2017

CALIFORNIA ANIMAL NUTRITION CONFERENCE

MAY 10 & 11, 2017
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Potential for feeding program to impact gut health and inflammation in dairy cattle

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INTRODUCTION

The classic role of the dairy cattle nutritionist is to formulate rations that meet animal performance requirements. However, as the delicate interplay between animal nutrition and health continues to unfold, we are beginning to understand more ways that nutritional programs impact the health of the digestive tract and consequently the health and performance of the animal. The goal of this paper is to provide a brief overview of gut structures and to discuss the potential for nutrition to negatively or positively impact gut health. Sub-acute ruminal acidosis (SARA) and yeast feeding will be used as specific examples to illustrate negative and positive effects of different feeding programs on gut health, respectively.

THE DIGESTIVE EPITHELIUM

The rumen epithelium serves as a selective barrier, allowing for absorption of short chain fatty acids (SCFA) while preventing entry and colonization by bacteria. Structurally the rumen epithelium consists of four layers, the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. In the healthy rumen, bacteria are loosely associated only with the stratum corneum. Tight junction proteins that regulate the permeability barrier are expressed most heavily in the stratum granulosum and to some extent in the stratum spinosum (Graham and Simmons, 2005). Connections among the stratum granulosum, stratum spinosum, and stratum basale allow for the transport of SCFA from the rumen contents to the basal lamina (Graham and Simmons, 2005).

The intestinal mucosa is made up of different classes of epithelial cells. The cells in the greatest numbers are the columnar epithelial cells responsible for the absorption of dietary nutrients. Tight junctions between columnar epithelial cells are crucial for forming the physical barrier between the tissue and digesta. Interspersed among the columnar epithelial cells are a variety of specialized cells that help to protect the tissue from bacteria and toxins within the gut lumen. Goblet cells produce mucus that adheres to the luminal side of epithelial cells and provides an additional physical barrier. The mucus also houses a variety of bactericidal and bacteriostatic agents that prevent colonization by microbes that penetrate the mucus barrier. Many of these antimicrobial agents originate from Paneth cells that produce different compounds in response to stimuli from gut contents or surrounding cells. These antimicrobial agents include peptide defensin molecules as well as antimicrobial enzymes. M cells are another specialized class of epithelial cell that sample particulates from the gut lumen and present them to underlying immune cells.

GUT IMMUNE SYSTEM

Gut associated lymphoid tissue (GALT) consists of structures of white blood cells that are found throughout the digestive tract in close association with the epithelial cells. These range in size

from large Peyer's patches to small isolated lymphoid follicles and consist of clusters of B and T lymphocytes interspersed with dendritic cells and phagocytes (Goto and Kiyono, 2012). Antigen presentation from M cells or dendritic cells causes B cells to be activated to IgA-secreting plasma cells. Secretory IgA then exits the columnar epithelial cells via transcytosis where it accumulates in the mucus to prevent attachment and translocation of target bacteria (Kamada et al., 2013). T cells in germinal centers within GALT are activated by receptor binding of microbial products and locally produced cytokines. In a healthy animal, tolerance of commensal gut microorganisms is facilitated by T cells with a regulatory phenotype that tend to suppress inflammatory responses by surrounding cells (Littman and Pamer, 2011).

Activities of lymphocytes and phagocytes within the healthy GALT respond to the microbiome to elicit appropriate responses: tolerance and local immunosuppression in response to commensal organisms and inflammation and immune activation in response to a microbial threat. Binding of bacterial components to pathogen recognition receptors such as toll like receptors (TLR) and NOD-like receptors is essential for homeostasis. Normal development of GALT structures is dependent upon functional pathogen recognition by these receptors. In addition to promoting proper GALT development, a symbiotic mix of commensal bacteria promotes mucus production and barrier function of the epithelium and inhibits colonization by competitive organisms (Kamada et al., 2013). The effects of the microbiome on GALT and mucosal functions are not only through direct interactions of microbial components with receptors but also through products of symbiotic microbes including short chain fatty acids (Brestoff and Artis, 2013).

Dysbiosis, an unhealthy shift in microbiome composition, can down-regulate these protective functions, stimulate mucosal inflammation, and potentiate colonization by pathogenic organisms. In humans, some disease states including inflammatory bowel disease are associated with a complete shift in intestinal population structure (Koboziev et al., 2014), and the shift in microbiome accompanying chronic inflammation diseases can drive intestinal T cells towards pro-inflammatory Th17 phenotypes (Littman and Pamer, 2011). Work in rodent models is beginning to demonstrate that chronic inflammatory diseases including metabolic syndrome, diabetes, and atherosclerosis are associated with shifts in intestinal microbiota (Caesar et al., 2012; Vieira et al., 2013). Although cause and effect are unclear, in certain instances transfer of healthy microflora to a sick individual can restore health and transfer of microbiota from a sick donor to a healthy recipient can cause disease. For example, mice lacking TLR5, a receptor that recognizes bacterial flagellin, developed metabolic syndrome and altered intestinal microbiome compared to wild-type mice (Vijay-Kumar et al., 2010). When their gut contents were transferred to healthy germ-free wild-type mice, the recipient mice developed characteristics of metabolic syndrome as well, indicating that the microbiome is important in both maintaining health and contributing to disease states. Such findings could be of relevance in the cow also, particularly in the transition period when cows experience some symptoms of metabolic syndrome including decreased insulin sensitivity, increased glucose tolerance, and increased mobilization of fatty acids.

DIET INDUCED INFLAMMATION IN DAIRY CATTLE – THE SARA EXAMPLE

The interaction between changes in gut microbiome and dairy cattle health is most well documented as it relates to rumen acidosis and sub-acute rumen acidosis (SARA). A switch to a

high grain diet or induction of SARA induces dramatic changes in the rumen fluid microbiome and in population structure of bacteria adhered to the rumen epithelium (Khafipour et al., 2009b; Chen et al., 2011). Sub-acute rumen acidosis-inducing diets also increase flow of fermentable carbohydrates to the intestines. This results in shifts in the intestinal microbiome as demonstrated by changes in fecal bacterial composition (Mao et al., 2012). These shifts in gastrointestinal bacterial communities in response to SARA are believed to be a key first step in the negative impacts of SARA on animal health and performance. In addition to the microbiome shift, acidosis also increases concentration of toxic and inflammatory compounds in the digesta (Ametaj et al., 2010; Li et al., 2012; Saleem et al., 2012) concurrent with a decrease in barrier function of rumen epithelium (Steele et al., 2011). Because the intestinal epithelium is composed of only a single layer of epithelial cells, systemic entry of bacteria or toxins in response to SARA may be more likely to occur in the intestinal mucosa than in the rumen. In fact, Khafipour et al. (2009a) found the timing of the presence of lipopolysaccharide in the blood following a SARA challenge suggested entry through the intestines instead of the rumen.

Shifts in the microbiome, accumulation of toxins in the digesta, and mucosal damage in response to SARA likely shift nearby GALT structures from an anti-inflammatory to a pro-inflammatory state, which, based on rodent models, may result in further damage to the mucosa and systemic inflammation. This may be a contributing factor to the increase in circulating acute phase proteins observed in response to grain-based SARA challenges (Plaizier et al., 2008). Systemic inflammation resulting from SARA likely contributes to the negative impacts of SARA on animal health.

MITIGATING GUT INFLAMMATION

Because SARA is one of the most recognized feeding problems that contribute to systemic inflammation, feeding strategies to minimize the risk of SARA should help to maintain gut health. Maintaining adequate NDF, particle size, and effective fiber and avoiding excessive starch or highly fermentable NFC are essential. Dietary buffers should be included in rations, and drier diets should be evaluated for their potential to contribute to sorting, which may induce SARA in some cows.

The permeability barrier function of the epithelium responds to changes in the animal or the digestive tract. For example, permeability is increased during oxidative stress or heat stress (Mani et al., 2012). Lactating and transition dairy cows are under considerable oxidative stress. Intracellular and extracellular antioxidants function to neutralize free radicals and prevent damage to cells and their membranes. Therefore, feeding to maintain adequate antioxidant status can help to ensure gut health. Nutrients with antioxidant activities include copper, manganese, and zinc which all serve as cofactors for superoxide dismutase, an enzyme responsible for converting superoxide into oxygen and hydrogen peroxide. Selenium also performs antioxidant functions primarily by acting as a cofactor for thioredoxin reductase enzymes that convert hydrogen peroxide to water. Dairy cattle selenium requirements are 0.3 mg/kg which is also the maximum supplementation level allowed (NRC, 2001). Though dietary selenium cannot be increased beyond this maximum, feeding selenium yeast instead of inorganic selenium provides a means to increase selenium absorption (Weiss and Hogan, 2005). Vitamins E and C also benefit gut and overall health through their antioxidant activities.

In addition to minerals and vitamins, other supplements may stimulate gut health. The effect of supplemental dietary fatty acids, in particular omega-3 fatty acids, on dairy cattle health is an area of active research. There is also some work evaluating the feeding of chicken egg IgY products from chickens vaccinated against enteric pathogens. Other supplements that can alter the gastrointestinal microbiome and potentially impact animal health include ionophores and essential oils. An area that has received significant attention is the potential use of probiotics and prebiotics, and the remainder of this paper will focus research related to feeding yeast.

Mitigating gut inflammation – yeast example

Feeding of prebiotics and probiotics to monogastric animals can shift the intestinal microbiome, alter activities of GALT, and improve animal health. Although determining those effects in ruminants is more complicated due to pregastric fermentation, it is likely that health benefits to feeding some prebiotics and probiotics can occur for dairy cattle as well.

Live yeast and yeast products have received a fair amount of study and have potential to be used as health promoting supplements in dairy cattle. Most studies have evaluated the effect of *Saccharomyces cerevisiae*, but there has been some work with other organisms including *S. boulardii* and *Aspergillus oryzae*. Studies indicate that feeding yeast supplements alters the rumen microbiome and can increase both fiber digestion and rumen pH (Chaucheyras-Durand and Durand, 2010; Pinloche et al., 2013). Yeast seem to function primarily as a prebiotic because similar changes in digestion occur whether yeast are alive or dead, although there may be a slight benefit to the live probiotic form (Oeztuerk et al., 2005; Oeztuerk, 2009). Work in monogastrics indicates that in addition to altering the intestinal microbiome, yeast supplementation can benefit host health through interaction of yeast carbohydrate moieties (primarily mannan oligosaccharides and β-glucans) with the gut mucosa. It is believed that these moieties can bind to pathogen receptors and, in so doing, alter functionality of the mucosa to increase barrier function and pathogen resistance (Munyaka et al., 2012).

In piglets, feeding of live *S. boulardii* reduced translocation of orally gavaged *Escherichia coli* across the gut mucosa and into the mesenteric lymph nodes (Lessard et al., 2009). In non-challenged broilers, feeding a commercial yeast derived product resulted in decreased mRNA levels of inflammatory cytokines in the ileum and cecum (Munyaka et al., 2012). In another broiler study, feeding of live *S. boulardii* increased goblet cell density in the jejunum, tight junction mRNA levels in the jejunum and ileum, and secretory IgA concentration in the jejunum (Rajput et al., 2013). Feeding of live yeast or yeast products to dairy cattle may have a similar ability to enhance animal health by increasing mucosal barrier function and altering GALT activity. Inclusion of *S. cerevisiae* into grain starter was shown to improve health of young calves (Magalhaes et al., 2008). In that experiment, scours was highly prevalent, and mortality was 12.1% in control calves and 7.5% in calves given the live yeast supplement. In a study using Holstein steers, we found that abomasal infusion of *S. boulardii* reduced fecal volatile fatty acid and pH changes following an abomasal oligofructose challenge, suggesting that *S. boulardii* improved the intestinal environment (Gressley et al., 2016). Finally, a study using transition cows fed cows 0, 30, 60, or 90 g/d of a combination live yeast and enzymatically hydrolyzed yeast product, and they found that increasing dose resulted in improved humoral immune

response to a model vaccination (Yuan et al., 2015). Interestingly, the moderate levels of yeast (30 and 60 g/d) also increased fecal IgA concentration, suggesting a direct impact of the supplement on gut health.

CONCLUSIONS

The gut is specialized to both absorb nutrients and to protect the animal from the microbes and toxins that exist within the digesta. Typically, gut immune cells are hyporesponsive, allowing for tolerance of commensal microorganisms and maintenance of the gut barrier function. Sub-acute ruminal acidosis is an example of a feeding situation that can disrupt this balance, leading to a breakdown of gut barrier function and localized and systemic inflammation. On the other hand, there exists potential for feeding strategies to improve gut health and barrier function. For example, probiotic and prebiotic supplements can stimulate shifts in the ruminal or intestinal microbiome that increase mucosal barrier integrity and boost systemic immunity.

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Potential for Feeding Program to Impact Gut Health and Inflammation in Dairy Cattle

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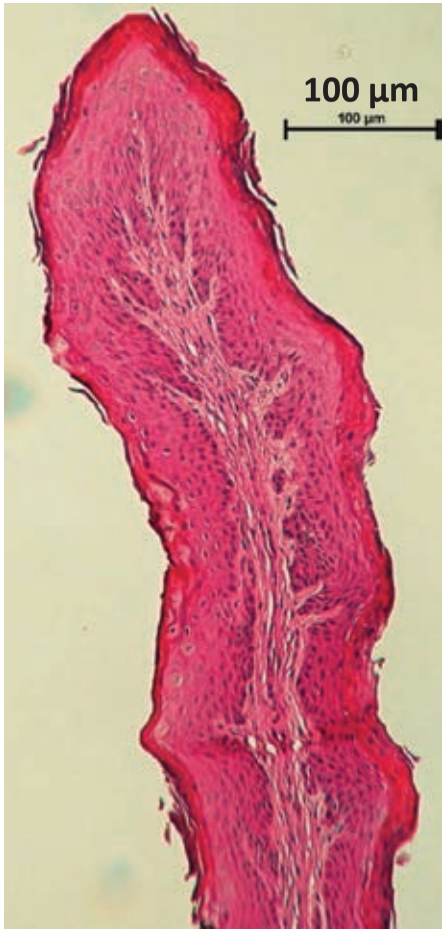
Department of Animal and Food Sciences

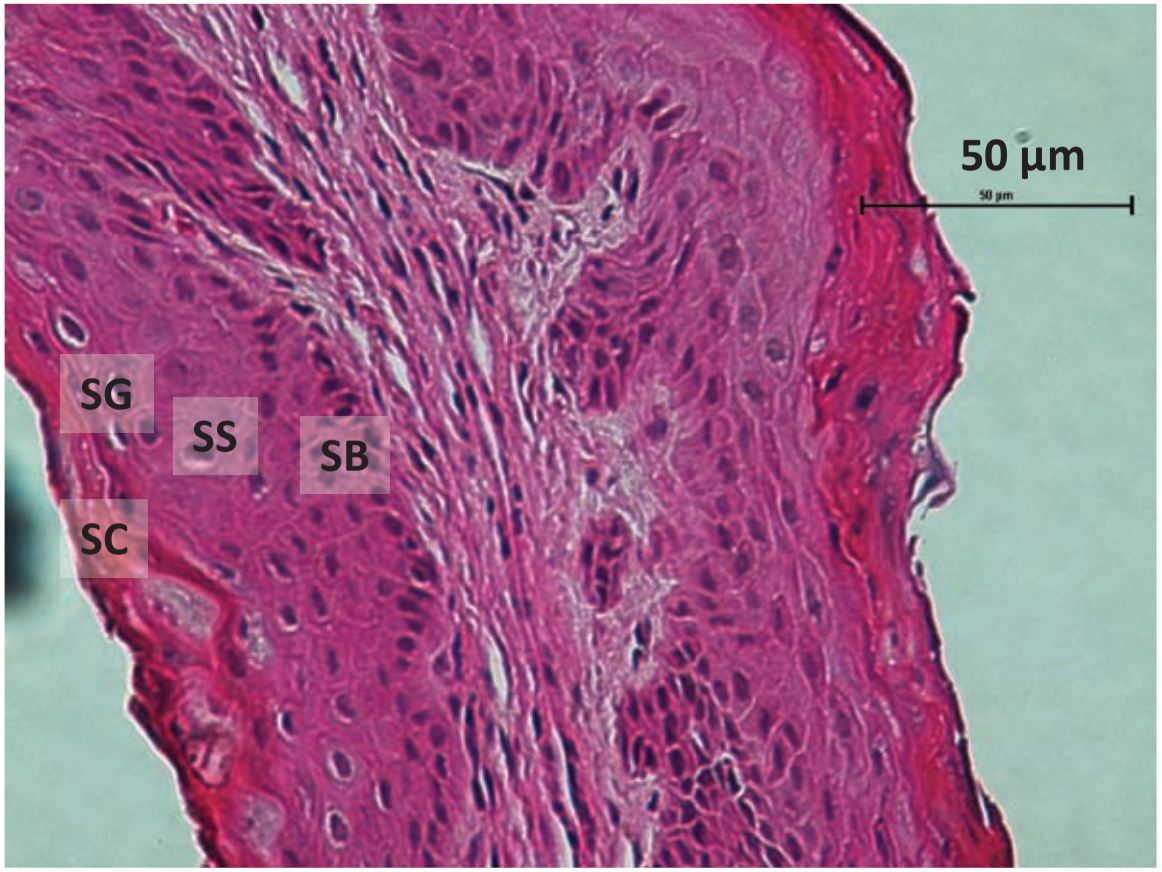
Outline

- Overview of gut morphology and gut health
- Gut health and inflammation
  - SARA as an example
- Dietary manipulation of gut health
  - Yeast as an example

Rumen Structure

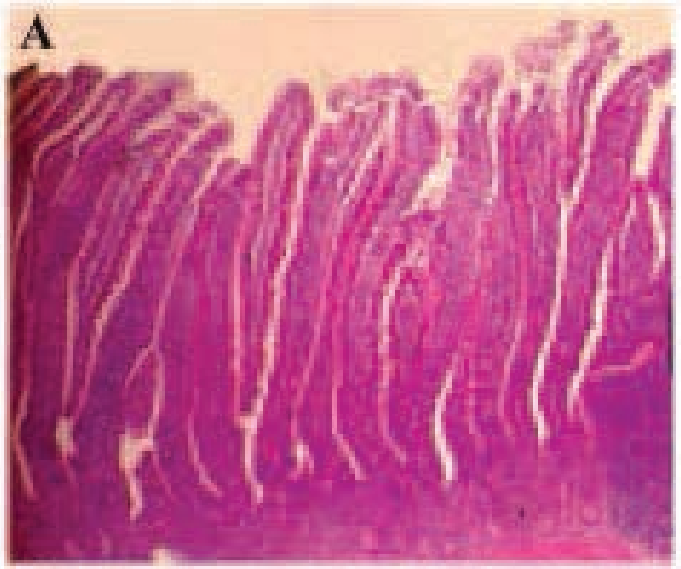


Healthy rumen papillus



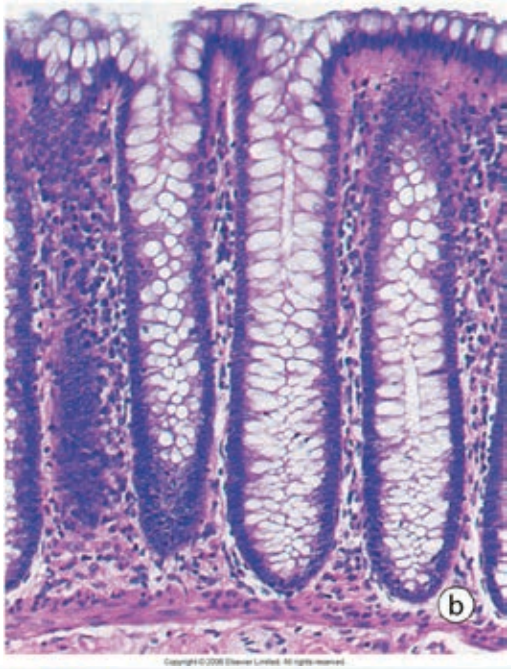
Intestinal Structure

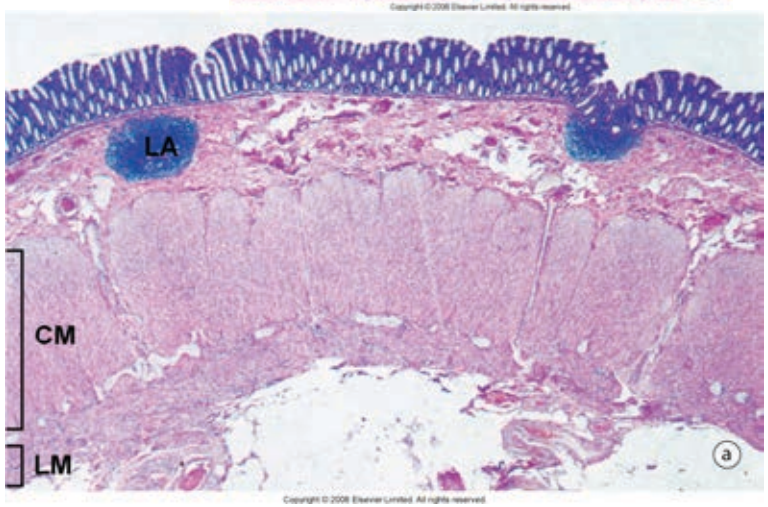
Jejunum



Ileum

Large Intestine



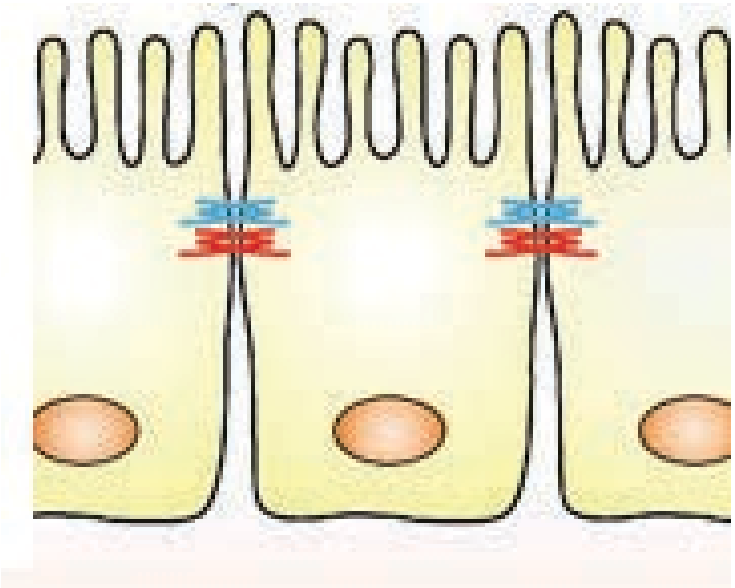


Intestinal Protection

- Animals are protected from toxins and microbes in their intestines via:
  - Tight junctions between cells
  - Cells specialized to protect animal from pathogenic microbes and their toxins
    - Goblet cells
    - M cells
    - Paneth cells

Tight Junctions

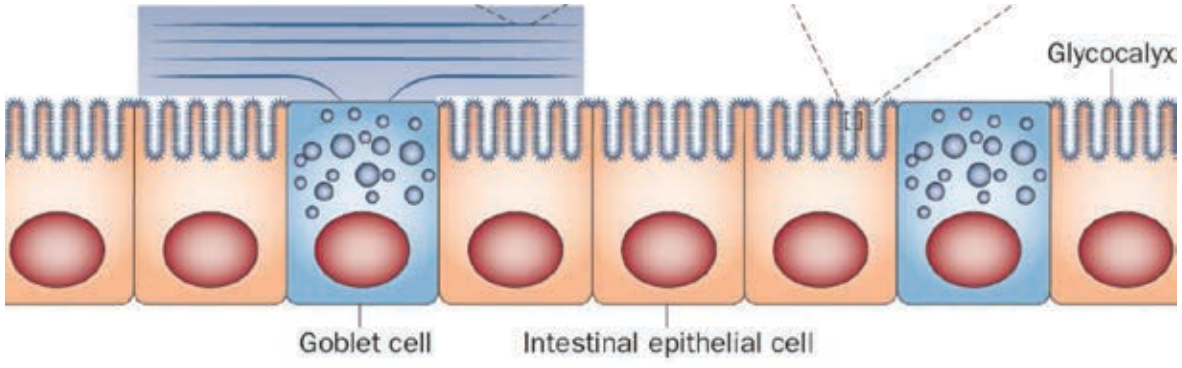
- Tight junction proteins link intestinal mucosa cells
  - Block entry of pathogens and dietary antigens



Suzuki 2013

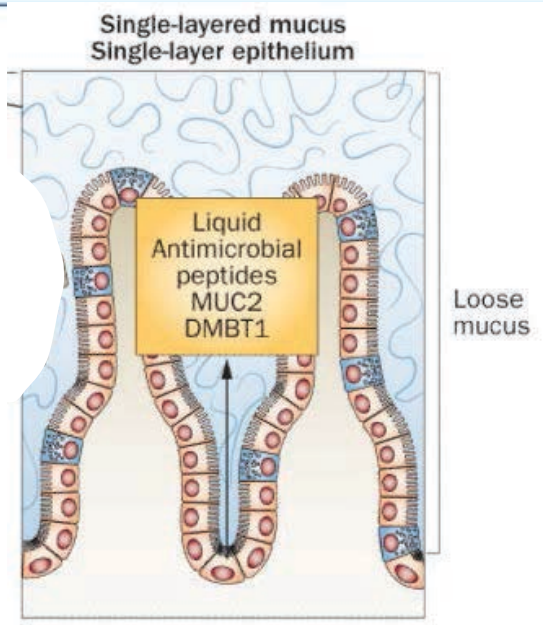
Goblet Cells

- Goblet cells secrete mucins found in loose and adhered mucus that protects the intestinal epithelium

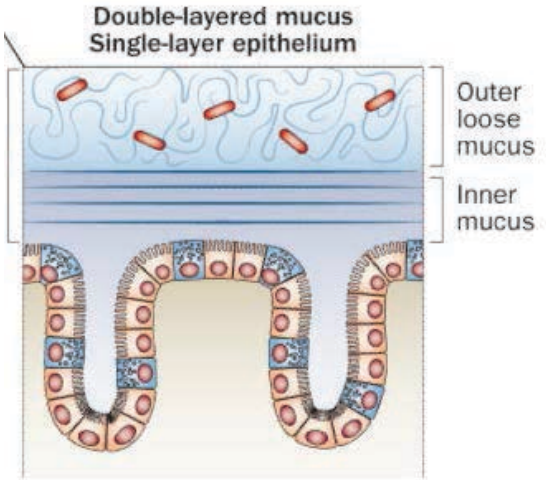


Johansson et al. 2013

Small Intestine

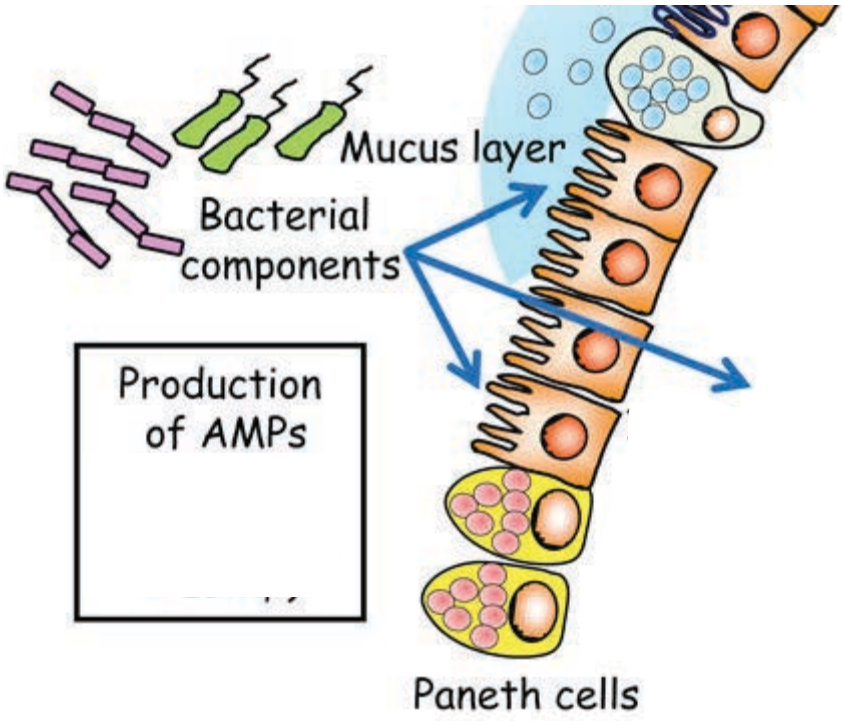


Large Intestine



Paneth Cells

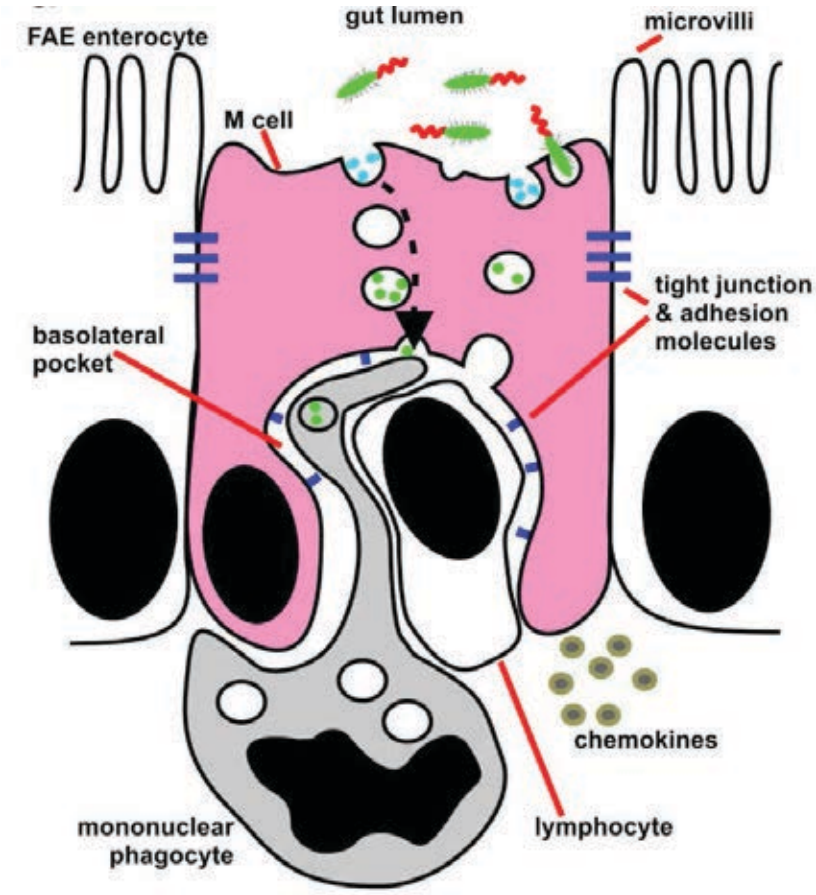
- Paneth cells secrete:
  - Antimicrobial peptides (AMPs)
  - Immunoregulatory molecules
- AMPs (stored in granules)
  - Defensins
  - Lysozymes
  - Cathelicidins



