78	4.11	4.73	4.22	3.88	0.64	1.1
Isobutyric acid, % DM	0.15	0.17	0.20	0.25	0.11	0.11	0.14	0.09	0.28	0.3
Ammonia, % CP	21.2	28.0	29.6	26.1	18.6	20.6	21.6	19.8	0.09	6.1

Gouvea et al. (2020)

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Haylage - Molasses

UF UNIVERSITY of FLORIDA

https://www.youtube.com/watch?v=9sTKjVxFmKQ&t=210s

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Haylage - Molasses

UF UNIVERSITY of FLORIDA

	Control	Molasses	*P* value
DM (%)	22	24	0.64
CP (%)	13.1	12.3	0.18
WSC (%)	0.4	1.0	<0.01
IVTD (%)	53	58	<0.01
pH	4.8	4.6	<0.01
Lactic acid (%)	2.7	3.6	<0.01
Acetic acid (%)	0.8	0.9	0.13
Ammonia (%)	8.3	9.8	0.15

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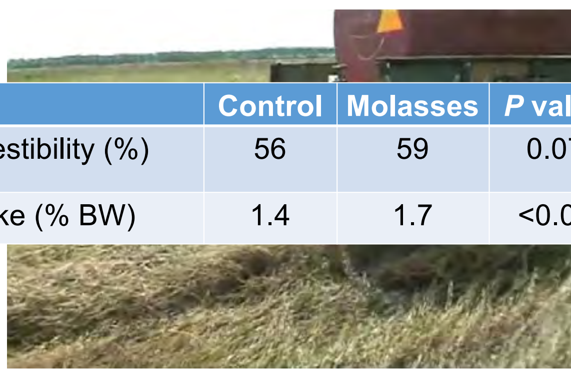
Haylage - Molasses

UF UNIVERSITY of FLORIDA



	Control	Molasses	*P* value
Digestibility (%)	56	59	0.07
Intake (% BW)	1.4	1.7	<0.01

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Haylage - Propionic Acid

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Haylage - Propionic Acid

UF | UNIVERSITY of FLORIDA

	Propionic Acid (% Green Forage)				SE
	0	0.5	1.0	0.5+ESA	
DM (%)	24	27	26	24	0.41
CP (%)	11.0	11.4	14.0	11.6	0.65
pH	5.1a	4.5b	4.3b	4.5b	0.14
Latic Acid (% DM)	0.1c	3.8b	5.3a	3.8b	0.60
Acetic Acid (% MS)	1.6a	0.8b	0.9b	0.9b	0.08
Propionic Acid (% DM)	0.5c	0.8bc	2.1a	0.9b	0.15
Butyric Acid (% DM)	4.5a	1.3b	0.1b	1.3b	0.06
Isobutyric Acid (% DM)	0.2a	0.05b	0.00b	0.05b	0.03
Ammonia (% N)	31.8a	16.6b	19.8b	19.8b	3.5

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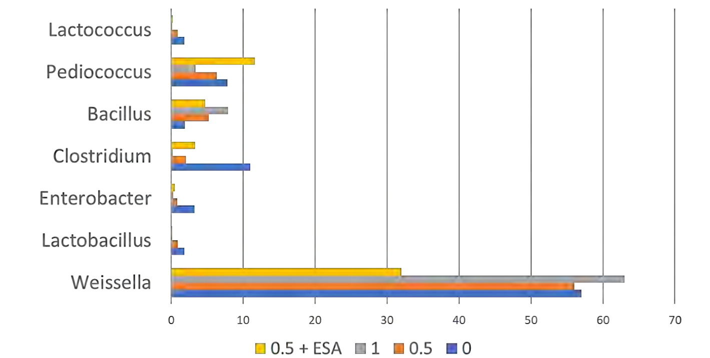
Haylage - Propionic Acid

UF | UNIVERSITY of FLORIDA



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Haylage - Propionic Acid

UF | UNIVERSITY of FLORIDA

Control (Untreated)

0.5% Propionic Acid

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Economics

UF | UNIVERSITY of FLORIDA

	Cost of Production	Dry matter	DM/bale	Custom Cost for harvesting and bailing	Custom Wrapping Cost	Total Cost	Cost per lb of DM
	$ / 700 lb (green weight) bale	(%)	(lb)	$ / 630 lb DM	$ / 630 lb DM	$ / 630 lb DM	$
Hay	35	90	630	20	--	55	0.08
Haylage	35	45	315	40	10	85	0.13

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Thank you

Joe Vendramini
UF Range Cattle
Research Center

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Make your herd resilient to hidden challenges

Ben Saylor, PhD, PAS
Dairy Technical Services Manager

Florida Ruminant Nutrition Symposium
May 9 – 11, 2022
Gainesville, FL

1

Presentation Outline

I. What does it mean to have a resilient herd?

II. Hidden challenges to resilience

III. The "3 Pillars" of a resilient animal

IV. The multiple functionalities of *Bacillus* for improving herd resilience

V. Effect of *Bacillus* supplementation on performance of dairy cattle

VI. Questions

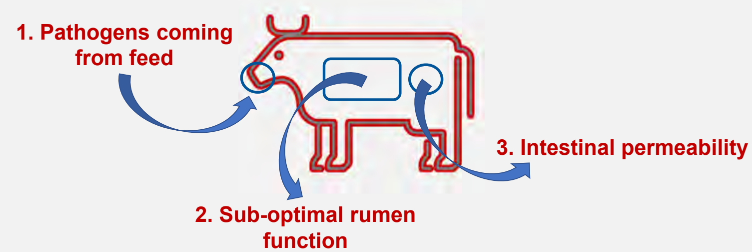
2

What does it mean to have a resilient herd?

- **Resilience** – "the capacity to recover quickly from difficulties" (Oxford Languages)
- **A resilient herd** can achieve consistent, high-level performance in the face of various pressures
- **Pressures:**
  - Nutritional – variation in feed quality, slug feeding, pathogens, mycotoxins
  - Environmental – heat stress, cold stress, wind
  - Social – over-crowding, pen moves, herd dynamics
  - Others?

3

Hidden challenges to resilience

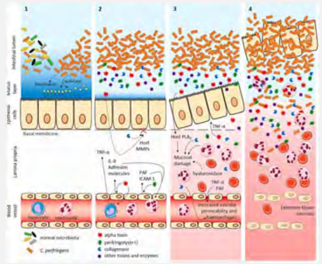


4

Hidden Challenge #1
Pathogens coming from feed

Subclinical → Clinical

- Clostridia, *Salmonella*, *E. coli*
- Clostridia are detected in **95% of TMR samples** (n=4,765)
  - Gram-positive, obligate anaerobes, spore-forming
  - Over 100 species
  - Toxin producing – *C. perfringens*
    - Known contributor to HBS in dairy cattle



Bretl et al. (2022) Abstr.
Goossens *et al. Vet Res (2017) 48:9*

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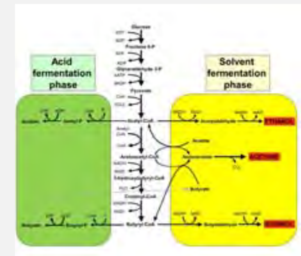
Hidden Challenge #1
Pathogens coming from feed

- Clostridia, *Salmonella*, *E. coli*
- Clostridia are detected in **95% of TMR samples** (n=4,765)
  - Gram-positive, obligate anaerobes, spore-forming
  - Over 100 species
  - Solvent producing – *C. beijerinckii*, *C. bifermentans*
    - Produce acetone, ethanol, butanol
    - Shown to negatively impact rumen function



6

Hidden Challenge #1
Pathogens coming from feed

- **Clostridial outgrowth** has been observed in TMR samples

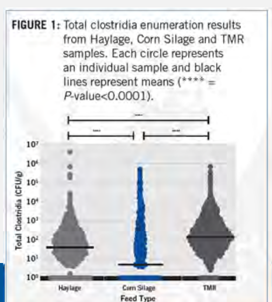


FIGURE 1: Total clostridia enumeration results from Haylage, Corn Silage and TMR samples. Each circle represents an individual sample and black lines represent means (**** = P-value<0.0001).

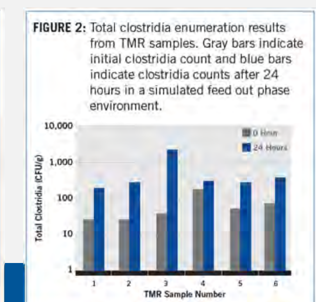


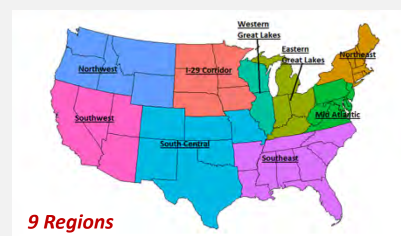
FIGURE 2: Total clostridia enumeration results from TMR samples. Gray bars indicate initial clostridia count and blue bars indicate clostridia counts after 24 hours in a simulated feed out phase environment.

Seemingly low clostridia counts in the ration can increase exponentially across a day's worth of feed.

7

ARM & HAMMER Pathogen Survey

The objective of this survey was to examine clostridia populations from concentrated dairy regions across the United States to identify regional similarities and differences.



