Evaluating the Effects of Zn Hydroxychloride on Gut Health

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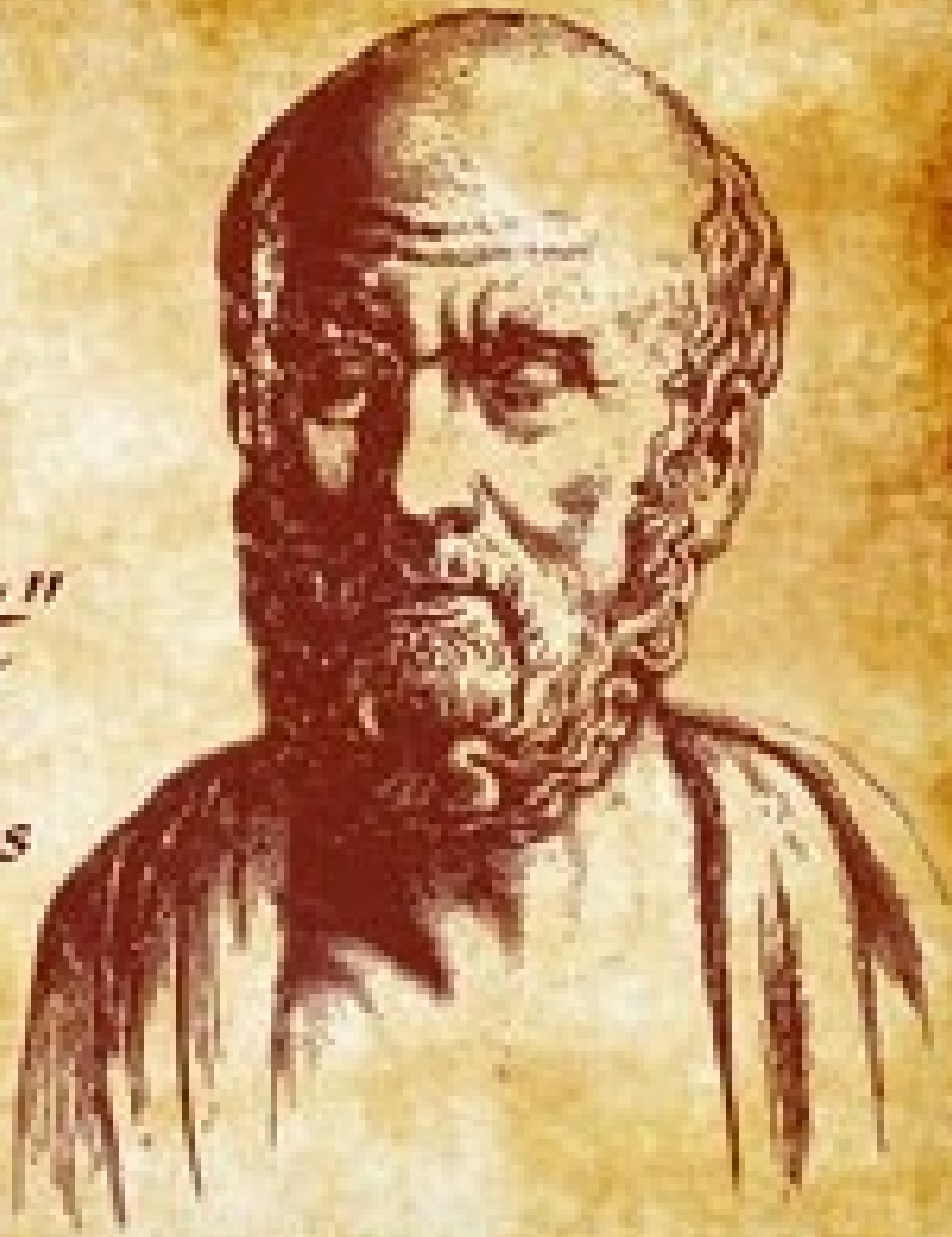
"All Disease begins in the gut"

~Hippocrates

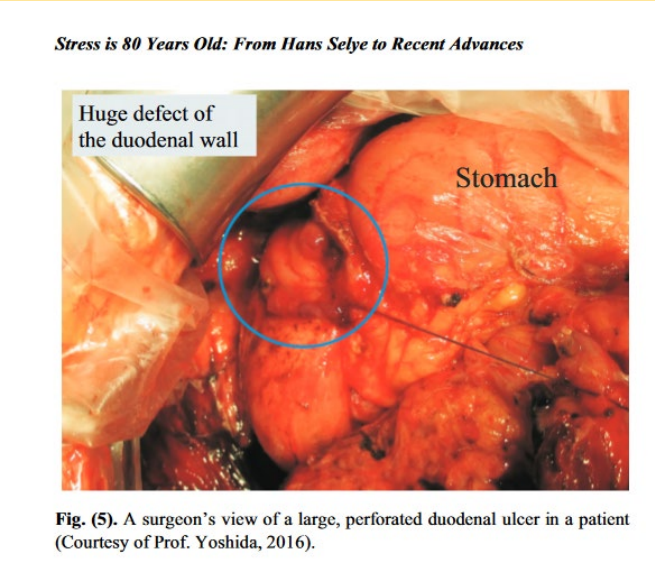


Stress

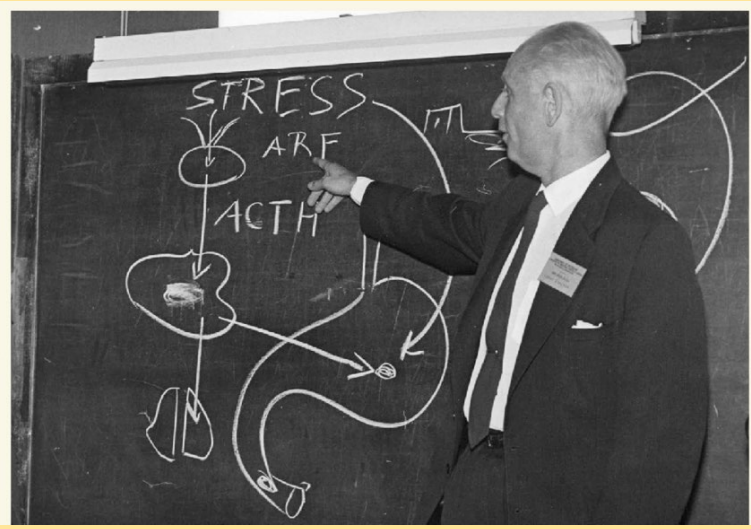
"The nonspecific response of the body to any demand."

"Maintaining an internal physiological balance through homeostasis can not by itself ensure the stability of body systems under stress."

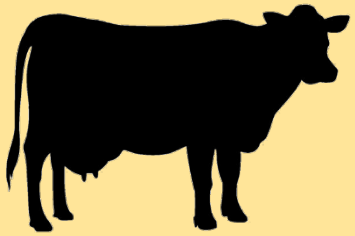
"Similar stress response to different potentially harmful stimuli."

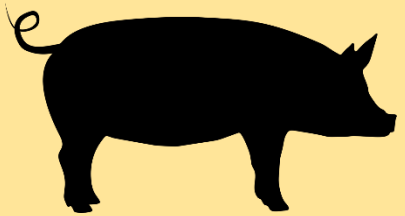


Hans Selye, 1936



Stressors: Intestinal Derived Immune Activation and Inflammation





Feed Restriction

Transition Period

Heat Stress

Rumen Acidosis

Hind Gut Acidosis

Mycotoxins & Enteric Pathogens

LEAKY GUT

Metabolic Response

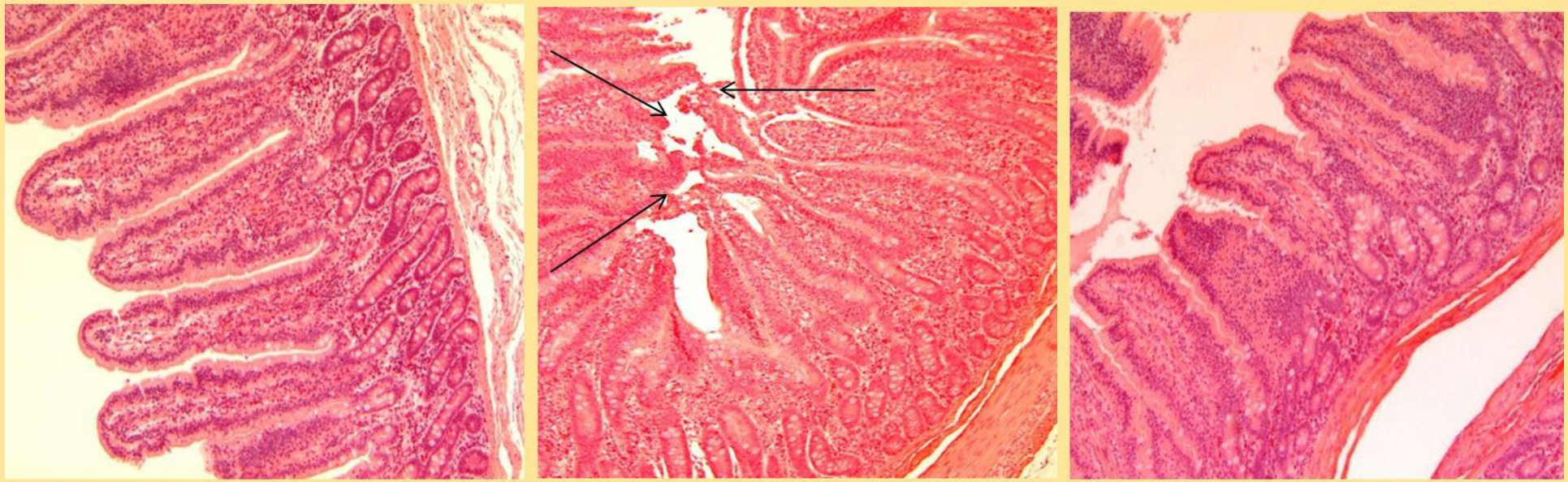
Immuno-metabolic Response

Endocrine Response

↓ Production (Meat, Milk, Eggs, etc.)

Biology of Heat Stress Symptoms

Intestinal Morphology



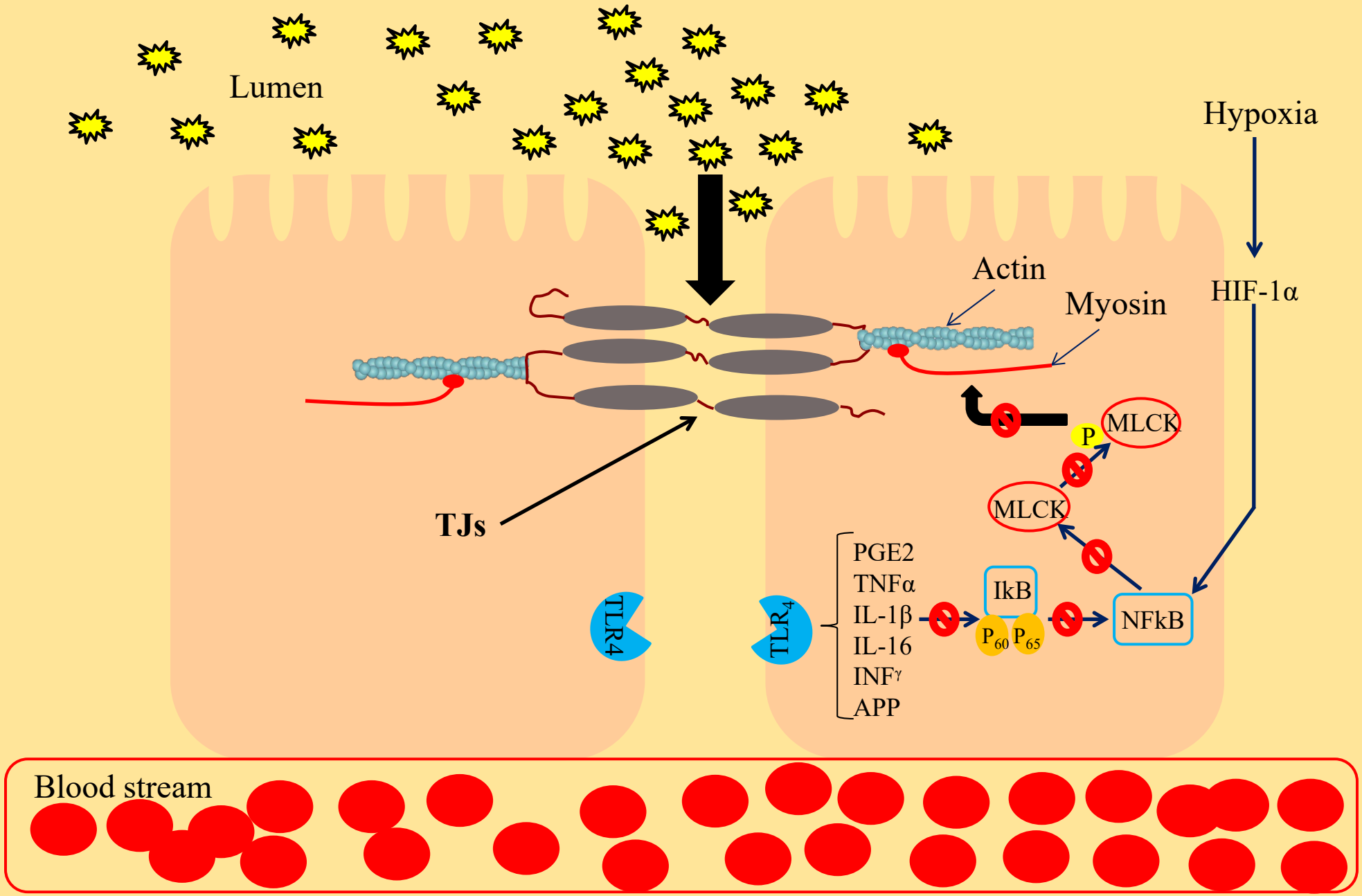
Thermal Neutral | Heat Stress | Pair-fed