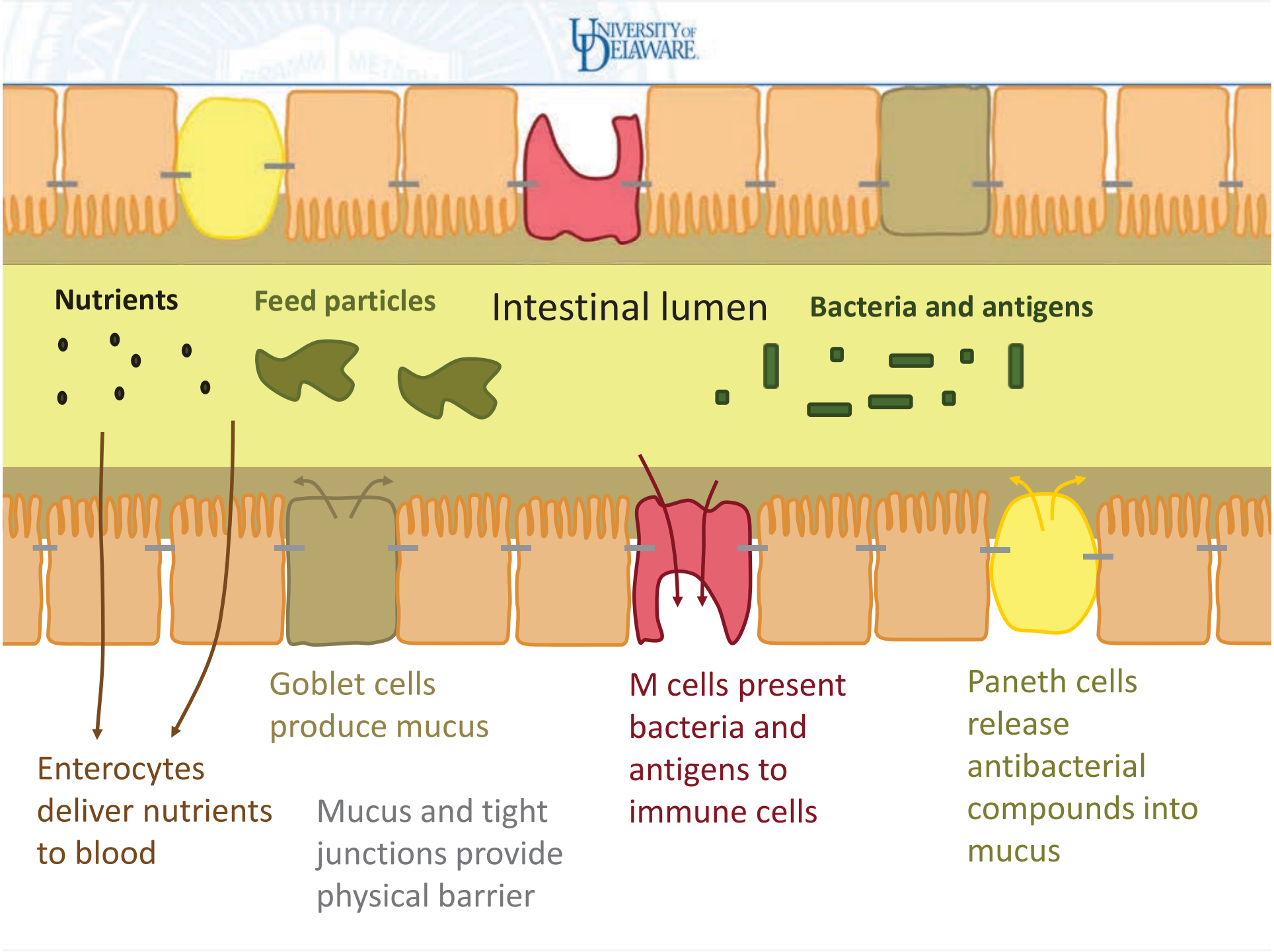
Kurashima et al. 2013

Microfold (M) Cells

- M cells transport macromolecules, antigens, and microorganisms to gut-associated lymphoid tissue (GALT)



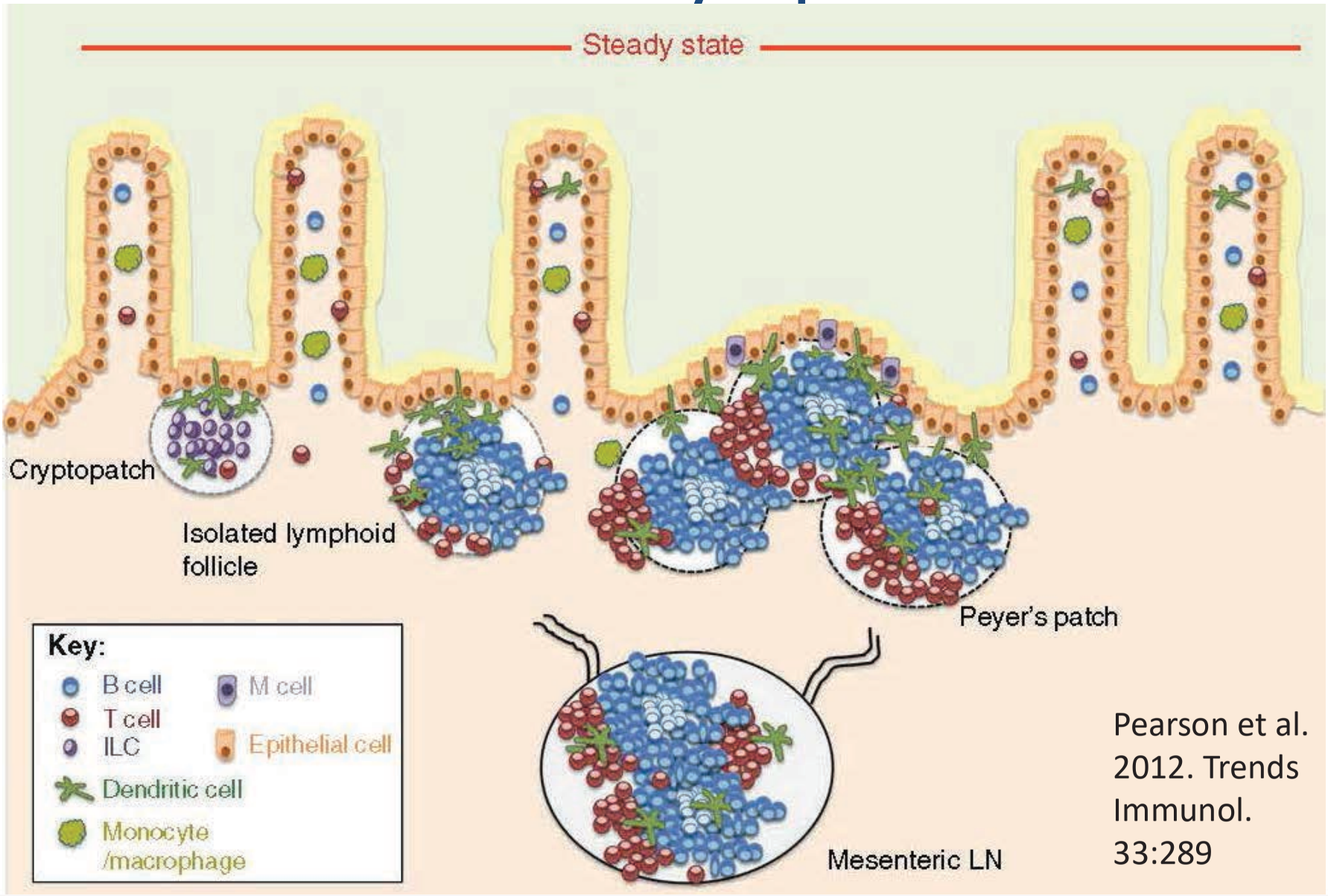
Mabbott et al. 2013


Nutrients
Feed particles
Intestinal lumen
Bacteria and antigens
Enterocytes deliver nutrients to blood
Goblet cells produce mucus
Mucus and tight junctions provide physical barrier
M cells present bacteria and antigens to immune cells
Paneth cells release antibacterial compounds into mucus

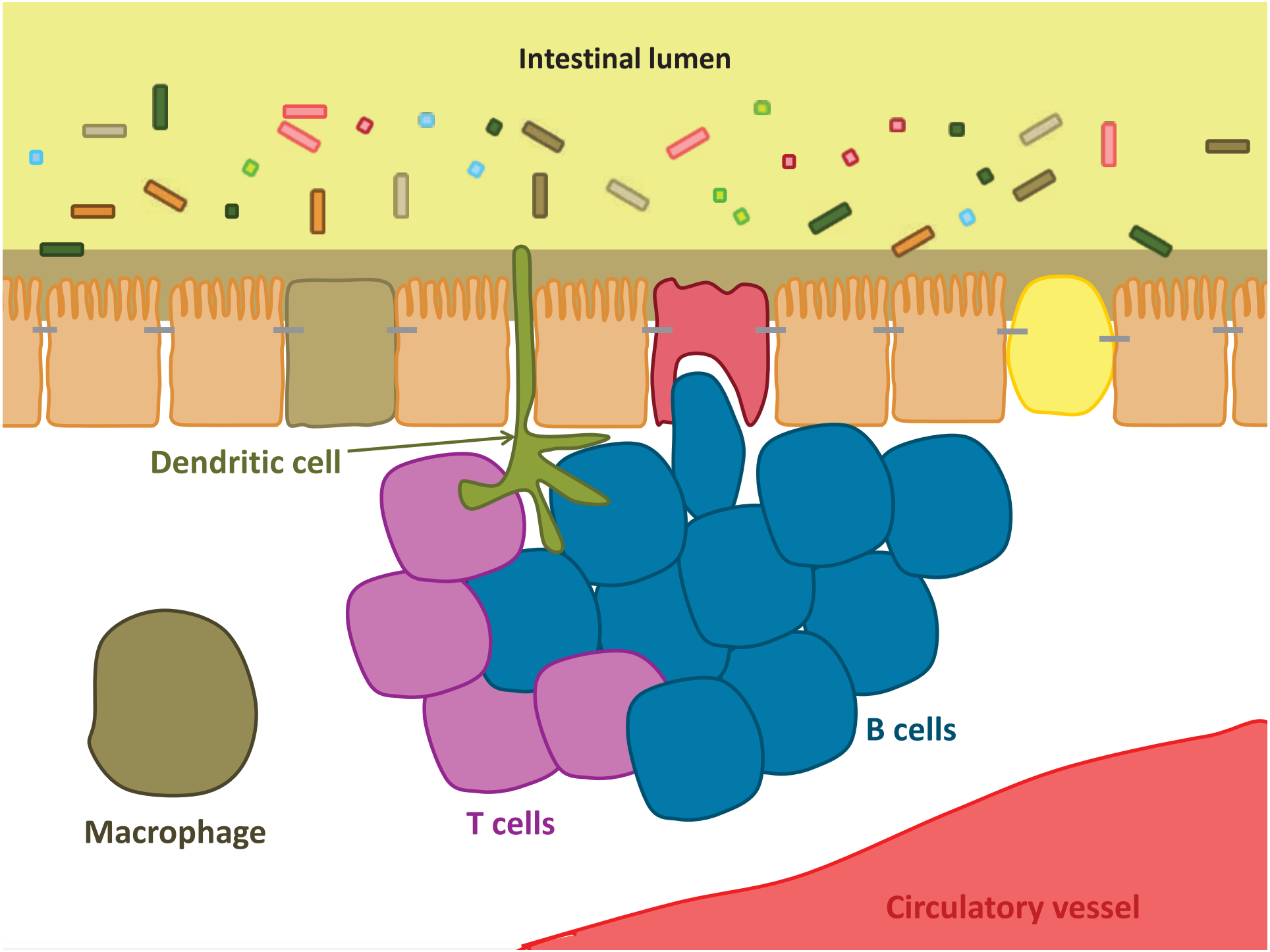
Sub-Epithelial Immune System

- Gut-associated lymphoid tissue (GALT)
  - Microstructures of white blood cells that lie just beneath the epithelium in the lamina propria
  - Respond to gut contents by maintaining homeostasis (e.g. presence of commensal bacteria) or activating immune response (e.g. presence of pathogenic organisms)
  - Regulate cell cycles of epithelial cells and “tightness” of tight junctions

Gut-Associated Lymphoid Tissue

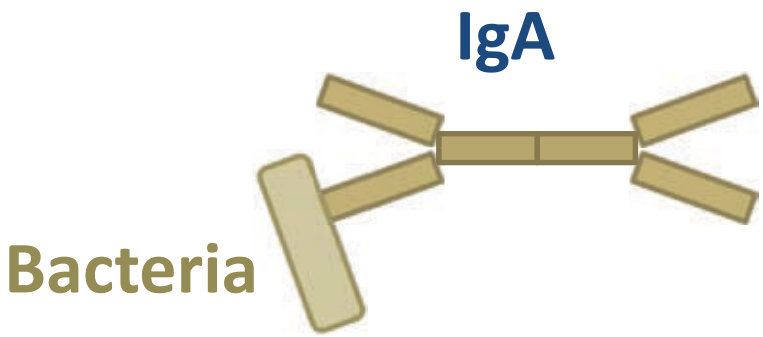


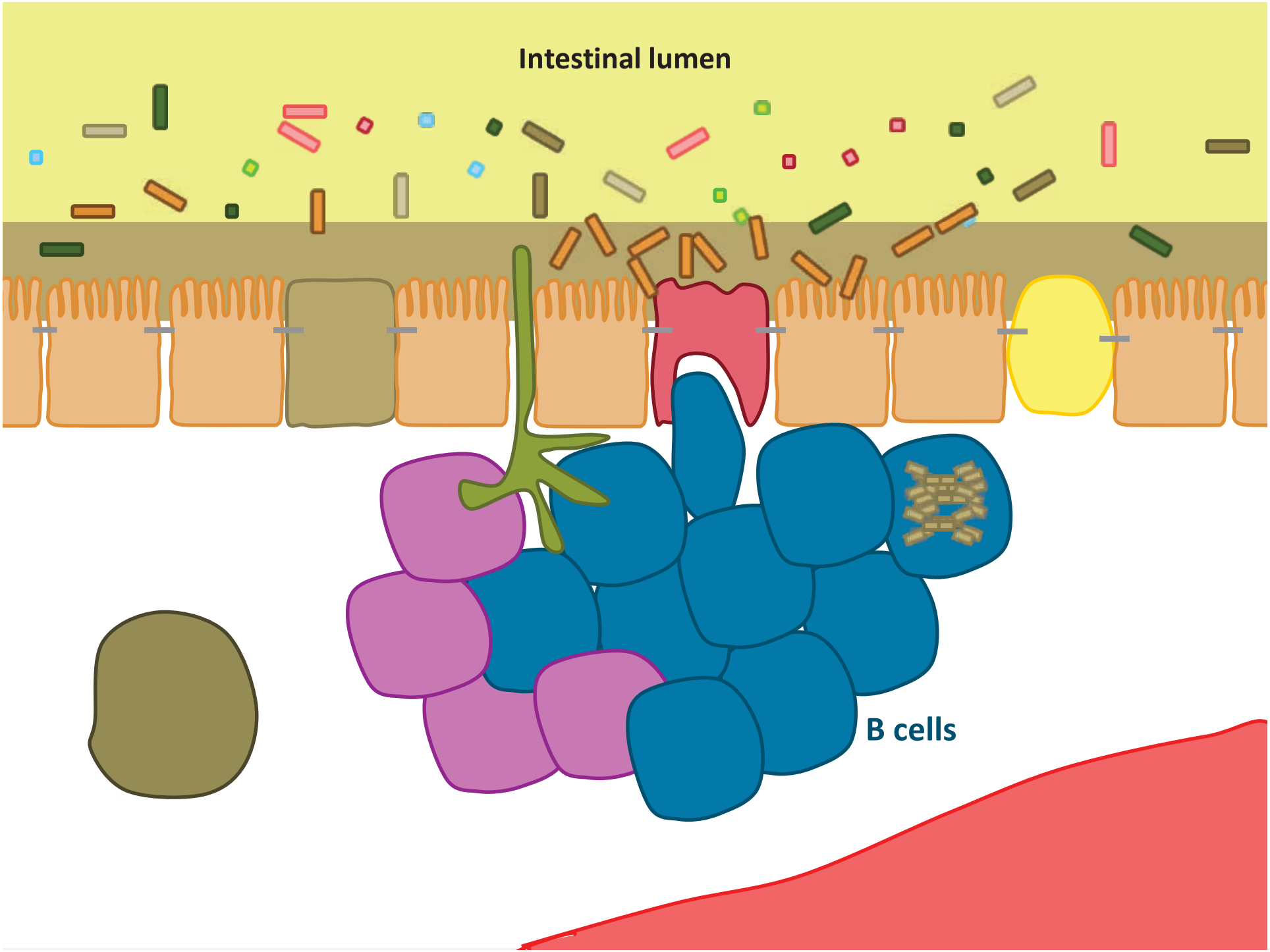
Pearson et al. 2012. Trends Immunol. 33:289


Intestinal lumen
Dendritic cell
Macrophage
T cells
B cells
Circulatory vessel

Immunoglobulin A

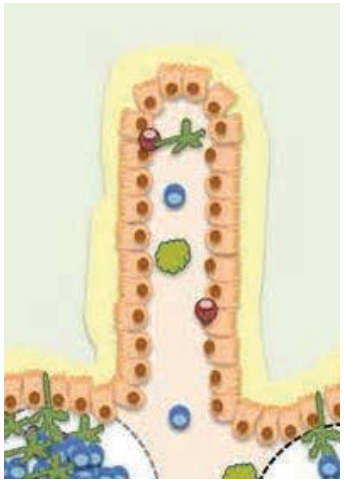
- Immunoglobulin A (IgA) is produced by activated B cells (plasma cells)
  - Can bind and deactivate bacteria and antigens
  - B cells produce many different IgA that are specific to a single organism or antigen

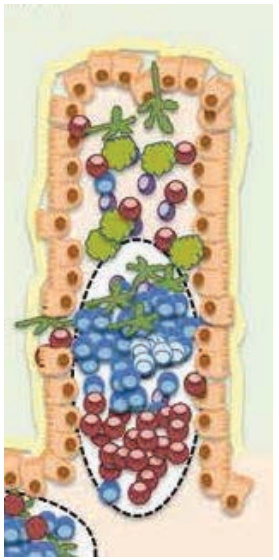



Intestinal lumen
B cells

Response to Dysbiosis

- Commensal bacteria in gut suppress inflammation
- Dysbiosis or pathogenic organism challenge results in:
  - Inflammation
  - Activation and recruitment of immune cells
  - Damage to epithelium
  - Passage of organisms and pathogens to sub-mucosal space





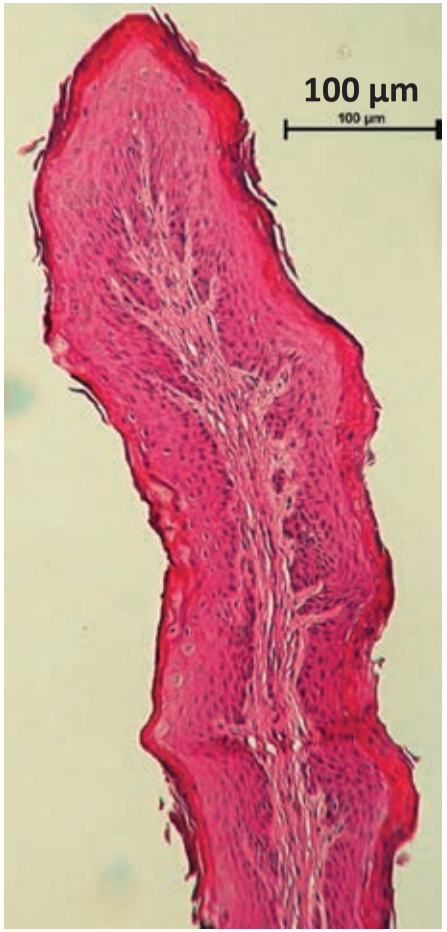
Pearson et al. 2012. Trends Immunol. 33:289

Findings from Monogastric Studies

- Crosstalk between microbes, intestinal cells, and GALT cells is essential for healthy epithelium
- A “healthy” mix of commensal microbes promotes mucin production and suppresses inflammation
- An “unhealthy” mix of gut microbes reduces mucin production and tight junction integrity, promotes systemic and local inflammation, and can induce sickness in healthy animals
- Chronic inflammatory disease states are associated with dysbiosis

Sub-Acute Rumen Acidosis (SARA)

Sub-Acute Rumen Acidosis



Healthy rumen papillus

↑ Fermentable CHO

↓

↑ Bacteria Growth Rate

↓

↑ VFA

↓

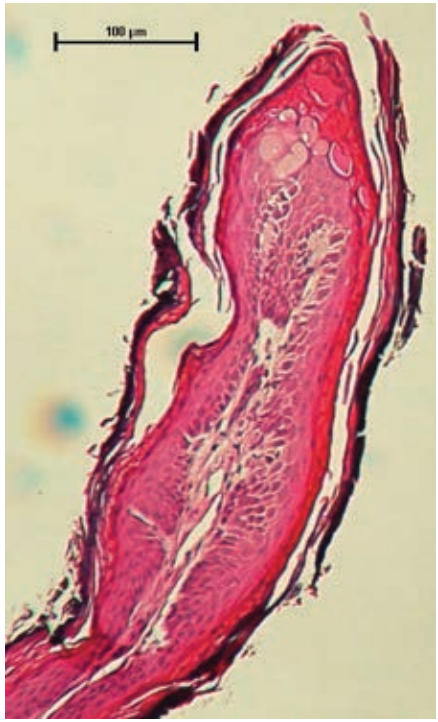
↓ pH (SARA)

↓

Shifts in Bacteria Populations
↑ Bacteria Toxins
Damage to GI Epithelium

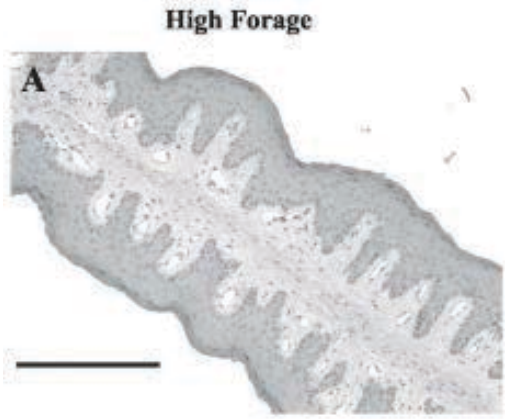
↓

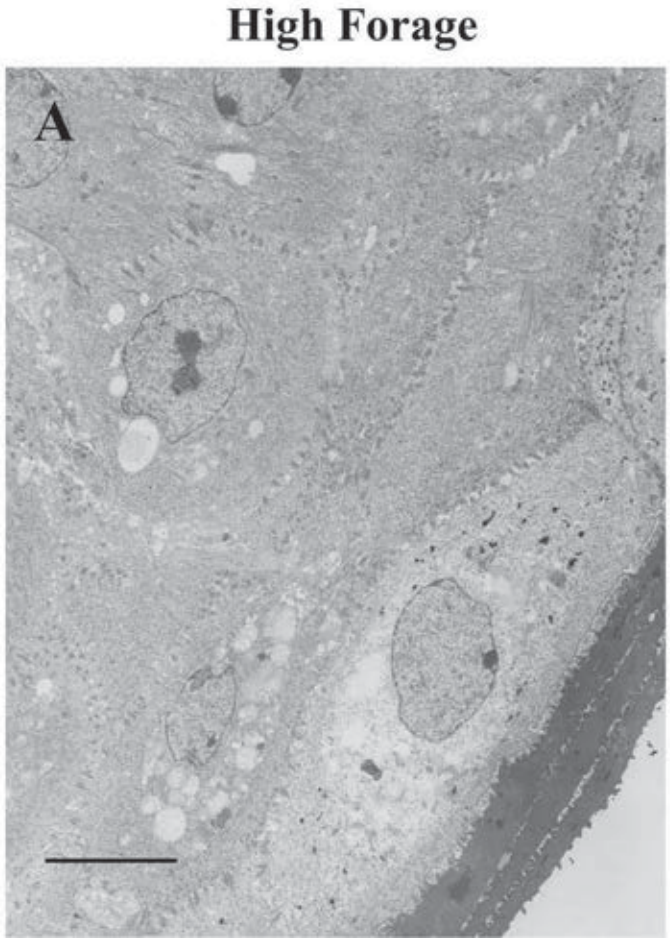
Systemic Immune Response
to SARA (Inflammation)