29,400 fecal samples from 786 farms in 30 states
Representing over 1.4 million dairy cows (15.9% of US dairy cows)

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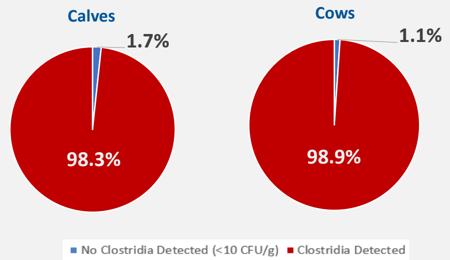
Hidden Challenge #1

Pathogens coming from feed

Percent of fecal samples that tested positive for clostridia



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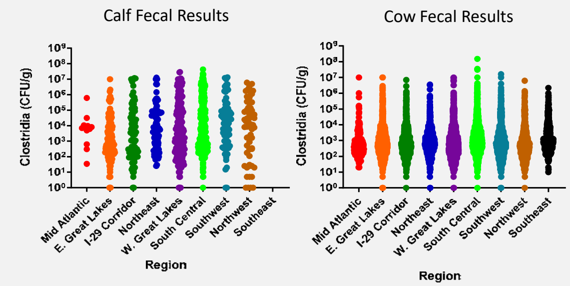
Hidden Challenge #1

Pathogens coming from feed



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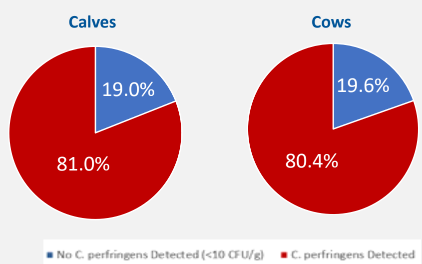
Hidden Challenge #1

Pathogens coming from feed

Percent of fecal samples that tested positive for *C. Perfringens*



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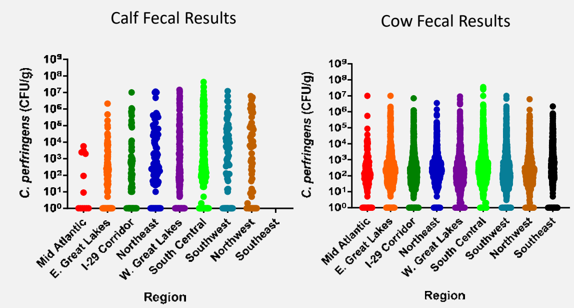
Hidden Challenge #1

Pathogens coming from feed



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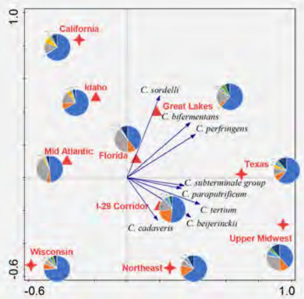
Hidden Challenge #1
Pathogens coming from feed



Clostridial control demands a REGIONAL APPROACH

Indicates that clostridial communities are significantly different

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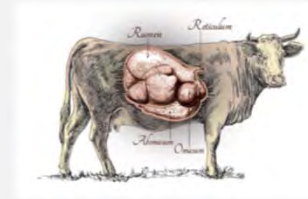
Hidden Challenge #2
Sub-optimal rumen function

- **30% - 50%** of cows experience sub-optimal rumen function
  - Low rumen pH
  - Reduced rumination activity
  - Reduced milk fat %
  - Poor manure consistency
- **Causes:**
  - Feeding highly fermentable diets w/ inadequate effective fiber
  - Slug feeding (summer heat, high stocking density)
  - Off-feed events (empty bunks, transition, environmental stress, disease)
  - Poor feed hygiene



Outcomes:
- Reduced capacity to digest fiber
- Reduced absorption of SCFA
- Reduced milk fat %

Bramley et al. (2008)
Beauchemin and Penner (2019)
Zhang et al. (2013)

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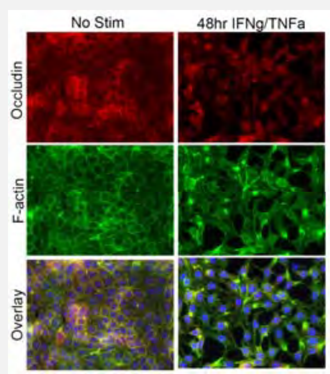
Hidden Challenge #3
Intestinal permeability

- The intestinal epithelium (gut lining) serves a dual purpose:
  - Nutrient absorption
  - Protection from pathogens and toxins in the GIT
- Intestinal barrier integrity can be negatively affected by various stressors:
  - Heat stress
  - Feed restriction (off-feed events)
  - Pathogenic challenges (*C. perfringens*)
  - Inflammation



Horst et al. (2021)
Kvidera et al. (2017)
Zhang et al. (2013)

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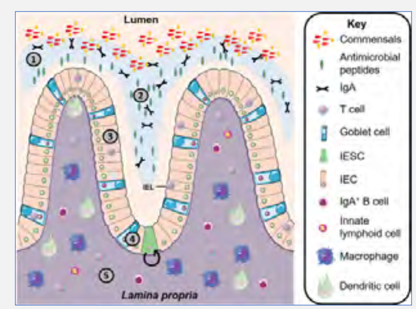
Hidden Challenge #3
Intestinal permeability

- When pathogens/toxins breach the intestinal barrier, immune cells and tissues react
- Lamina propria is home to estimated 70-80% of the immune system
- Immune response (**INFLAMMATION**)
  → Health issues/Productivity losses
- Proper barrier function is **ESSENTIAL** for optimal nutrient absorption and effective protection.



Horst et al. (2021)
Kvidera et al. (2017)
Mucosal Immunol **4**, 31–42 (2011)

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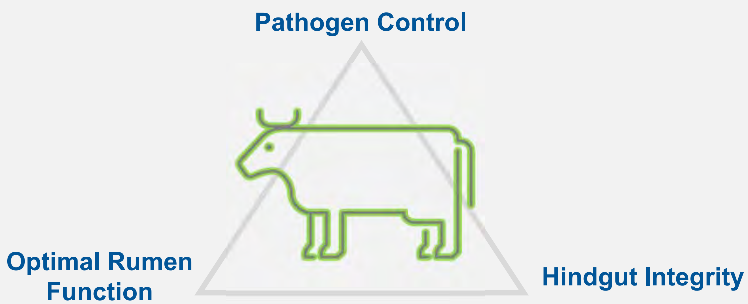
Three pillars of a resilient animal



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The multiple functionalities of *Bacillus* for improving herd resilience

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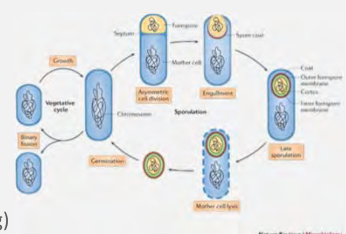
Intro to *Bacillus*

- Over 300 species and sub-species of *Bacillus*
- *Bacillus* on AAFCO Generally Recognized as Safe (GRAS) list:
  - *Bacillus amyloliquefaciens*
  - *Bacillus coagulans*
  - *Bacillus lentus*
  - *Bacillus licheniformis*
  - *Bacillus pumilus*
  - *Bacillus subtilis*
- Spore-forming
  - Highly resistant to processing (heat and pelleting)
- Enter vegetative cycle with adequate moisture and nutrients



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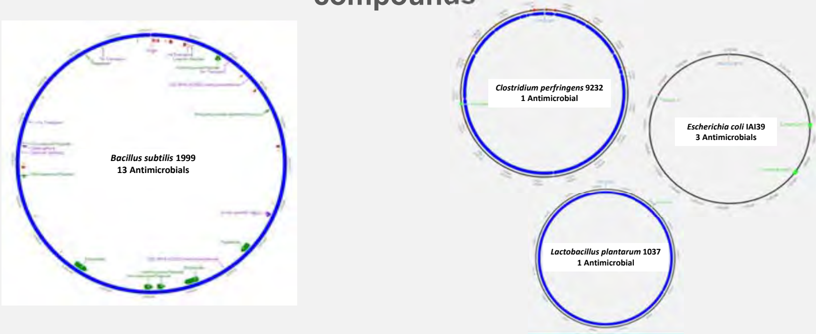
Improving resilience with *Bacillus*

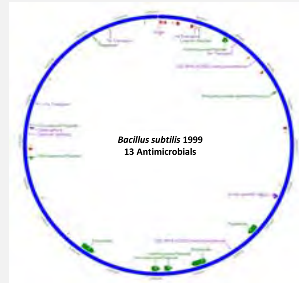
20

Bacillus produce numerous antimicrobial compounds



21

Bacillus produce numerous antimicrobial compounds



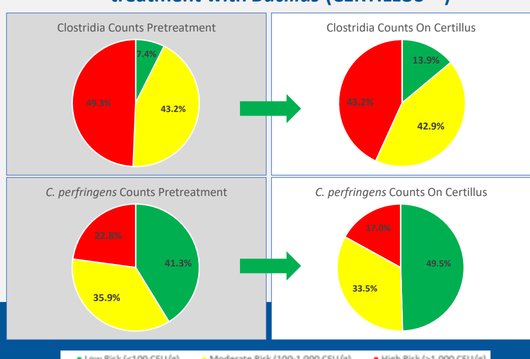
- Example antimicrobials:
  - **Lipopeptides** – disrupt pathogen membrane function.
  - **Polyketides** – inhibit protein synthesis in pathogens.
  - **Siderophores** – scavenge iron away from pathogens.

22

Using *Bacillus* to shift the pathogen landscape

Shift in risk associated with total clostridia and *C. perfringens* before and after treatment with *Bacillus* (CERTILLUS™)

77 Dairies
230,000 cows
18 states



***89% increase** in low-risk samples*

***13% decrease** in high-risk samples*

***20% increase** in low-risk samples*

***26% decrease** in high-risk samples*

23

With *Bacillus*, strains matter.