Pearce et al., 2011

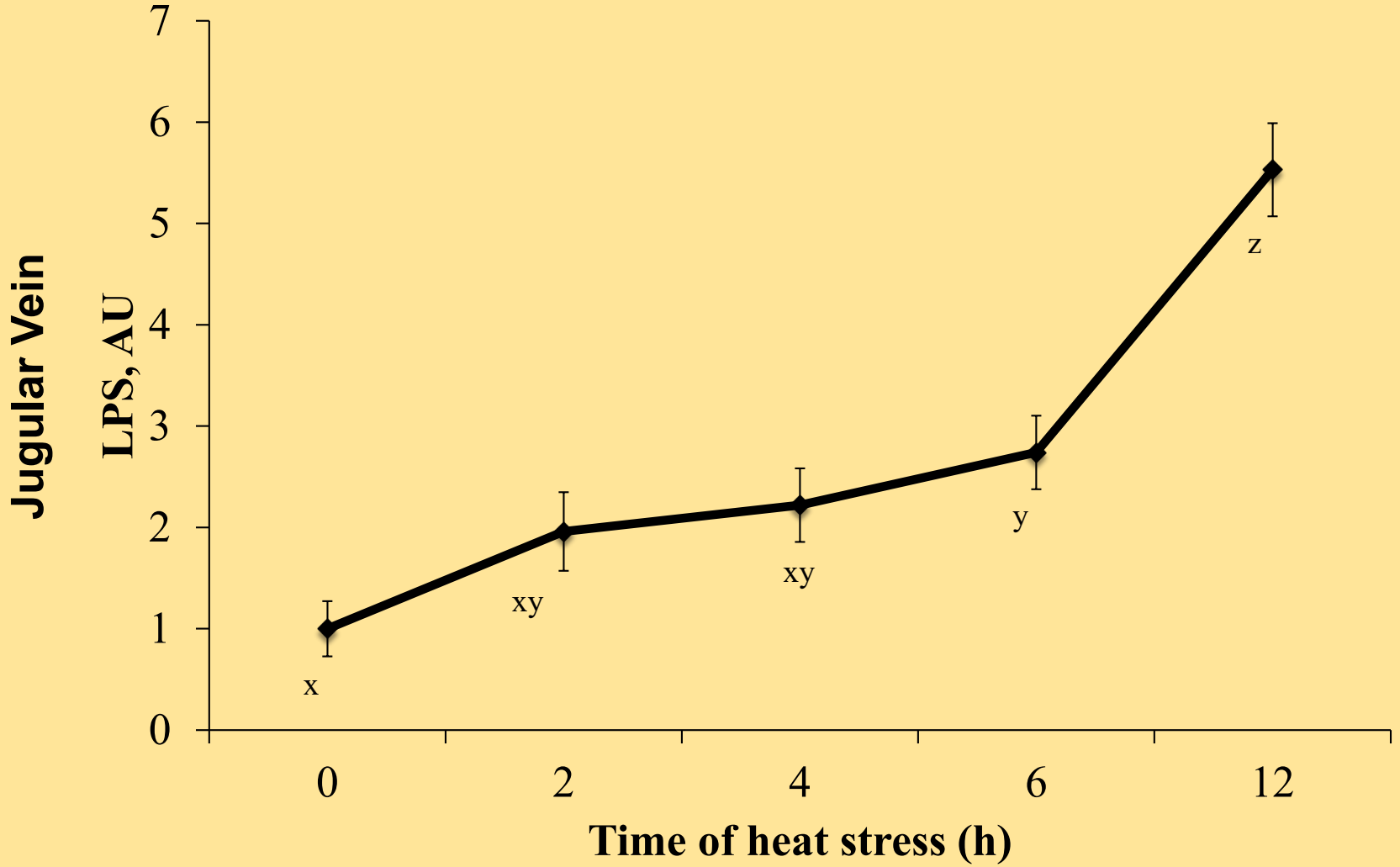
Heat Stress and Gut Health

- Lipopolysaccharide (LPS) stimulates the immune system

- LPS promotes inflammation production….catabolic condition
  - TNFα, IL-1 etc..
    - Reduced appetite
    - Stimulates fever
    - Causes muscle breakdown
    - Induces lethargy
    - ....reduces productivity

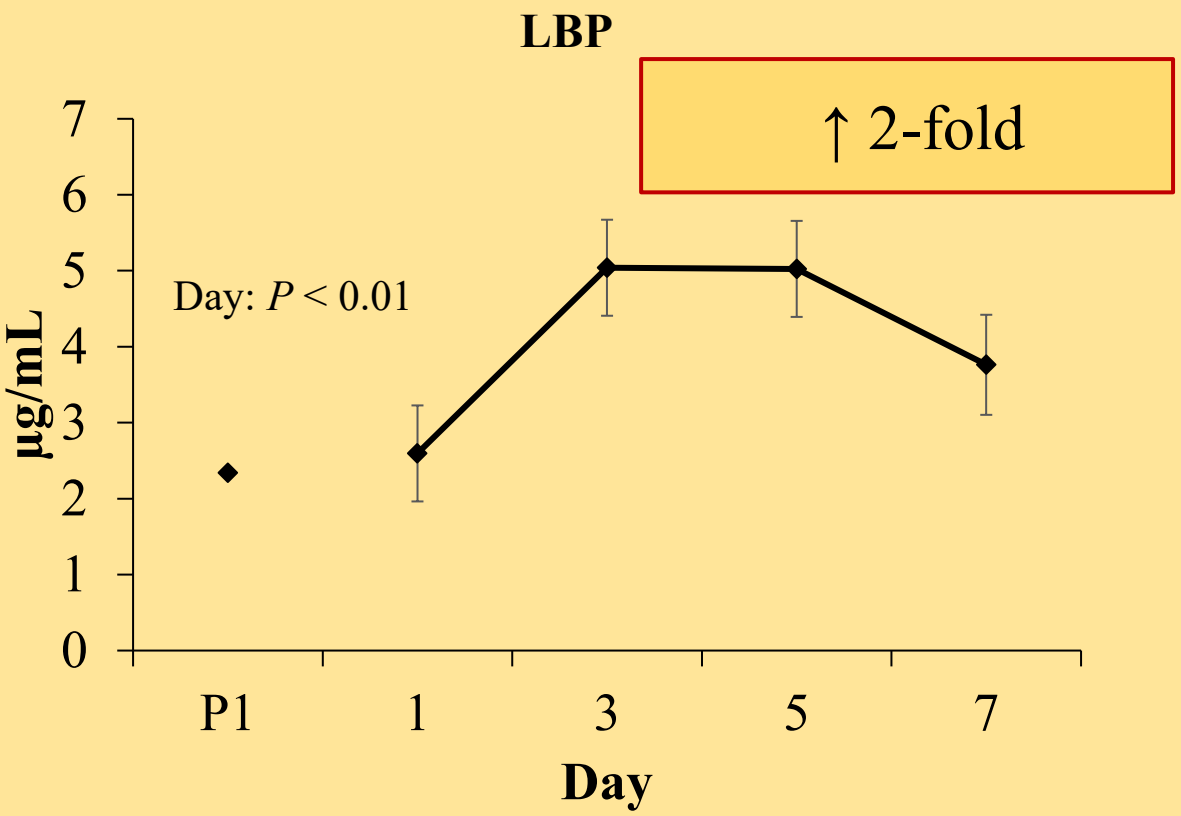

Lumen
Hypoxia
Actin
Myosin
HIF-1α
TJs
MLCK
P
MLCK
TLR4
TLR4
PGE2
TNFα
IL-1β
IL-16
INFγ
APP
IkB
P60 P65
NFkB
Blood stream
Submucosa

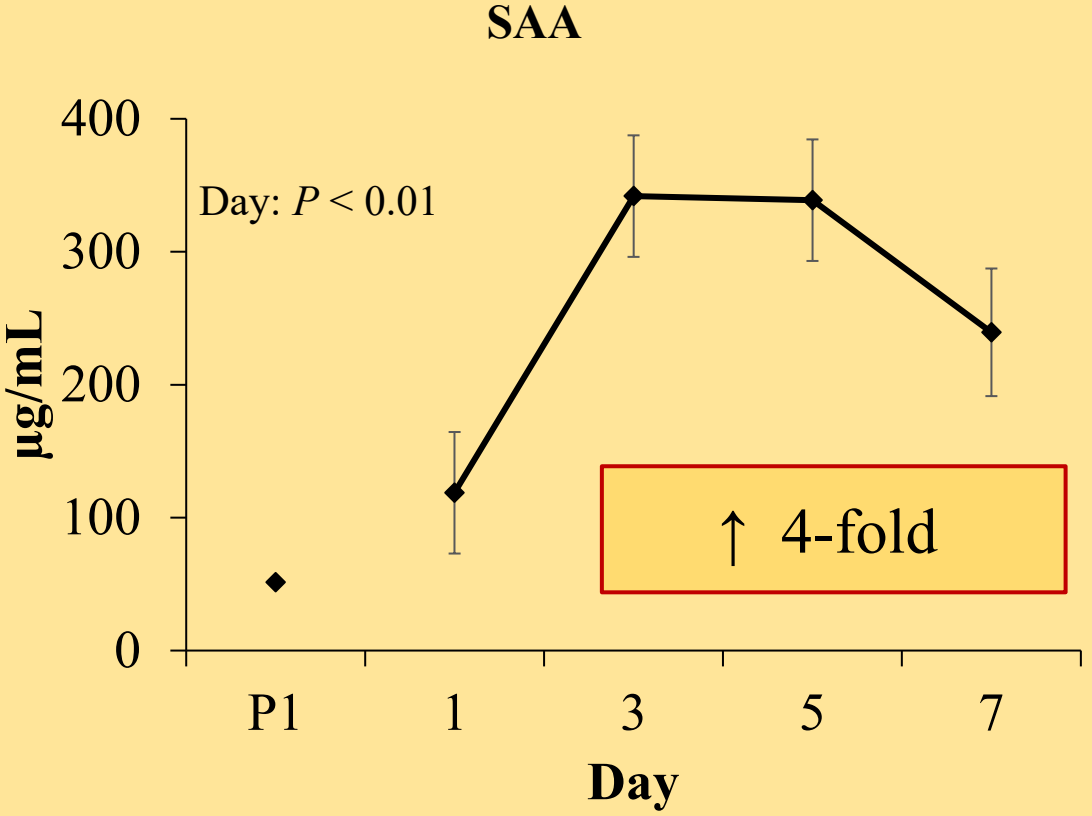
The Effects of Heat Stress are Rapid!



Pearce et al., 2015

Acute Phase Proteins Increase During Heat Stress





Heat Stress Summary

- Leaky gut
  - Shown in multiple species (ruminants and monogastrics)
  - Demonstrated with different approaches
- Inflammation and acute phase protein response
- Heat stress is <u>essentially immune activation</u>
  - Physiologically similar to mastitis, metritis, pneumonia etc.

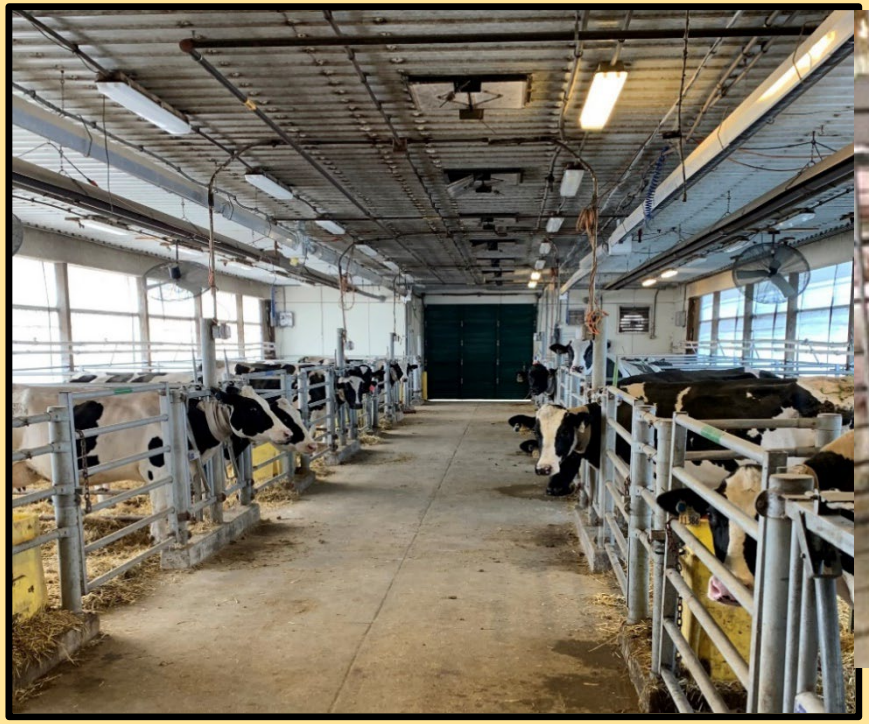
Objectives:

Determine Feed Restriction’s Impact on Leaky Gut

“Out of Feed Events” are common in EVERY animal agriculture industry

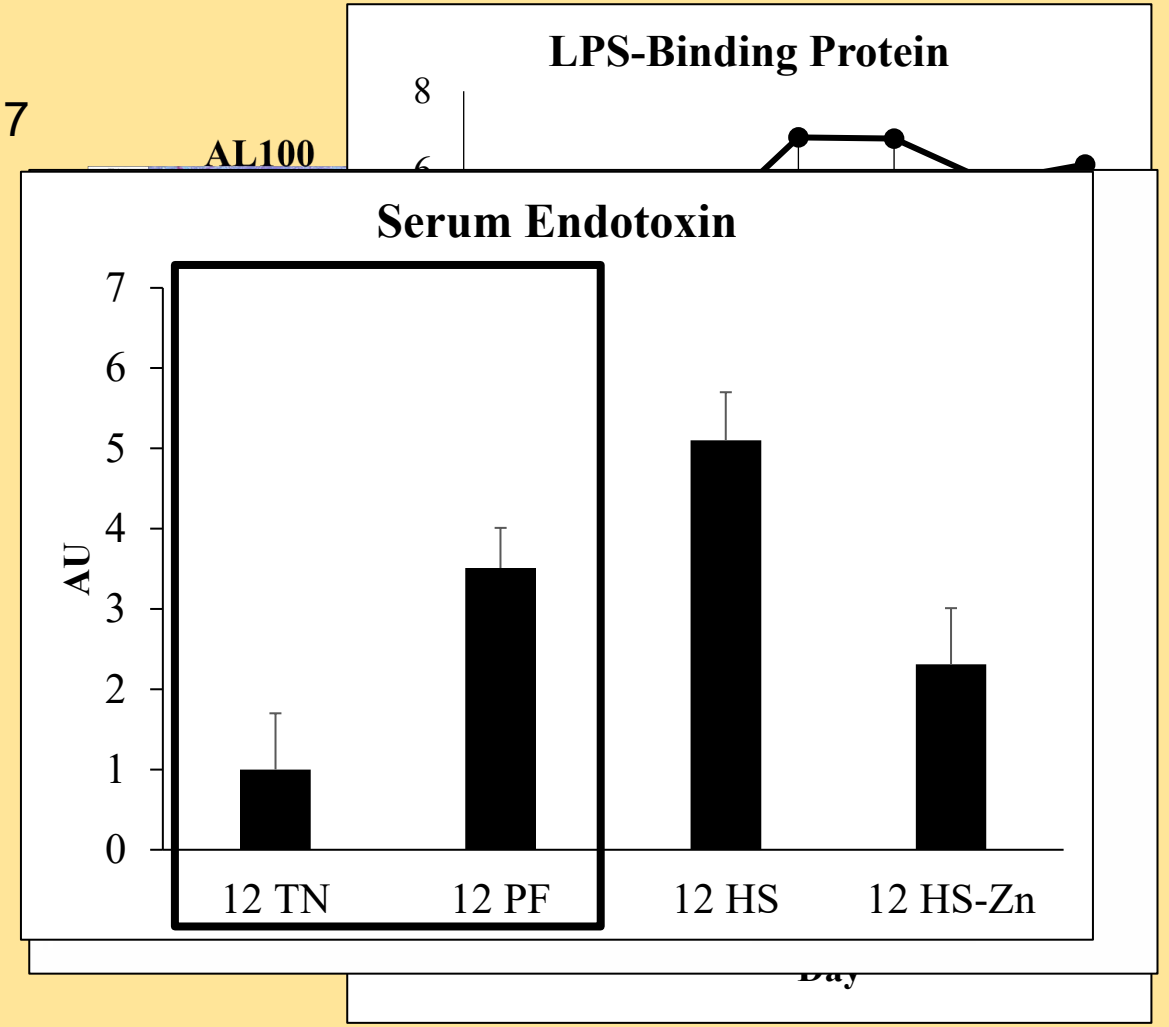
Insufficient Nutrient Intake

- Feed intake is frequently inadequate to meet nutrient requirements:
  - Off-feed events
  - Out of feed events
  - Shipping
  - Overcrowding
  - Transition period
  - Heat stress
  - Abrupt weaning
  - Gestating sows

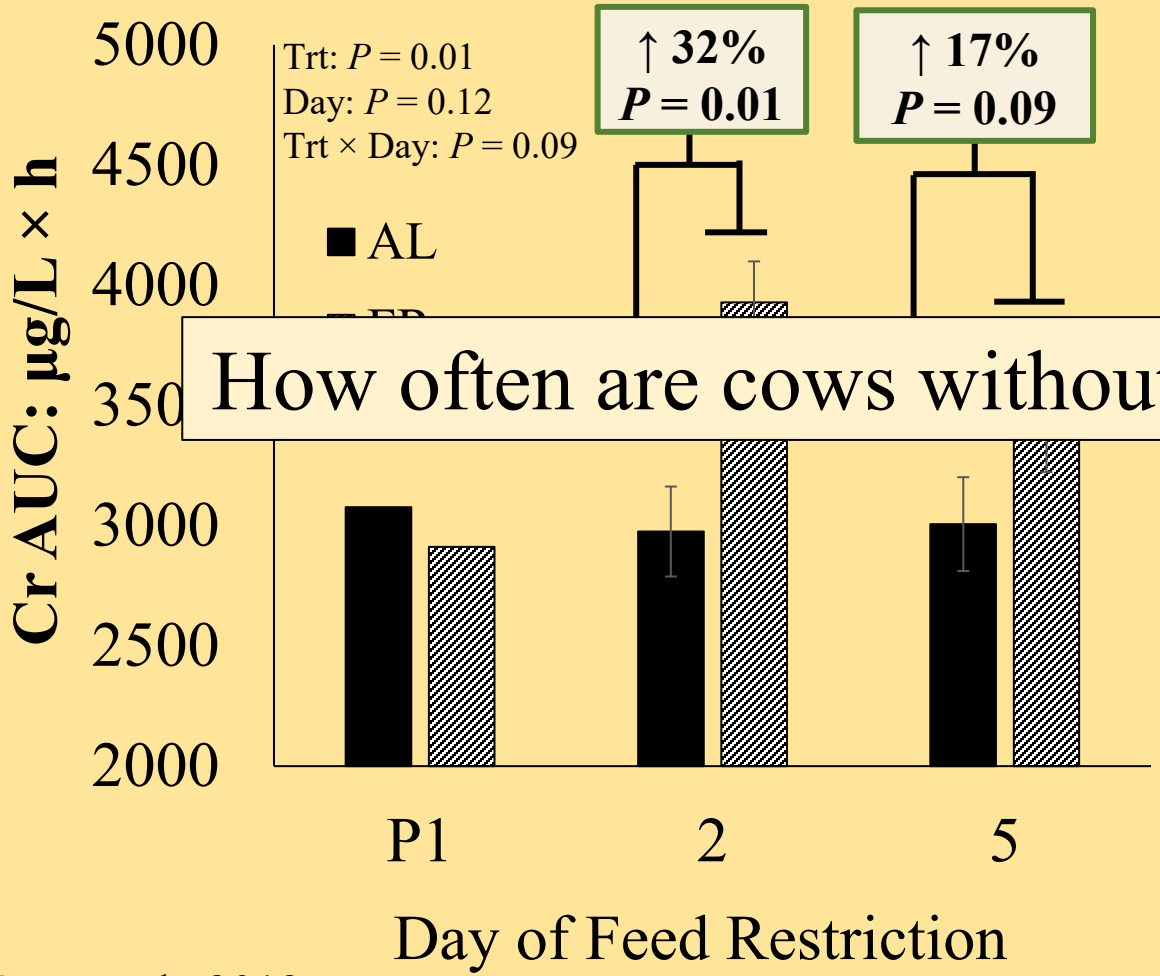


Consequences of Feed Restriction-Gut Health

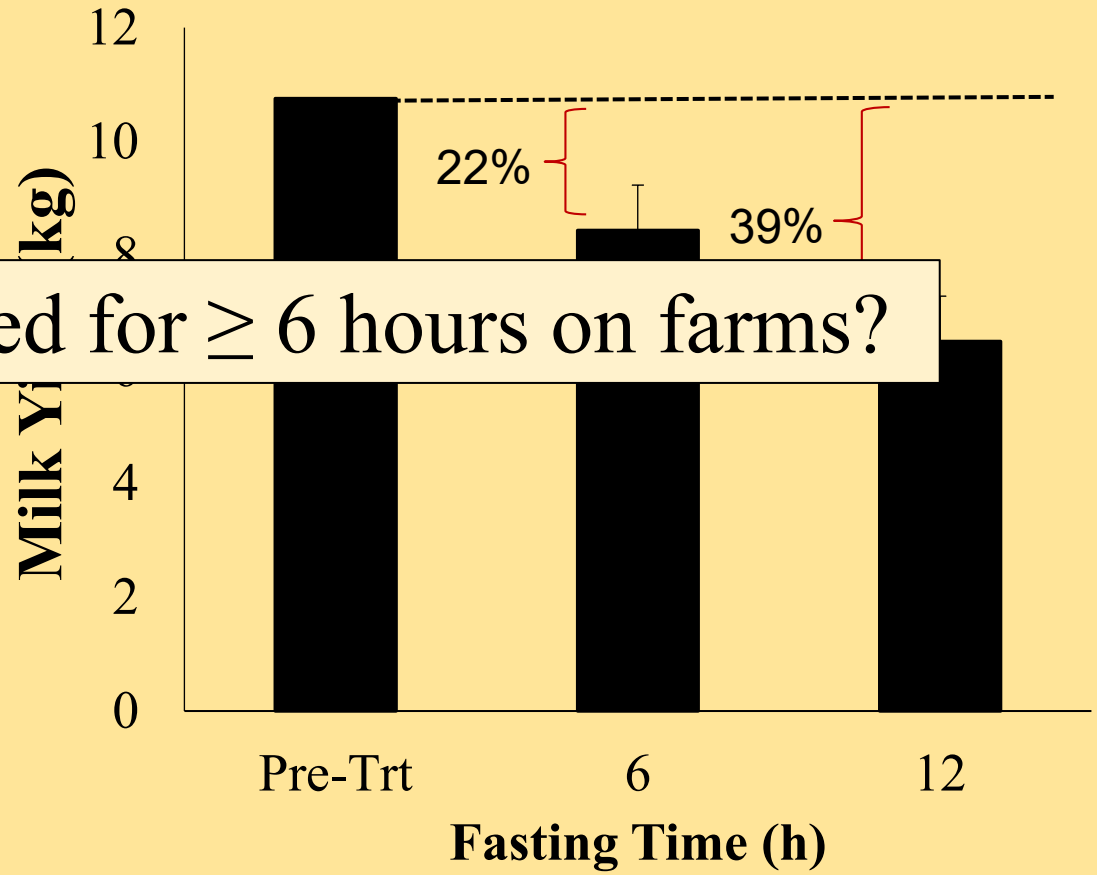
- Alters intestinal architecture
  - Ueno et al., 2011; Pearce et al., 2015; Kvidera et al., 2017
- Reduced transepithelial resistance
  - Pearce et al., 2015
- Increased total tract permeability
  - Zhang et al., 2013
- Increased circulating lipopolysaccharide
  - Pearce et al., 2015
- Increased acute phase proteins
  - Kvidera et al., 2017



Feed Restriction Causes Intestinal Hyperpermeability and Production Losses in Cattle



How often are cows without feed for ≥ 6 hours on farms?