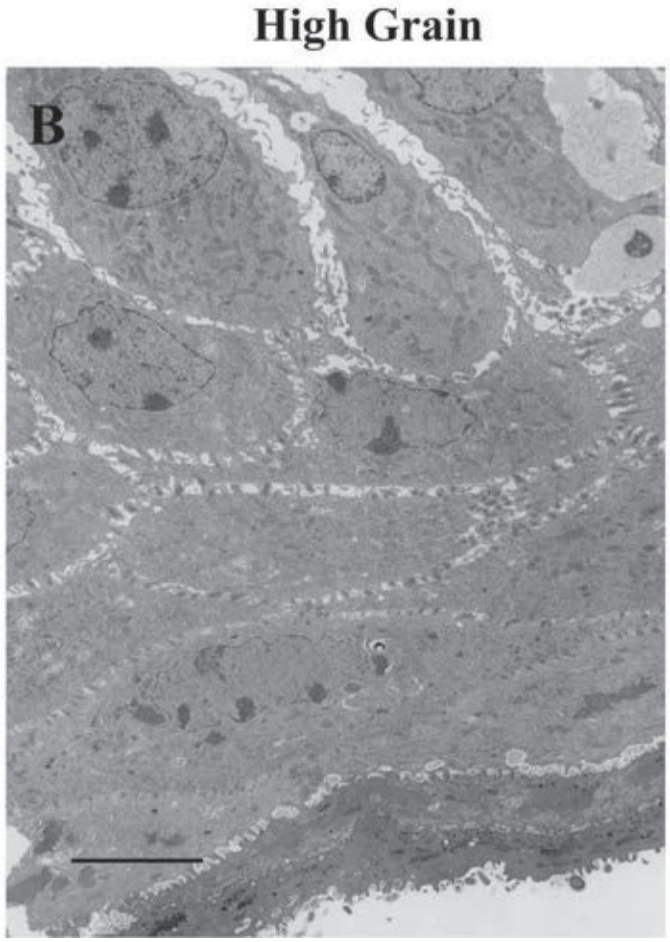


Papillus following SARA challenge

Rumen Epithelium Response to SARA

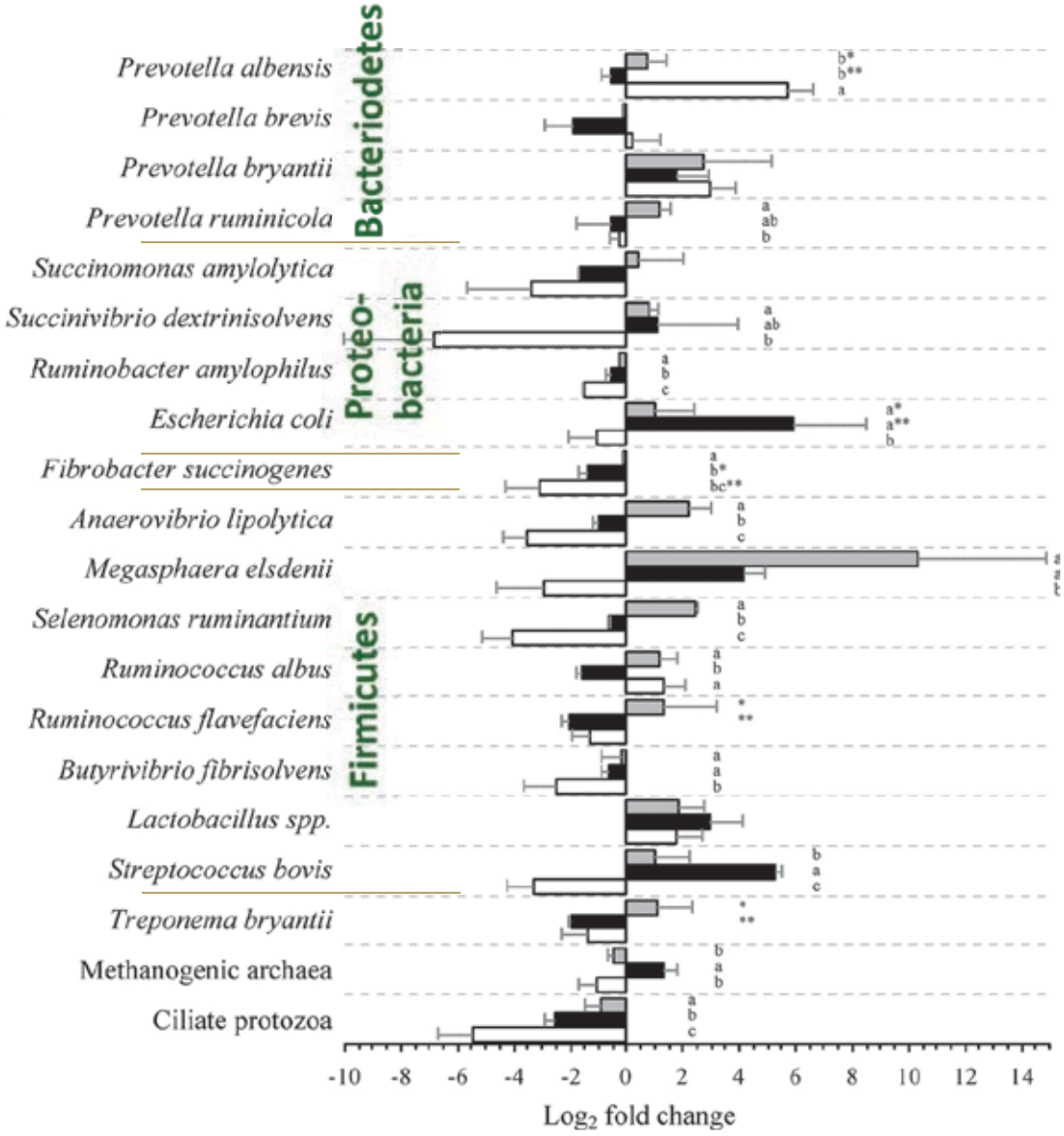






Univ. of Guelph, Univ. of Manitoba, UNC
Steele et al. 2011. Am. J. Physiol.- Reg. I. 300:R1515

Rumen Fluid Bacteria Shifts during SARA



Alfalfa pellet-induced SARA

↑ ***Prevotella*** **(Bacteriodetes)**

Severity of SARA and degree of inflammation highly correlated with E. coli abundance

Severe grain-induced SARA

↑ ***E. coli*** **(Proteobacteria)**

Mild grain-induced SARA

↑ ***M. elsdenii*** **(Firmicutes)**

Severe grain-induced SARA

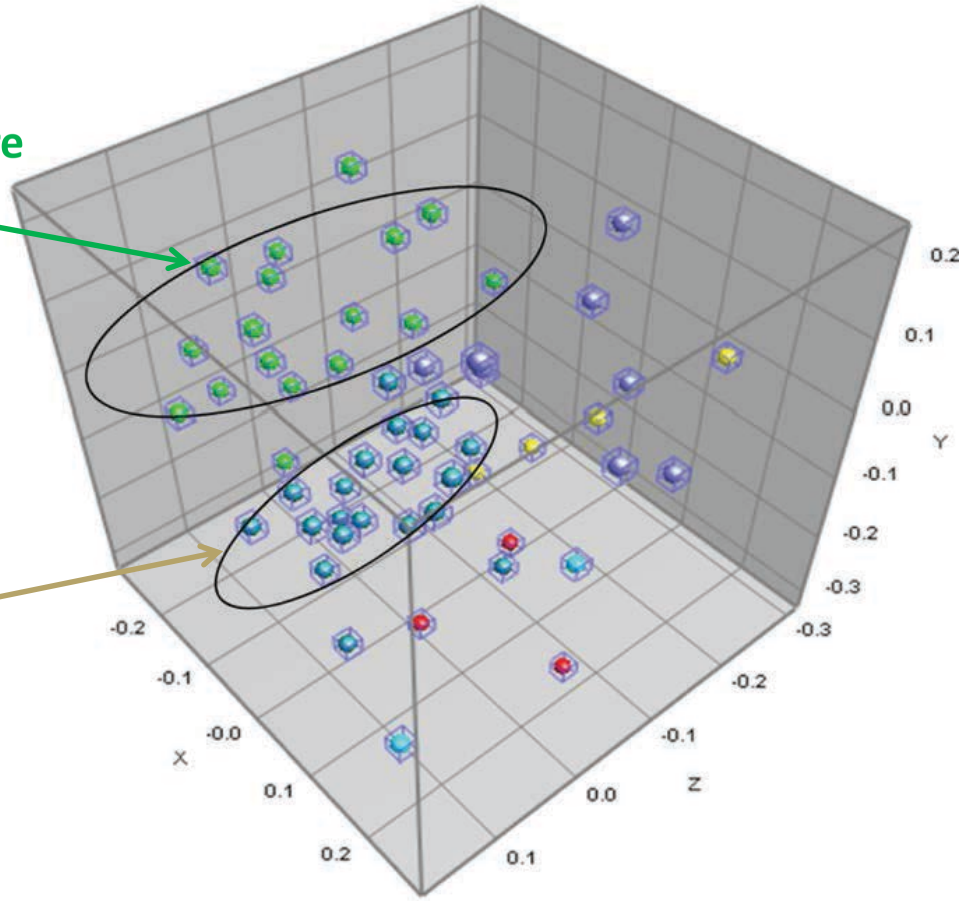
↑ ***S. bovis*** **(Firmicutes)**

University of Manitoba
Khafipour et al. 2009. Appl. Environ. Microbiol. 75:7115

Papillae Adherent Bacteria Shifts during High Grain Feeding

These heifers were fed 72% grain or 89% grain diet

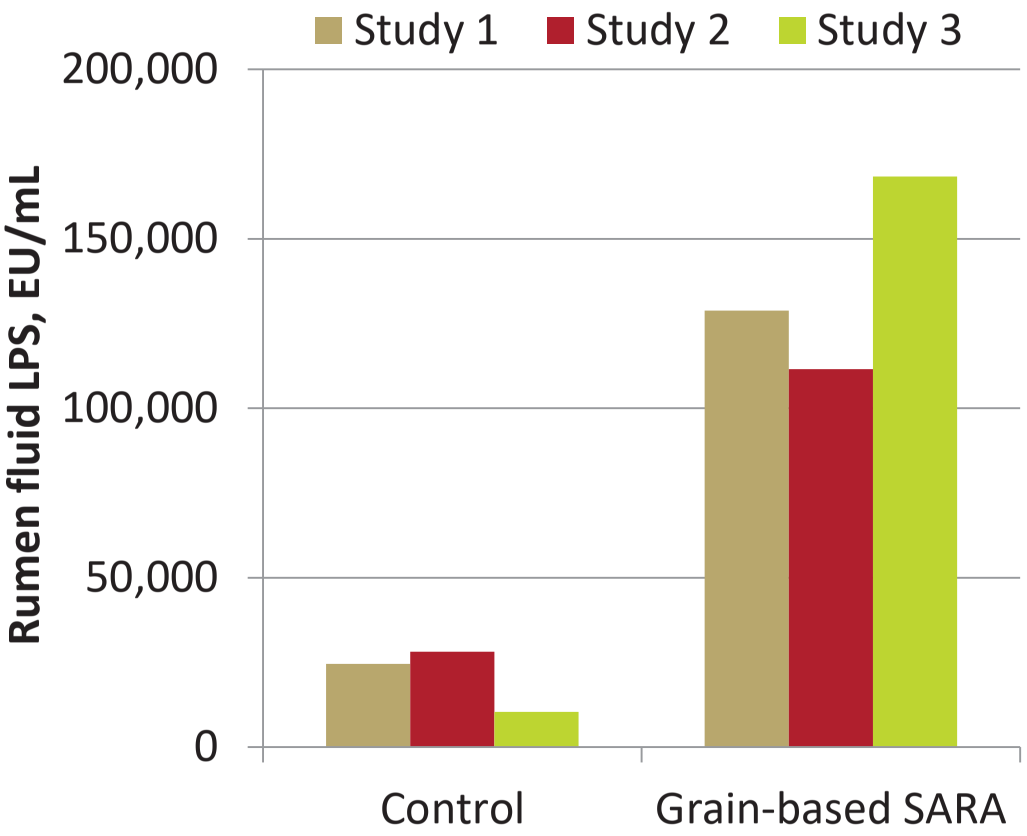
75% of these heifers were fed 97% hay diet



***Treponema* sp., *Ruminobacter* sp., and *Lachnospiraceae* sp. Were detected only when fed the high grain diets**

University of Alberta
Chen et al. 2011. Appl. Environ. Microbiol. 77:5770

Rumen Fluid Endotoxin (LPS)



Other biologically active compounds that increase during SARA:

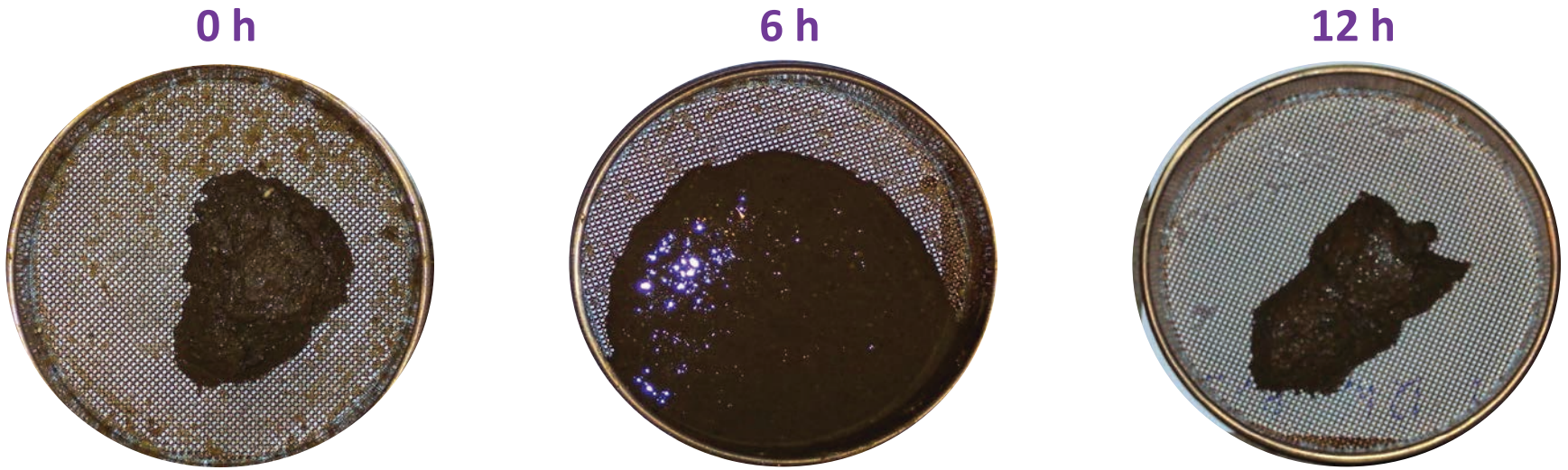
- **Methylamine**
- **Putrescine**
- **Ethanolamine**

Saleem et al. 2012.
JDS 95:6606

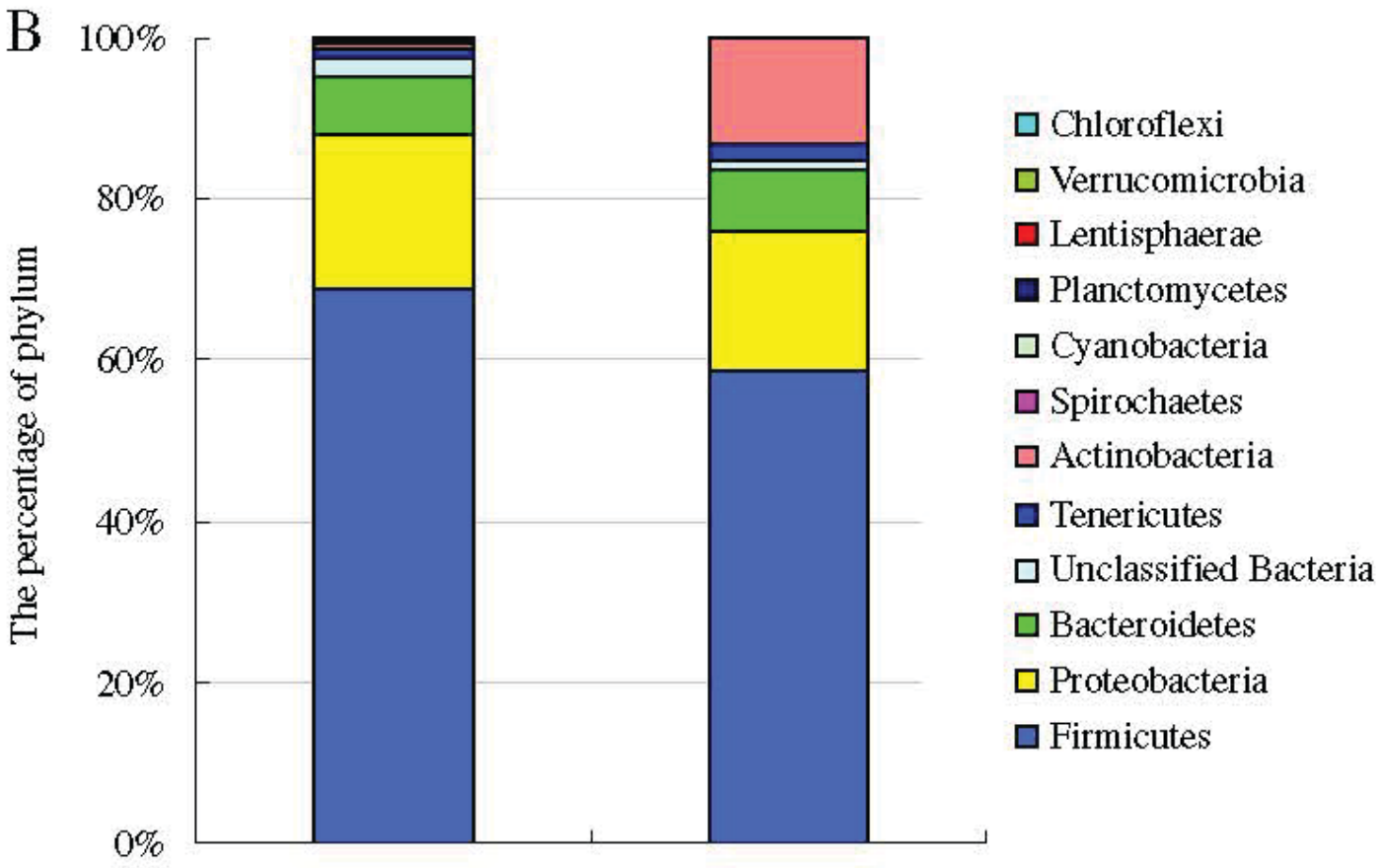
Study 1: Gozho et al. 2007. JDS 90:856; reverse log transformed
Study 2: Khafipour et al. 2009. JDS 92:1060; mean of 3 time points
Study 3: Li et al. 2012. JDS 95:294

SARA Impacts on the Intestines

- Excessive carbohydrate fermentation in the rumen mirrored by changes in the intestines
- Fecal indicators of SARA
  - Diarrhea, frothy feces, increased fecal particle size, mucin casts



Fecal Bacteria Shifts During SARA



Mao et al. 2012. BMC Vet. Res. 8:237

LPS in the Large Intestine

SARA feeding challenge

	Control	Grain-induced SARA	Alfalfa-induced SARA
Rumen LPS (EU/mL)	10,405[a]	118,522[c]	30,715[b]
Feces LPS (EU/g)	12,832[a]	93,154[b]	17,326[a]

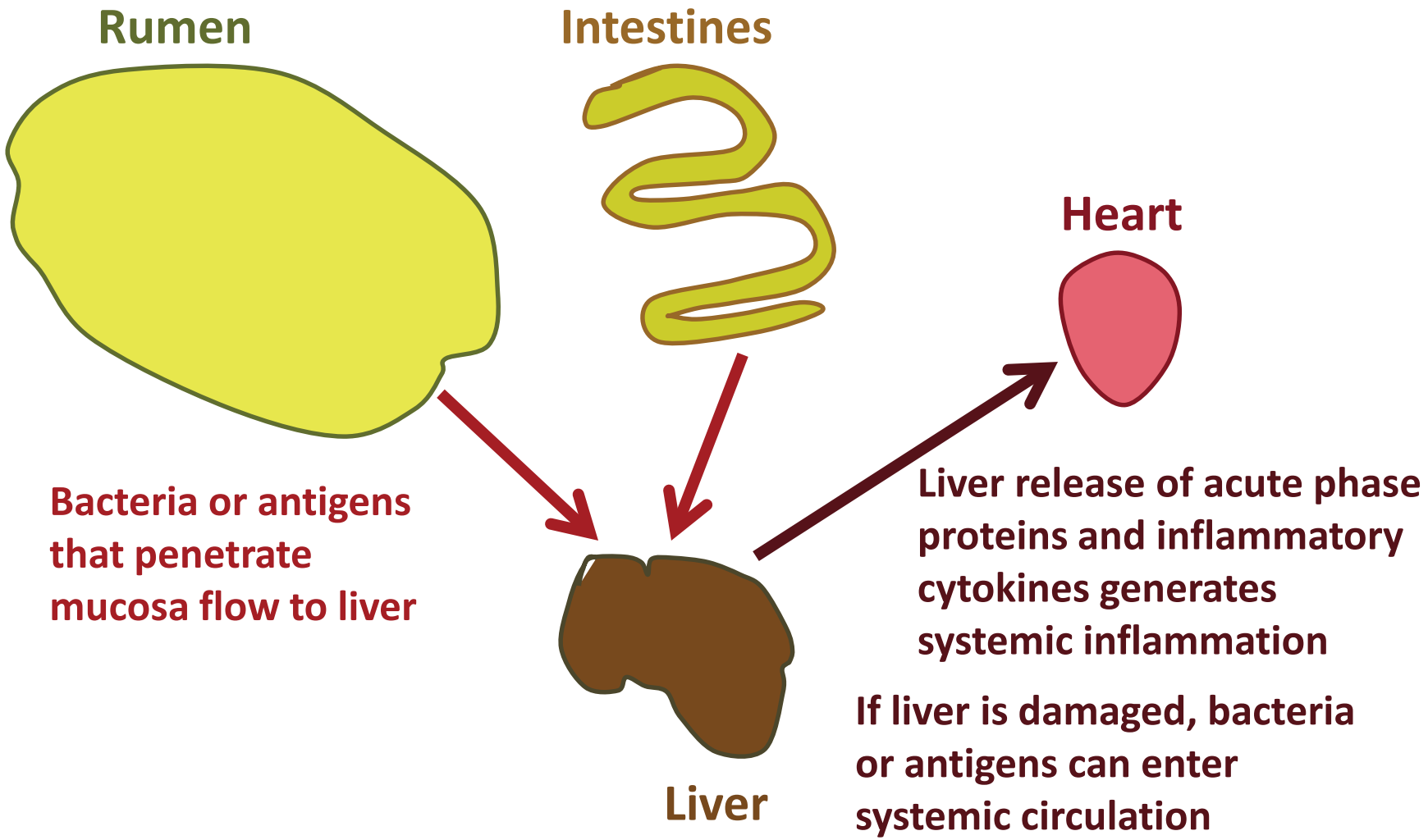
Li et al. 2012. JDS 95:294

Abomasal oligofructose (OL) or starch (ST) challenge

	Control	OL1	OL4	ST1	ST4
Feces LPS (EU/g), average over 42 h	2,449[a]	7,341[b]	21,345[c]	6,415[b]	5,324[b]

Gressley et al. 2016. JAS 94:284

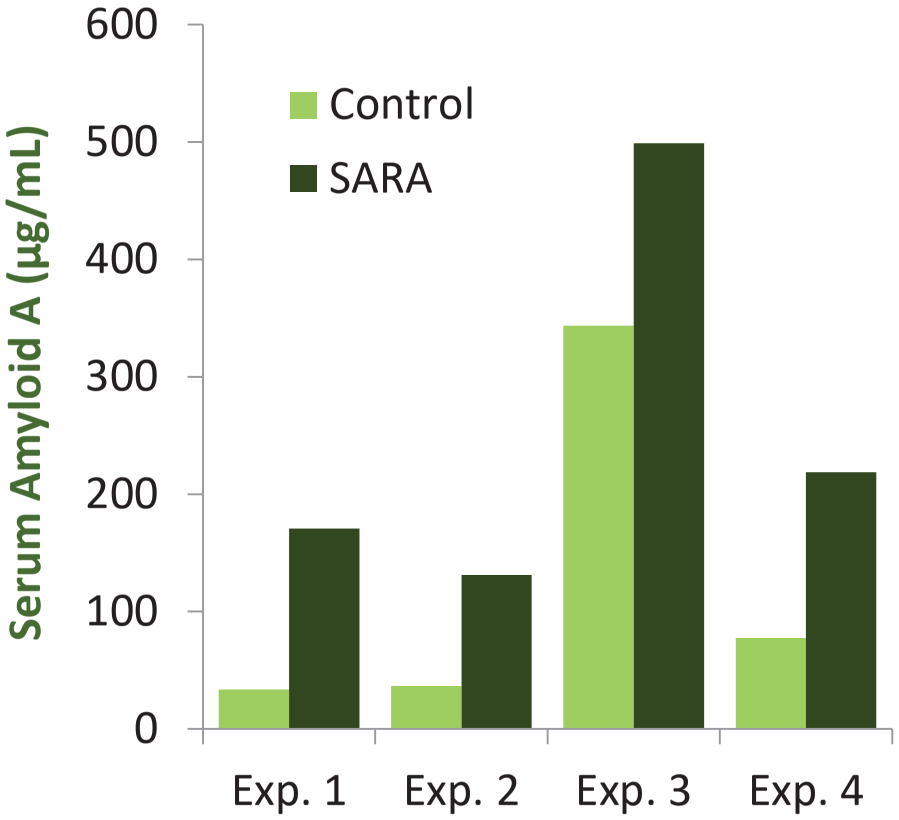
Inflammatory Response to SARA

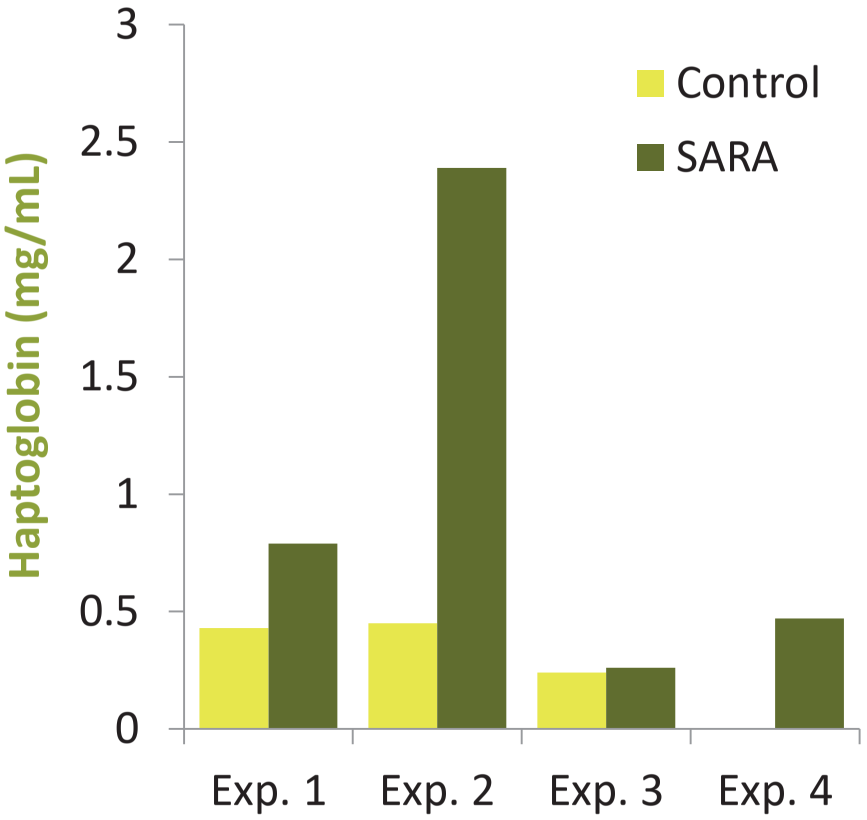


Liver Acute Phase Proteins

- Main ones are α-1 acid glycoprotein, haptoglobin (Hp), LPS-binding protein (LBP), and serum amyloid A (SAA)
  - Produced by liver and other tissues in response to an inflammatory trigger
- They moderate the inflammatory response, recovery, and tissue repair
- Produced in response to grain-induced SARA, indicating grain-induced SARA causes inflammation

SARA and Blood Acute Phase Proteins





Plaizier et al. 2008. Vet. J. 176:21

Summary thus far

- In healthy animals, gut structures and the gut microbiome interact to suppress inflammation and maintain animal health
- As illustrated by the SARA example, disruptions in gut homeostasis can result in local tissue damage and systemic inflammation → reduced performance

Mitigating Gut Inflammation

- Prevent SARA
  - Adequate NDF, particle size, and effective fiber
    - 19-21% forage NDF (Beauchemin and Penner)
  - Avoid excessive starch and highly fermentable NFC
    - 23-28% starch (de Ondarza, Grant, Linn)
    - 30-44% NFC, depending on availability (de Ondarza, Grant, Linn)
  - Adequate moisture
  - Include buffers

Mitigating Gut Inflammation

- Ensure adequate antioxidant status
  - 12-15 ppm Cu, 33-42 ppm Mn, 0.3 ppm Se, 40-50 ppm Zn (Weiss)
  - Vitamin E: 500 IU/d lactating, 1,000 IU/d dry, potentially more prefresh (Weiss)
- Avoid heat stress