	Growth Inhibition by *Bacillus* Strain									
Isolate	**747**	**839**	**1104**	**1541**	**1704**	**1781**	**1999**	**2018**	**2084**	**15533**
Clostridium perfringens	86	100	63	25	99	86	80	68	60	38
Clostridium perfringens	91	100	90	50	100	90	91	91	84	76
Clostridium perfringens	63	99	31	69	100	54	55	38	25	20
Clostridium perfringens	68	100	61	97	100	62	67	66	50	36
Clostridium butyricum	86	100	84	97	100	85	85	87	73	78
Clostridium bifermentans	61	100	12	1	96	53	62	7	2	29
Clostridium bifermentans	91	100	46	13	98	86	88	55	58	6
Clostridium bifermentans	43	98	32	100	98	20	0	37	0	12
Clostridium beijerinckii	33	98	15	94	98	23	0	32	0	15
Clostridium beijerinckii	90	100	64	50	100	85	84	89	68	100
Pathogenic *E. coli*	90	100	59	49	98	98	91	56	58	10
Pathogenic *E. coli*	84	100	29	1	82	52	68	30	46	33
Pathogenic *E. coli*	99	100	16	3	99	95	98	17	31	11
Pathogenic *E. coli*	62	99	8	1	86	58	74	8	11	15
Pathogenic *E. coli*	99	99	30	21	98	90	77	31	43	17
Pathogenic *E. coli*	100	100	34	23	99	99	95	37	51	12
Pathogenic *E. coli*	100	100	35	14	99	100	98	36	51	16

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ARM & HAMMER's approach to pathogen control

- Library of 30,000 *Bacillus* strains (15 commercialized strains)
- More than 200,000 isolates of clostridia, *E. coli*, and *Salmonella* against which we can test inhibition activity of our *Bacillus*
- **Regional** and **Custom** formulations
- **Routinely validated and updated** based on regional pathogen sampling

Microbial Terroir

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Improving resilience with *Bacillus*

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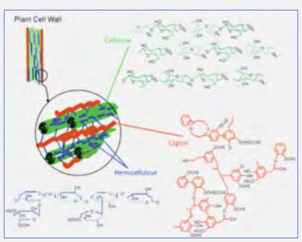
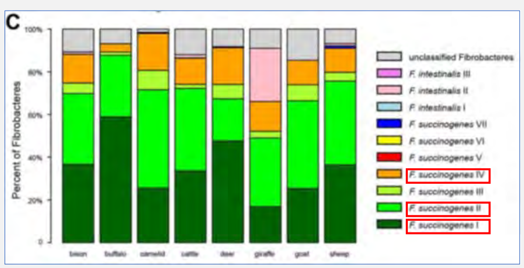
Fibrobacter succinogenes and fiber degradation.





Neumann et al. (2017)

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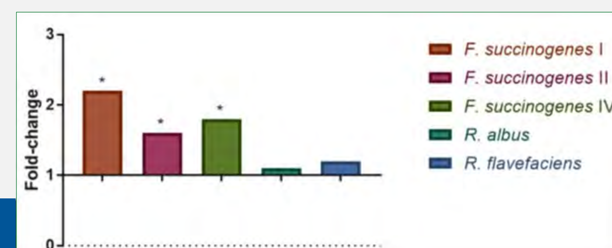
Using *Bacillus* to increase populations of fiber digesting bacteria

- Data from in vivo field demonstration at commercial dairy
- Rumen fluid samples collected after 12 weeks of supplementation with 2 billion CFU/hd/d of *Bacillus* (CERTILLUS™).



*Indicates a significant ($P \leq 0.05$) fold-change

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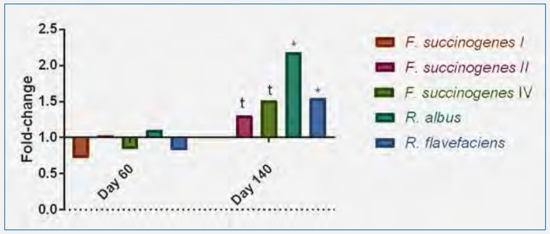
Using *Bacillus* to increase populations of fiber digesting bacteria

- Data from in vivo field demonstration at commercial dairy
- Treated animals supplemented with 2 billion CFU/hd/d of *Bacillus* (CERTILLUS™).



*P ≤ 0.05
ᵗ0.05 < P ≤ 0.10

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Rumen function and animal resilience

- Increasing ability of the rumen to digest fiber has potential to:
  - Increase energy supply from fibrous feedstuffs (forages, high-fiber byproducts)
  - Improve the rumen's ability to handle fluctuations in forage quality and digestibility
  - Improve animal **EFFICIENCY** and **RESILIENCE**

Oba and Allen (1999)
Adesogan et al. (2019)

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Improving resilience with *Bacillus*

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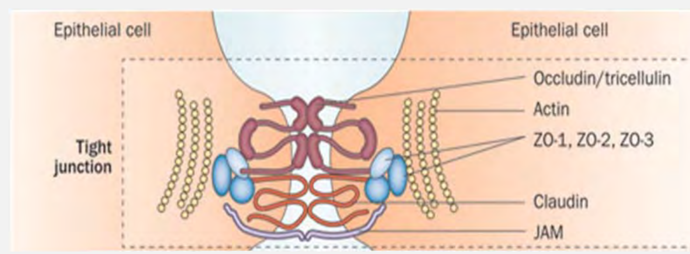
Using *Bacillus* to increase hindgut integrity

- *Bacillus* have been shown to increase expression of tight junction proteins



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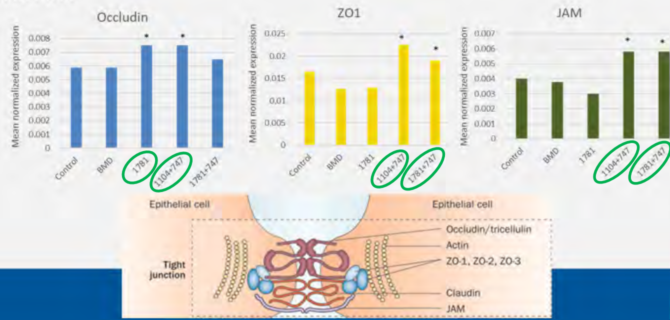
Using *Bacillus* to increase hindgut integrity

- *Bacillus* have been shown to increase expression of tight junction proteins

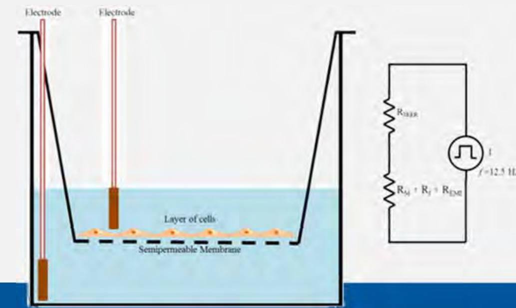


Gadde et al. (2017)

33

Measuring integrity of epithelial tight junctions

- Transepithelial/transendothelial electrical resistance (TER).



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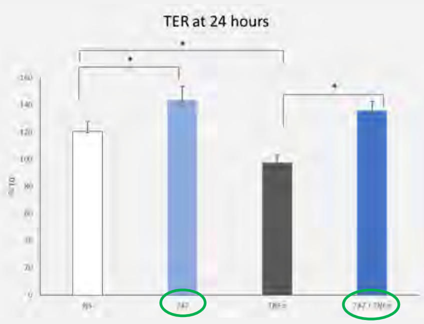
Bacillus have been shown to increase TER



TNFα = Immune Stimulus

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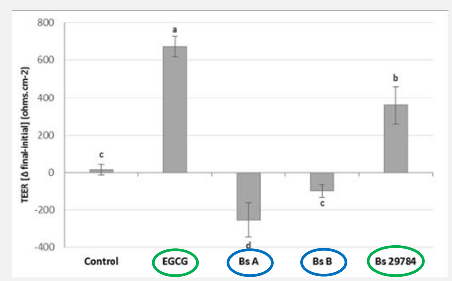
Effect of *Bacillus* on barrier function are strain dependent



Front. Immunol. 10:564. doi: 10.3389/fimmu.2019.00564

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CERTILLUS

Powered By Microbial Terroir

Effects of *Bacillus* supplementation on dairy cow performance

An indication of a resilient animal

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Oklahoma State University

Study Overview

- 28 Holstein cows (primiparous and multiparous)
- Continuous lactation trial (25 weeks)
- CON vs. **2 billion CFU/hd/d of *Bacillus*** (CERTILLUS)
- Cows housed together but fed individually using electronic feeders
- Herd had LOW pathogen challenge
- Measured effects of *Bacillus* supplementation on feeding behavior and performance

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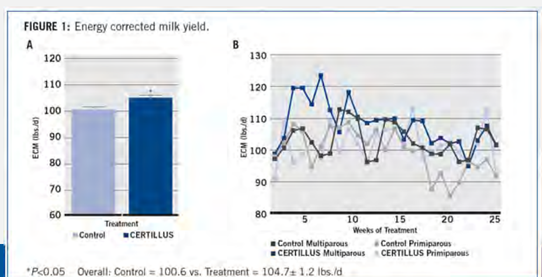
Oklahoma State University

- ECM: **+ 4.1 lbs./cow/d**
- Fat %: 4.02% (CON) vs. **4.41%** (*Bacillus* supp.)



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Oklahoma State University

Item	*Bacillus* Response	*P*-value
Feeding events	**- 14.5%**	**< 0.05**
Feed intake	**- 13%**	**< 0.05**
Feed efficiency	**+ 16.9%**	**< 0.05**

*No effect of *Bacillus* supplementation on BW/BCS

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Oklahoma State University

Conclusions

- *Bacillus* supplementation (2 billion CFU/hd/d):
  - Increased milk fat % and ECM yield ($P < 0.05$)
  - Decreased feeding events and DMI ($P < 0.05$)
  - Improved efficiency ($P < 0.05$)
- We hypothesize that *Bacillus* supplementation influenced nutrient availability via rumen fermentation efficiency and/or nutrient absorption from the lower gut

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New York Commercial Dairy

Study Overview

- Randomized, controlled pen trial (135 d)
- 2,302 cows split into groups by lactation and MY and randomly allocated to pens (90 lb. herd)
- CON vs. **2 billion CFU/hd/d of *Bacillus*** (CERTILLUS)
- Herd had LOW pathogen challenge
- Measured effects of *Bacillus* supplementation on performance and inflammatory markers in blood

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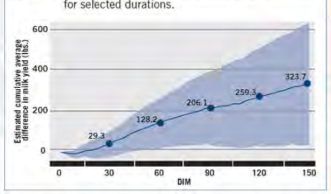
Results – *Performance*

TABLE 1 Estimated average differences in cumulative milk yield (lbs.) for selected durations.