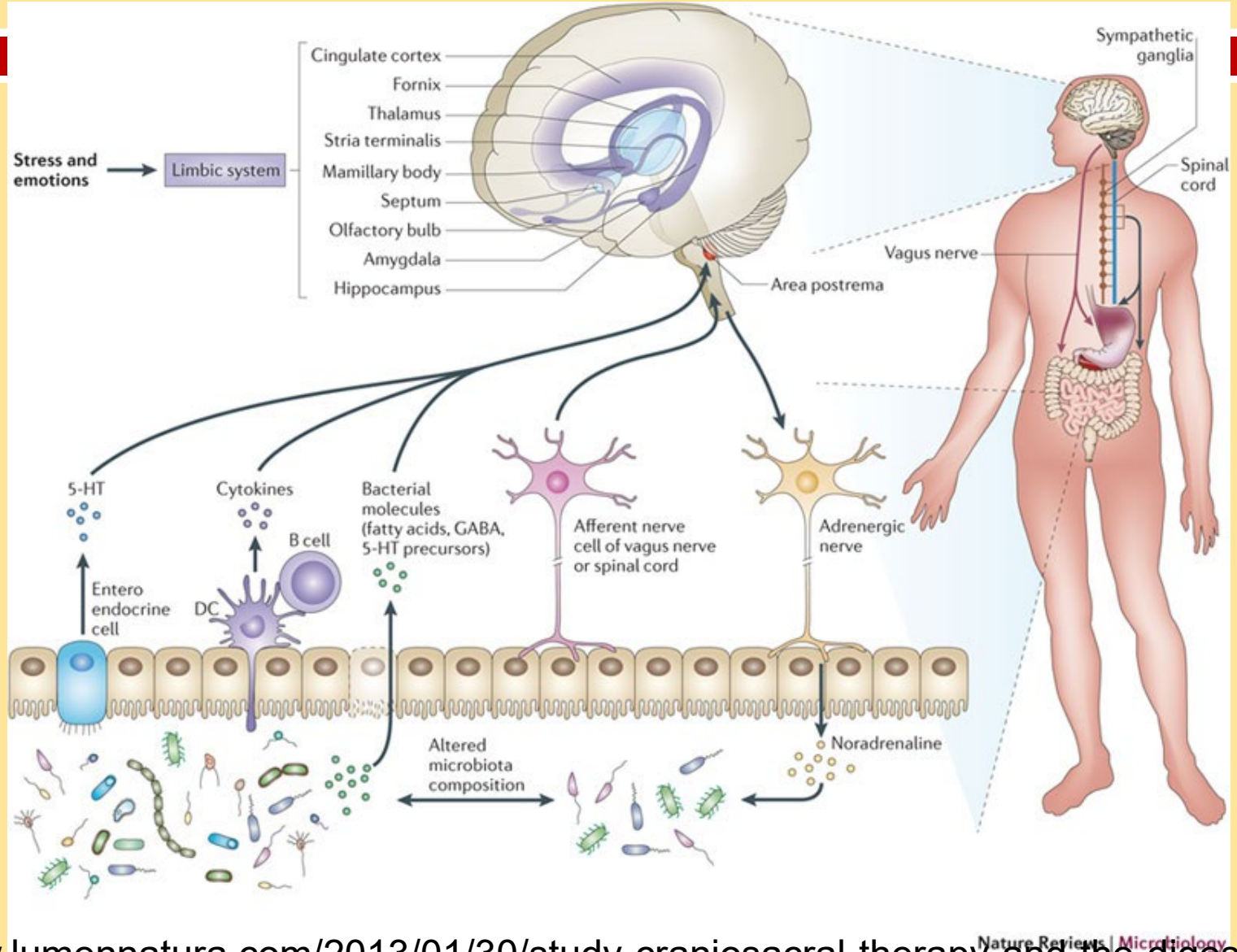
Horst et al., 2019

Documented Stressors that Cause Leaky Gut

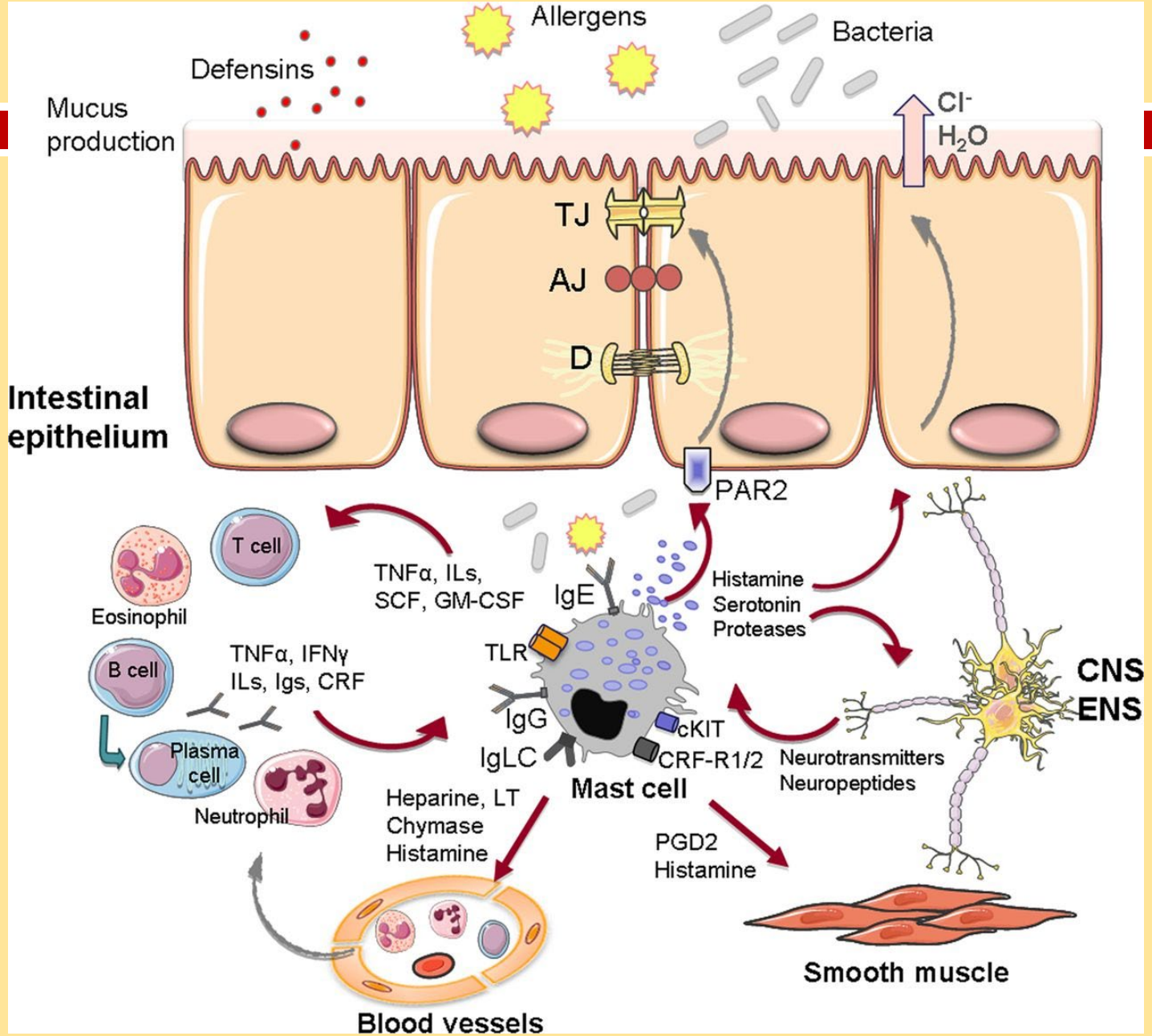
- Heat
- Weaning
- Hunger/feed restriction
- Noise
- Public Speaking
- Cold
- Neonate-Maternal Separation

- How can such different stressors all cause leaky gut???
- They are all psychological

The GIT is enervated by both the CNS and ENS
There's more neurons on the gut than in the brain



https://www.lumennatura.com/2013/01/30/study-craniosacral-therapy-and-the-digestive-system/



http://gut.bmj.com/content/65/1/155

Stress Increases GIT Pathogens

ELSEVIER

CrossMark

Institut Pasteur

Microbes and Infection 17 (2015) 173–183

www.elsevier.com/locate/micinf

Review

How bacterial pathogens colonize their hosts and invade deeper tissues

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Abstract

Bacterial pathogens have evolved a wide range of strategies to colonize and invade human organs, despite the presence of multiple host defense mechanisms. In this review, we will describe how pathogenic bacteria can adhere and multiply at the surface of host cells, how some bacteria can enter and proliferate inside these cells, and finally how pathogens may cross epithelial or endothelial host barriers and get access to internal tissues, leading to severe diseases in humans.

Keywords: Bacterial invasion; Bacterial adhesion; Microbiota; Host barrier; Host–pathogen interactions; *Listeria*

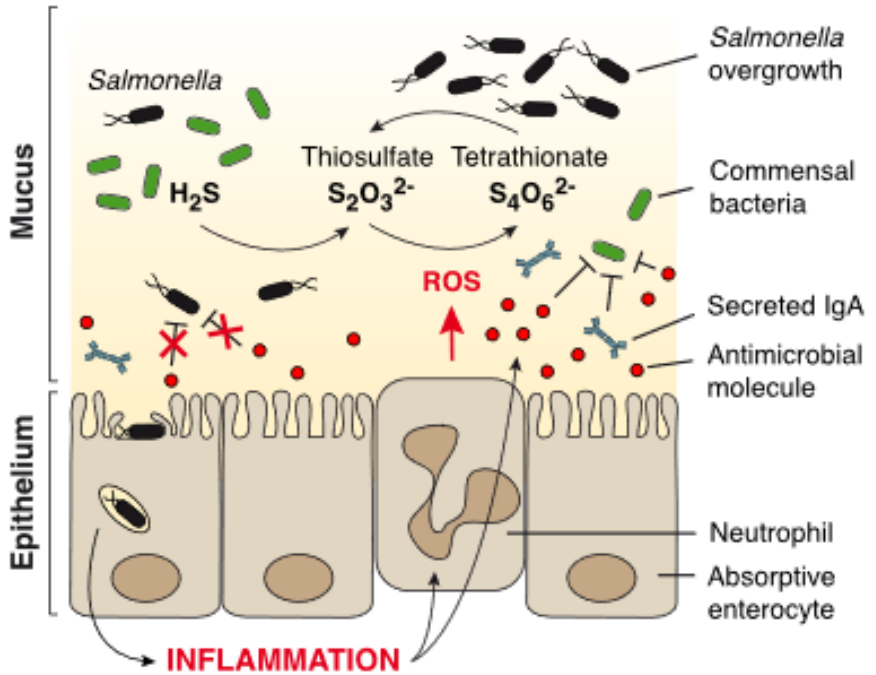

Fig. 2. Schematic representation of events leading to *Salmonella* overgrowth in the intestine. Invasion of intestinal epithelial cells by *Salmonella* triggers an inflammatory response leading to the release of antimicrobial peptides and the production of ROS (*R*eactive *O*xygen *S*pecies) by neutrophils. H_2S, a fermentation end product generated by commensal bacteria, is oxidized into thiosulfate by the colonic epithelium and then into tetrathionate by ROS. In contrast to fermenting bacteria of the microbiota, *Salmonella* can use this tetrathionate as a terminal electron acceptor to support growth in anaerobic conditions. The use of tetrathionate, in addition to *Salmonella* resistance to antimicrobial molecules, allow this pathogen to out-compete commensal bacteria in this inflamed context.

Intestinal epithelium (mainly neutrophils and macrophages) create an oxidative environment (via redox) as a defensive strategy. This kills commensal bacterial….but the pathogens have developed oxidative evasion techniques

Journal of Animal Science, 2022, **100**, 1–11
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Advance access publication 17 December 2021
Housing and Management

Effects of postweaning supplementation of immunomodulatory feed ingredient on circulating cytokines and microbial populations in programmed fed beef heifers

Keelee J. McCarty,[†] Jessie E. Tipton,[†] Ralph E. Ricks,[†] Jessica Danielo,[†] Jesse S. Thompson,[‡] Elliot Block,[‡] Scott L. Pratt,[†] and Nathan M. Long[†,1]

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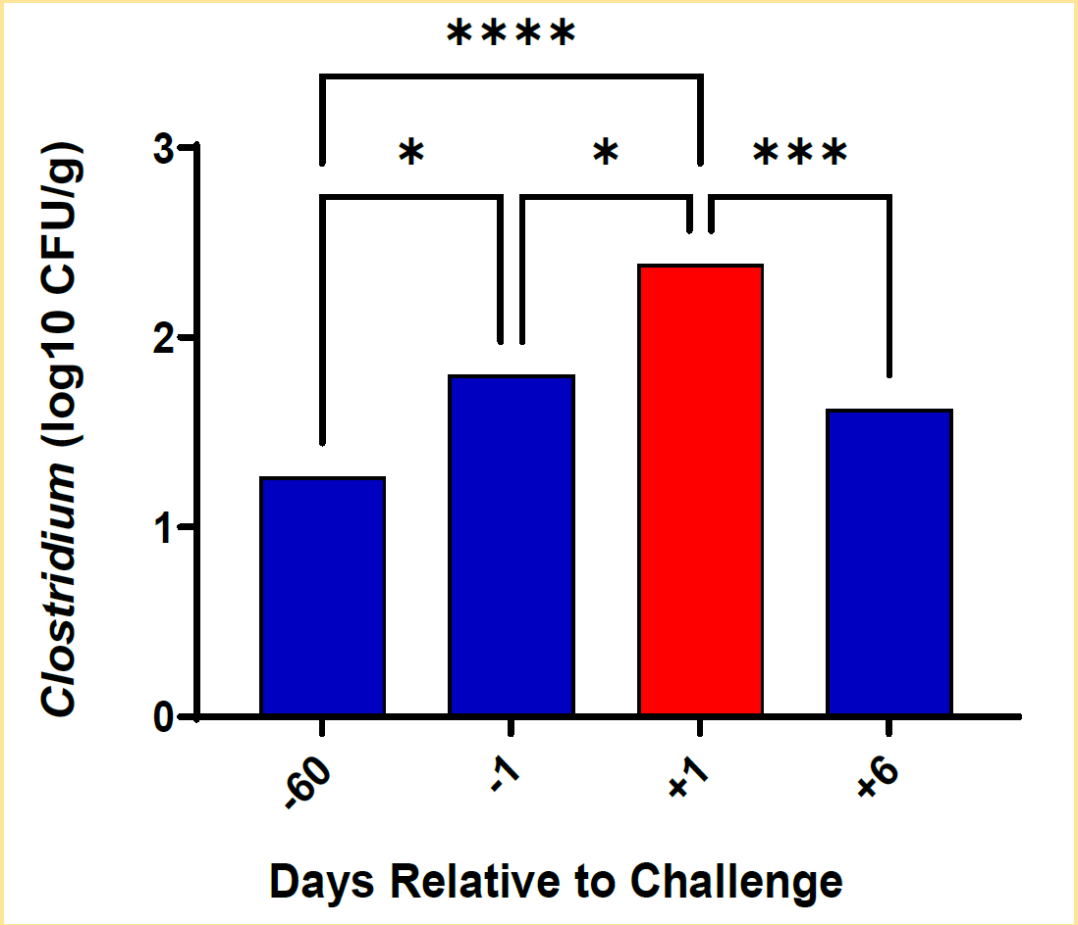
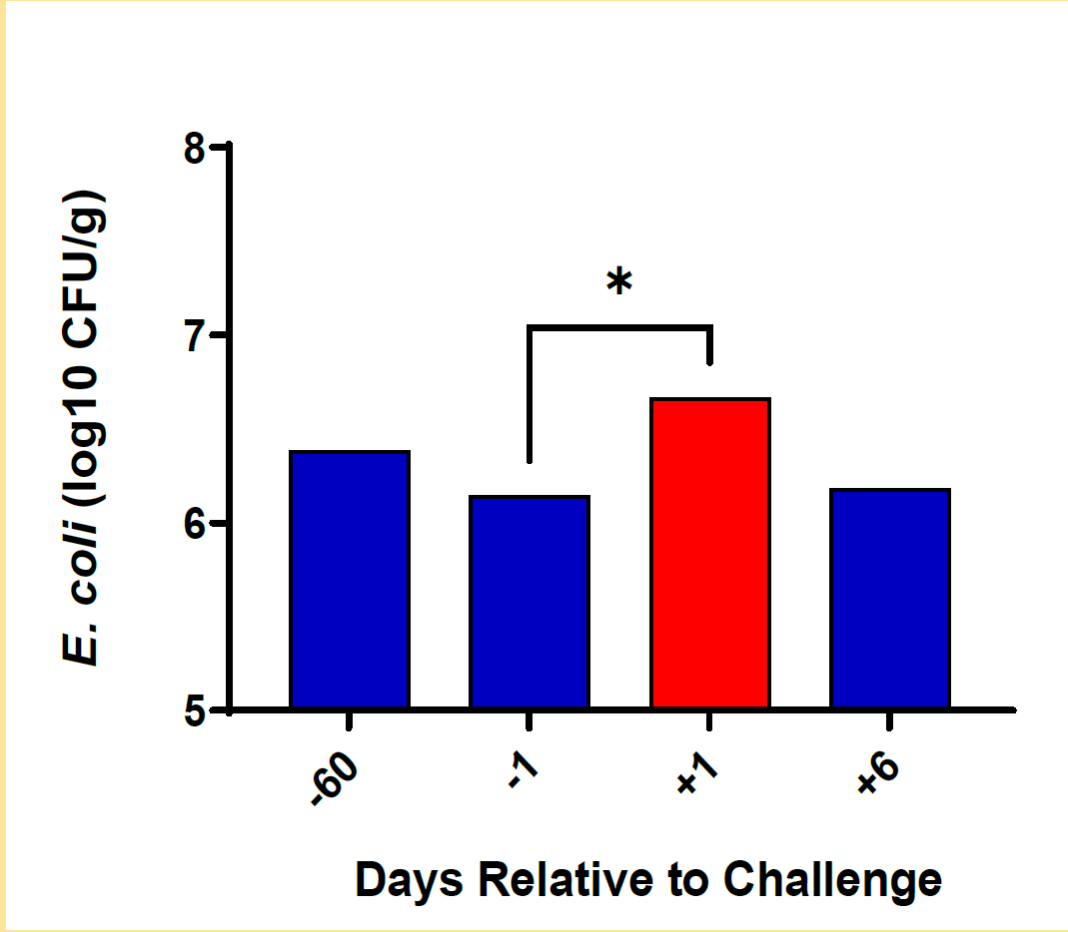
Transport stress increases *Clostridia* and *E. coli* Shedding in Cattle