Mitigating Gut Inflammation

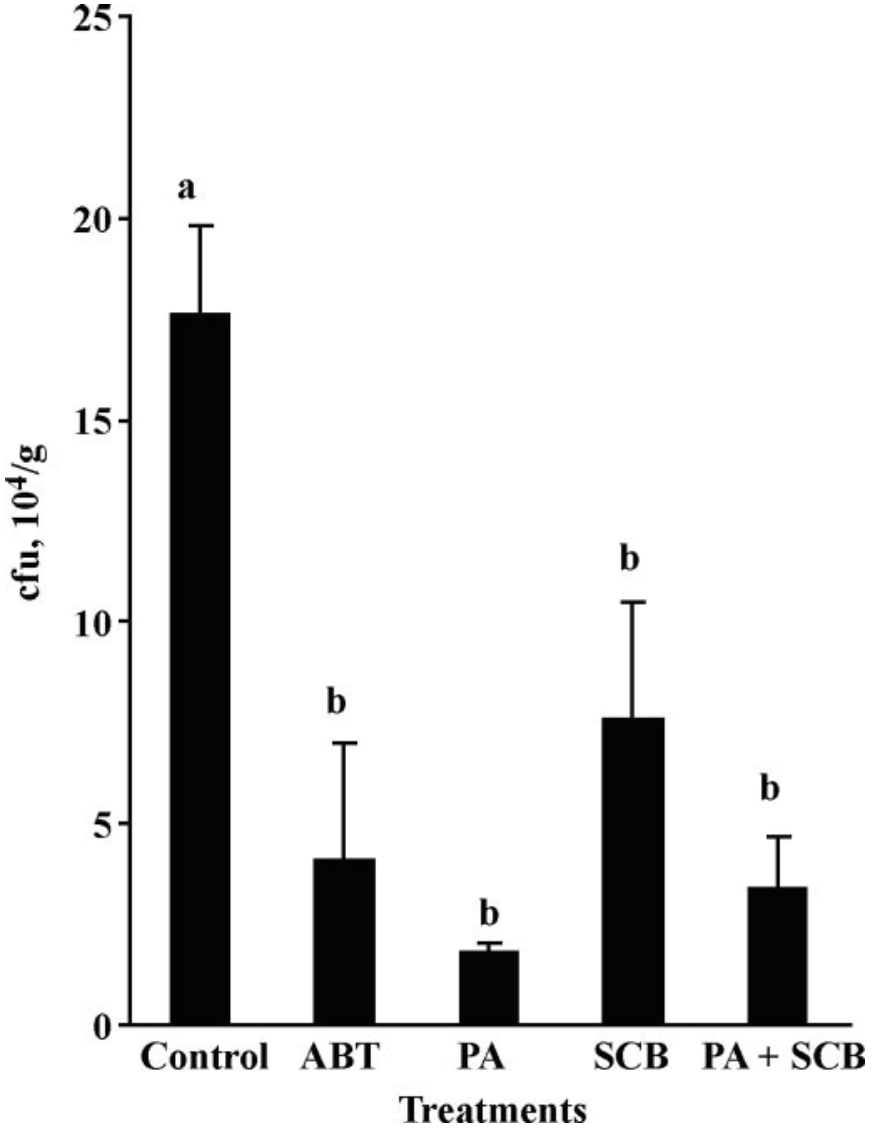
- Supplements with potential benefit
  - Omega 3 fatty acid supplements
  - Ionophores
  - IgY
  - Essential oils
  - Prebiotics or probiotics
    - Yeast

Yeast

- Prebiotic and probiotic activities
  - Increases fiber digestion and rumen pH
  - Increases digestive efficiency
  - May help prevent SARA
- Gut immunomodulating activity
  - Mannan oligosaccharides and β-glucans can bind pathogen receptors on mucosa
  - Response to yeast moiety binding
    - Increased barrier function
    - Increased mucus production
    - Increased pathogen resistance

Piglet Study, *S. boulardii*

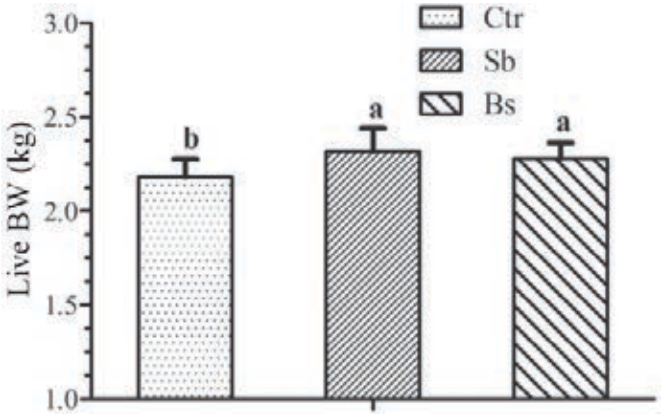
- Dietary supplements were antibiotics (ABT), *Pediococcus acidilactici* (PA) or *Saccharomyces cerevisiae boulardii* (SCB)
- Measured transfer of bacteria to mesenteric lymph nodes following an oral *E. coli* challenge

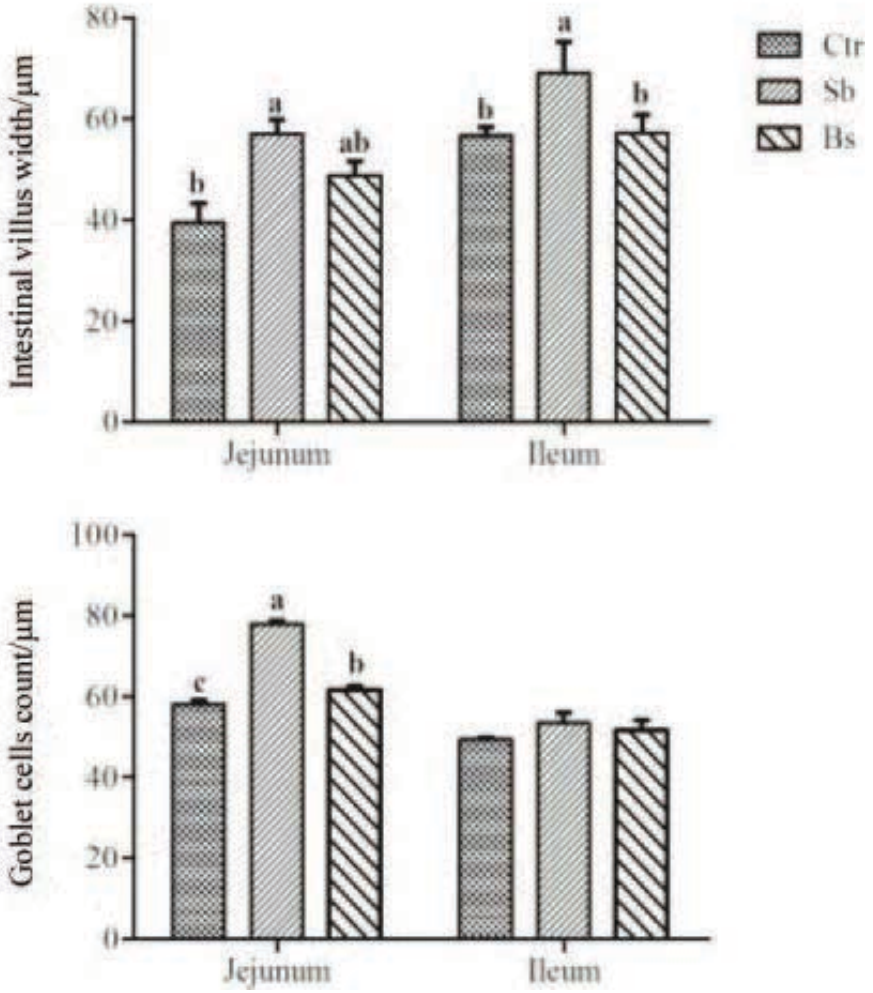


Lessard et al. 2009. JAS. 87:922-934

Broiler Study, *S. boulardii*

Control (Ctr)
S. boulardii (Sb)
Bacillus subtilis B10 (Bs)

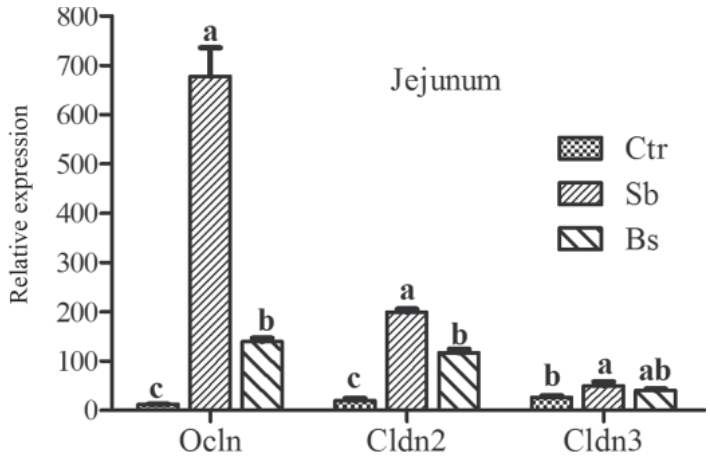


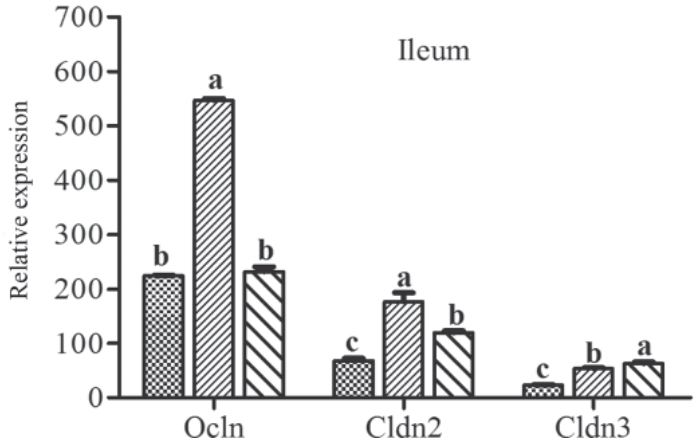


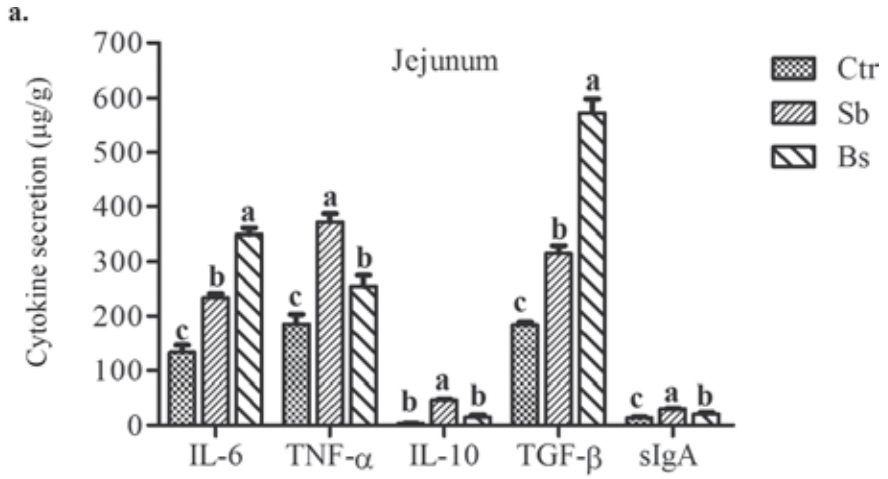
Rajput et al. 2013. Poultry Sci. 92:956-965

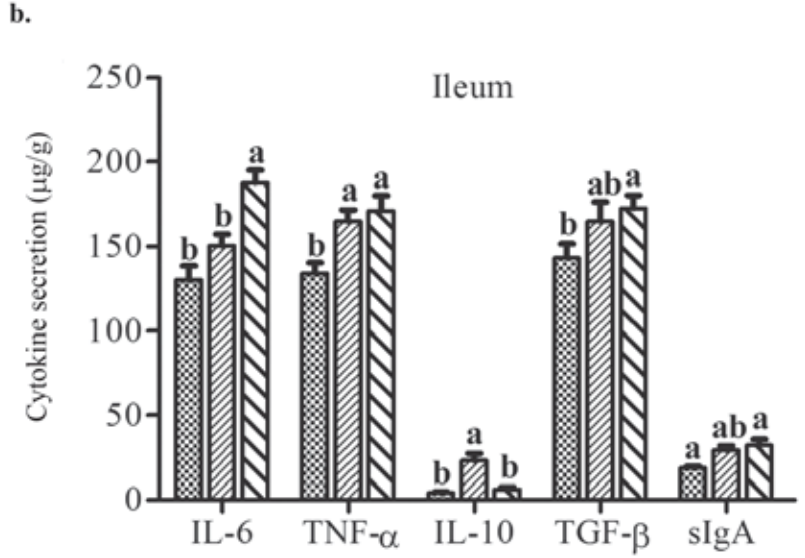
Broiler Study, *S. boulardii*

Control (Ctr) *S. boulardii* (Sb) *Bacillus subtilis* B10 (Bs)







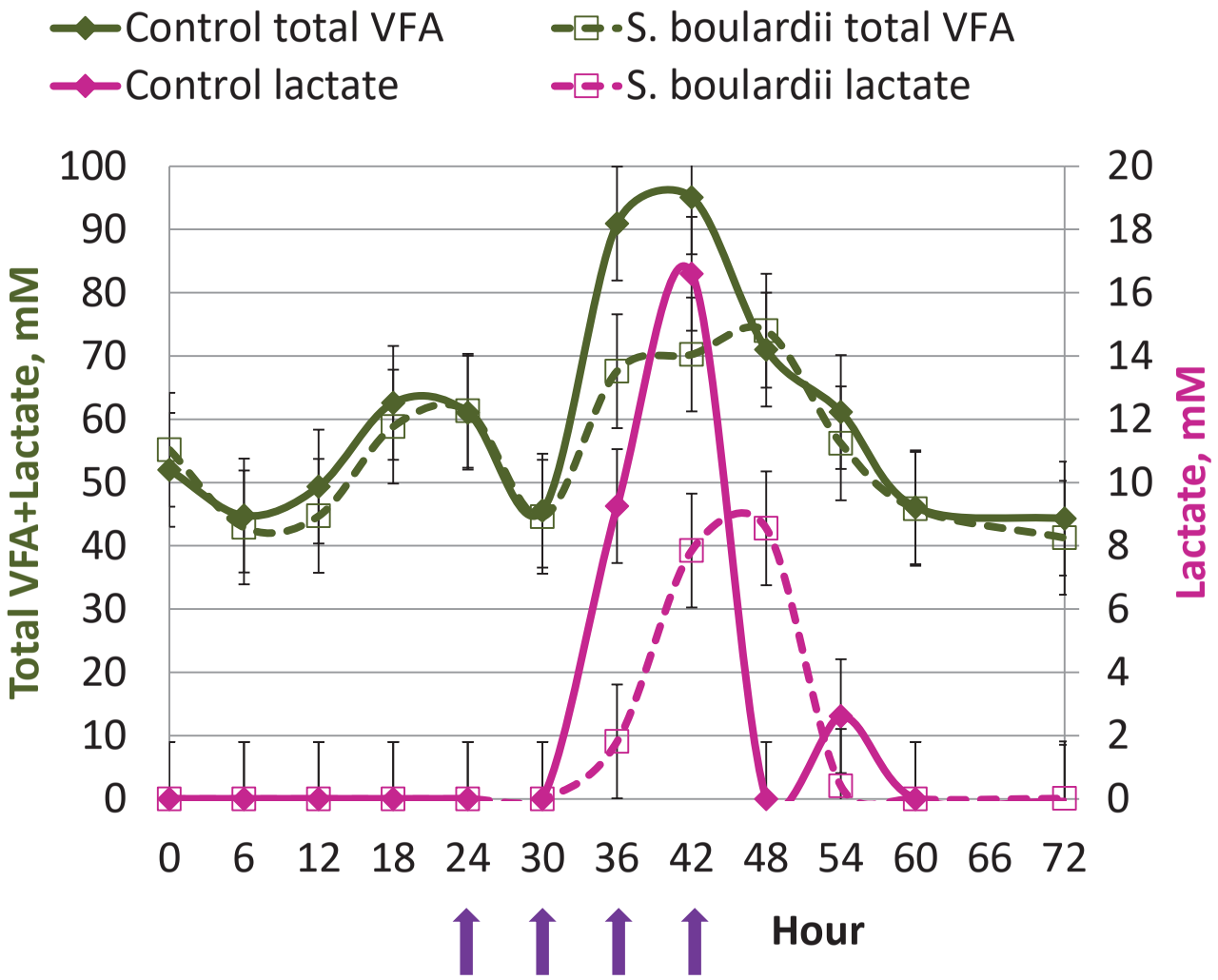


Rajput et al. 2013. Poultry Sci. 92:956-965

S. boulardii in steers

- All steers received 4 abomasal pulse doses of 0.25 g/kg BW oligofructose every 6 h beginning at 24 h
  - Designed to induce digestive upset
- Treatments
  - Abomasal water
  - Abomasal *S. boulardii* (10 g/d of 2 × 10^{10} cfu/g)

S. boulardii in steers

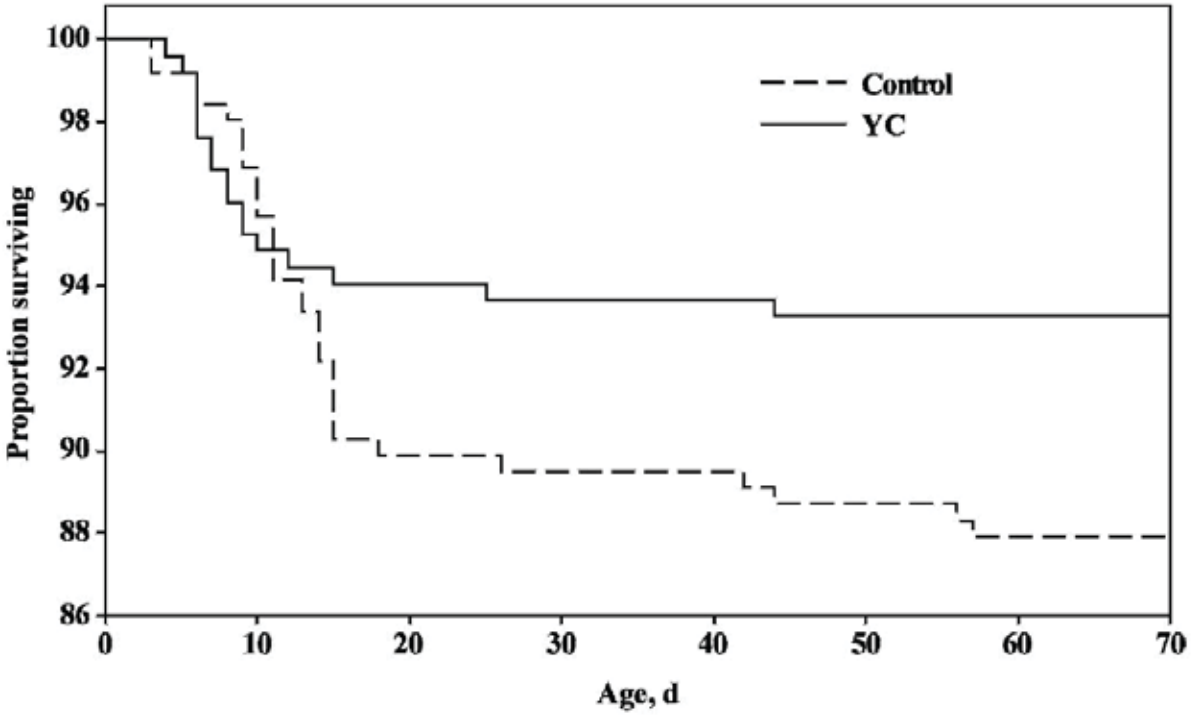


= 0.25 g/kg BW abomasal oligofructose

Gressley et al. 2016. JAS 94:284

Yeast and Calves

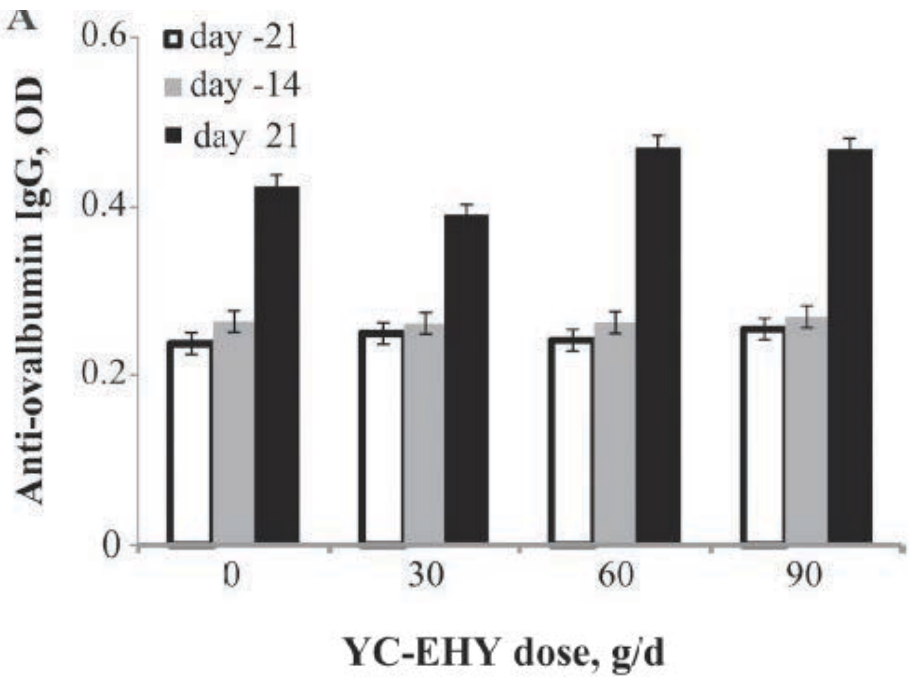
- 512 calves, 2 treatments:
  - Control starter grain
  - Starter grain with 2% yeast culture (Diamond V XP)

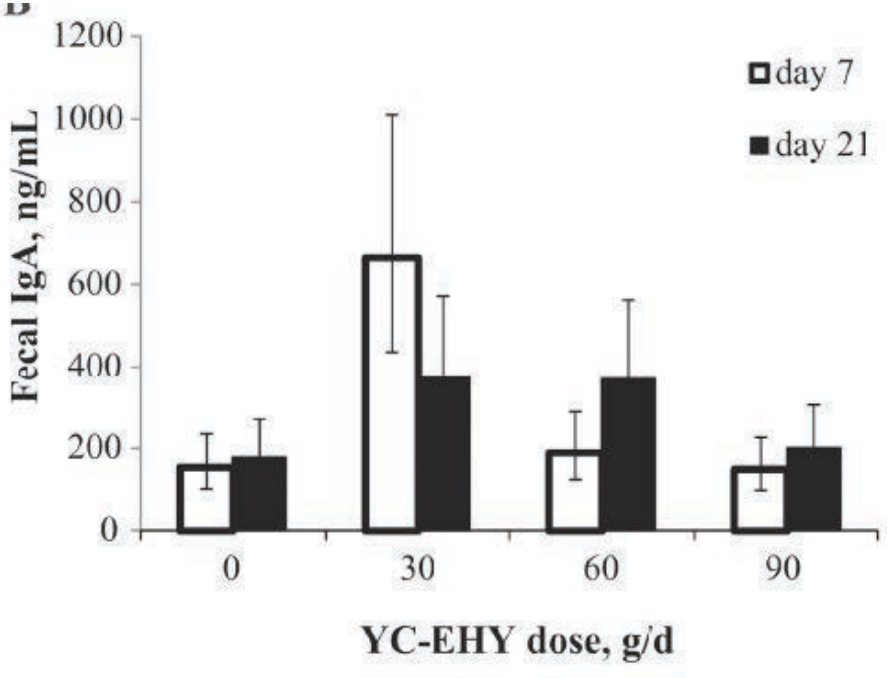


Magalhães et al., 2008, JDS, 91:1497

Yeast and Cows

- 40 transition cows
  - Fed from 21 d prepartum to 42 d postpartum with 0, 30, 60, or 90 g/d yeast culture plus enzymatically hydrolyzed yeast (Celmanax)





Take Home Messages

- Gut tissues are complex and changes in digesta and digestive microbiome impact the host
- The diet impacts gut health and local and systemic inflammation
  - SARA example
- Feeding strategies may provide a useful tool to alter the gut microbiome, fortify the gut epithelium, and reduce inflammation
  - Yeast example

Thank You!

IOWA STATE UNIVERSITY

Department of Animal Science

Effects of stress and entodoxins on intestinal integrity and function in pigs

Nicholas Gabler

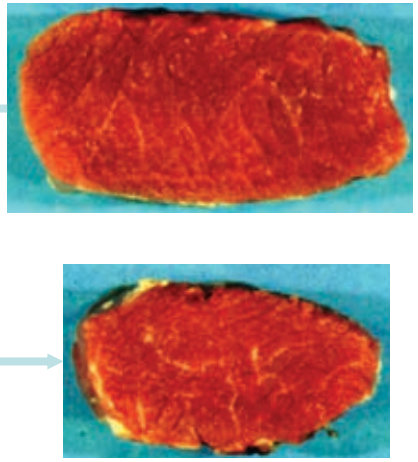
Associate Professor

WNF 2016

Introduction

- Intestinal function, Stress, Immune and Inflammatory challenges impact
  - Feed intake
  - Growth rates
  - Wellbeing
  - Mortality
  - Profitability

Growth Potential is Reduced
Altered Metabolic Demand
Reduced Efficiency



Why focus on the GI tract?