Duration	(CERTILLUS-Control)	SE	*P*-Value
First 30 days	29.3	32.2	0.363
First 60 days	128.2	63.1	0.042
First 90 days	206.1	93.9	0.028
First 120 days	259.3	124.7	0.038
First 150 days	323.7	155.3	0.037

+ 3.3 lbs. of ECM w/ *Bacillus* supplementation

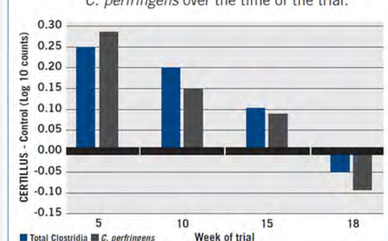
FIGURE 1: Average differences in cumulative milk yield (lbs.) for selected durations.



43

Results – *Microbials*

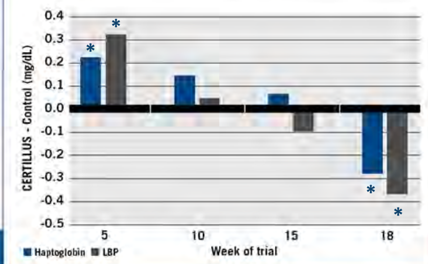
FIGURE 4: Differences between CERTILLUS and Control-fed cows in total clostridia and *C. perfringens* over the time of the trial.



44

Results – *Inflammatory markers*

Differences between CERTILLUS and Control-fed cows for haptoglobin and lipopolysaccharide binding protein over the time of the trial.



*$P \leq 0.05$

45

New York Commercial Dairy

Conclusions

- *Bacillus* supplementation (2 billion CFU/hd/d):
  - Increased cumulative daily milk yield ($P < 0.05$)
  - Increased ECM milk yield ($P < 0.05$)
  - Reduced counts of total clostridia and *C. perfringens*
  - Decreased markers of inflammation ($P < 0.05$)

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Conclusions

- **A resilient herd** can achieve consistent, high-level performance in the face of various pressures
- **Hidden challenges to resilience**
  - Pathogens coming from feed
  - Sub-optimal rumen function
  - Hindgut permeability
- ***Bacillus* supplementation** can increase herd resilience (and performance) by:
  - Controlling pathogens
  - Increasing populations of ruminal fibrolytic bacteria
  - Improving hindgut integrity

47

Questions?

Benjamin.Saylor@churchdwight.com

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The Role of Animal Production on the Environment

Robin R. White
rrwhite@vt.edu

VIRGINIA TECH | Animal and Poultry Sciences

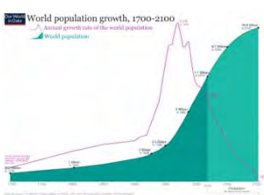
1

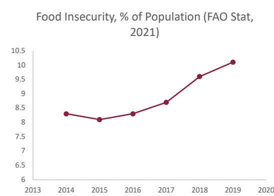
A Roadmap

- Food Sustainability
  - A Snapshot of Sustainability
    - Defining Goals/Targets
      - Future Perspectives

2

What is a sustainable food system?



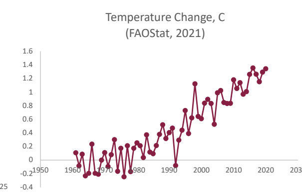


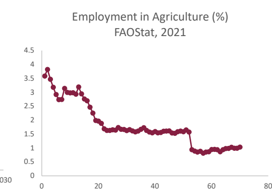
One that contributes to food security and nutrition for all in such a way that the economic, social, cultural, and environmental bases to generate food security and nutrition for future generations are safeguarded.

- Von Braun et al. 2021 (https://www.un.org/sites/un2.un.org/files/scgroup_food_systems_paper_march-5-2021.pdf)

3

What is a sustainable food system?





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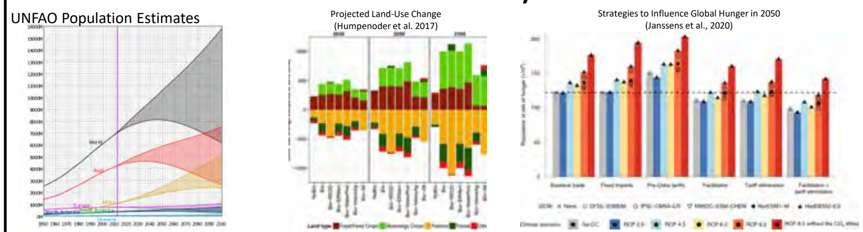
4

What is a sustainable food system?



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- Von Braun et al. 2021 (https://www.un.org/sites/un2.un.org/files/scgroup_food_systems_paper_march-5-2021.pdf)

5

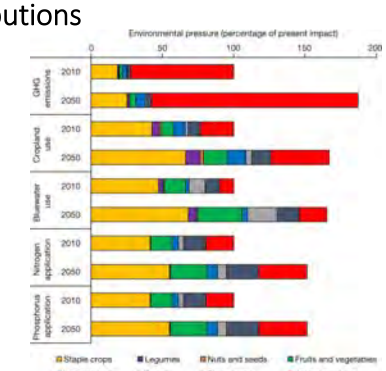
Sustainability is Here to Stay

6

7

Prioritize Problems, Identify Current Contributors & Limit Contributions

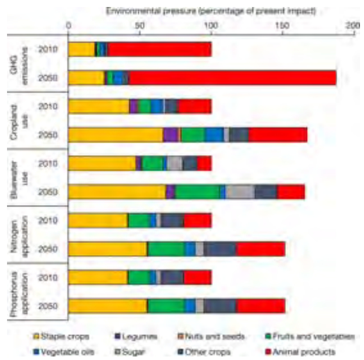
Rockstrom et al., 2009



Springmann et al., 2018

8

Global Context Matters



Springmann et al., 2018

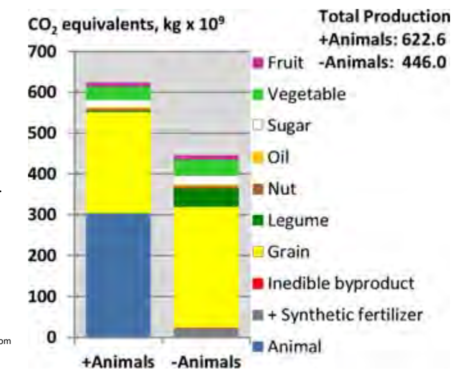
Source	USA	Global
Livestock, % agriculture	41.8%	58.0%
Livestock, % total	3.89%	14.5%
Agriculture, % total	9.3%	25%

9

Practicality Matters

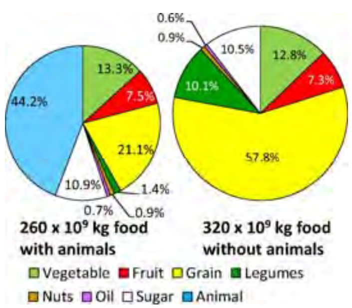
- 28% reduction in agricultural GHG, not the 50% associated with animals
- Regardless of accounting of fertilizer synthesis and byproduct disposal emissions, <3% change in total U.S. emissions

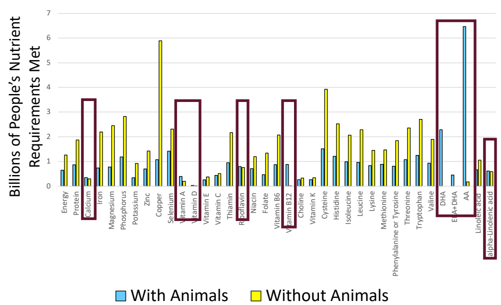
White, R. R., & Hall, M. B. (2017). Nutritional and greenhouse gas impacts of removing animals from US agriculture. *Proceedings of the National Academy of Sciences, 114*(48), E10301-E10308.



10

Unintended Consequences Should Be Considered

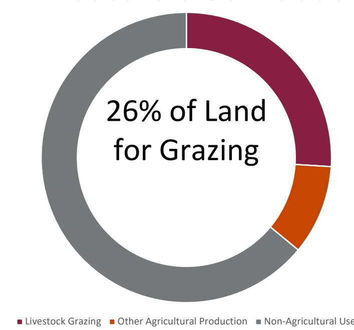




White, R. R., & Hall, M. B. (2017). Nutritional and greenhouse gas impacts of removing animals from US agriculture. *Proceedings of the National Academy of Sciences, 114*(48), E10301-E10308.

11

Timescale also needs to be considered



"each year, 13 billion hectares of forest are lost due to land conversion for agricultural uses [such] as pastures or cropland"

UNFAO: http://www.fao.org/3/ar591e/ar591e.pdf

Global 1997 - 2012

12

Timescale also needs to be considered

Land Use Type	2005 (1000 ha)	2018 (1000 ha)	Change (ha/y)
Urban Areas	71,037	74,904	297,000
Herbaceous Crops	1,221,098	1,235,250	1,089,000
Grassland	3,009,450	3,001,984	-571,000
Tree-covered Areas	4,977,582	4,976,059	-117,000
Mangroves	20,010	19,939	-5,500
Shrub-covered Areas	1,363,597	1,370,606	539,000
Aquatic/Flooded Areas	98,964	107,518	658,000
Snow and Glaciers	1,215,561	1,219,624	312,000

UNFAO: http://www.fao.org/faostat/en/#data/LC

13

Timescale also has practical components

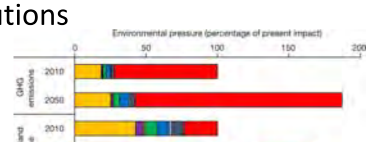
Food Type	EAT-Lancet
	g/d
Grains	232
Tubers	50
Vegetables	300
Fruit	200
Dairy	250
Meat (Red + Poultry)	43
Eggs	13
Fish	28
Legumes	50
Nuts	25
Oils	52
Sweeteners	31

[1]% Used refers to the percentage of current production (FAOStat, 2019) that would need to go for human consumption if 10 billion people consumed this average diet.