McCarty et al., 2022: JAS 100: 1-11

J. Dairy Sci. 103:1541–1552
https://doi.org/10.3168/jds.2019-17350

Effect of enzymatically hydrolyzed yeast on health and performance of transition dairy cattle

H. Stefenoni,[1] J. H. Harrison,[1,2*] A. Adams-Progar,[1] and E. Block[3]
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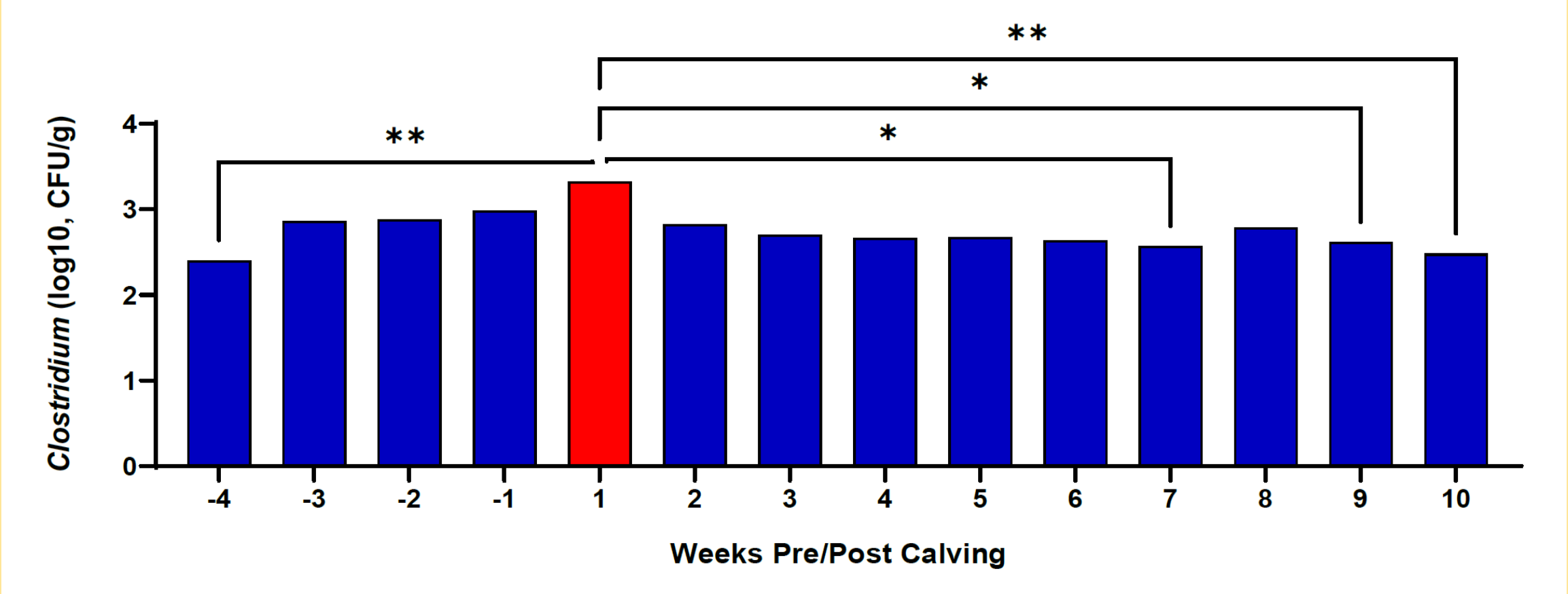
[2]Department of Animal Sciences, Washington State University, Puyallup 98731
[3]Church and Dwight Animal Nutrition, Princeton, NJ 08543

Transition Cow *Clostridia* shedding



Stefenoni et al., 2020; JDS 103: 1541-1552

Journal of Animal Science, 2024, **102**, skae073
https://doi.org/10.1093/jas/skae073
Advance access publication 14 March 2024
Metabolism and Metabolomics

Calcium trafficking and gastrointestinal physiology following an acute lipopolysaccharide challenge in pigs

Julie Opgenorth,[†] Edith J. Mayorga,[†] Megan A. Abeyta,[†] Brady M. Goetz,[†] Sonia Rodriguez-Jimenez,[†] Alyssa D. Freestone,[†] Chad H. Stahl,[‡] and Lance H. Baumgard[†,1]

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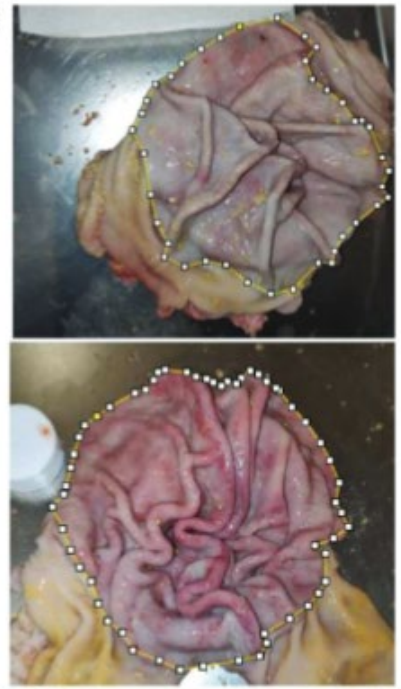
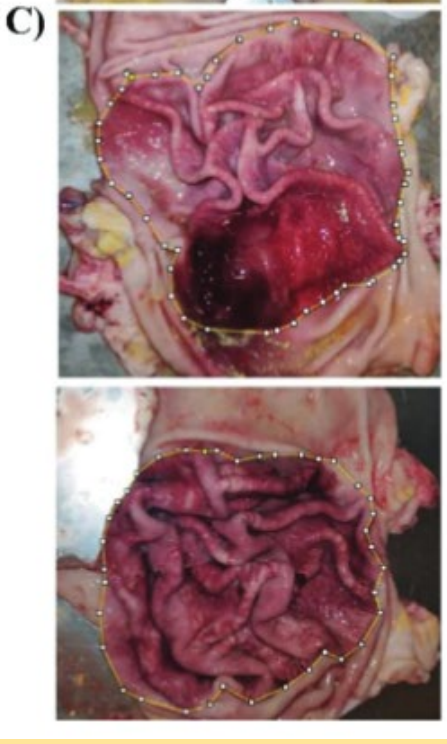
I.V. Saline Administration



Immune activation has enormous effects on GIT motility (not shown) and causes gastric hemorrhage.

What are the implications to dietary strategies??

I.V. LPS Administration



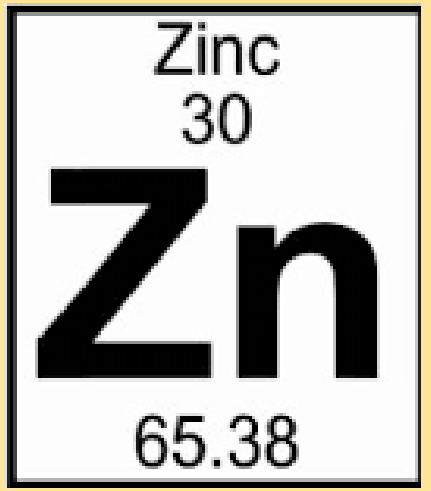
$$ Billion Euro Question/Opportunity $$

- Can the Feed or Animal Health Industry do anything about leaky gut…..especially without dietary antibiotics???

- Targets:
  - Direct action at intestine
  - Indirect via:
    - Increased feed intake
    - Rumen acidosis prevention
      - Hind gut acidosis prevention
    - Improved immune function

Zinc (Zn)

- Essential nutrient for all living organisms
  - Structural component
  - Catalytic factor
  - Signaling mediator
- Improves cellular function of immune cells (Haase and Rink et al., 2014; Wessel and Cousins, 2015; Mayorga et al., 2017)
  - Altered cytokine production
  - Acute phase protein production
  - Oxidative burst
- Enhances epithelial barrier integrity (mammary, intestinal [and lung??])
  - Decreased plasma lactose (Weng et al., 2017)
  - Decreased SCC (Kellogg et al., 2004; Nayeri et al., 2014)



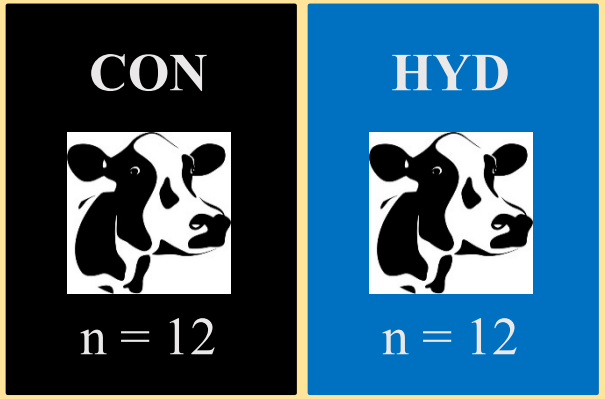
Human Gut Health and Zinc

- Alam et al., 1994. Enteric protein loss and intestinal permeability changes in children during acute shigellosis and after recovery: effect of zinc supplementation
- Rodriguez et al., 1996. Intestinal paracellular permeability during malnutrition in guinea pigs: effect of high dietary zinc. Gut. 39:416-422.
- Sturniolo et al., 2001. Zinc supplementation tightens “leaky gut” in Crohn’s disease. Inflamm. Bowel Dis. 7:94-98.
- Finamore et al., 2008. Zinc deficiency induces membrane barrier damage and increases neutrophil transmigration in Caco-2 cells. J. Nutr. 138:1664-1670
- Peterson et al., 2008. Moderate zinc restriction affects intestinal health and immune function in lipopolysaccharide-challenged mice. J. Nutr. Biochem. 19:193-198.
- Mahmood et al., 2009. Zinc carnosine, a health food supplement that stabilizes small bowl integrity and stimulates gut repair processes. Gut 56:168-175

Could a more Bioavailable Zinc Improve GIT Barrier Parameters in Farm Animals?

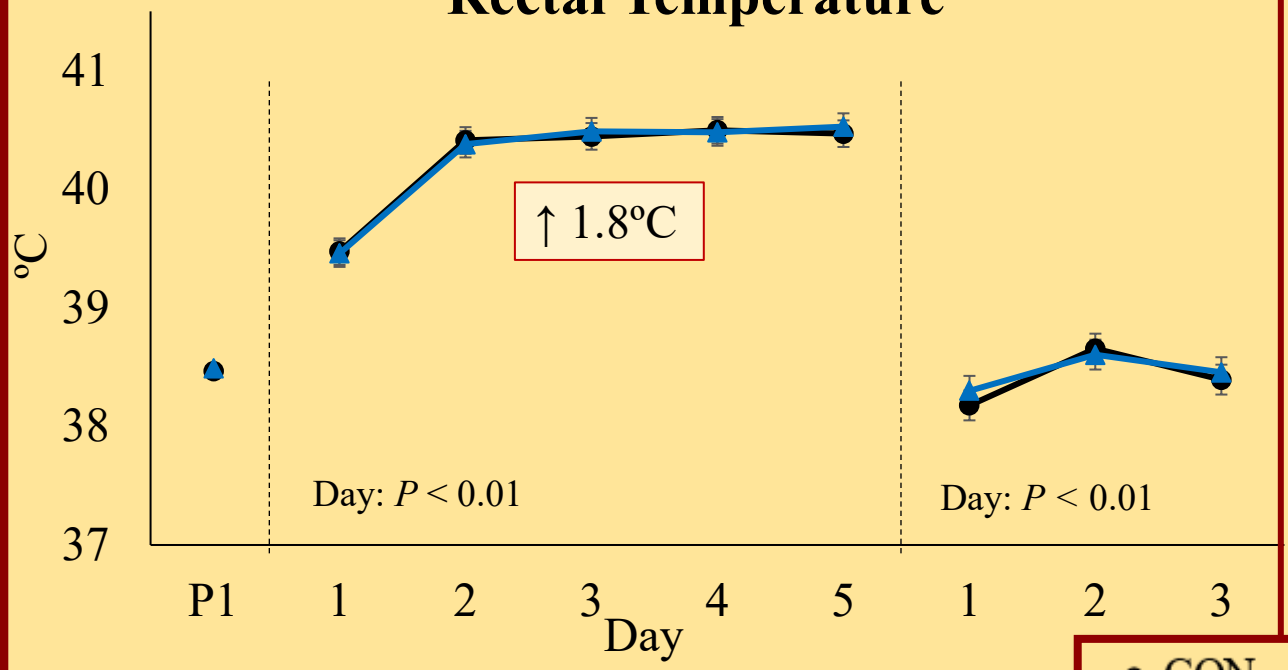
Heat Stress: Materials and Methods

- Twenty-four lactating Holstein cows (680 ± 63 kg BW; 130 ± 34 DIM; Parity 2.0 ± 0.5)