- Important for digestion and absorption
- Large immune organ
- Intestinal barrier necessary for preventing pathogen translocation and maintaining homeostasis
- Susceptible to hypoxia and inflammation
- GI tract integrity in pigs can be compromised due to weaning stress, enteric disease, nutrition (mycotoxin etc…) and climate stress

→ Reduced pig performance and wellbeing

Function of the GI tract

- The GI system reflects a complex and cooperative network of various organs
- Cellular specialization
- Highly efficient, interactive and redundant
- The circulatory features of the GI tract and liver set them apart from other organs
- Many functions of the GI tract are governed by the enteric nervous system

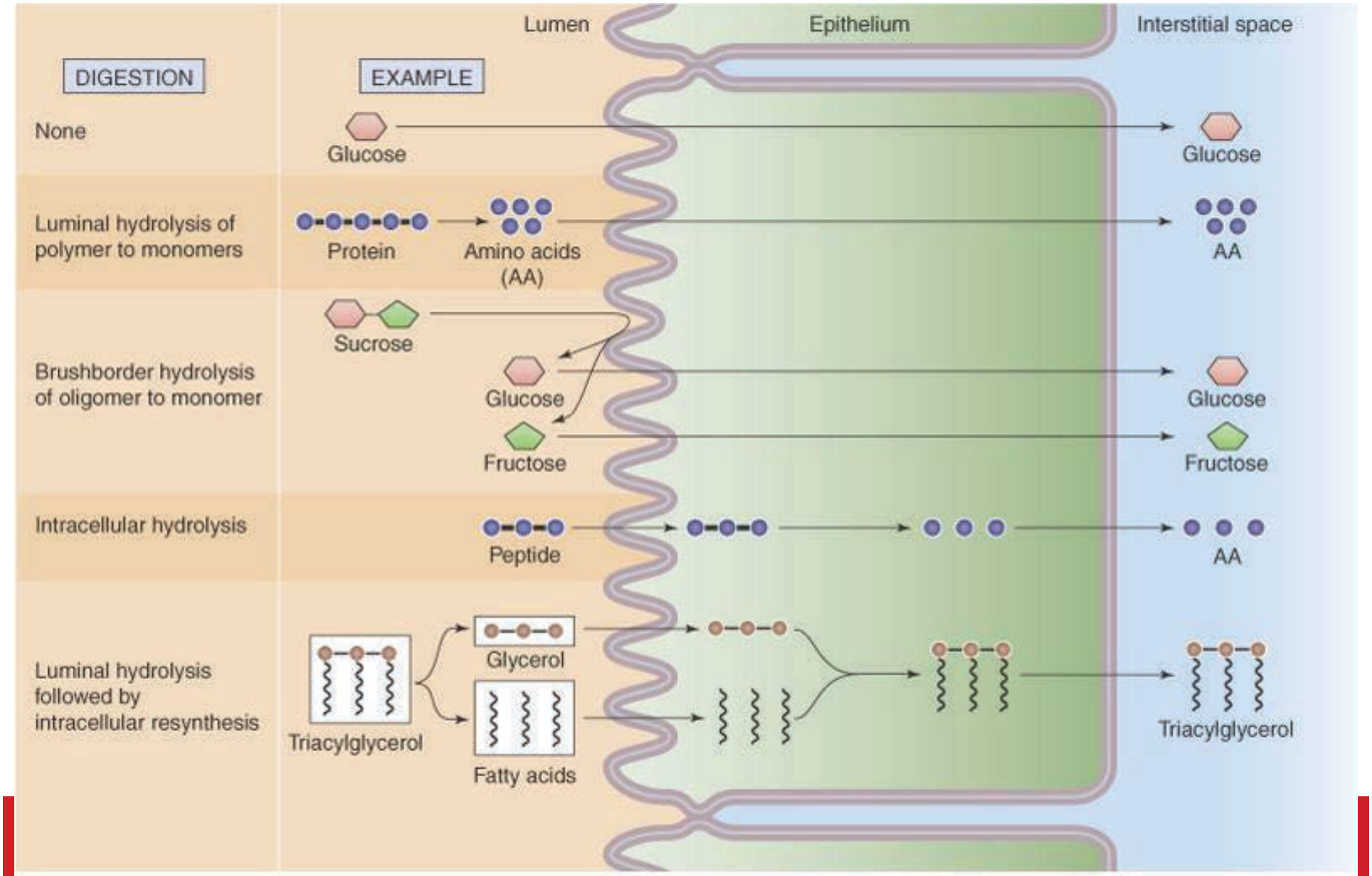
Function of the GI tract

- Key processes of the GIT
  1. Motility
  2. Secretion
  3. Digestion
  4. Absorption
  5. Excretion
  6. Host defense

Organization of the GI tract

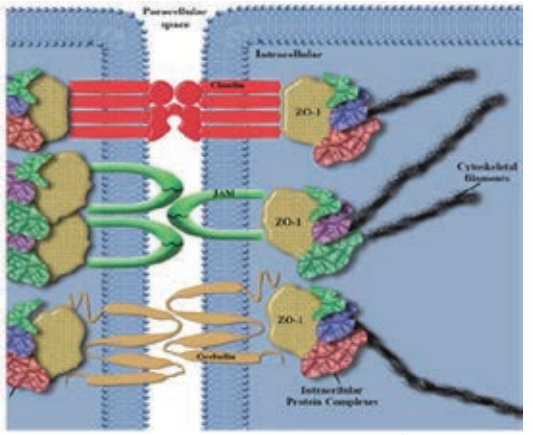
- The primary function of the GI tract is to transfer nutrients, water and electrolytes from the foods we eat into the body's internal environment
- Also contributes to appetite control
- The act of eating does not automatically make the preformed organic molecules in food available to the body cells as a source of fuel of building blocks
  - Fats consumed as TAG but absorbed as MAG and FFA
  - Protein consumed as proteins and large peptides but absorbed as AA and small peptides
  - CHO is present in the diet as starch, disaccharides and monosaccharide but absorbed as monosaccharide

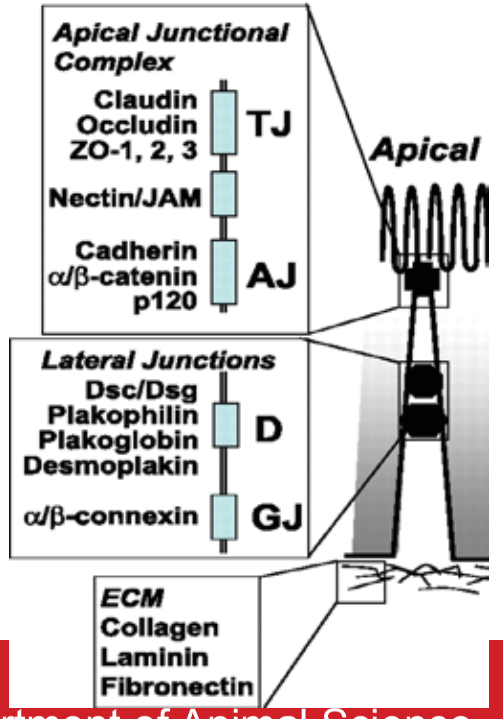
Digestion and Absorption



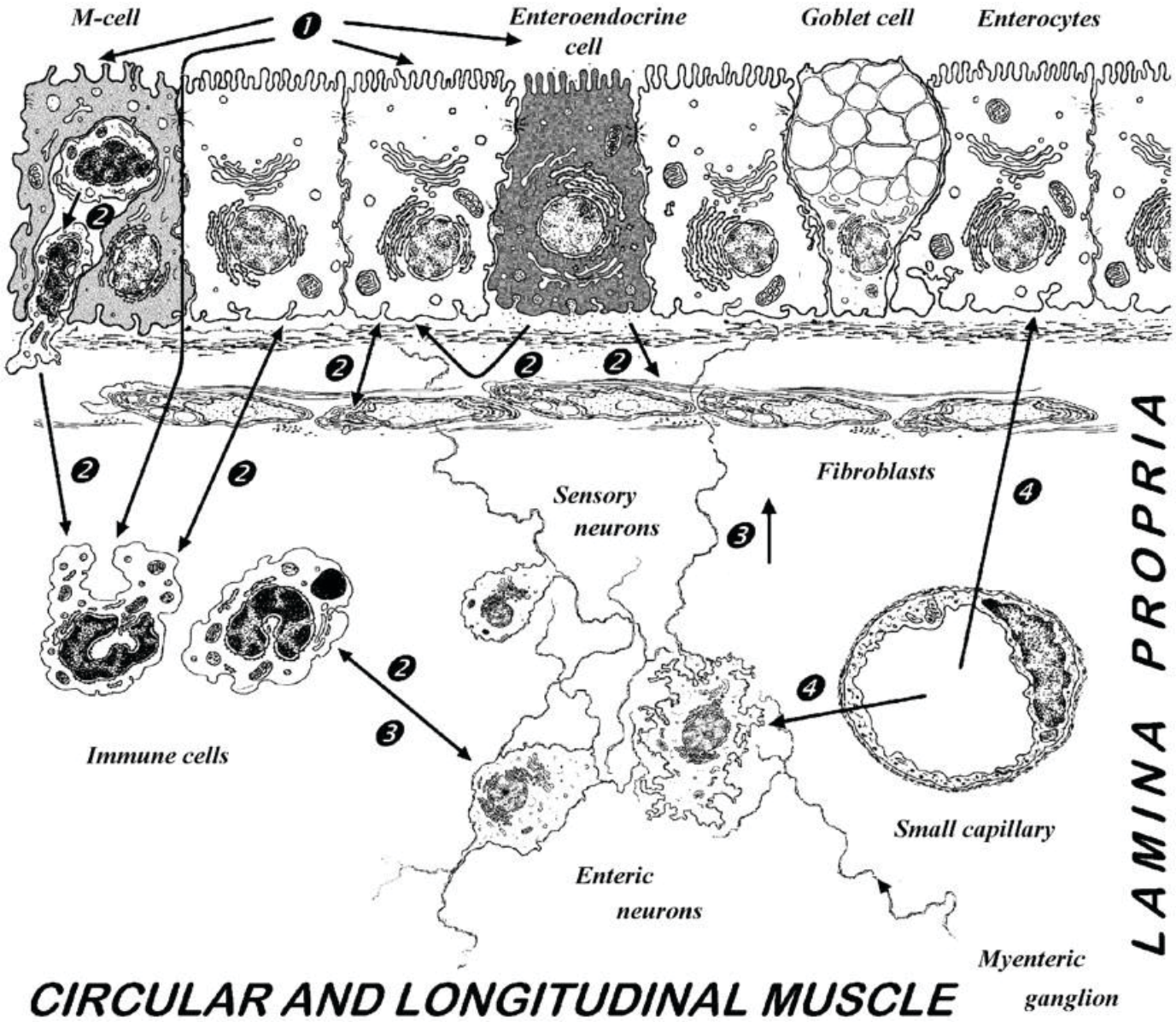
Intestinal Barrier Organization

- Protein-protein interactions
- Secretory products
- Polarized epithelial cells
  - Adhesion complexes form the epithelial barrier
    - Tight junctions (TJ)
    - Adhesion junctions (AJ)
    - Desmosomes (D)
    - Gap junctions (GJ)
- This barrier can be compromised due to stress, inflammation and damage

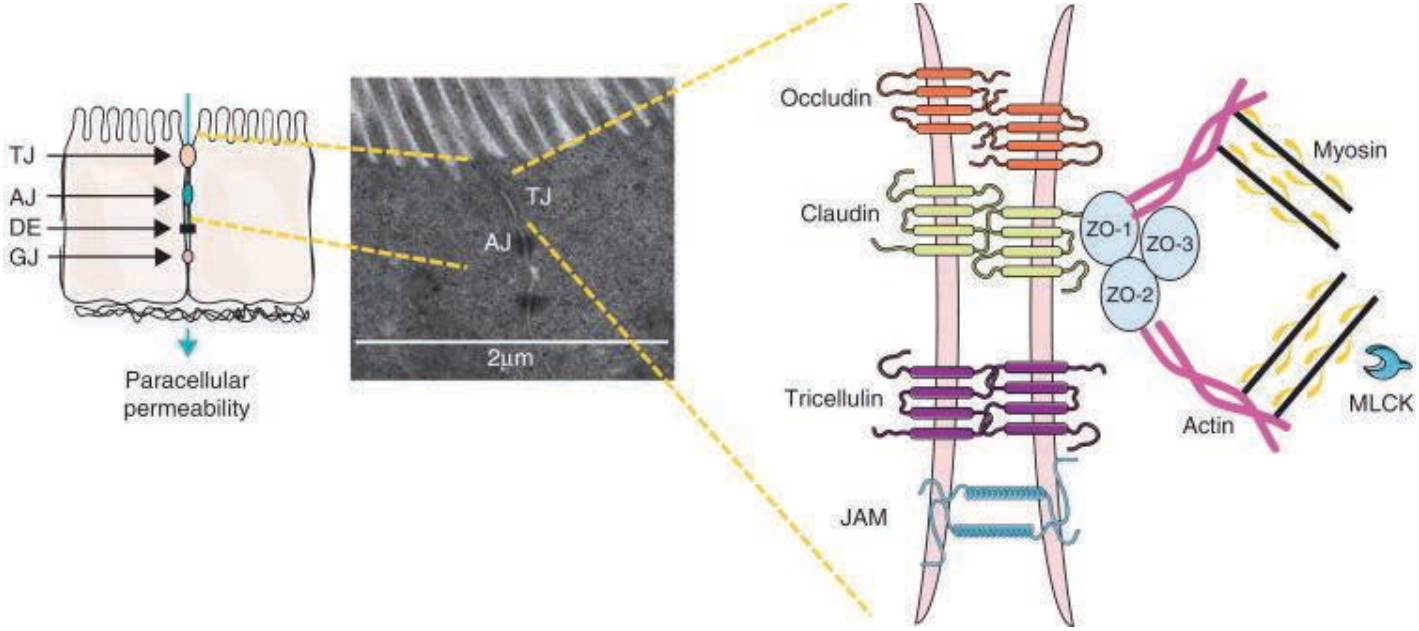




Halbleib J M et al. Mol. Biol. Cell 2007;18:4261-4278

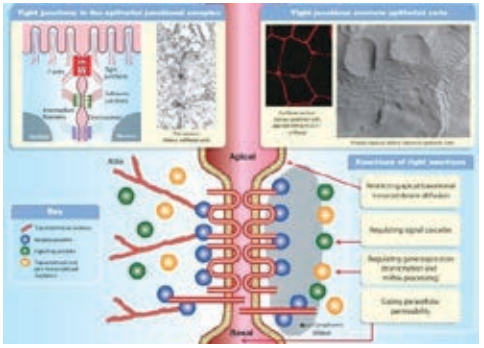


Pácha J Physiol Rev 2000;80:1633-1667



- Four transmembrane families of proteins (occludin, claudins, junctional adhesion molecule (JAM) and tricellulin) contribute to TJ formation.
- The intracellular domains of these transmembrane proteins interact with cytosolic scaffolding proteins like zonula occludens (ZO) proteins, which in turn anchor the transmembrane proteins to the perijunctional actinomyosin ring.
- The interaction of TJ proteins with the actin cytoskeleton is vital to the maintenance of TJ structure and function.
  - TJ interaction with the acinomyosin ring permits the cytoskeletal regulation of the TJ barrier integrity.
  - Contraction of the actinomyosin ring is regulated by the myosin light chain kinase (MLCK) which phosphorylates the myosin light chain.

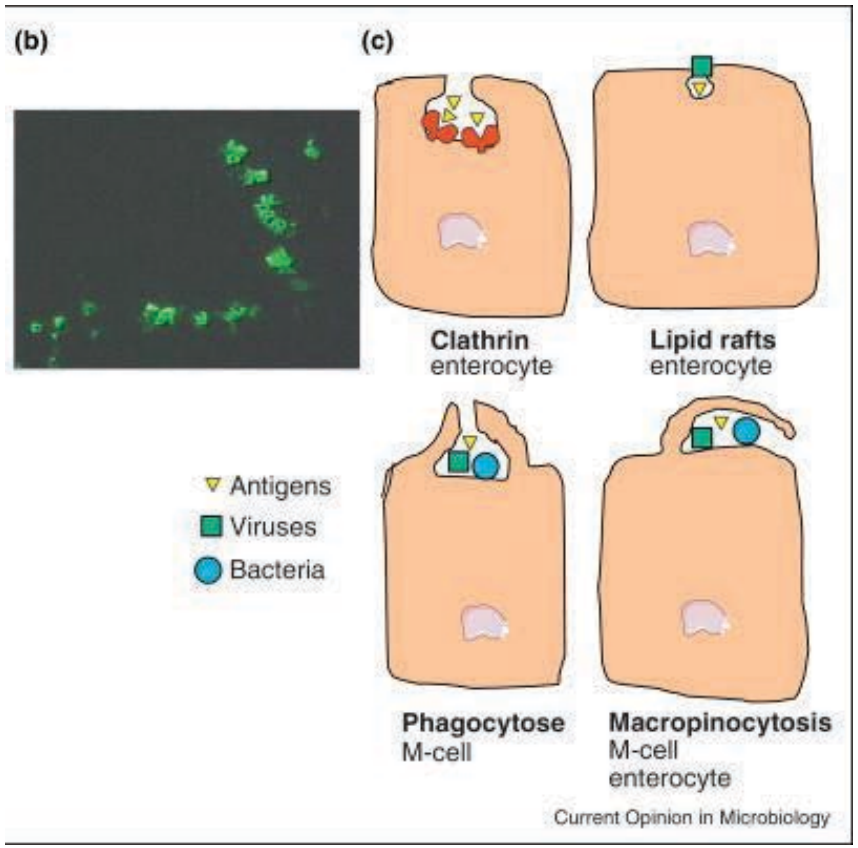
Balda M S , Matter K J Cell Sci 2008;121:3677-3682



Tight Junction Proteins

- Claudin
  - ~27 family member proteins
  - Are considered to be the primary seal forming proteins.
  - Claudin 1, 2, 3, 4, 5, 7, 8, 12 and 15 are expressed in the intestines.
  - They can exhibit three types of selectivity:
    i. Anion selectivity
    ii. Cation selectivity
    iii. Water selectivity

- Occludin
  - Transmembrane spanning
  - Phosphorylation of occludin regulates occludin localization and TJ permeability



Mechanisms of transcellular permeability

- **(b)** M-cells transport luminal antigens and bacteria toward the underlying immune cells which are the actors of the immune response

- **(c)** The transcellular route across M-cells and other epithelial cells exhibits two classical pathways depending on the particle's size.

A compromised intestine reduces performance and health

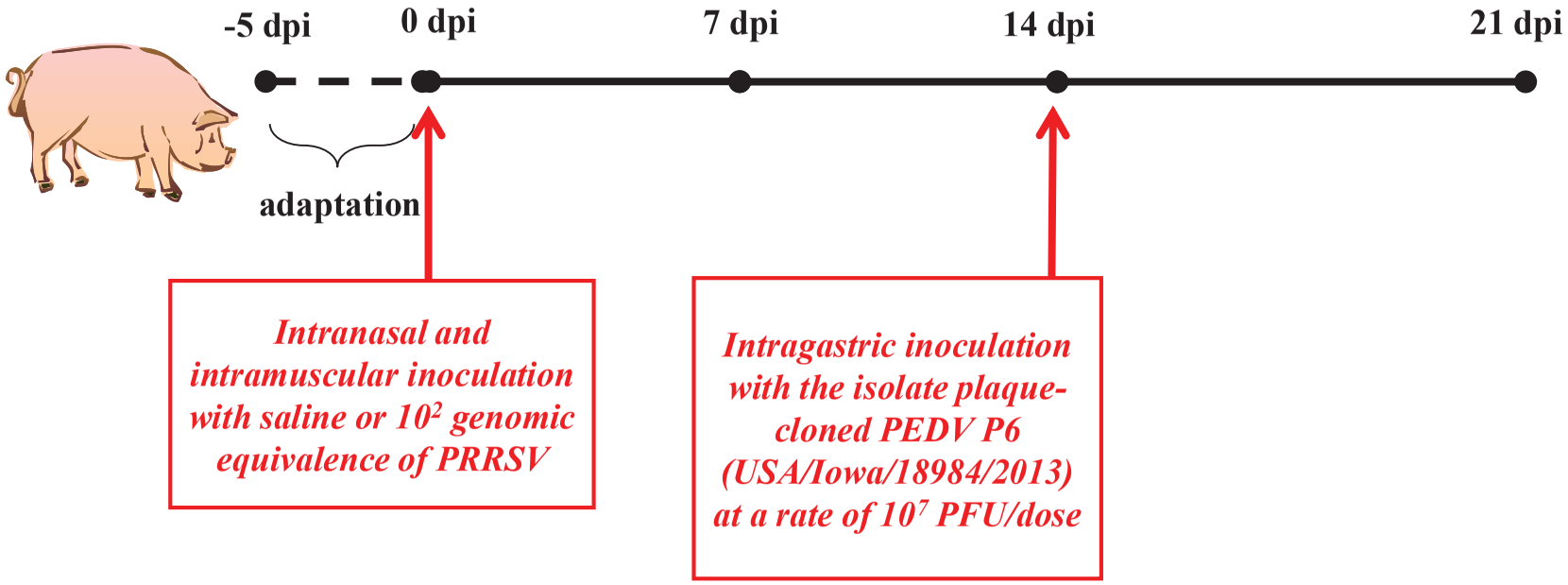
- Reduced intestinal integrity
- Reduced appetite
- Reduced function

  - Increased intestinal permeability to pathogens and toxins (i.e. mycotoxins and endotoxin)

THE IMPACT PRRS AND PED VIRUS CO-INFECTION ON NURSERY-GROWER PIG PERFORMANCE AND INTESTINAL FUNCTION

Schweer et al, (2016) J Anim Sci. 94:2: 514-522
Schweer et al, (2016) J Anim Sci. 94:2: 523-532

Methodology



- 16 kg BW pigs
- Body weights and feed intake recorded weekly
- Blood samples collected weekly
- Total tract fecal collections 17-20 dpi
- Euthanized pigs for tissue and digesta collections at 21dpi

Diet

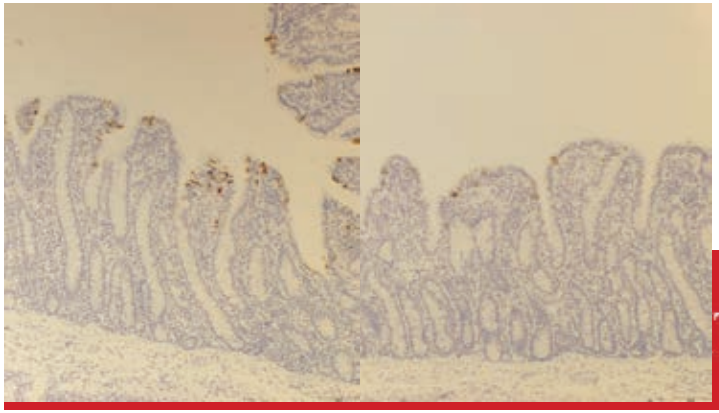
Ingredient	%
Corn	60.93
SBM	30.00
DDGS	5.00
Soybean oil	1.00
Lime	0.94
Lysine	0.50
Salt	0.35
Vitamin & Mineral premix	0.30
Monocalcium phosphate 21%	0.55
DL-Methionine	0.19
L-Threonine	0.22
Heat Stable Optiphous 2000	0.02
Titanium dioxide	0.40
Calculated	
ME, Mcal/kg	3.39
NE, Mcal/kg	2.43
SID Lysine, %	1.33

Clinical Diagnostic

Parameter	Control	PRRSV	PEDV	PRRSV + PEDV	SEM	P-value
Log+1 QPCR PRRS titer						
14 dpi	neg	5.2	neg	4.9	0.31	0.64
21 dpi	neg	3.9	neg	3.4	0.36	0.36
PRRSX3 EIA titer						
14 dpi	neg	1.2	neg	1.4	0.14	0.10
21 dpi	neg	1.1	neg	1.5	0.16	0.003

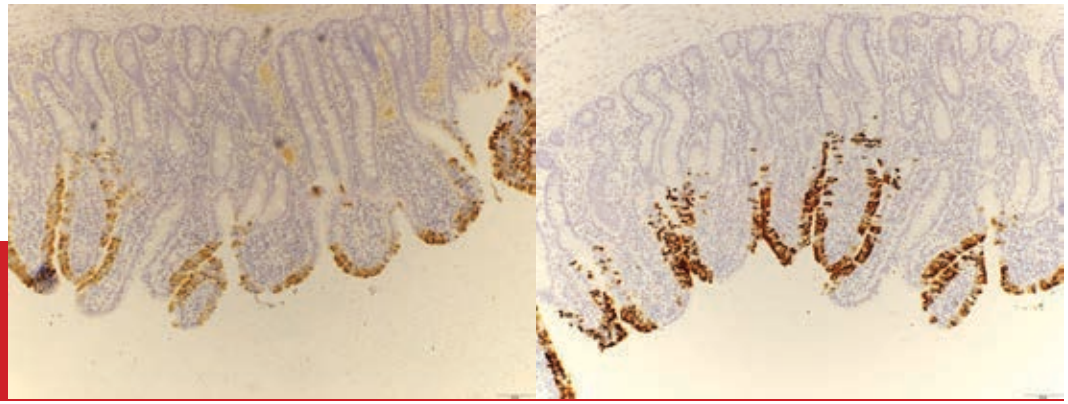
PEDv only

ISH IHC



PEDv + PPRSv

ISH IHC



What is the impact on growth performance?

Performance

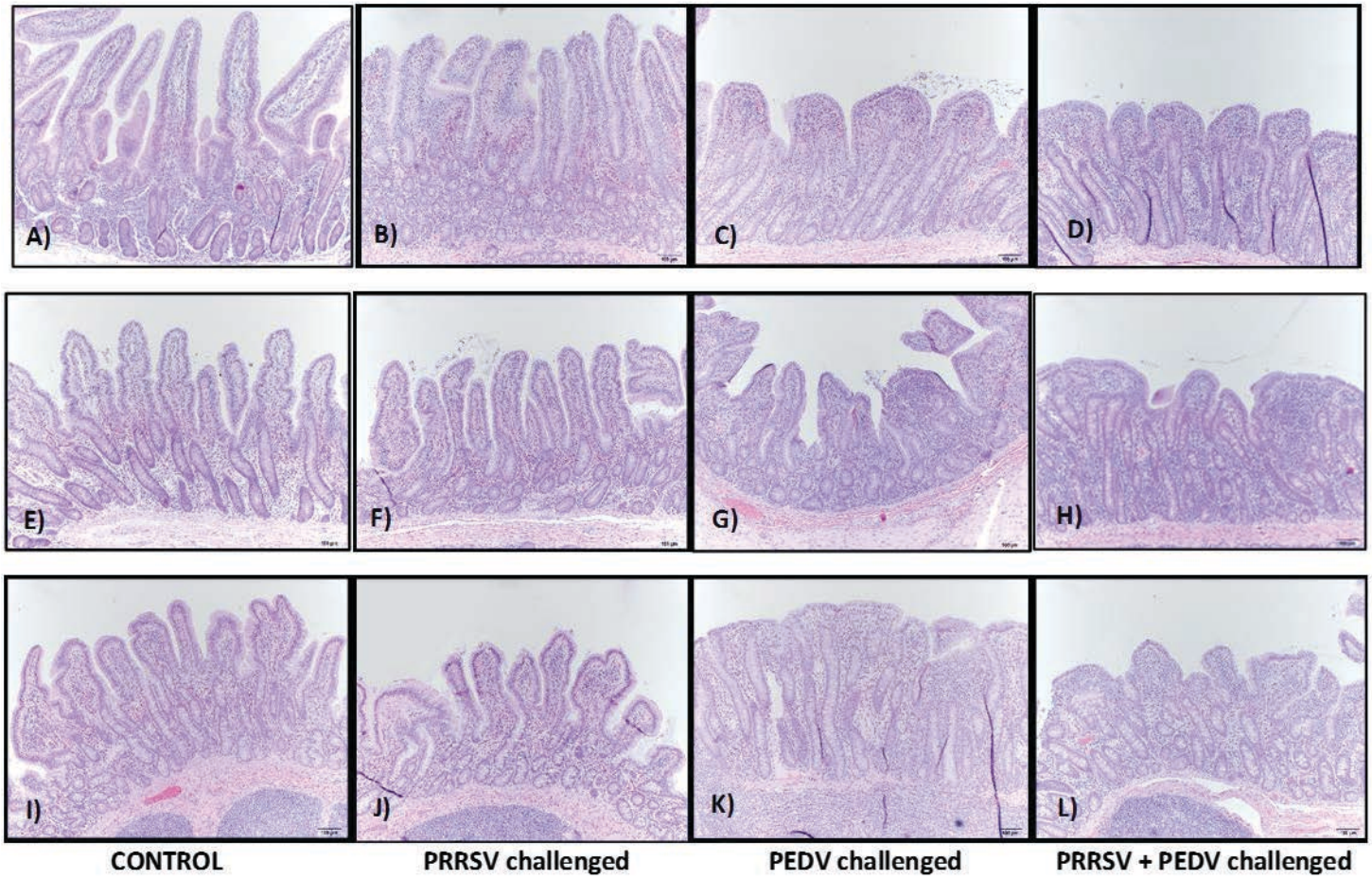
Parameter	Control[1]	PRRS[2]	PED[2]	PRRS + PED[2]	SEM	*P*-value			
						Overall	*PRRS[3]*	*PED[4]*	*Infection[5]*
0-14 d Performance									
ADG, kg	0.62[a]	0.35[b]	0.61[a]	0.41[b]	0.03	<0.001	<0.001	0.506	0.003
ADFI, kg	0.95[a]	0.67[b]	0.94[a]	0.76[b]	0.05	0.001	<0.001	0.441	0.032
G:F	0.65[a]	0.53[b]	0.65[a]	0.55[ab]	0.03	0.020	0.003	0.861	0.095
15-21 d Performance									
ADG, kg	0.66[a]	0.63[a]	0.35[b]	0.20[b]	0.04	<0.001	0.083	<0.001	0.001
ADFI, kg	1.22[a]	1.02[a]	0.88[ab]	0.67[b]	0.07	0.003	0.026	<0.001	0.005
G:F	0.54[ab]	0.62[a]	0.39[bc]	0.31[c]	0.05	0.001	1.000	<0.001	0.189
0-21 d Performance									
ADG, kg	0.63[a]	0.44[b]	0.51[ab]	0.34[c]	0.04	<0.001	<0.001	0.001	<0.001
ADFI, kg	1.04	0.78	0.92	0.73	0.04	0.002	<0.001	0.076	0.002
G:F	0.61[a]	0.56[a]	0.56[a]	0.47[b]	0.02	0.005	0.006	0.008	0.019

[1]n=3 pens per treatment; healthy, virus naïve (Control)
[2]n=6 pens per treatment; PRRSV infected (PRRS), PEDV infected (PED), co-infected (PRP)
[3]Orthogonal contrast for PRRSV naïve vs PRRSV infected
[4]Orthogonal contrast for PEDV naïve vs PEDV infected
[5]Orthogonal contrast of virus naïve vs virus challenged
[a,b,c]$P < 0.05$ represents treatment differences

- ADG reduced 20-50%
- ADFI reduced 10-30%
- GF reduced 10-20%

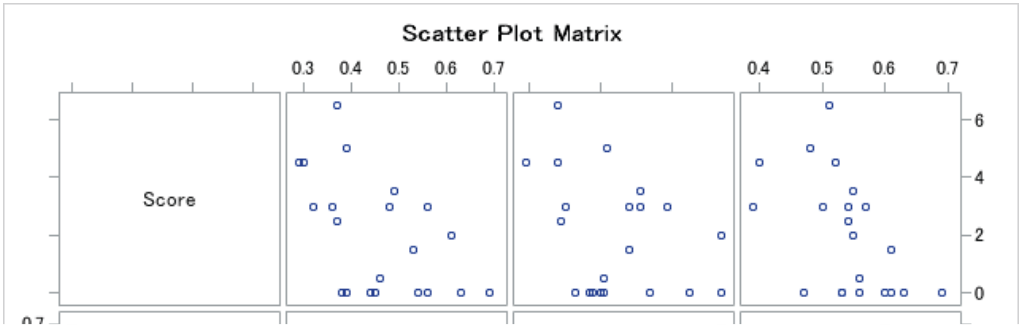
What is the impact on intestinal morphology and function?