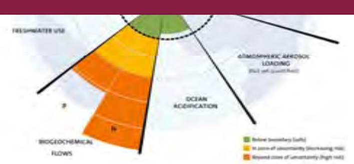
Can the agricultural system sustain this increase in legume and nut production globally?

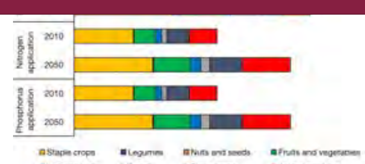
14

Prioritize Problems, Identify Current Contributors & Limit Contributions



A major challenge at a national or international level





Rockstrom et al., 2009

Springmann et al., 2018

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A Roadmap

Sustainability is a complex, multifaceted concept and a moving target – but one that we need to start tackling

- Food Sustainability
  - A Snapshot of Sustainability
    - Defining Goals/Targets
      - Future Perspectives

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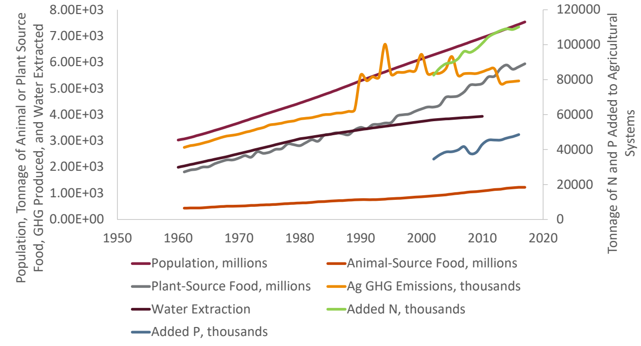
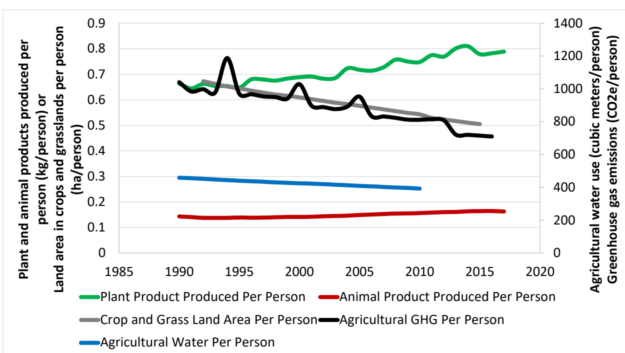
Are Impacts Getting Worse Over Time?



Data from UN-FAO, downloaded Feb 2019

18

Are Impacts Getting Worse Over Time?



Data from UN-FAO, downloaded Feb 2019

19

Evaluating Current Considerations

Historical Trends

Current Provision

Theoretical Omission

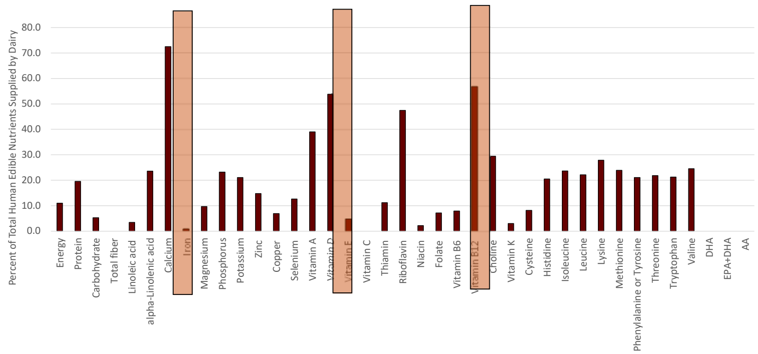
20

Historical Success in Sustainability Improvements

- Improving efficiency of production systems contributes to enhanced environmental footprints
- Linking enhanced environmental footprints to social acceptance is a moving target

21

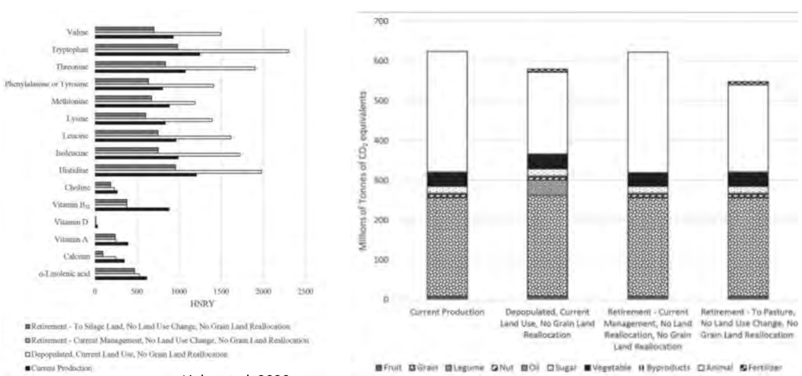
Nutrients Supplied by Dairy in the U.S.



Liebe et al. 2020

22

Theoretical Omission



Liebe et al. 2020

23

A Roadmap

Food Sustainability

Sustainability is a complex, multifaceted concept and a moving target – but one that we need to start tackling

A Snapshot of Sustainability

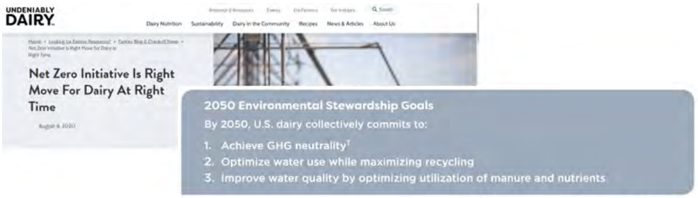
Based on most approaches to assessment, dairy industry sustainability metrics are promising

Defining Goals/Targets

Future Perspectives

24

The Goals are Already Set



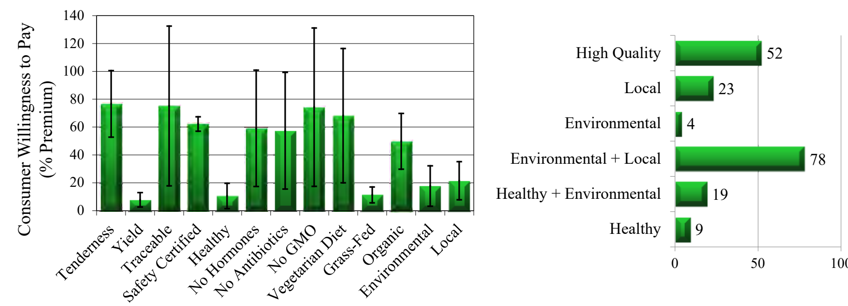
The U.S. dairy industry is leading by example with a commitment to environmental sustainability, working toward a set of goals that include cleaner water with maximized recycling and carbon neutrality by 2050.

ALISHA STAGGS

Dairy Program Manager for TNC's North America Agriculture Program

25

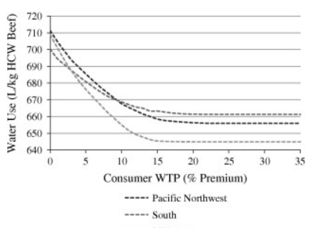
An Industry Approach: Why does it matter?



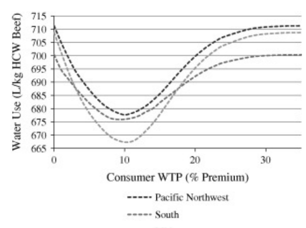
White and Brady, 2014

26

An Industry Approach: Why does it matter?



Water use efficiency returns to increasing consumer WTP



Water use efficiency returns to increasing consumer WTP, when considering probability of purchase (i.e., market share)

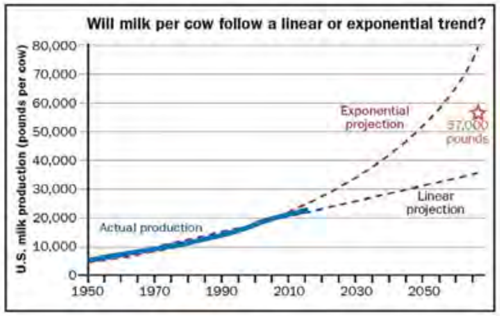
White and Brady, 2014

27

Continuing these trends becomes the responsibility of the industry as a whole…

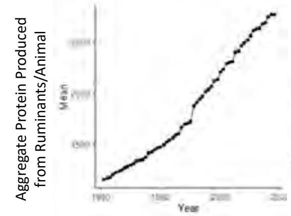
28

How do we work toward continued productivity?