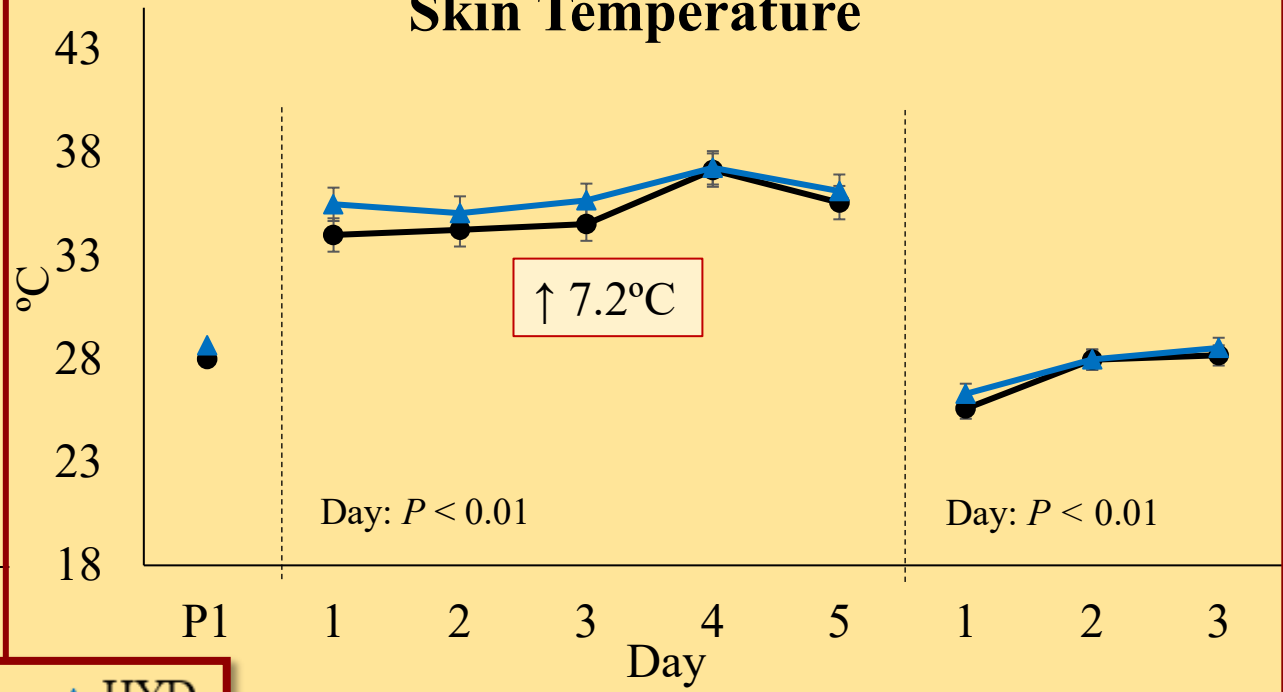


- Prefeeding phase of 16 d, 2 treatments:
  - Control (**CON**): diet contained 75 ppm supplemental Zn from $ZnSO_4$
  - Treatment (**HYD**): diet contained 75 ppm supplemental Zn from Zn HYD (IntelliBond Z, Selko, Indianapolis, IN)
- Three experimental periods:
  - Period 1 (5 d) - Thermo-neutral conditions (**TN**)
    - Collection of baseline measurements
  - Period 2 (5 d) - Heat stress induction
    - Electric heat blankets (**EHB**) implementation
  - Period 3 (3 d) - Recovery period - TN