Total Histological Disease Activity Scores



CONTROL	PRRSV challenged	PEDV challenged	PRRSV + PEDV challenged
Lesion Score = 0 No morphological changes	Lesion Score = 0.1 No morphological changes	Lesion Score = 2.6 Mild villus atrophy and fusion, some inflammatory infiltrates	Lesion Score = 4.42, Moderate villus atrophy and fusion, mild inflammatory infiltrates

Correlations between 0-21 dpi performance and histological disease activity scores



Performance was negatively correlated with the histology disease activity score

Pearson Correlation Coefficients

	Score	ADG	ADFI	GF
Score	1.00	-0.52 (*P* = 0.015)	-0.37 (*P* = 0.103)	-0.53 (*P* = 0.013)
ADG		1.00	0.91 (*P* = 0.0001)	0.82 (*P* = 0.0001)
ADFI			1.00	0.54 (*P* = 0.003)
GF				1.00

PRRS and PED differentially alter intestinal function

Parameter	Control	PRRS	PED	PRRS+ PED	SEM	*P*-value			
						Overall	*PRRS*[2]	*PED*[3]	*Infection*[4]
Ex vivo FD4[5], AU	39	98	77	171	34.1	0.092	0.036	0.120	0.103
Transepithelial resistance[6], AU	1.0[a]	1.1[a]	0.6[b]	1.7[c]	0.06	<0.001	<0.001	0.019	0.084
Glucose transport[6], AU	4.45[b]	10.01[a]	1.84[b]	2.82[b]	1.28	<0.001	0.024	0.001	0.795
Glutamine transport[6], AU	0.97[b]	1.74[ab]	3.08[a]	0.88[b]	0.48	0.009	0.148	0.199	0.120
Lysine transport[6], AU	0.75[b]	2.08[a]	1.44[ab]	0.52[b]	0.34	0.023	0.581	0.242	0.196
Lactase[7]	2.2[ab]	4.5[a]	1.8[ab]	1.4[b]	0.74	0.033	0.235	0.032	0.656
Maltase[7]	15.5	21.7	12.1	13.4	2.54	0.064	0.150	0.032	0.926
Sucrase[7]	6.6[b]	13.5[a]	2.4[b]	4.2[b]	1.17	<0.001	0.002	<0.001	0.938
Aminopeptidase[8]	11.9	13.1	12.7	12.8	0.50	0.446	0.219	0.647	0.137
Na^+/K^+-ATPase[9]	329	370	265	388	37.2	0.064	0.035	0.546	0.811

[1]n=6 pigs per treatment; PRRSV infected=PRRS; PEDV infected=PED; Co-infected=PRP (PRP)
[2]Orthogonal contrast for PRRSV naïve vs PRRSV infected
[3]Orthogonal contrast for PEDV naïve vs PEDV infected
[4]Orthogonal contrast of virus naïve vs virus challenged
[5]FD4, Fluorescein isothiocyanate-dextran 4.4 kDa mucosal to serosal permeability conducted in modified Ussing chambers
[6]Normalized ex vivo measurement conducted in modified Ussing chambers
[7]Enzyme activity = liberated glucose, µmol per min per g protein
[8]Aminopeptidase activity = µmol per min per g protein
[9]Na^+/K^+-ATPase activity = µmol P_i liberated per mg protein per h
[a,b,c]$P < 0.05$

Apparent Digestibility

Parameter	Control[1]	PRRS[2]	PED[2]	PRP[2]	SEM	*P*-value			
						Overall	*PRRS[3]*	*PED[4]*	*Infection[5]*
ATTD									
Dry matter	88.7[a]	87.5[a]	81.8[b]	80.7[b]	0.90	<0.001	0.234	<0.001	0.001
Organic Matter	92.4[ab]	92.7[a]	90.3[ab]	89.2[b]	0.78	0.033	0.643	0.010	0.174
Gross Energy	87.2[a]	86.4[a]	77.3[b]	76.8[b]	0.90	<0.001	0.462	<0.001	<0.001
Nitrogen	85.9[a]	84.7[a]	80.1[ab]	74.9[b]	1.90	0.014	0.100	0.004	0.045
AID									
Dry matter	59.3	57.4	50.9	48.8	3.63	0.305	0.596	0.073	0.218
Organic matter	77.7	75.8	71.2	69.0	2.45	0.208	0.409	0.042	0.137
Nitrogen	68.0	70.3	62.0	65.1	4.11	0.423	0.534	0.291	0.730
Total amino acids	68.7	69.9	64.5	66.8	3.74	0.651	0.641	0.430	0.769

[1]n=3 pens per treatment; healthy, virus naïve (Control)

[2]n=6 pens per treatment; PRRSV infected (PRRS), PEDV infected (PED), co-infected (PRP)

[3]Orthogonal contrast for PRRSV naïve vs PRRSV infected

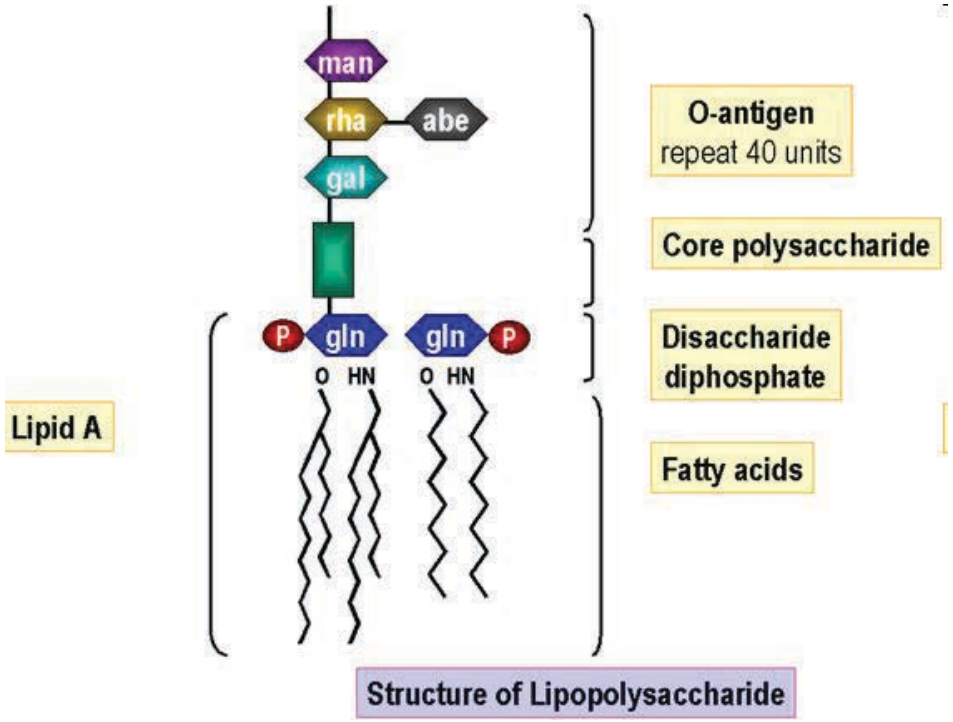
[4]Orthogonal contrast for PEDV naïve vs PEDV infected

[5]Orthogonal contrast of virus naïve vs virus challenged

[a,b]$P < 0.05$ represents treatment differences

Apparent Digestibility

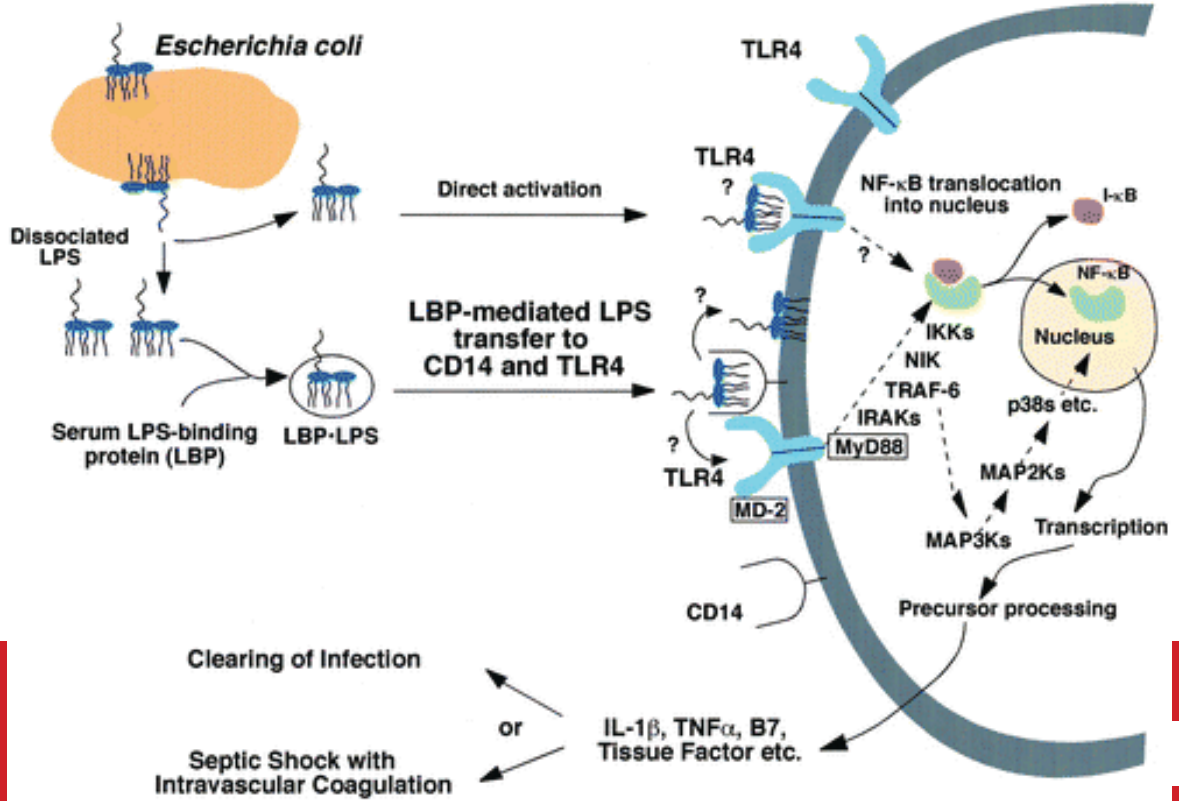
- PEDV reduces apparent total tract digestibility of nutrients and energy
  - Lesser impact of AID
- Enteric PEDV health challenges may differ from systemic (i.e. PRRS)
- These data may be confounded by feed intake differences
- Contribution of endogenous losses unknown


man
rha
abe
gal
P
gln
gln
P
O HN
O HN
Lipid A
O-antigen
repeat 40 units
Core polysaccharide
Disaccharide
diphosphate
Fatty acids
Structure of Lipopolysaccharide

ENDOTOXIN

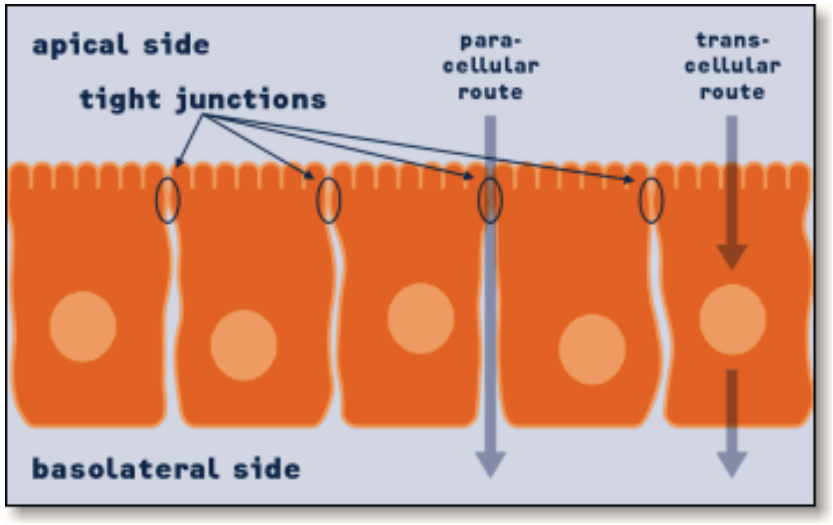
What is endotoxin?

- Endotoxin is derived from gram negative bacteria such as E. coli and Salmonella
- Incredibly potent initiator of immune cascades
  - Activates receptor mediated inflammatory processes
  - Gut-derived bacterial endotoxin plays an important role in the development of local and systemic inflammation



Raetz and Whitfield (2002)

How does enteric derived endotoxin enter circulation?



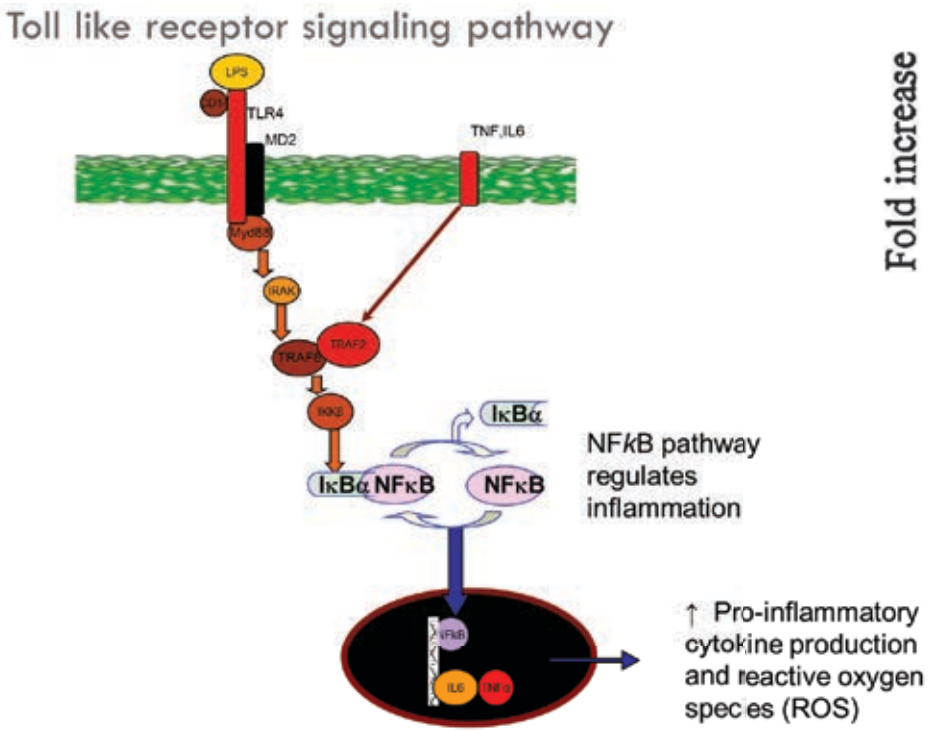
1. Paracellular transport through tight junctions
   - "Leaky gut"
   - Passive diffusion
2. Transcellular transport through the enterocyte
   - Receptor mediated endocytosis (TLR4)
   - Micelle Mediated transport during fat absorption

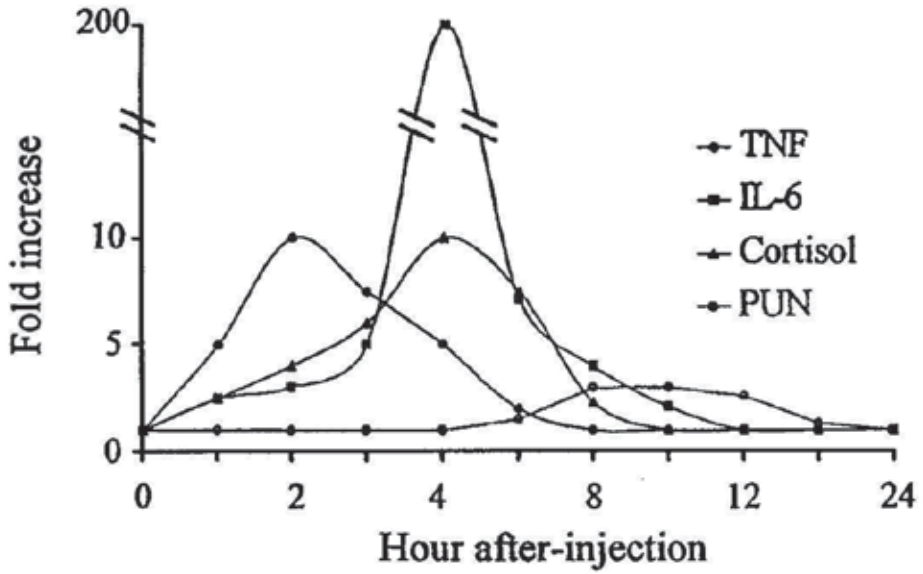
A compromised intestine reduces performance and health

- Reduced intestinal integrity
- Reduced appetite
- Reduced function

    - Increased intestinal permeability to pathogens and toxins (i.e. mycotoxins and endotoxin)

Classical innate immune response to LPS

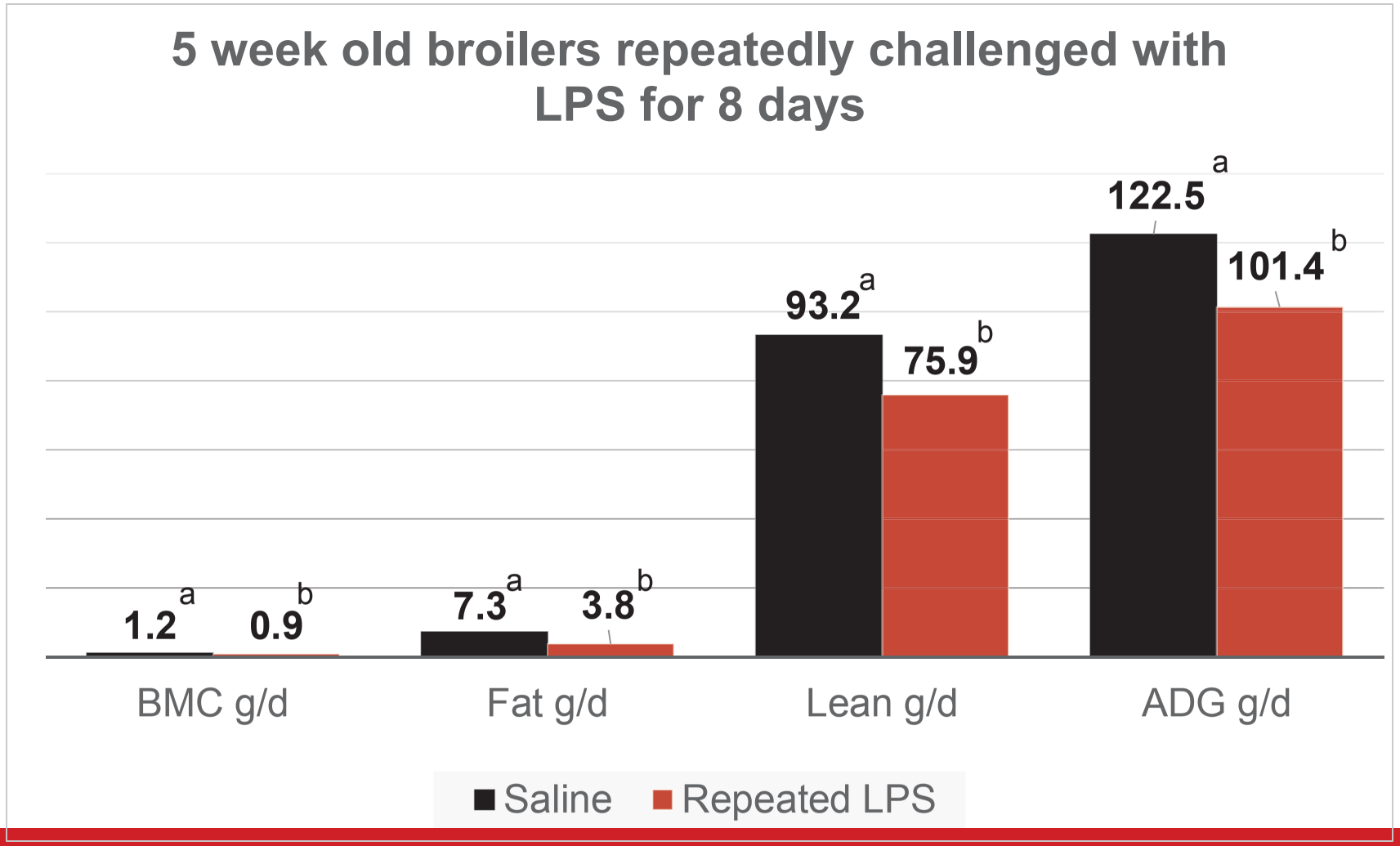




- Time course evaluation of Lipopolysaccharide
- Injection (5 μg LPS/kg BW) challenge in nursery pigs
- Note the three-fold increase in plasma urea nitrogen (PUN) at 8-12 hours

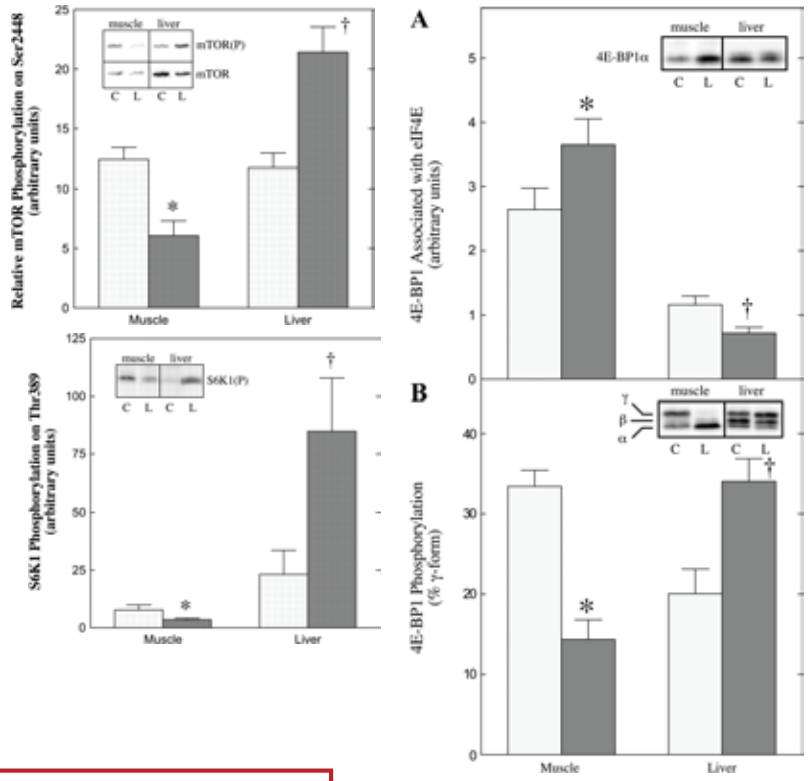
Webel et al., 1997

Why do we care about endotoxins?

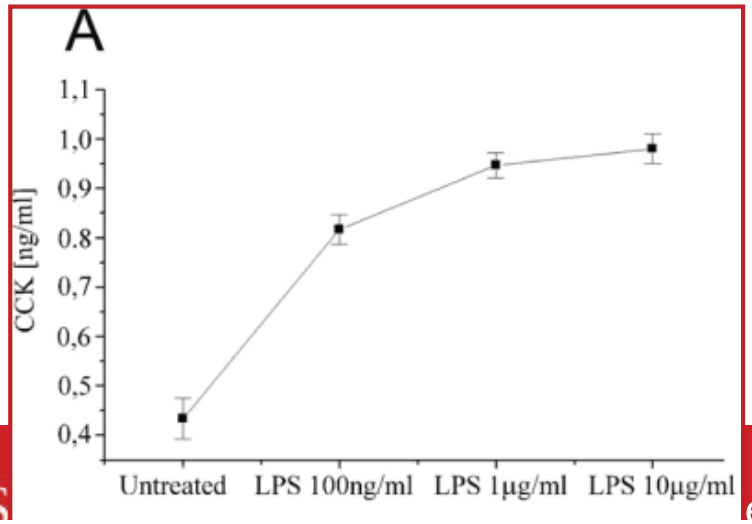


Why do we care ?

- Development of intestinal dysfunction
- Peripheral tissue catabolism
  - Inhibits skeletal muscle protein synthesis
  - Lipolysis and protein catabolism
- Disease
  - Metabolic diseases/dysfunction
  - Type II diabetes
  - Atherosclerosis
- Suppresses appetite

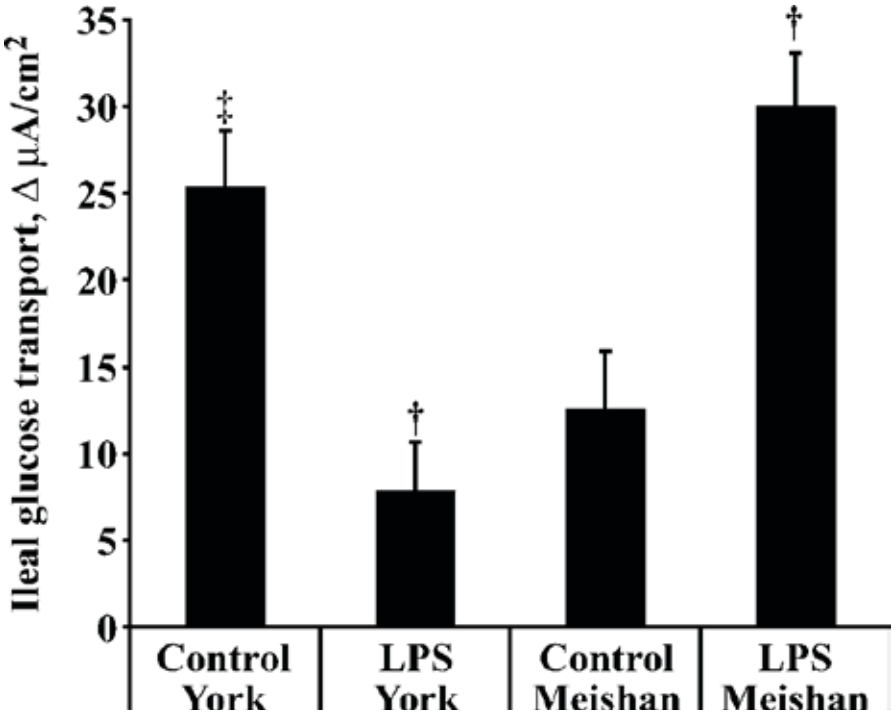


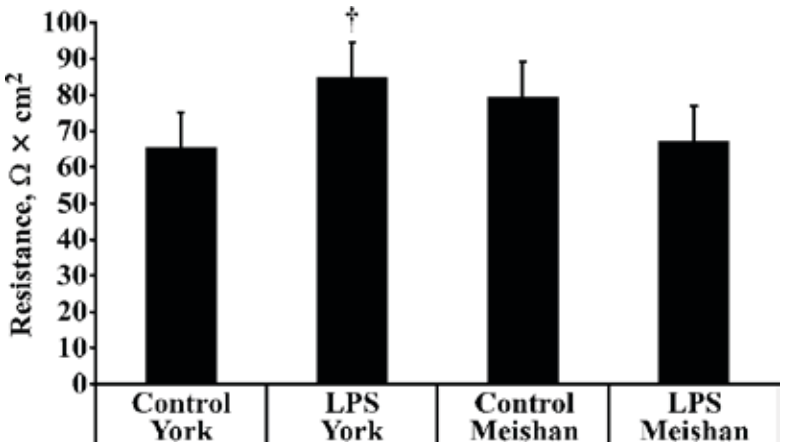
Kimball, S. R. et al. (2003)

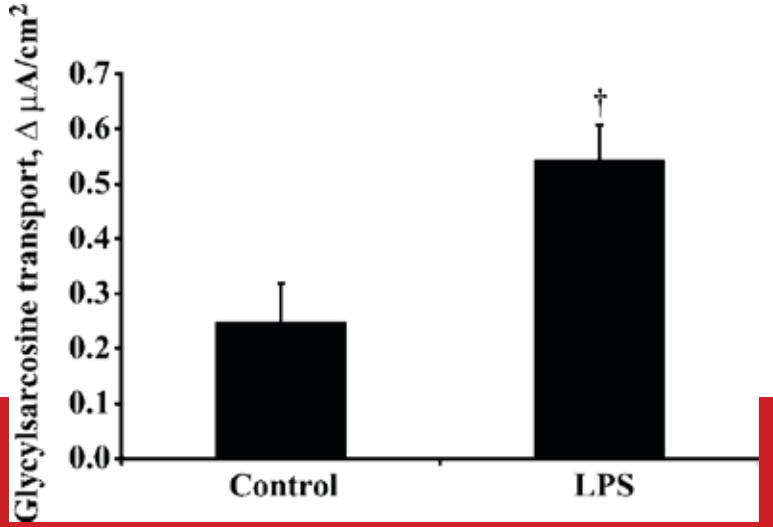


Bogunovic M et al. (2007). Murine STC-1 enteroendocrine cells

Small intestinal function differentially responded to LPS and breed (Albin et al., 2007)





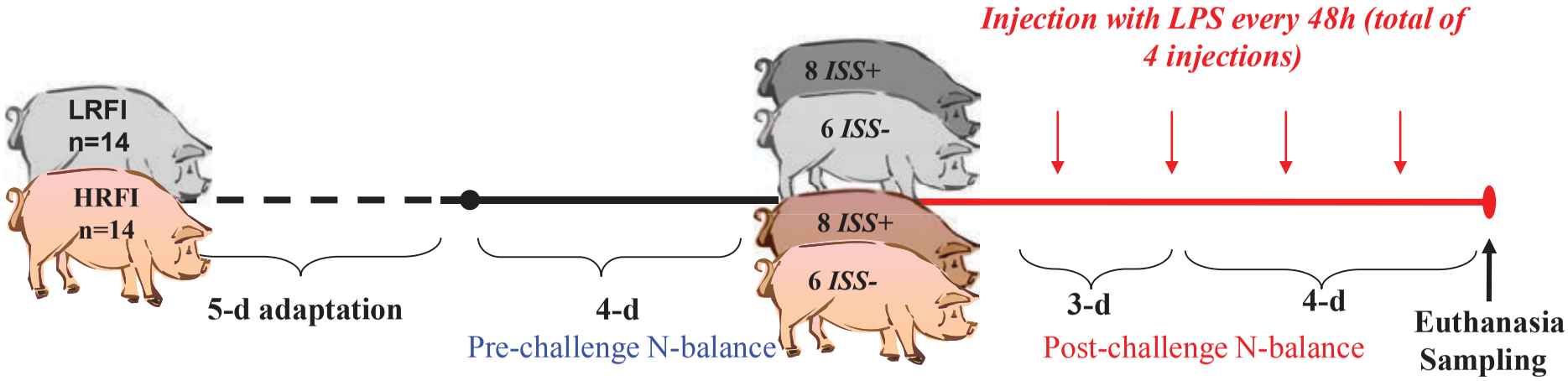


Ileal Na-dependent glucose transport as affected by pig breed and 4 h of LPS exposure (interaction; $P < 0.05$).