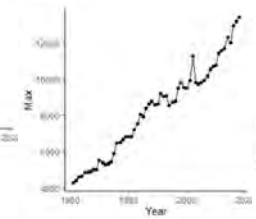


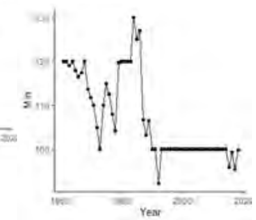
29

The trends are good, but distributions should also be considered



The best producers are getting better

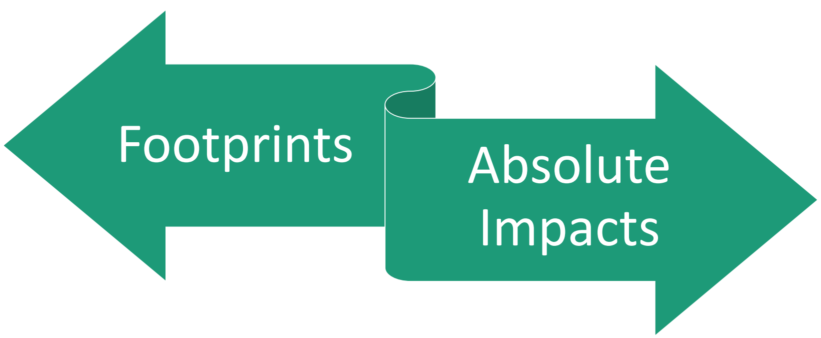




those that struggle continue to struggle

30

Goals and Targets



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Four "ways" to reduce footprints

- More Product
- Less Time
- Fewer Animals
- Reduced Inputs

32



Strategies for reducing absolute impacts

- Fewer Animals
- Less Time
- Reduced Impacts (i.e., direct mitigation)
- Reduced Inputs

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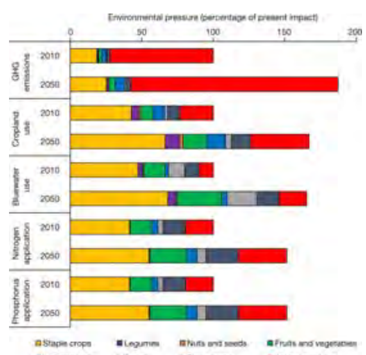
A Roadmap

- Food Sustainability
  - Sustainability is a complex, multifaceted concept and a moving target – but one that we need to start tackling
- A Snapshot of Sustainability
  - Based on most approaches to assessment, dairy industry sustainability metrics are promising
- Defining Goals/Targets
  - The messaging is there, much of the science is in place, work is still needed on exploring social and economic dimensions
- Future Perspectives

34

Carbon Emissions Are A Major Topic

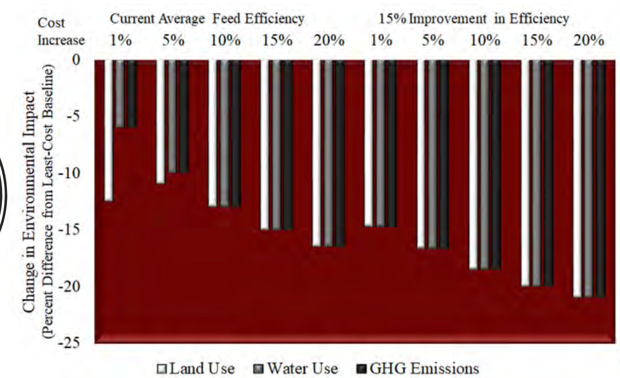
Rockstrom et al., 2009



Springmann et al., 2018

35

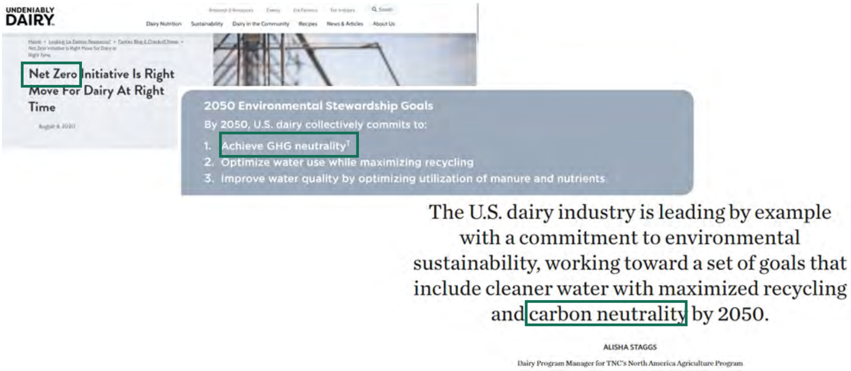
Complementary Objectives



White, R.R. 2016. Effects of improving energy and protein use efficiency on opportunities to improve environmental impact of dairy production. Ag. Syst. 146:20-29

36

Wording matters…



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Emerging/Recirculating Challenges

Methane

Offsets

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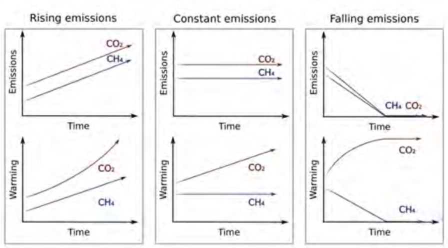
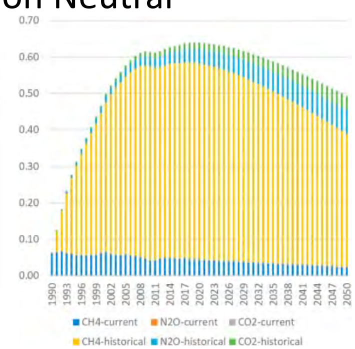
Warming Neutral vs Carbon Neutral



Mitigating methane emissions has major impact on global warming potentials.

Cain et al., 2019



Radiative forcing-based climate footprint of the Australian sheep meat sector projected to 2050

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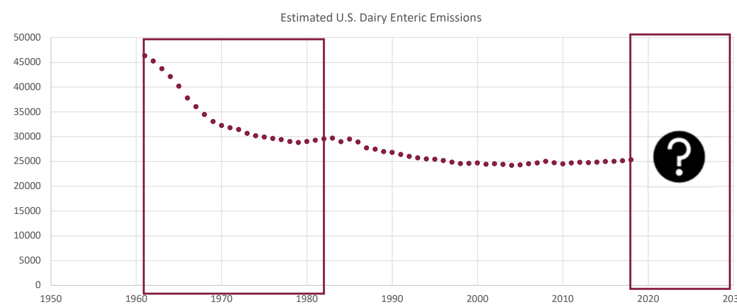
Ruminant Methane and GHGe



40

Emerging/Recirculating Challenges

Methane

Offsets

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Carbon offsets

Not compatible with LCA approach

Do not include all life cycle phases

Do not account for (negative) environmental and social impacts

Arendt, Rosalie, Vanessa Bach, and Matthias Finkbeiner. "Carbon Offsets: An LCA Perspective." *Progress in Life Cycle Assessment 2019*. Springer, Cham, 2021. 189-212.

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A Roadmap

Food Sustainability

Sustainability is a complex, multifaceted concept and a moving target – but one that we need to start tackling

A Snapshot of Sustainability

The messaging is there, much of the science is in place, work is still needed on exploring social and economic dimensions

Based on most approaches to assessment, dairy industry sustainability metrics are promising

Defining Goals/Targets

Future Perspectives

Keeping an eye on how to handle emerging challenges like CH4 quantification, carbon credits, and other similar "method" consideration

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Questions/Comments

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May 11, 2022

Climate neutrality for U.S. cattle production: What does it mean?

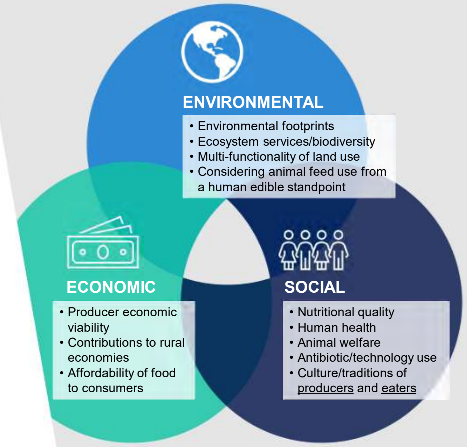
Sara Place, PhD
Chief Sustainability Officer

1

Sustainability is complex & full of value judgments, yet the issue that dominates today is climate change

Questions that society is asking:

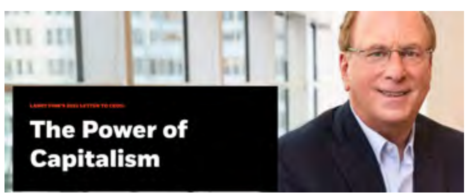
- What should we be eating?
- How should food be grown/produced?
- Can beef/dairy be a part of a sustainable diet?



2

Consumers are asking questions, but so are investors – ESG expectations are shaping the landscape

"Our question to these companies is: what are you doing to disrupt your business? How are you preparing for and participating in the net zero transition? As your industry gets transformed by the energy transition, will you go the way of the dodo, or will you be a phoenix?"
-Larry Fink, CEO of Blackrock



$9.5 trillion assets under management

"We focus on sustainability not because we're environmentalists, but because we are capitalists and fiduciaries to our clients."

 https://www.blackrock.com/corporate/investor-relations/larry-fink-ceo-letter

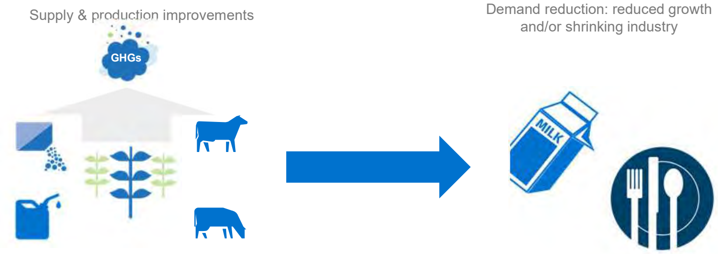
3

Simple reality: We can achieve progress on the supply side or the demand side

It's up to the cattle industry to determine if supply side alone can achieve societal expectations. We have knowledge gaps, economic barriers, and implementation challenges ahead

Supply & production improvements

Demand reduction: reduced growth and/or shrinking industry

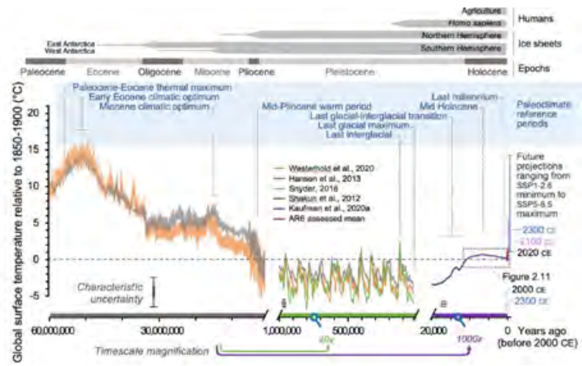


4

Climate in Context

5

Earth's Recent Surface Temperature History



Cross-Chapter Box 2.1, Figure 1: Global mean surface temperature (GMST) over the past 60 million years relative to 1850-1900 shown on three time scales.