Rectal Temperature

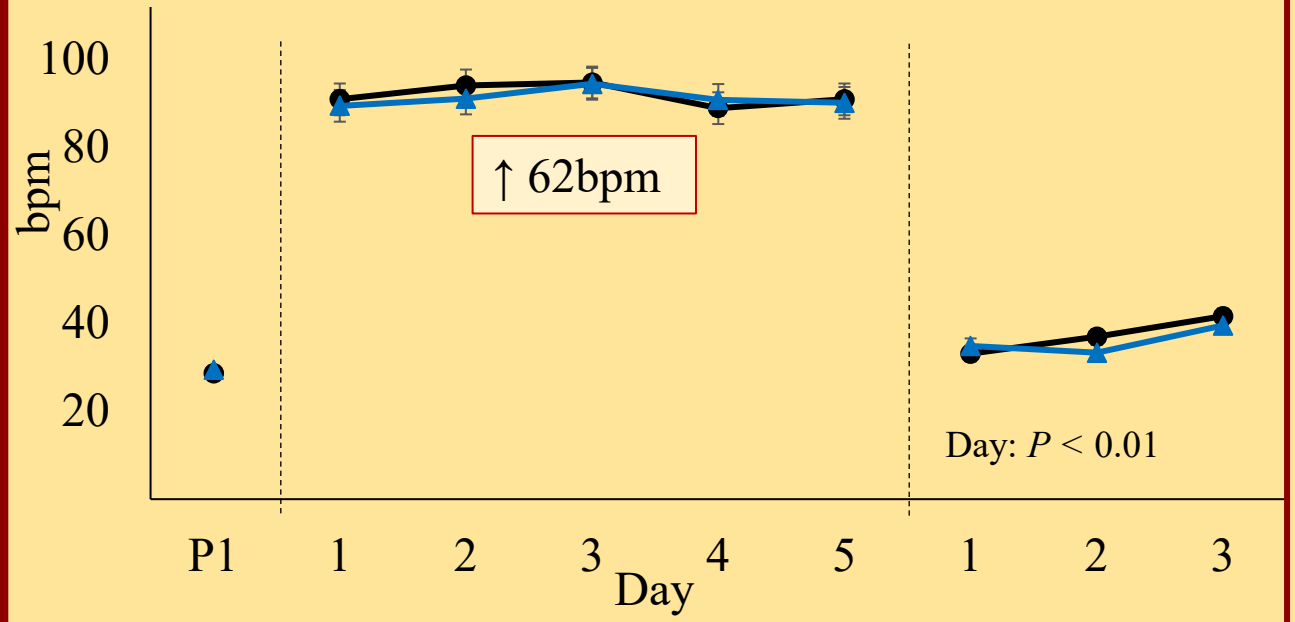


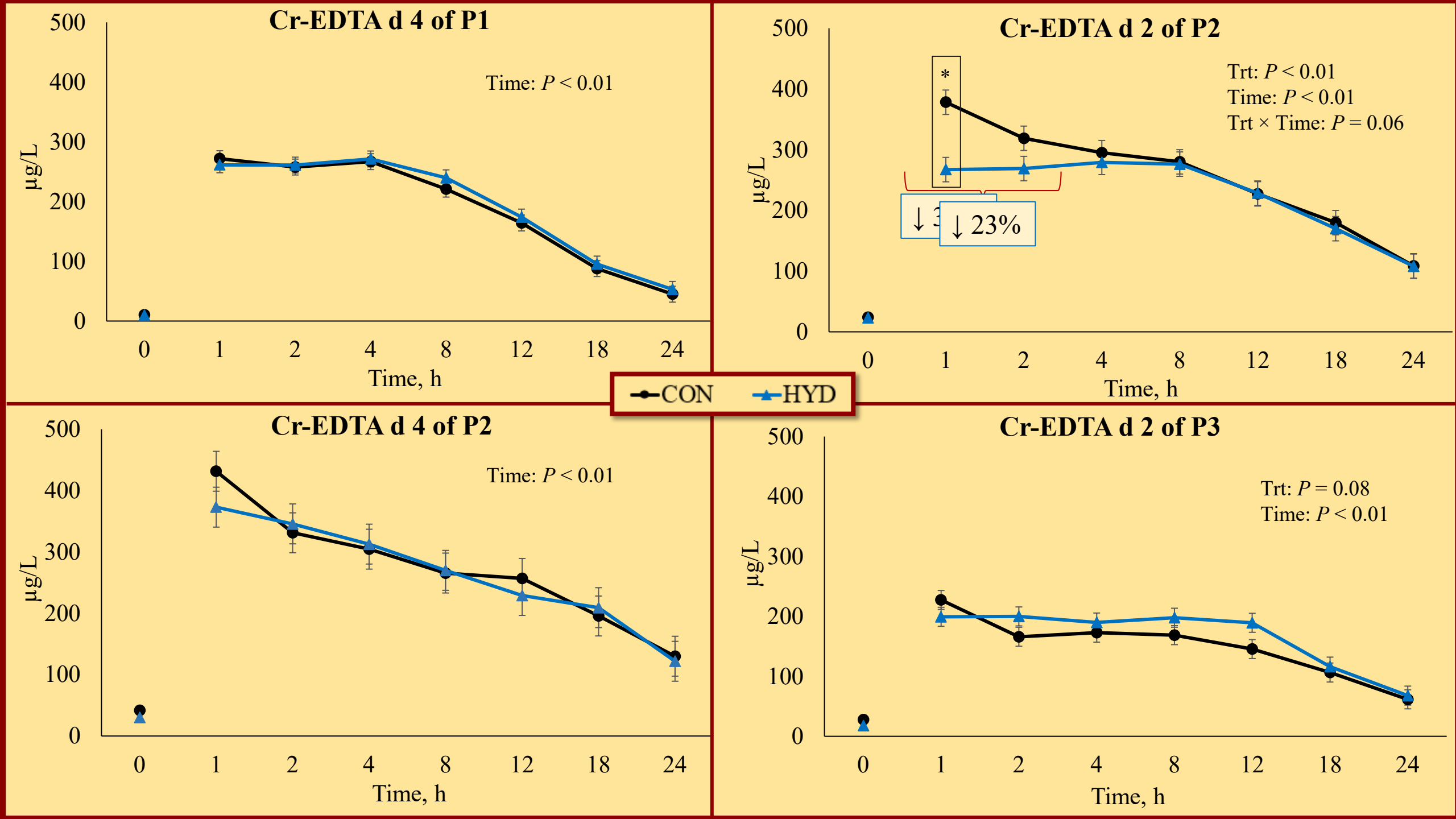
Skin Temperature



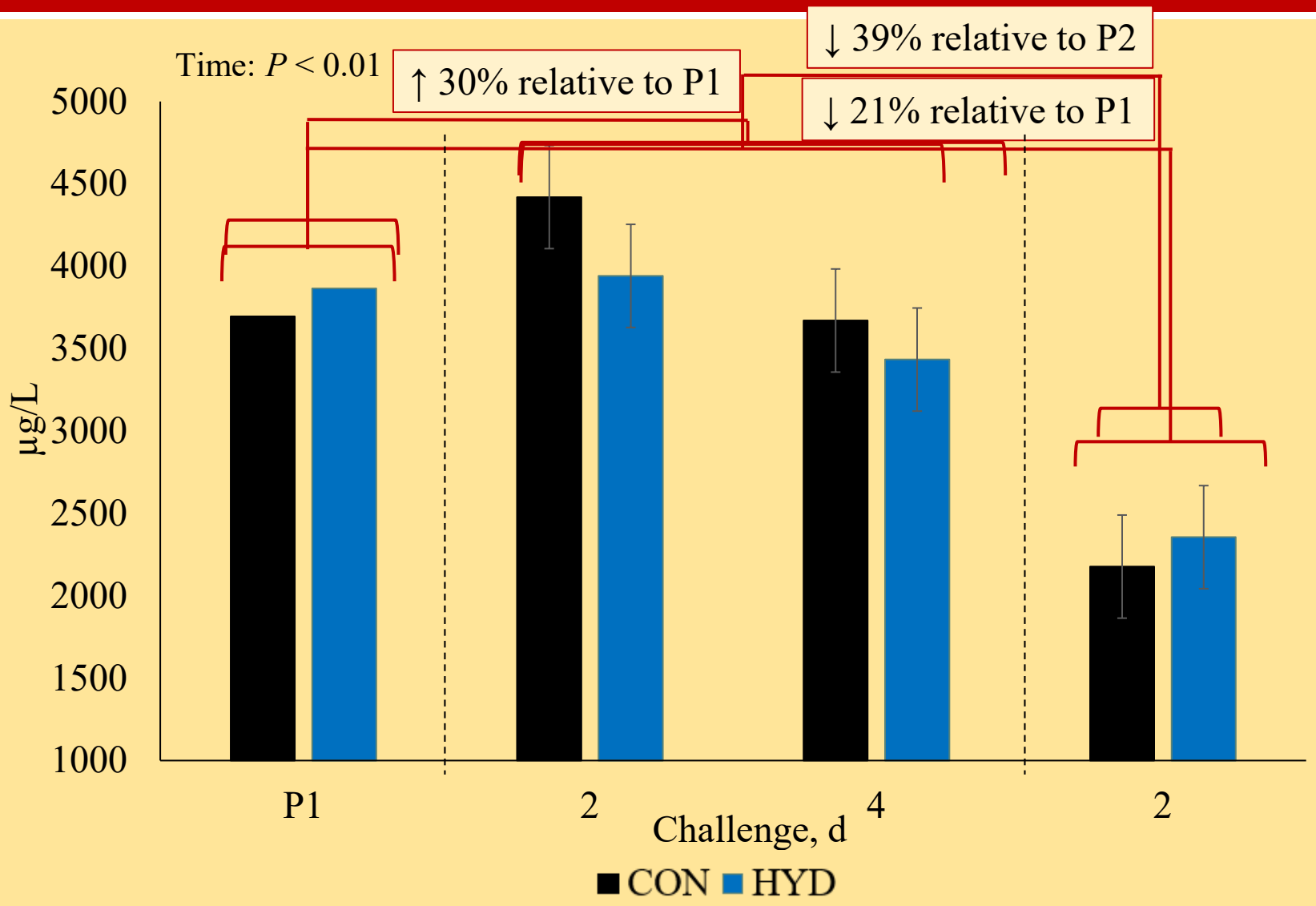
CON HYD

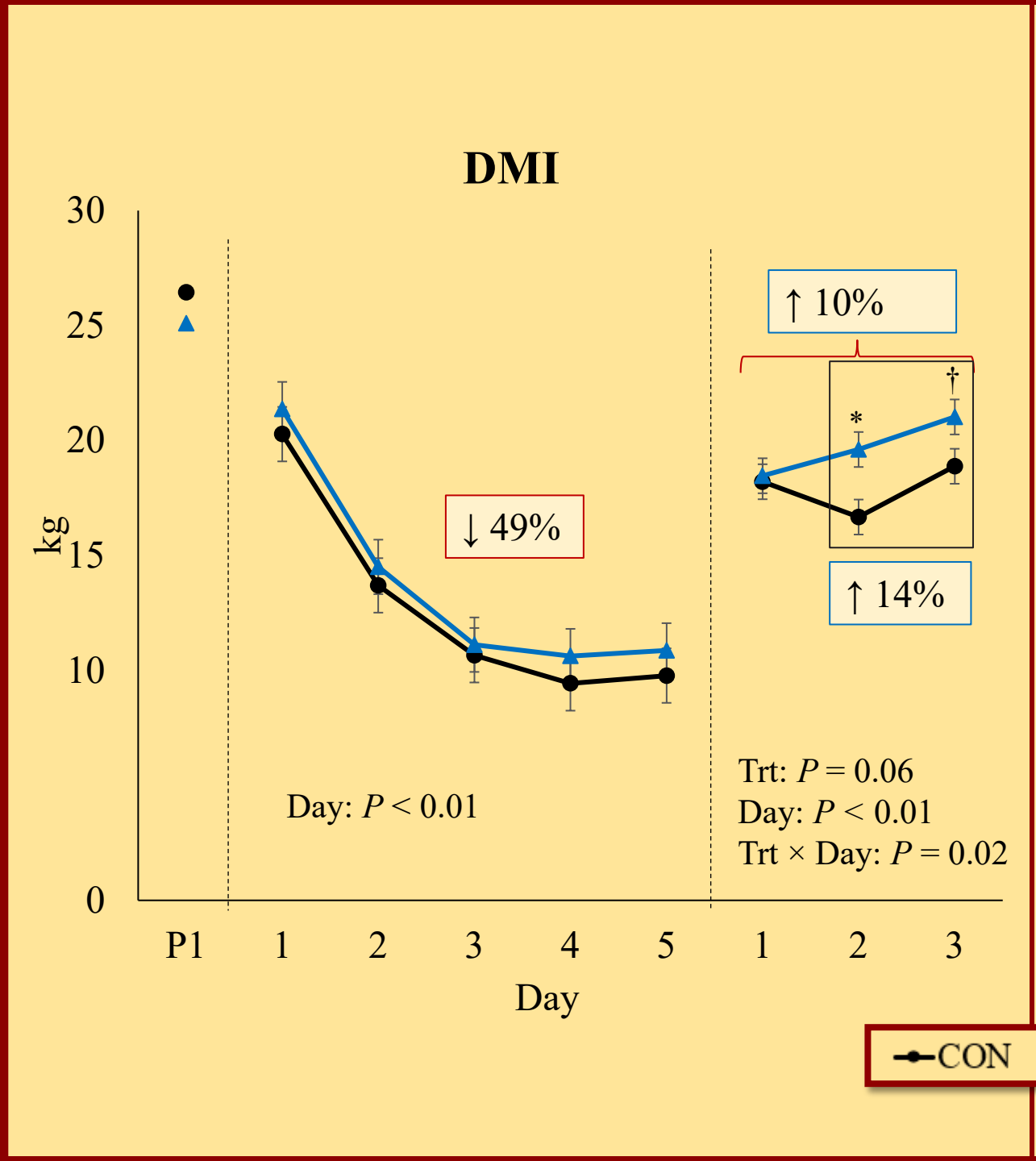
Respiration Rate

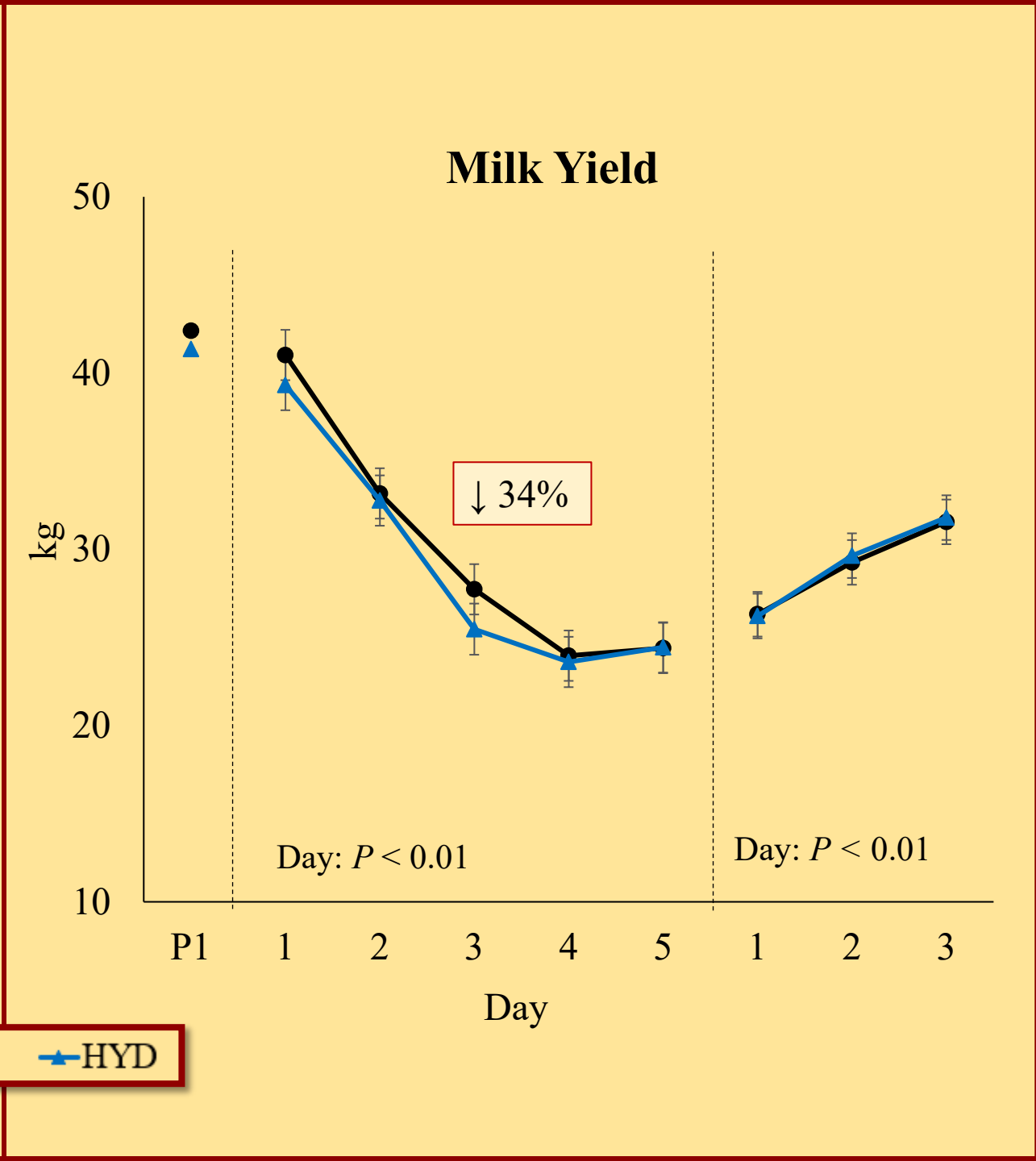



Cr-EDTA d 4 of P1
Time: P < 0.01
µg/L
Time, h
Cr-EDTA d 2 of P2
Trt: P < 0.01
Time: P < 0.01
Trt × Time: P = 0.06
*
↓ 23%
Cr-EDTA d 4 of P2
Time: P < 0.01
Cr-EDTA d 2 of P3
Trt: P = 0.08
Time: P < 0.01
CON
HYD

Cr-EDTA: AUC


Time: $P < 0.01$
↑ 30% relative to P1
↓ 39% relative to P2
↓ 21% relative to P1
5000
4500
4000
3500
3000
2500
2000
1500
1000
µg/L
P1
2
4
2
Challenge, d
CON
HYD


DMI
30
25
20
15
10
0
kg
↓ 49%
↑ 10%
*
†
↑ 14%
Day: P < 0.01
Trt: P = 0.06
Day: P < 0.01
Trt × Day: P = 0.02
P1
1
2
3
4
5
1
2
3
Day


Milk Yield
50
40
30
20
10
kg
↓ 34%
Day: P < 0.01
Day: P < 0.01
P1
1
2
3
4
5
1
2
3
Day

CON
HYD

J. Dairy Sci. 103:11911–11929
https://doi.org/10.3168/jds.2020-18860

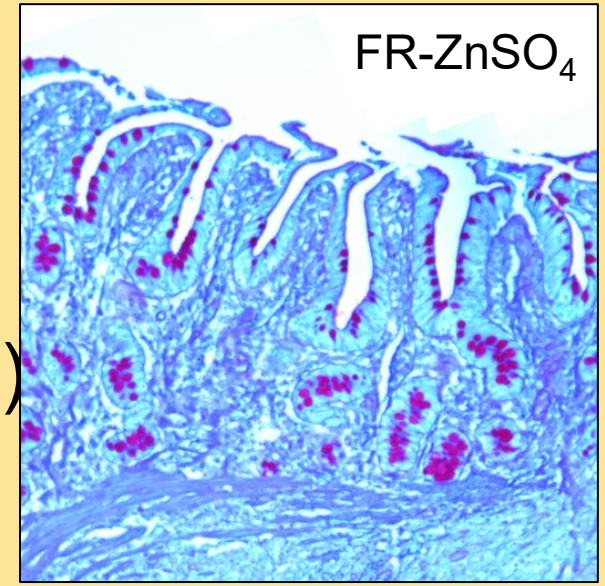
Evaluating effects of zinc hydroxychloride on biomarkers of inflammation and intestinal integrity during feed restriction

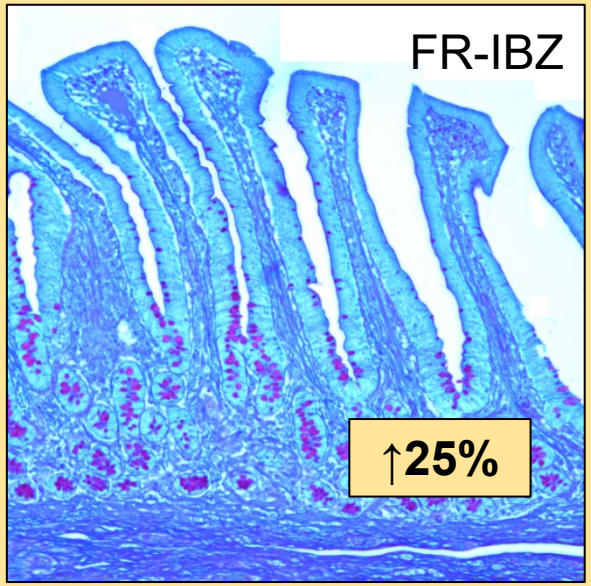
E. A. Horst,[1] E. J. Mayorga,[1] M. Al-Qaisi,[1] S. Rodriguez-Jimenez,[1] B. M. Goetz,[1] M. A. Abeyta,[1] P. J. Gorden,[2] S. K. Kvidera,[3] and L. H. Baumgard[1]*

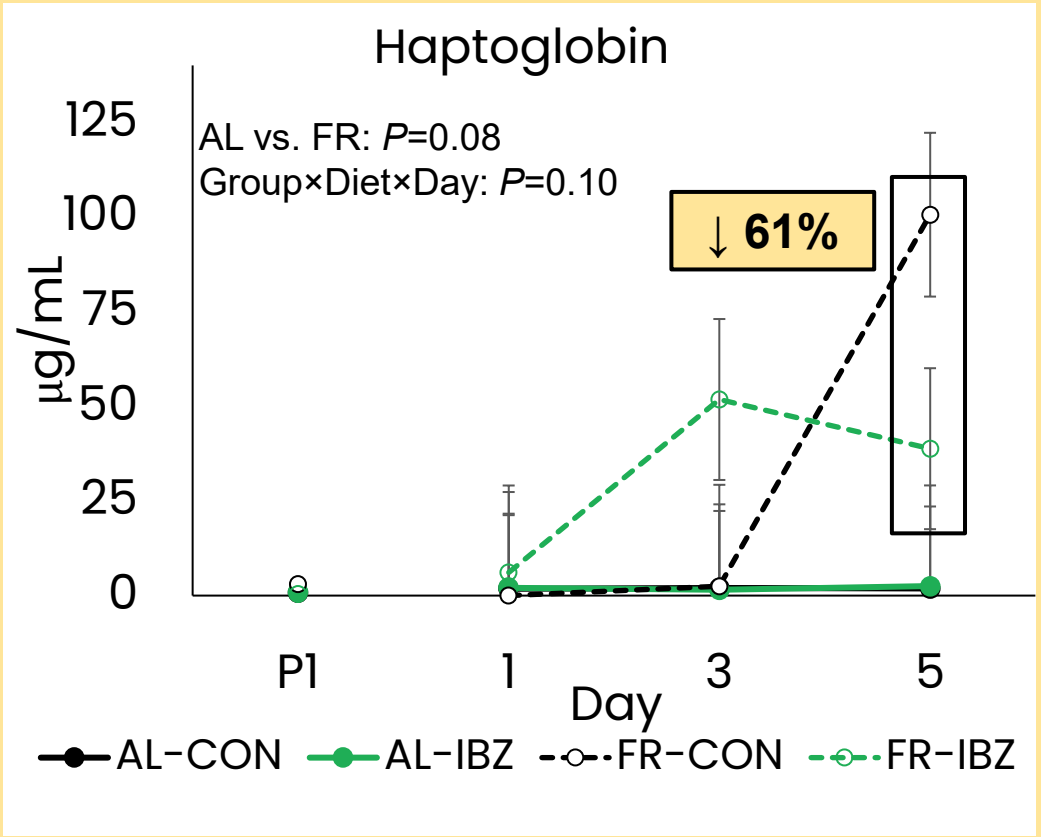
[1]Department of Animal Science, Iowa State University, Ames, 50011
[2]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, 50011
[3]Micronutrients USA LLC, Indianapolis, IN 46241