Swine repeated LPS challenge model

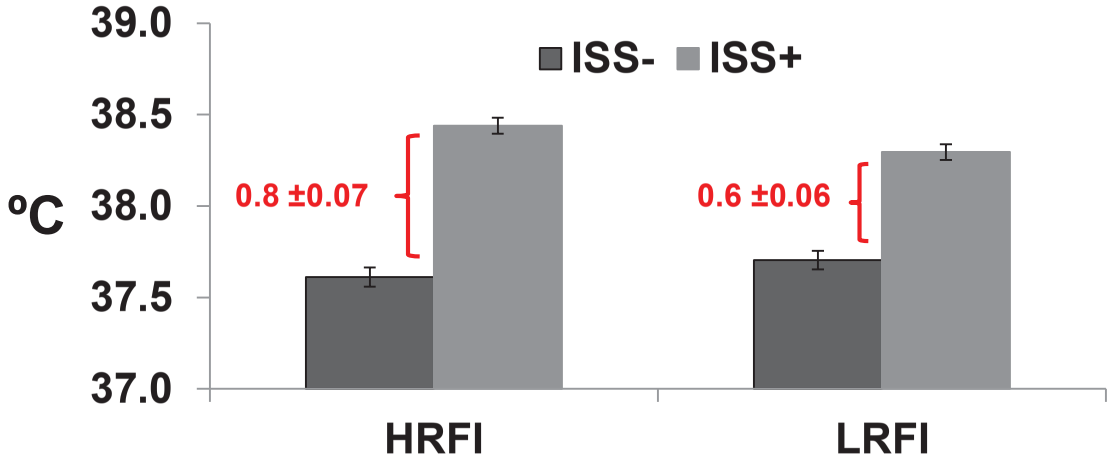
(Rakhshandeh et al.,)

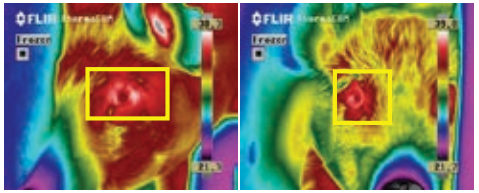
- ***General design***
  - **Immune system stimulation (ISS+)**
    - ✓ Repeated intramuscular injection of increasing amounts of lipopolysaccharide (LPS) every 48h *(initial dose of 30 µg.kg^{-1} BW, increase by 20, 30 and 40 %)*
    - ✓ *Control saline*



Results – Febrile Response

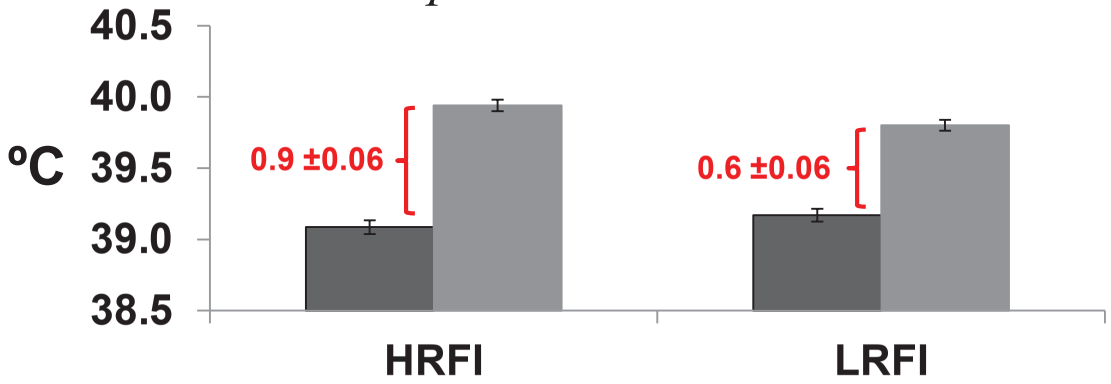
Eye temperature





Effect	P values
Line	0.60
ISS	< **0.001**
Line×ISS	0.01

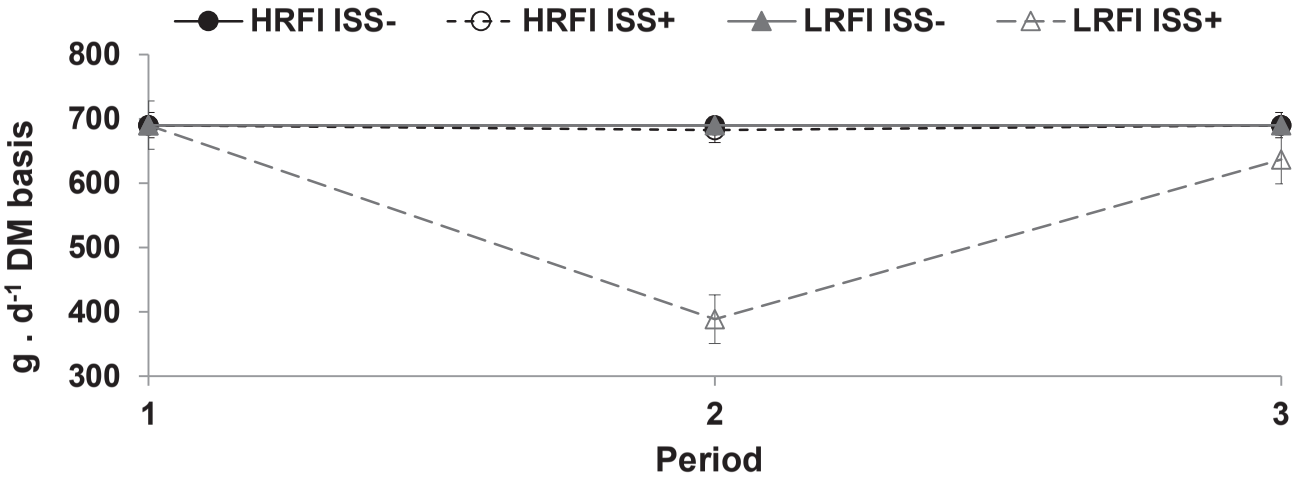
Rectal temperature



Effect	P values
Line	0.52
ISS	< **0.001**
Line×ISS	**0.01**

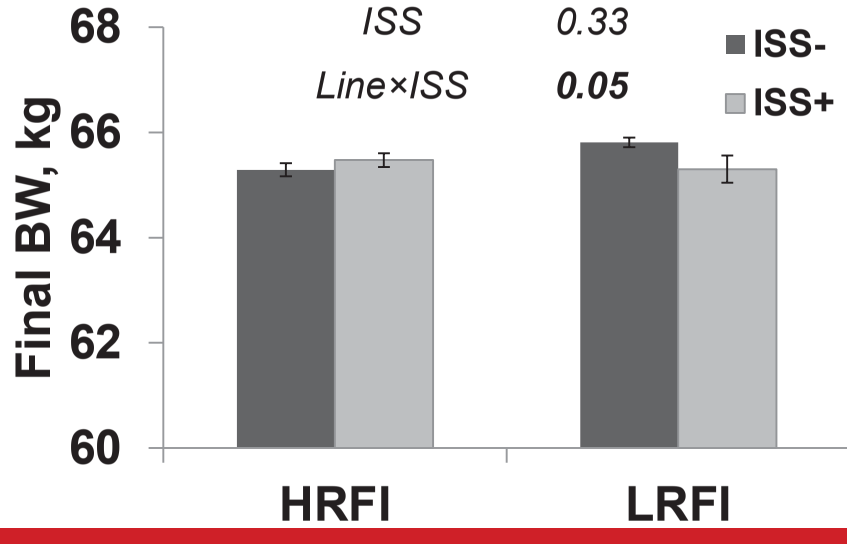
LPS and pig performance

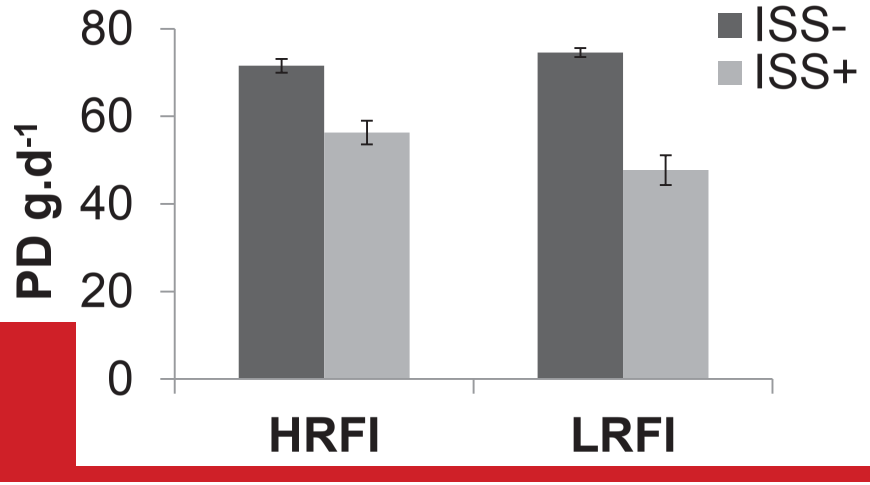
Feed intake



Effect	*P values*
Line	***< 0.01***
ISS	***< 0.01***
Line×ISS	***< 0.01***

Effect	*P values*
Line	*0.30*
ISS	*0.33*
Line×ISS	***0.05***

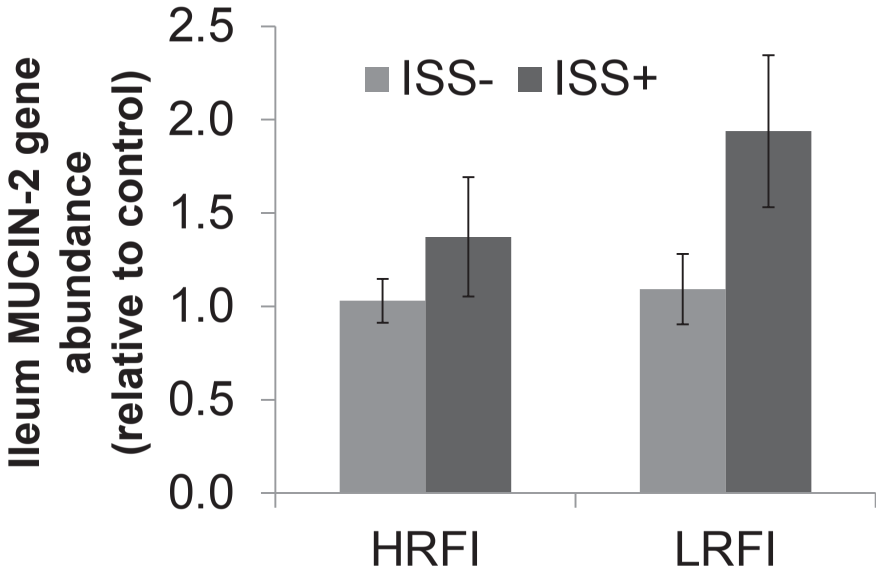




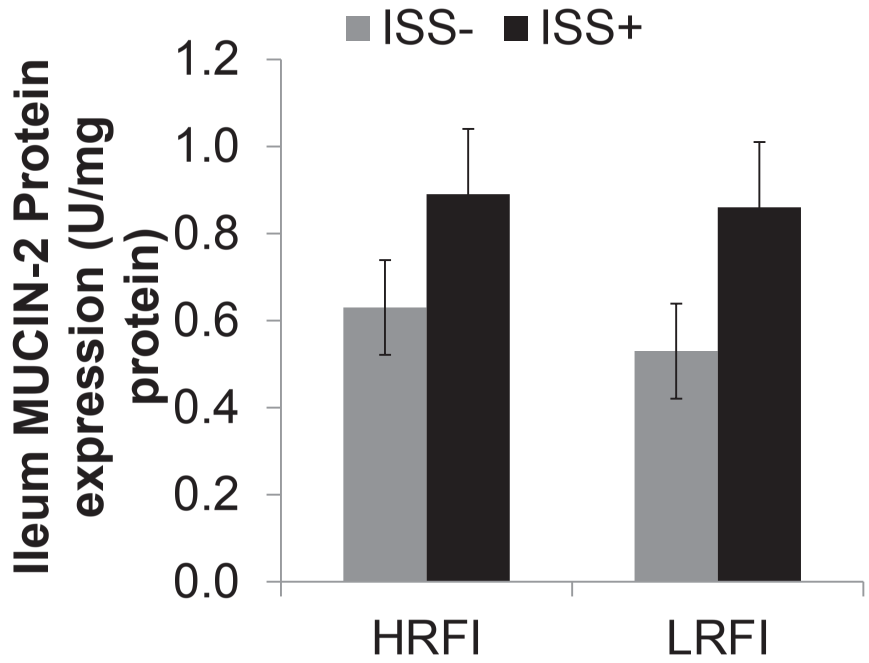
LPS and Digestibility

	HRFI			LRFI			*P value*		
	ISS-	**ISS+**	**SE**	**ISS-**	**ISS+**	**SE**	***Line***	***ISS***	***Line×ISS***
AID, %									
Crude protein	82	79	1.1	81	76	3.9	*0.38*	***0.03***	*0.65*
Organic matter	89	80	6.3	87	79	5.2	*0.70*	***0.07***	*0.90*
ATTD, %									
Energy	87	86	0.65	88	87	0.24	***0.02***	*0.17*	*0.23*
Crude protein	80	78	1.1	82	79	0.6	***0.01***	***0.04***	***0.02***
Organic matter	90	90	0.3	91	90	0.2	***0.01***	*0.21*	***0.06***

- ISS with LPS reduces ileal and total tract digestibility



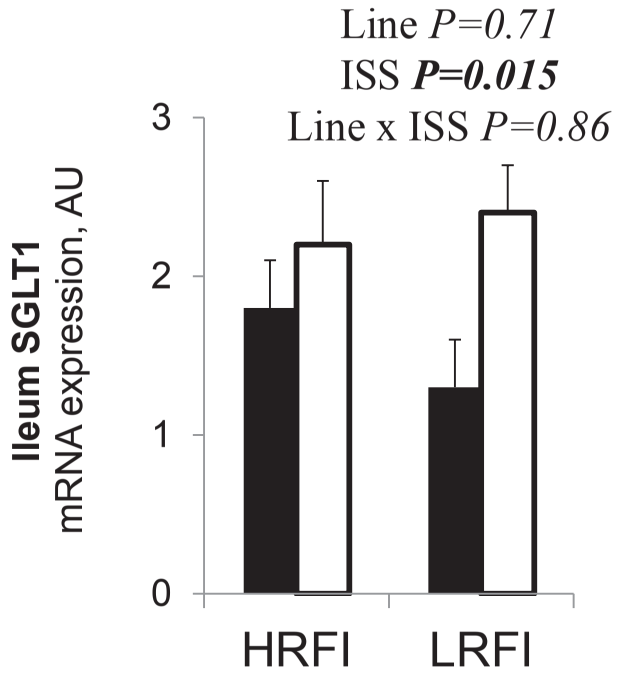
Effect	P values
Line	0.28
ISS	**0.05**
Line×ISS	0.38

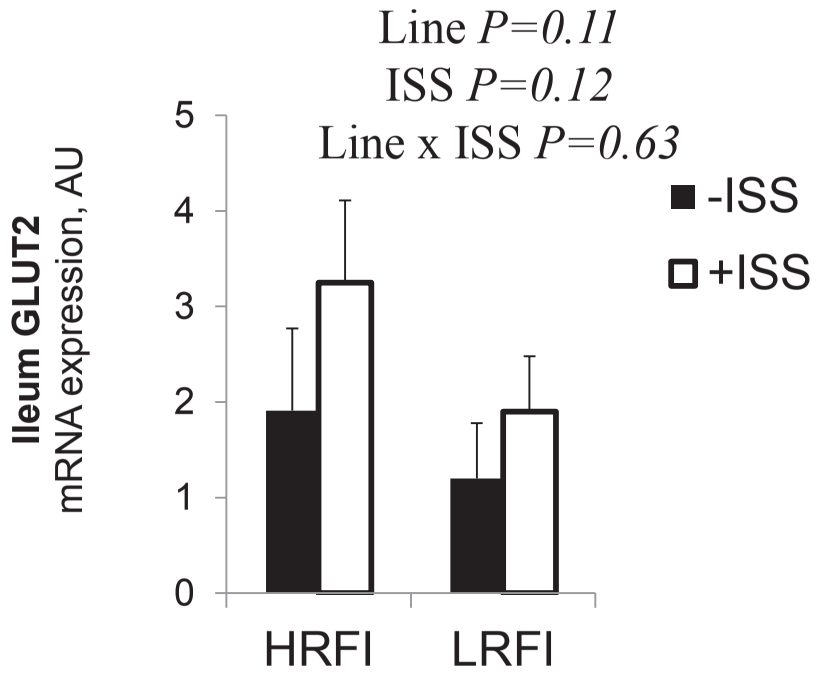


Effect	P values
Line	0.55
ISS	**0.015**
Line×ISS	0.78

Nutrient Transport and Blood data

	HRFI			LRFI			P value		
	ISS-	ISS+	SE	ISS-	ISS+	SE	*Line*	*ISS*	*Line×ISS*
Nutrient transport, *ΔμA/cm²*									
Glucose	7.0	10.0	2.00	6.0	12.0	2.00	*0.74*	***0.05***	*0.49*
Glutamine	1.0	1.6	0.94	0.5	0.6	0.20	*0.17*	*0.56*	*0.70*
Ileum TER, *Ωcm²T*	110	126	15	135	139	29	*0.33*	*0.60*	*0.60*



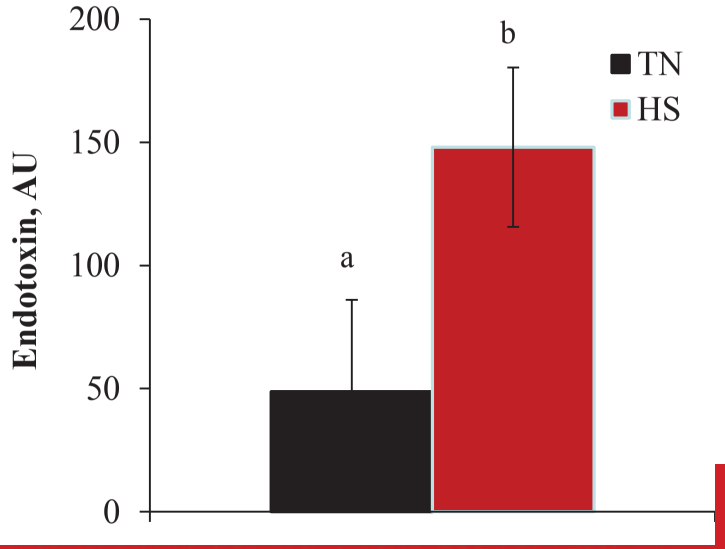


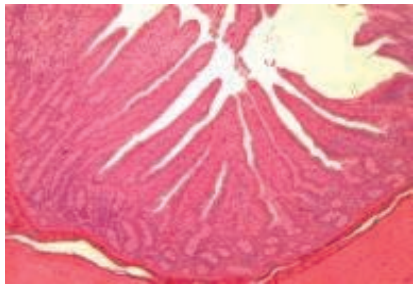
Heat stress reduces intestinal integrity and increases intestinal endotoxin permeability

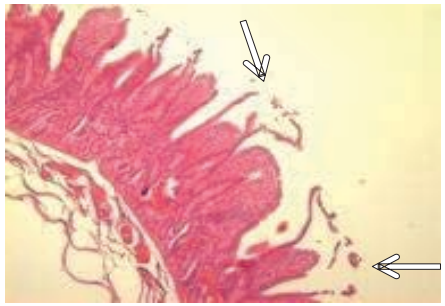
Parameter	Environment		*P-value*
	TN	HS	
Ileum TER[1], Ω x cm^2	182 ± 17.4	88 ± 18.6	<0.01
Colon TER[1], Ω x cm^2	133 ± 7.2	102 ± 8.3	0.01
Ileum FD4 permeability[2]	3.6 ± 0.93	7.9 ± 1.08	0.01
Colon FD4 permeability[2]	2.7 ± 3.55	15.7 ± 3.55	0.02

[1]Transepithelial electrical resistance

[2]FITC-Dextran 4.4 kDa permeability, µg/mL/min/cm

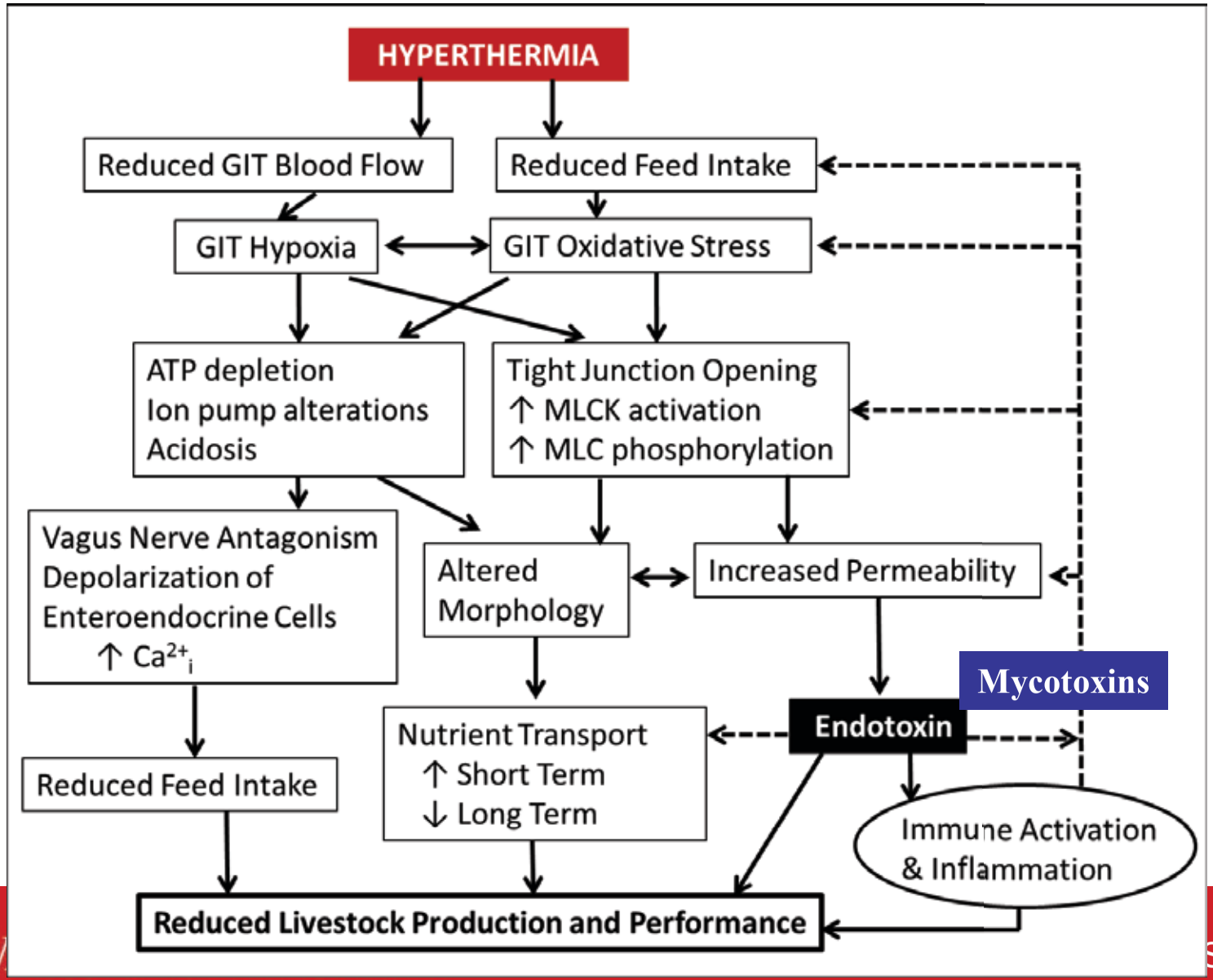




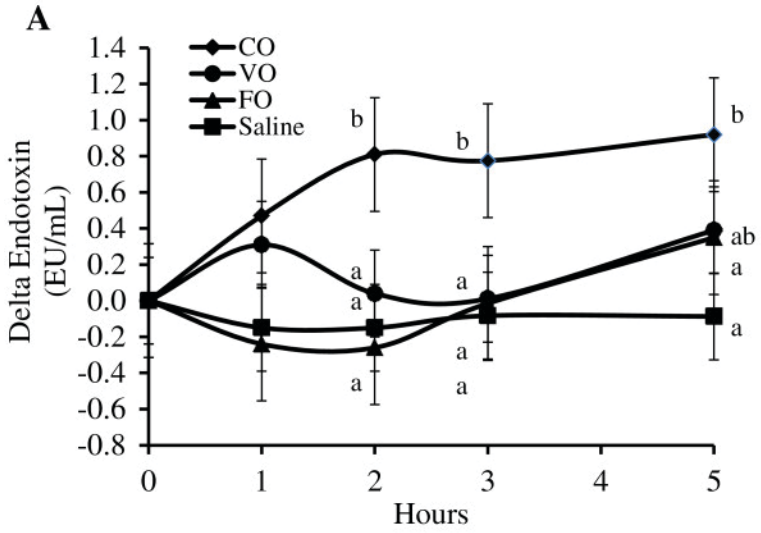


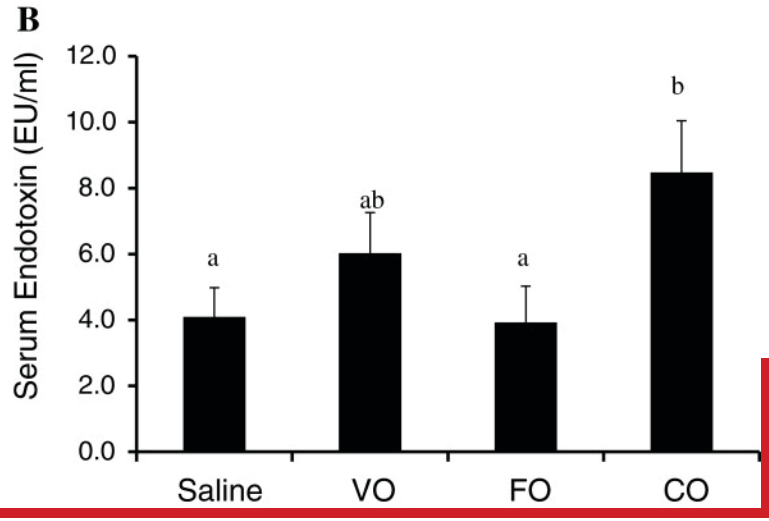
~50 kg pigs, constant 35° C for 24 h, 25-40% RH.