6

Greenhouse effect keeps global average temperature at ~57 °F.

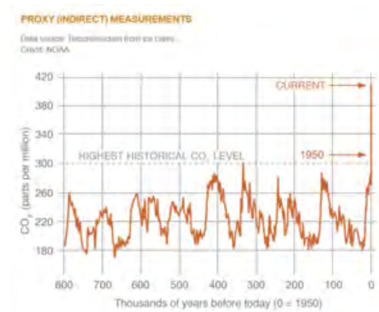
Without the greenhouse effect, global average temperature would be less than 0°F.

Available at: https://climate.nasa.gov/climate_resources/188/graphic-the-greenhouse-effect/

7

Carbon dioxide (CO_2) concentrations within the atmosphere have increased rapidly in the past few decades

"Over the past 171 years, **human activities have raised atmospheric CO2 concentrations by 48%** above pre-industrial levels found in 1850. This is **more than what had happened naturally over a 20,000 year period** (from the Last Glacial Maximum to 1850, from 185 ppm to 280 ppm)."

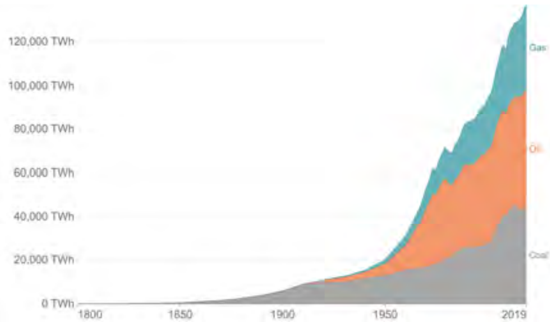


Available at: https://climate.nasa.gov/vital-signs/carbon-dioxide/

8

Global Fossil Fuel Consumption

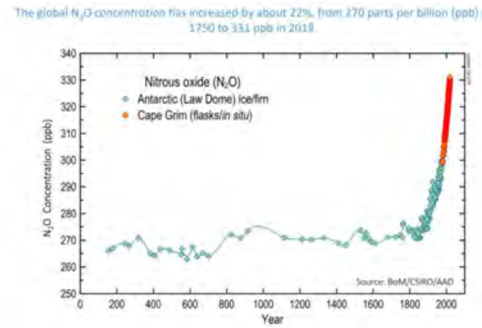
Global primary energy consumption by fossil fuel source, measured in terawatt-hours (TWh)



Source: Vaclav Smil (2017), Energy Transitions: Global and National Perspective & BP Statistical Review of World Energy
OurWorldInData.org/fossil-fuels/ • CCBY

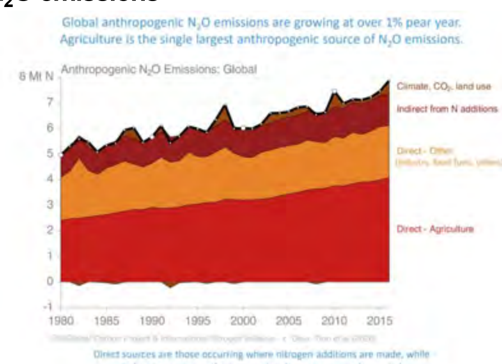
Available at: https://ourworldindata.org/fossil-fuels

The global N_2O concentration has increased by about 22%, from 270 parts per billion (ppb) in 1750 to 331 ppb in 2018.



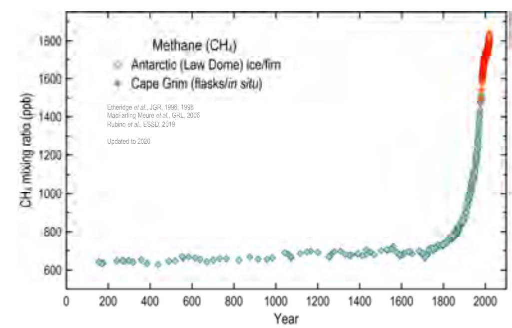
Available at: https://www.globalcarbonproject.org/nitrousoxidebudget/20/files/GCP_NitrousOxideBudget_2020.pdf

Agriculture is the main disrupter of the global N cycle and is a major contributor to N_2O emissions

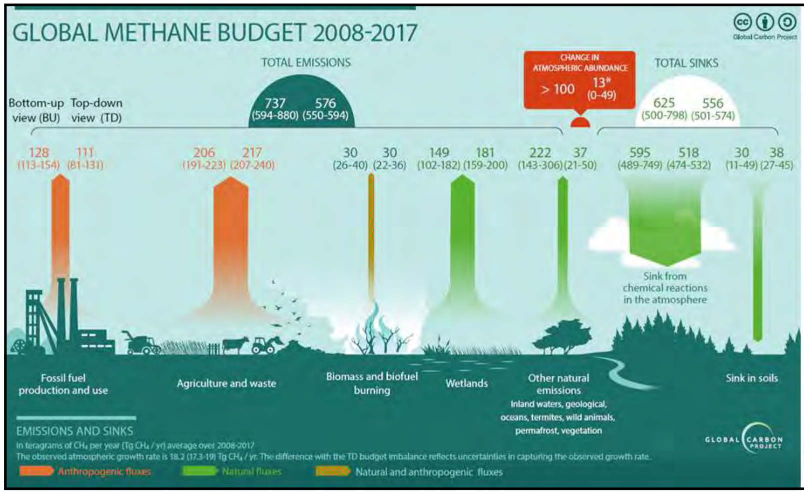


Available at: https://www.globalcarbonproject.org/nitrousoxidebudget/20/files/GCP_NitrousOxideBudget_2020.pdf

Methane (CH_4) is the second most important anthropogenic GHG and has also increased atmospheric concentrations since the Industrial Revolution (up 150% since 1750)



Available at: https://www.globalcarbonproject.org/methanebudget/20/presentation.htm



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Greenhouse Gas Emissions in Context

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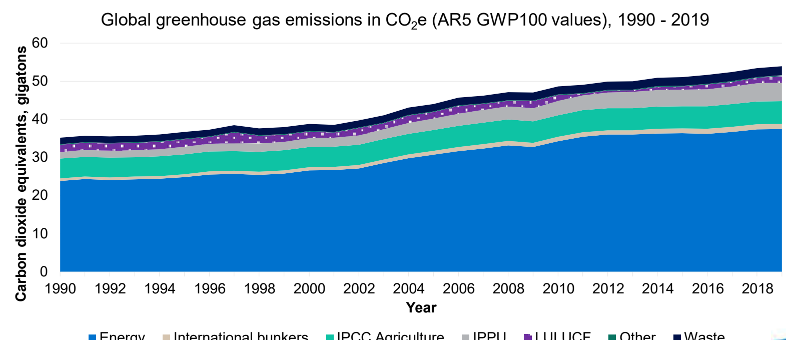
Since 1990, global GHG emissions have grown 53%, while agriculture + ag land use emissions are down 3% (population is up 45%)



Source: UN FAOSTAT database, available at: https://www.fao.org/faostat/en/#data

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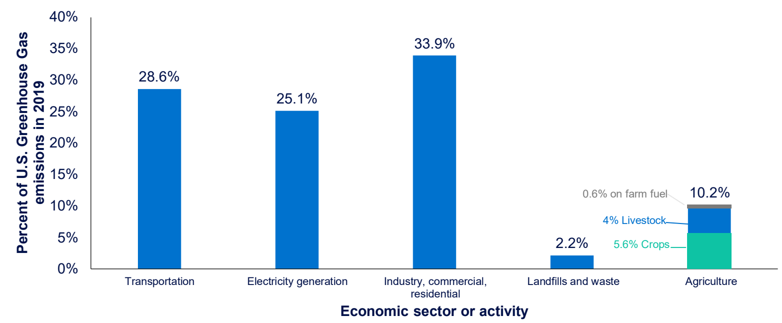
U.S. Greenhouse Gas Emissions in 2019



United States Department of Agriculture. National Agriculture Statistics Service. Available at: https://quickstats.nass.usda.gov/
US Environmental Protection Agency. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990 – 2019. 2021. available at: https://www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks-1990-2019

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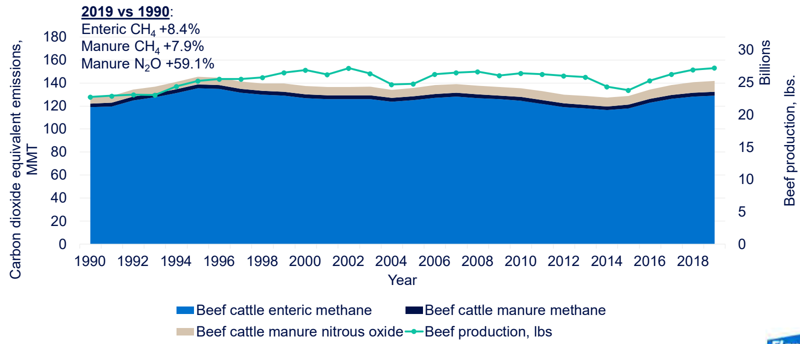
Emissions intensity of US beef is down 7.5%, beef production is up 19.7%, & absolute emissions are up 10.7% since 1990



United States Department of Agriculture. National Agriculture Statistics Service. Available at: https://quickstats.nass.usda.gov/
US Environmental Protection Agency. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990 – 2019. 2021. available at: https://www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks-1990-2019

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The Climate Balance Sheet for US Beef Cattle Production

EMISSIONS SOURCES (% OF TOTAL[1]):

Enteric methane emissions (56%)
- Cow-calf production = 77% of enteric methane emissions
- Opportunities: improved production efficiency, reduced mortality, increased digestibility of feedstuffs, new innovations to inhibit methane