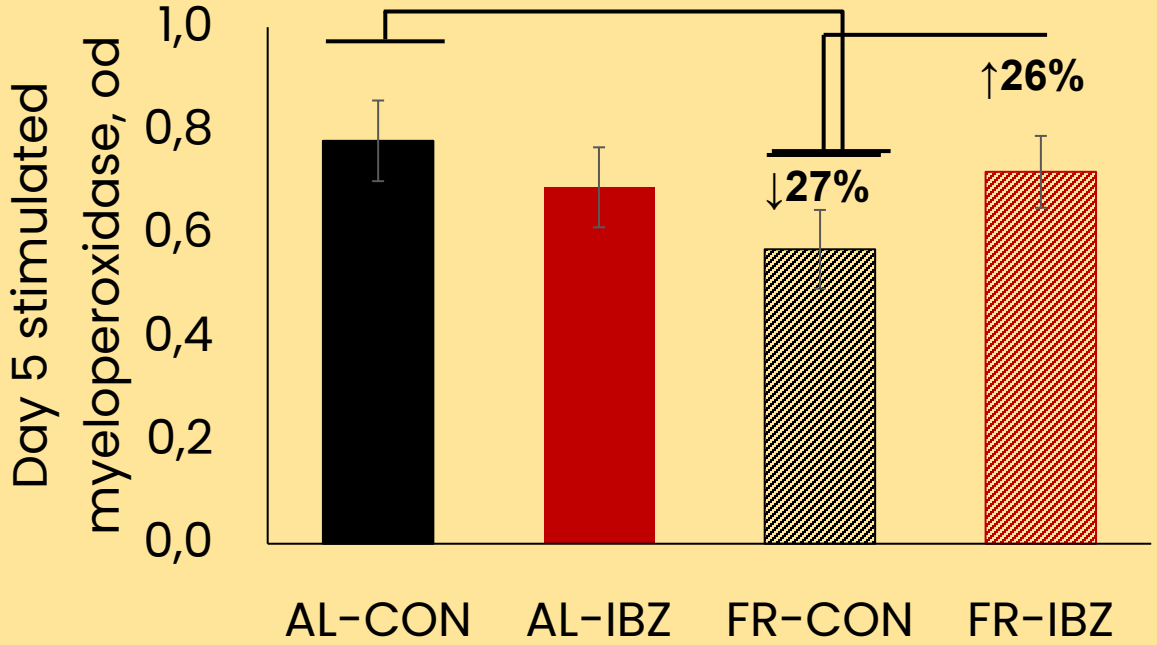
- 24 Holstein cows; 159 DIM
- 2×2 factorial:

1. Ad-libitum fed or feed restricted
2. $ZnSO_4$ or IntelliBond Z (at 75 ppm)







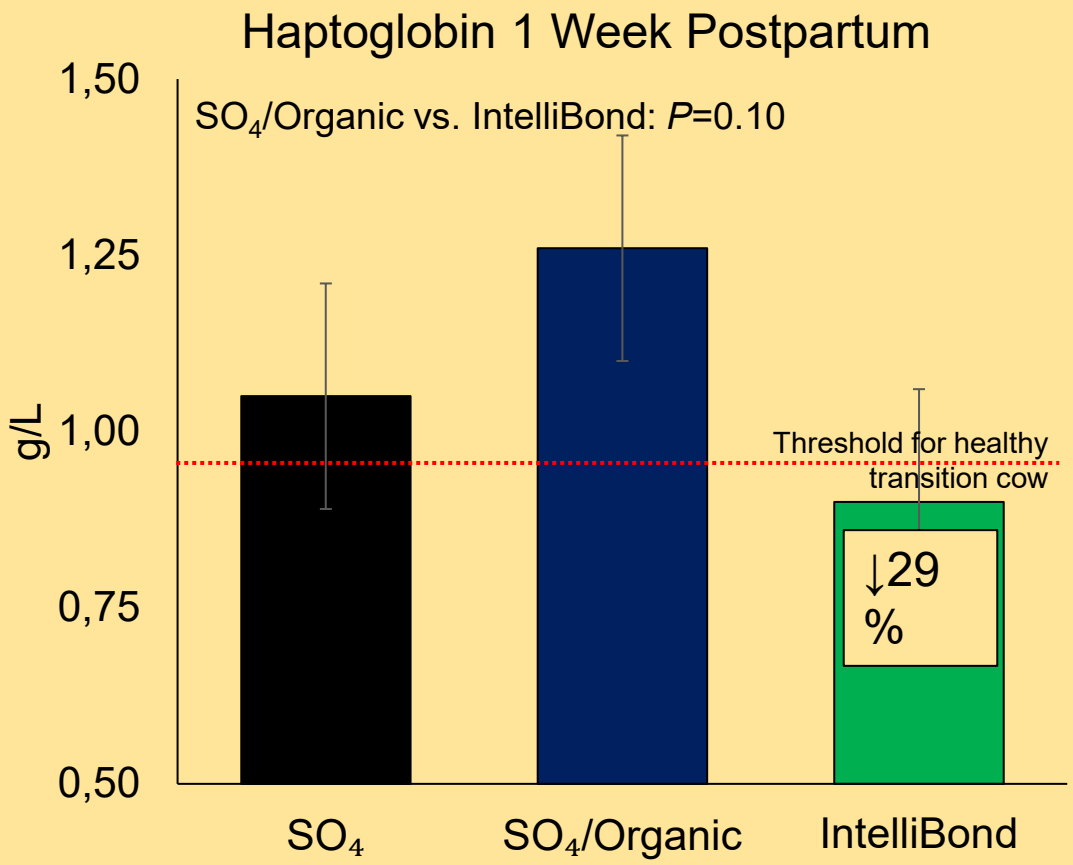


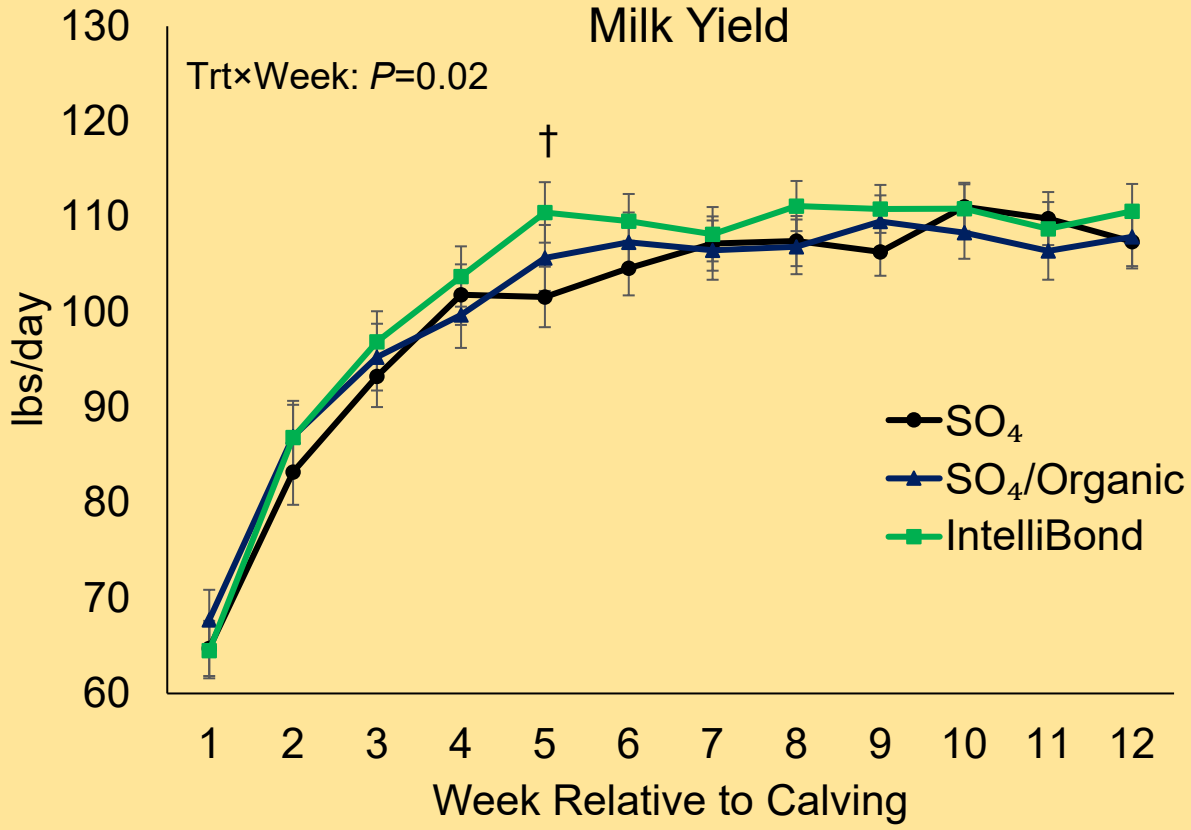
AL-CON vs. FR-CON: *P*=0.07
FR-CON vs FR-IBZ: *P*=0.15

Horst et al., 2020. J. Dairy Sci.

- 60 multiparous cows; -28 to 84 DIM
  Housed in tie stalls; treatments were top-dressed once daily

Mineral	Inorganic	Combination	IntelliBond
Copper	$CuSO_4$	75% $CuSO_4$/ 25% organic	IntelliBond C
Zinc	$ZnSO_4$	75% $ZnSO_4$/ 25% organic	IntelliBond Z
Manganese	$MnSO_4$	75% $MnSO_4$/ 25% organic	IntelliBond M





Yasui et al., 2014. J. Dairy Sci. 97:3728-3738

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Short Communication
Animal Nutrition and Farm Systems

Effect of zinc source (zinc sulfate or zinc hydroxychloride) on relative abundance of fecal *Treponema* spp. in lactating dairy cows

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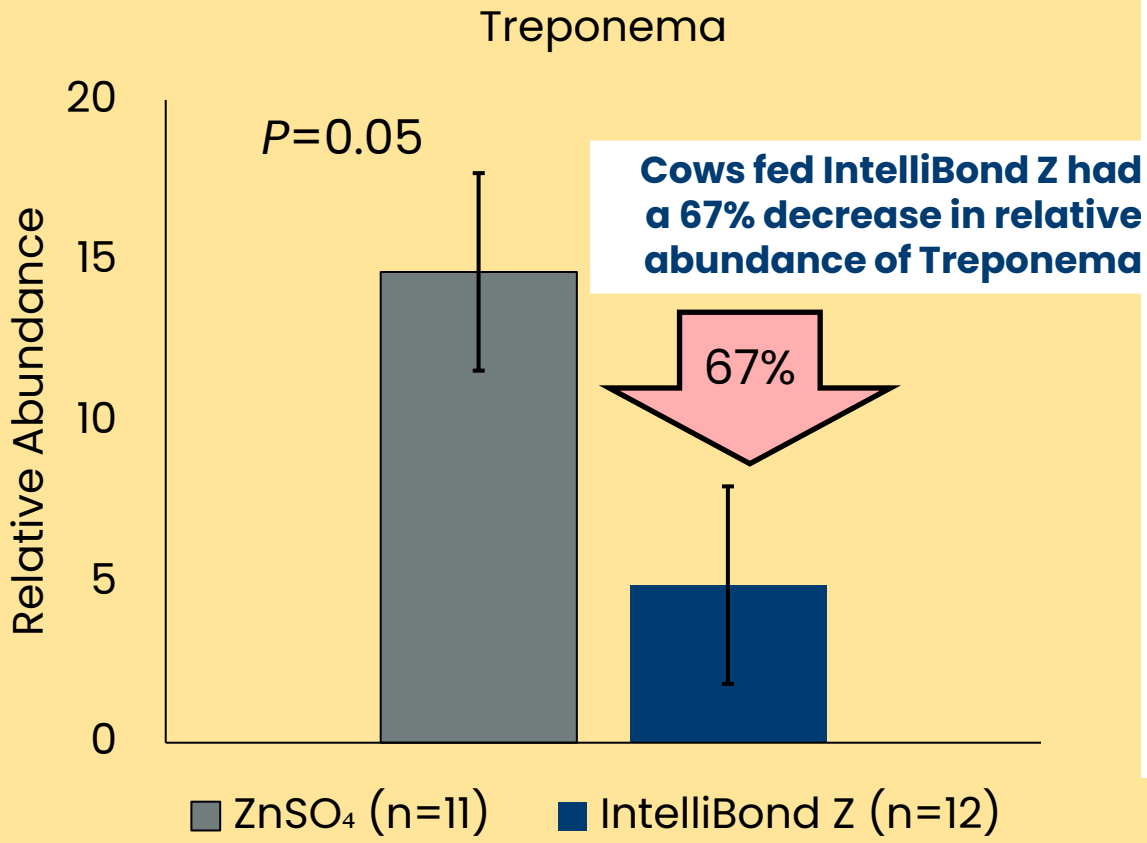

Cows fed IntelliBond Z had a 67% decrease in relative abundance of Treponema

*All cows fed ad-libitum

Table 1. Relative abundance of 16S rRNA gene sequences for recovered *Treponema* spp. and diversity indices for fecal microbiome sequences of cows fed either Zn sulfate or Zn hydroxychloride

Amplicon sequencing variant	CON[1]	HYD	SE	*P*-value[2]
Treponema	14.7	4.9	3.1	0.05
Treponema 2	14.1	4.6	3.0	0.05
Treponema porcinum	0.354	0.043	0.103	0.06
Uncultured *Treponema*	0.185	0.312	0.056	0.13
Gut metagenome *Treponema*	0.009	0.017	0.005	0.24
Diversity metric				
Richness	567	700	66	0.16
Chao1	573	714	67	0.16
Shannon's diversity	7.50	7.96	0.21	0.13
Inverse Simpson	16.3	19.5	1.3	0.11
Pielou's evenness	0.826	0.848	0.016	0.33
Good's coverage	0.999	0.999	0.0004	0.25
Faith's phylogenetic diversity	30.8	34.7	1.7	0.12

[1]Treatments consisted of either 75 mg/kg supplemental $ZnSO_4$ (CON) or 75 mg/kg supplemental Zn hydroxychloride (HYD).

[2]*P*-values reported for the effects of HYD.

GIT Stress & Hydroxy-Zn Summary

- Heat stress caused gastrointestinal tract [GIT] hyperpermeability
  - Feeding Hydroxy-Zn ameliorated leaky gut and the temporal pattern suggests the effects are in the small intestine
- Feed Restriction caused intestinal inflammation
  - Feeding Hydroxy-Zn increased villi height
  - Decreased circulating haptoglobin
  - Increased GIT neutrophil infiltration
  - Increased feed intake post-feed restriction
- During the transition period
  - Feeding Hydroxy-Zn decreased haptoglobin
  - Tended to increase milk yield
- Fecal bacterial load
  - Feeding Hdroxy-Zn decreased Treponema (lameness causing pathogen)

Seminar Summary

- Many common on-farm situation cause “leaky gut” and
  - Probably stems from the general “stress” response in the gut
    - Explains why diarrhea/vomiting often occurs during stress
- Primary strategy should be to prevent or minimize stress
  - This is primary management
- Dietary Strategies
  - Avoid rumen and hind gut acidosis
  - Keep fermentation in the rumen
  - Dietary strategies like bioavailable Zinc to prevent leaky guy

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