Feed/soil emissions (24%)
- Mostly soil nitrous oxide
- Opportunities: improvements in crop yields, optimized fertilizer use, integration of cattle & crops

Fossil fuel & input emissions (17%)
- Equipment, fertilizer, electricity, lime
- Opportunities: energy efficiency, optimized fertilizer use

Manure emissions (3%)
- Manure nitrous oxide & methane
- Opportunities: Manure management strategies and innovations customized to operations (e.g., composting, anaerobic digestion where relevant)

[1]Rotz, CA, Asem-Hiablie, S, Place, S, Thoma, G. Environmental Footprints of Beef Cattle Production in the United States. Agricultural Systems [Internet]. 2019 Feb [cited 2020 Aug 13]. 169:1-13. https://www.sciencedirect.com/science/article/pii/S0308521X18305675

CARBON SEQUESTRATION:

Pasture and rangelands
- Opportunities: Maintain soil C stores, increase soil where possible via management & re-establishment on degraded/highly erodible croplands

Row crops fed to cattle
- Opportunities: increase no-till/reduced tillage, cover crops, integration with cattle & other livestock

REDUCE EMISSIONS
+
MAINTAIN & ENHANCE SINKS
=
NET ZERO CLIMATE IMPACT

US Environmental Protection Agency. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990 – 2018. 2020. available at: https://www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks-1990-2018

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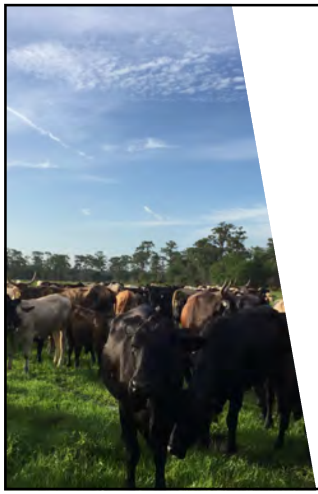

US Cattle Emissions

- Both beef and dairy are dominated by methane (enteric + manure)
- Critically important to understand the implications of different climate metrics & how different metrics relate to climate goals

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Accounting for Short-lived GHG Emissions Separately to Better Link Emissions to Warming

Article | Open Access | Published: 04 September 2019

Improved calculation of warming-equivalent emissions for short-lived climate pollutants

Michelle Cain, John Lynch, Myles R. Allen, Jan S. Fuglestvedt, David J. Frame & Adrian H Macey

npj Climate and Atmospheric Science **2**, Article number: 29 (2019) | Cite this article

2813 Accesses | **64** Altmetric | Metrics

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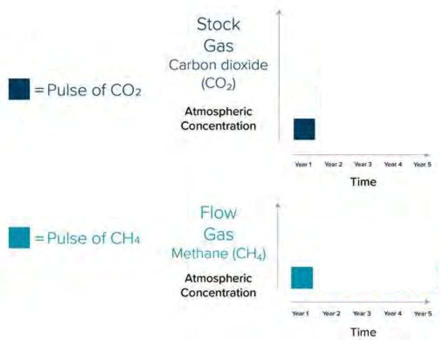

Stock gases will accumulate over time, because they stay in the environment.

If emissions and sinks are in balance

Flow gases will stay stagnant, as they are destroyed at the same rate of emission.

Based on research by Myles R. Allen, Keith P. Shine, Jan S. Fuglestvedt, Richard J. Millar, Michelle Cain, David J. Frame & Adrian H. Macey. Read more here: https://rdcu.be/bfl75

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EM-US-20-0258

The "So-what" of New Climate Metrics for Short-lived Gases

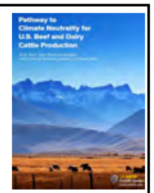
Better reflects reality of how emissions impact temperature
- This is what we actually care about

Highlights that methane emissions do not have to be zero to reach "climate neutrality"
- Climate neutrality defined here as not contributing to additional warming or achieving net zero warming

Important for beef/cattle as methane is the largest GHG in profile
- But, it's not the only GHG associated with cattle production!

If the Goal is Climate Neutrality for US Cattle

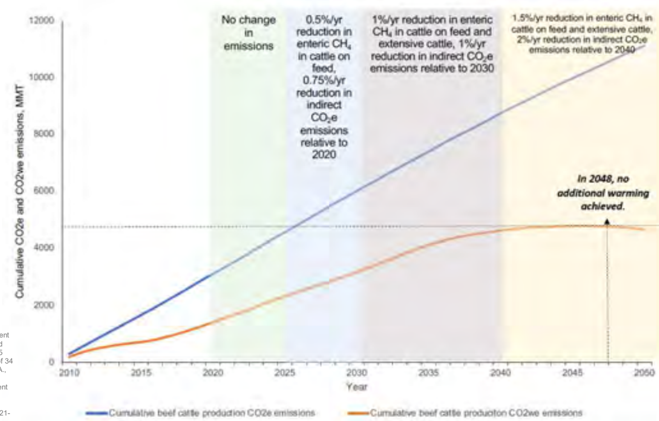
What Could that Look Like?



Assumptions in Scenario to Reach Climate Neutrality for Beef

Item	2020	2050	% change from 2020
Total non-dairy cattle, Jan. 1	**79,766,700**	**79,549,600**	**-0.3%**
Beef production, billion lbs.	**27.1**	**31.2**	**+15%**
Beef cattle enteric CH_4, Tg CO_2e[1]	175.5	136.0	-23%
Feedlot cattle enteric CH_4/d, g/hd	127	96	-24%
Beef cow enteric CH_4/d, g/d	262	204	-22%
Indirect GHG emissions, Tg CO_2e[1]	101.4	72.3	-28%
Carbon footprint, kg CO_2e/kg beef carcass[1,2]	23.72	15.70	-34%
Total GHG emissions, Tg CO_2e[1]	291.3	222.4	-24%

[1]Carbon dioxide equivalents (CO_2e) using GWP100 values of 34 and 298 for methane and nitrous oxide, respectively
[2]The carbon footprint here does not allocate emissions to or from dairy cattle, but rather only accounts for enteric and manure emissions directly attributed to non-dairy cattle within the U.S. EPA GHG inventory. For comparison, Rotz et al. (2019) found a U.S.-wide carbon footprint for beef cattle production of 21.3 kg CO2e/kg carcass weight using GWP100 values of 28 and 265 for CH4 and N2O, respectively. The 2020 footprint reported here would be 21.04 kg CO2e/kg carcass weight using those GWP100 values



[1]Carbon dioxide warming equivalent (CO_2we) emissions are calculated using 20-year time horizon & AR5 GWP100 values for CH_4 & N_2O of 34 and 298, respectively. Smith, M.A., Cain, M. & Allen, M.R. Further improvement of warming-equivalent emissions calculation. npj Clim Atmos Sci 4, 19 (2021). https://doi.org/10.1038/s41612-021-00169-8

Figure 6. *Cumulative carbon dioxide equivalent (CO_2e) or carbon dioxide warming equivalent (CO_2we) for US beef cattle production from 2010 to 2050 for the case study scenario. Assumed changes in emissions by time period are indicated on the graph. The point at which annual CO_2we emissions do not add to further warming is indicated on the graph.*

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What Would Be Needed To Reach Climate Neutrality While Maintaining Herd and Production Growth

Need to reduce absolute emissions, not just per lb. of beef & milk

Enteric methane is a major "lever" to pull for beef:

- Genetics (feed intake, methane directly)
- Feed additives, feeding strategies
  - Challenge how to deliver to grazing cattle where ~82% of the methane emissions come from?
- Other innovations (e.g., vaccine?)

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What Would Be Needed To Reach Climate Neutrality While Maintaining Herd and Production Growth

Unlikely reducing enteric methane will get cattle production to climate neutrality alone, so need other reductions and/or increase C sinks

Other reduction examples:

- Reducing feed emissions (e.g., soil N_2O emissions)
- Reducing energy/fuel emissions

Carbon sequestration

- Potential to increase is likely highly dependent upon climate & land's prior use
- **Consideration: if carbon sold as an offset to buyers outside supply chain, can beef or dairy claim as well??**

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Carbon value of beef – will it be a cost or a revenue opportunity?

$9.3 billion carbon value in beef cattle production @ $40 per metric ton $CO_{2}e$

		Beef cattle production GHG emissions, MMT CO_2e	Total carbon value @$40/t CO_2e, USD	@ 25% reduction & 50% market share, MMT CO_2e	Carbon offset value @$40/t CO_2e, USD
Enteric	CH_4	129.1	$5,162 mil	-16.1	$645.3 mil
Manure	CH_4	3.40	$136 mil	-0.4	$17.0 mil
Manure	N_2O	9.39	$375 mil	-1.2	$46.9 mil
Feed, fuel, other indirect[1]	CO_2e	90.0	$3,600 mil	-11.3	$450 mil
			$0.34/lb. of beef[2]		$1,159 mil $0.04/lb. beef[2]

[1]Using GWP100 value for methane of 25, for nitrous oxide 298, & estimate of feed, fuel, & other indirect based on Rotz et al., 2019 & source: US EPA GHG Inventory for year 2019. Available at: https://www.epa.gov/sites/default/files/2021-04/documents/us-ghg-inventory-2021-annexes.pdf
[2] Using 2019 beef production as base production from USDA-NASS Quick Stats database

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Bottom Line

Climate neutrality for beef cattle production in the USA is likely possible and technically feasible

- But, it requires new innovations

We cannot lose focus of other aspects of sustainability

- First and foremost, need **economic viability**
- Cattle production is critical source of nutrition & ruminant benefits to sustainability are substantial (optimum land use, upcycling, wildfire suppression, etc.)

Societal perceptions are driving conversation & expectations are high

- Future pathways to tangibly achieve action are needed
- Gaps to fill in knowledge, implementation, economic feasibility, and people!

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Thank You

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