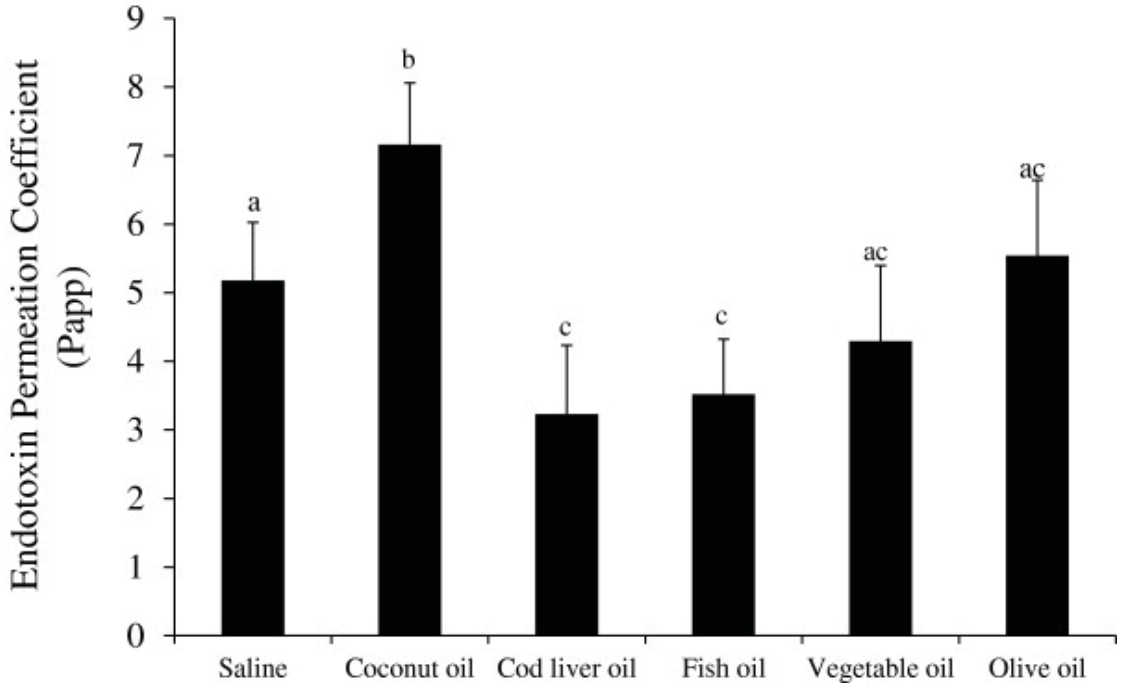
Pearce et al., 2013 PLoS ONE 8(8): e70215.


HYPERTHERMIA
Reduced GIT Blood Flow
Reduced Feed Intake
GIT Hypoxia
GIT Oxidative Stress
ATP depletion
Ion pump alterations
Acidosis
Tight Junction Opening
↑ MLCK activation
↑ MLC phosphorylation
Vagus Nerve Antagonism
Depolarization of
Enteroendocrine Cells
↑ Ca^{2+}_i
Altered
Morphology
Increased Permeability
Mycotoxins
Endotoxin
Nutrient Transport
↑ Short Term
↓ Long Term
Reduced Feed Intake
Immune Activation
& Inflammation
Reduced Livestock Production and Performance

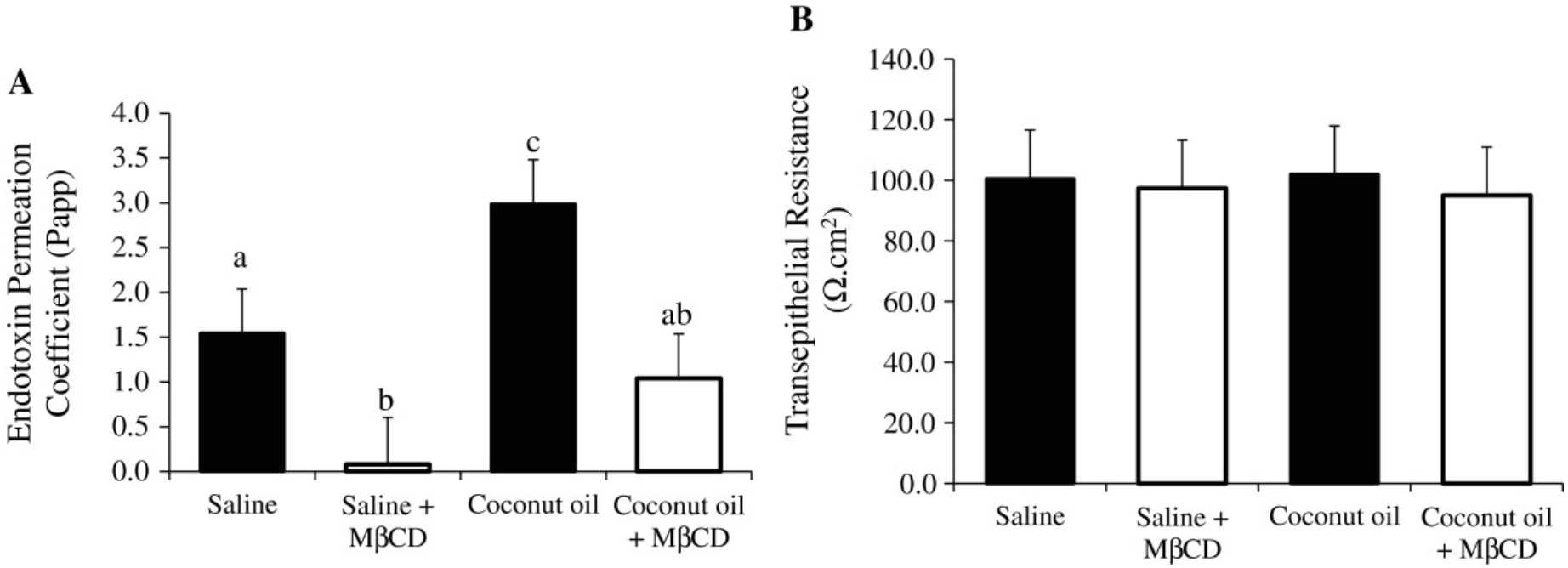
Dietary oil composition differentially modulates intestinal endotoxin transport and postprandial endotoxemia Mani et. Al., 2013 *Nutrition & Metabolism* **10**:6



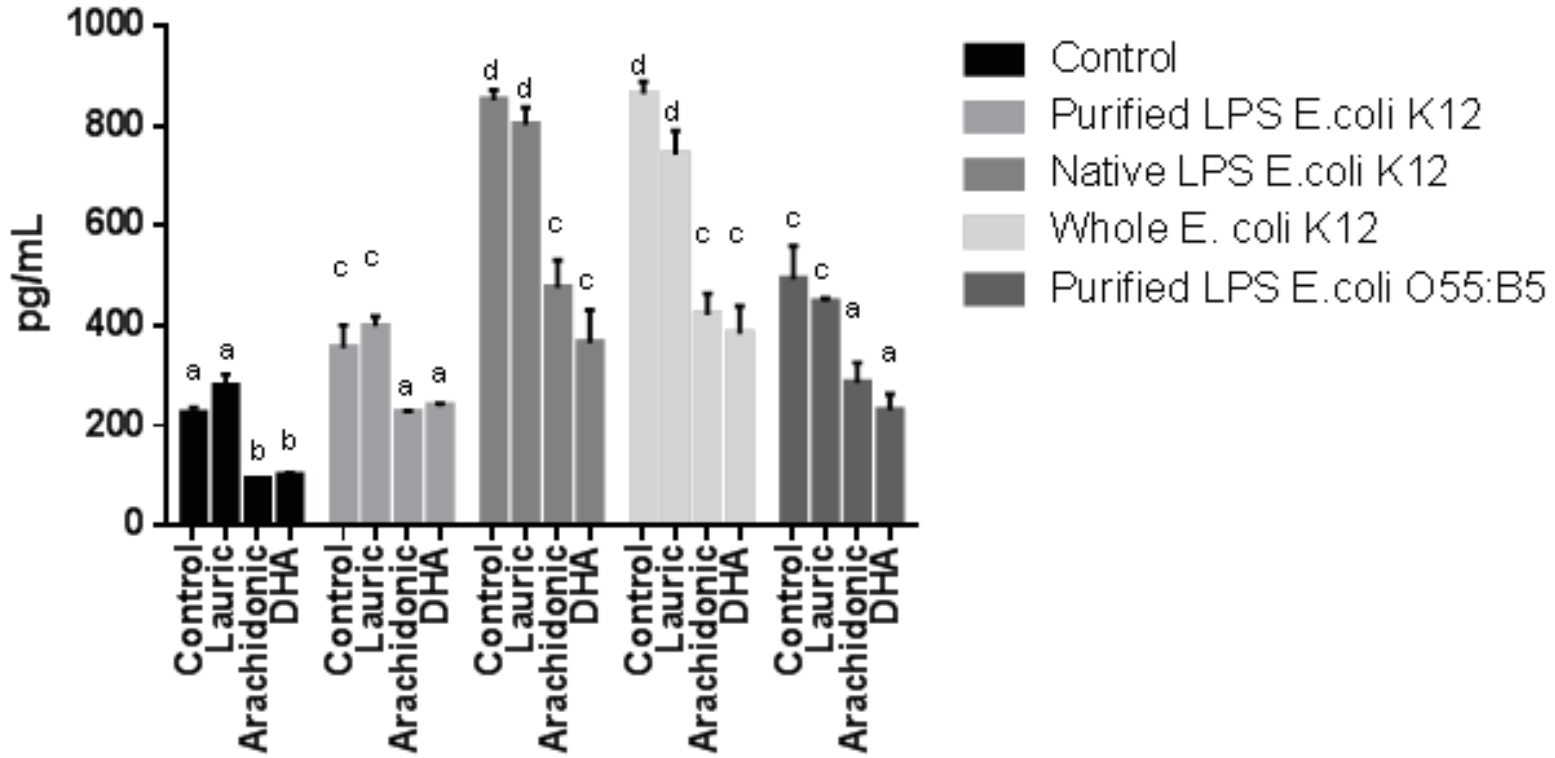




Dietary oil composition differentially modulates intestinal endotoxin transport and postprandial endotoxemia Mani et. al., 2013 *Nutrition & Metabolism* **10**:6



IL-8 secretion from IPEC-J2 treated apically with different LPS molecules



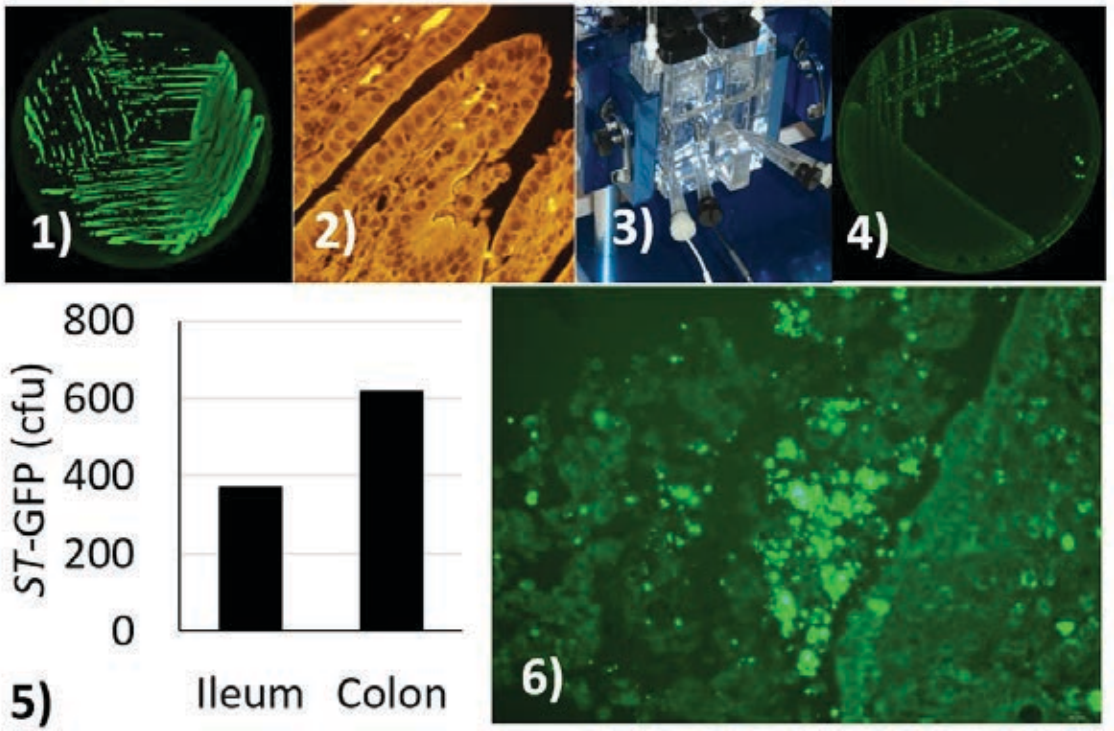
- Whole and lysed bacteria are potent immunogens to pig intestinal epithelial cells

Bacterial translocation model

- *Ex vivo* model using the Ussing Chambers
  - "Screening tool"

- Labelled bacteria
  - *E. Coli K12 or* F18 - pmCherry
  - *Salmonella typhimurium* – GFP (ATCC)

- Bacterial → host
  1. Adherence
  2. Internalization
  3. Translocation



Dietary fat content alters bacteria translocation

Effects of dietary fat and beta-methyl cyclodextrin raft inhibitor on ex vivo jejunum serosal *E. coli* K12 flux.

	Low Fat -Cont	Low Fat -raft	High Fat -Cont	High Fat -raft	SEM	*P*-value Diet	*P*-value Inhibitor	*P*-value D x Inh.
cfu	187^{a}	183^{a}	1373^{c}	969^{b}	139.1	<0.001	0.143	0.048

Effects of dietary fat and a TLR-4 specific antagonist (LPS from *Rhodobacter sphaeroides*on) on ex vivo jejunum serosal *E. coli* K12 flux.

	Low Fat -Cont	Low Fat -TLR4	High Fat -Cont	High Fat -TLR4	SEM	*P*-value Diet	*P*-value Inhibitor	*P*-value D x Inh.
Cfu	188^{ab}	85^{a}	1609^{c}	303^{b}	105.8	<0.001	<0.001	<0.001

- Pigs consuming High Fat diets composing of lard and soybean oil have increased ex vivo intestinal *E. coli* translocation in a TLR4/raft mediated manner

Conclusions

- Depending on the environmental and genetic conditions
  - Breed differences
  - Pre- and post-absorptive effects
  - Enteric verses systemic pathogens
  - Endotoxin/LPS verses Gram negative bacteria
  - Paracellular (“leaky”) verses transcellular enter

- Gram negative pathogenic bacteria/components antagonize livestock health and performance
  - Inhibition of endotoxin/bacteria absorption across the gastrointestinal tract and attenuation of inflammation
    - Dietary fat
    - Probiotics/Prebiotics/fiber
    - Phytogenic compounds
    - Binders

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 # 2014-67017-21778 (Fatty acids)

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  - Dr. Brian Kerr
- Iowa Pork Producers Association (IPPA)

MYCOTOXIN EFFECTS: MAKING SENSE OF COMPLEX BIOLOGICAL INTERACTIONS

Duarte Diaz Ph.D.
Department of Animal and Comparative Biomedical Sciences
University of Arizona

Why have mycotoxin concerns increased?

- Better analytical methods

Mycotoxin Analysis

Analytical Methods

- Fully quantitative

- Semi-quantitative

- Rapid monitoring

Mycotoxin Specific Sampling Protocols

The variability measured by the variance associated with a 0.91 kg sample, 50 g subsample, measuring aflatoxin in 1 aliquot by immunoassay in a lot of shelled corn at 20 ppb aflatoxin.

	Variance	Ratio %
Sample = 0.91 kg	268.1	75.5
Sub S^2, 50g	56.3	15.9
Immunoassay, 1 aliquot	30.4	8.6
Total	354.8	100

Sampling, sample preparation and analysis errors account for about 75.5, 15.9 and 8.6% of the total error, respectively.

Whitaker et al. 2005

Sampling Recommendations

Table 6. Effect of increasing sample size on reducing the sampling variability[1].

	Sample size (kg)	
	0.91 kg	*4.54 kg*
Variance	266.5	53.3
Subsample[2], 50 g	56.3	56.3
TLC, 1 aliquot	27.9	27.9
Total	**350.7**	**137.5**
Range	20 ± 37	20 ± 23

Increasing sample size by a factor of five from 0.91 to 4.54 kg will cut the sampling variance in by a factor of five from 266.3 to 53.3 (80%). The total variance is reduced from 350.7 to 137.5 (60%).

Whitaker et al. 2005

Mold and Toxin Distribution

Protein

12	13	12	14
13	13	14	12
15	11	12	12
13	14	11	9
13	12	12	13

Mean Protein Concentration 13%

(USDA)

Aflatoxin

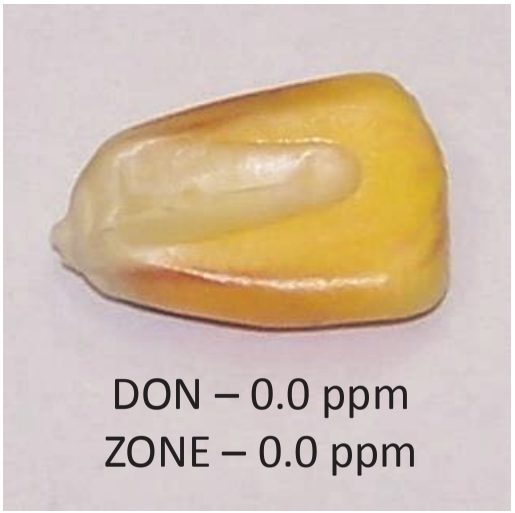
0	0	0	0
0	0	200	0
0	0	0	0
0	0	0	0
0	0	0	0

Mean Aflatoxin Concentration 10ppb

(USDA)

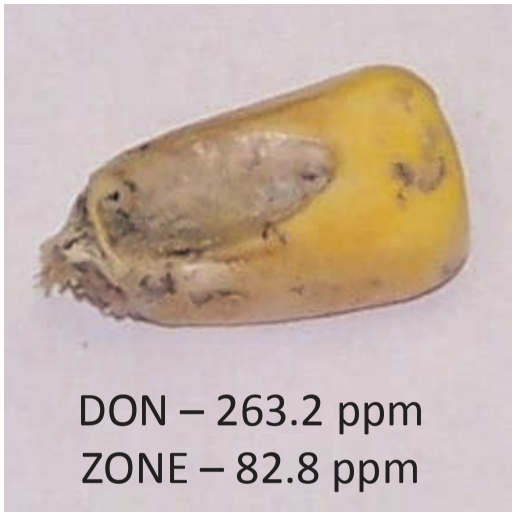
Visual Biases

DON – 7.0 ppm
ZONE – 0.0 ppm



DON – 0.0 ppm
ZONE – 0.0 ppm

DON – 2.1 ppm
ZONE – 4.4 ppm



DON – 263.2 ppm
ZONE – 82.8 ppm

DON – 1.8 ppm
ZONE – 0.3 ppm

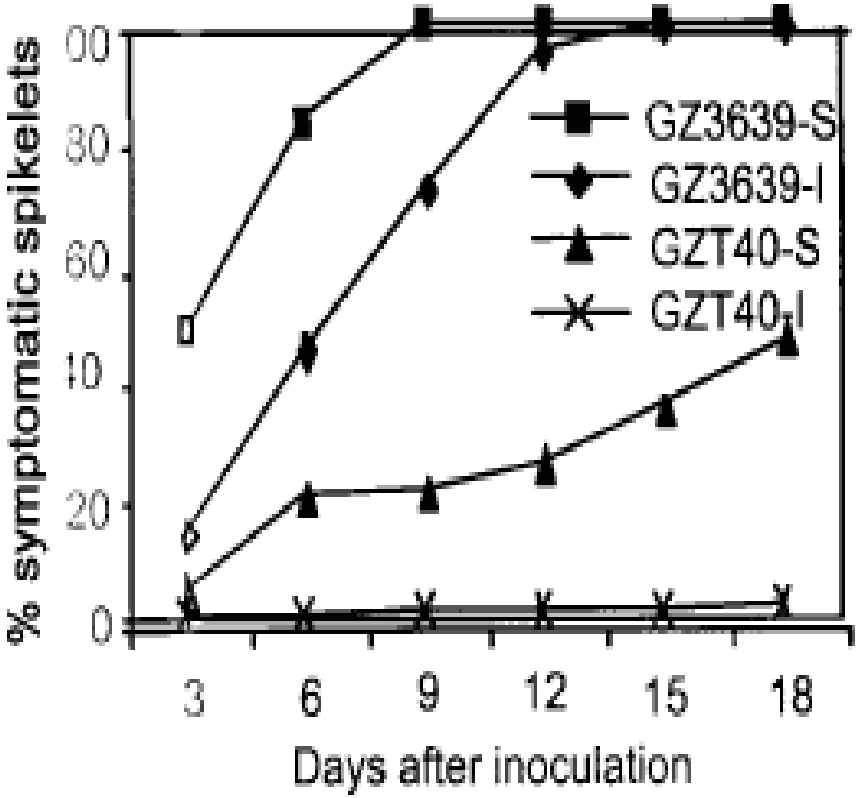
Courtesy of Trilogy Labs

Why have mycotoxin concerns increased?

- Better analytical methods
- Understanding of their occurrence and effects?

Biological significance

- Response to stress
- Competitive advantage (ecological)
- Mechanisms for propagation



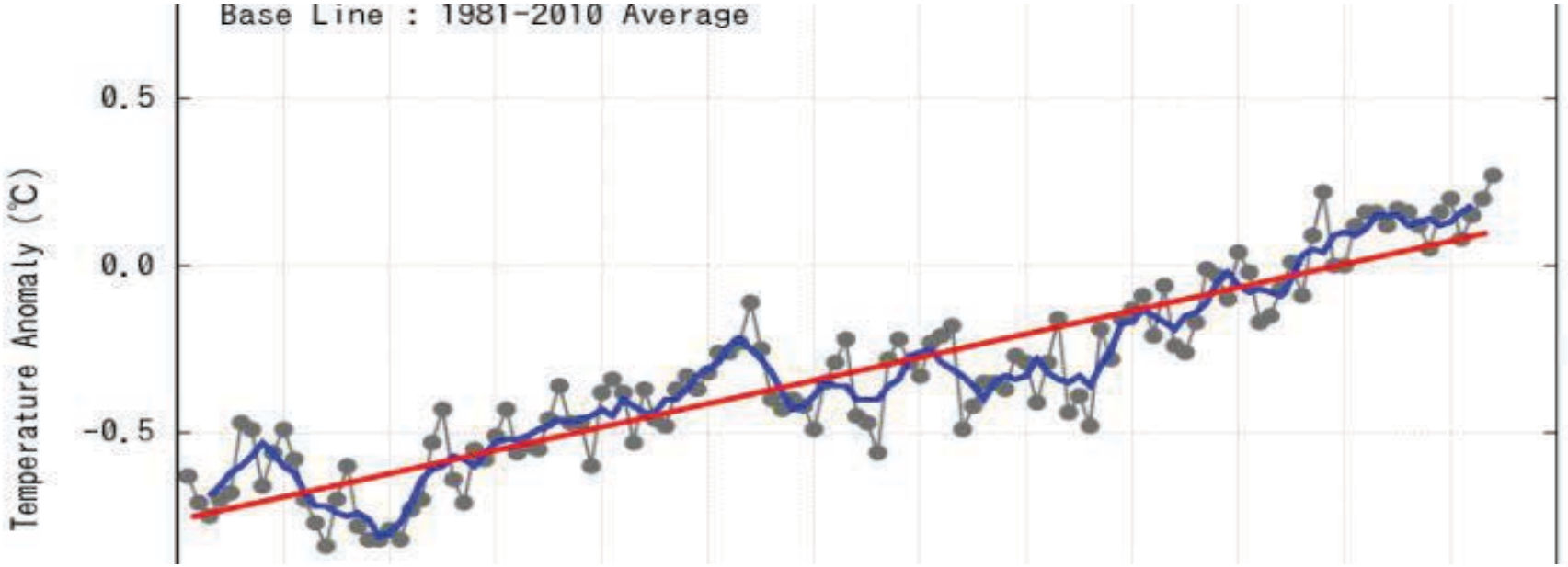
Bai et. al Mycopathologia 2001

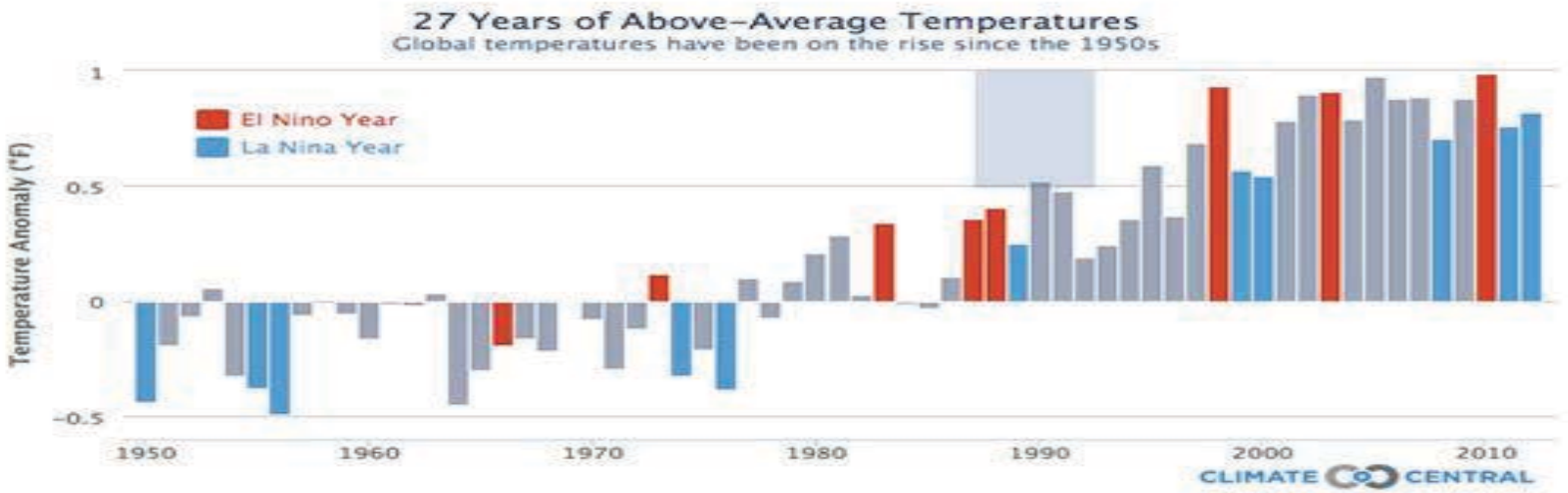
Why have mycotoxin concerns increased?

- Better analytical methods
- Understanding of their occurrence and effects
- Increased incidence in some years

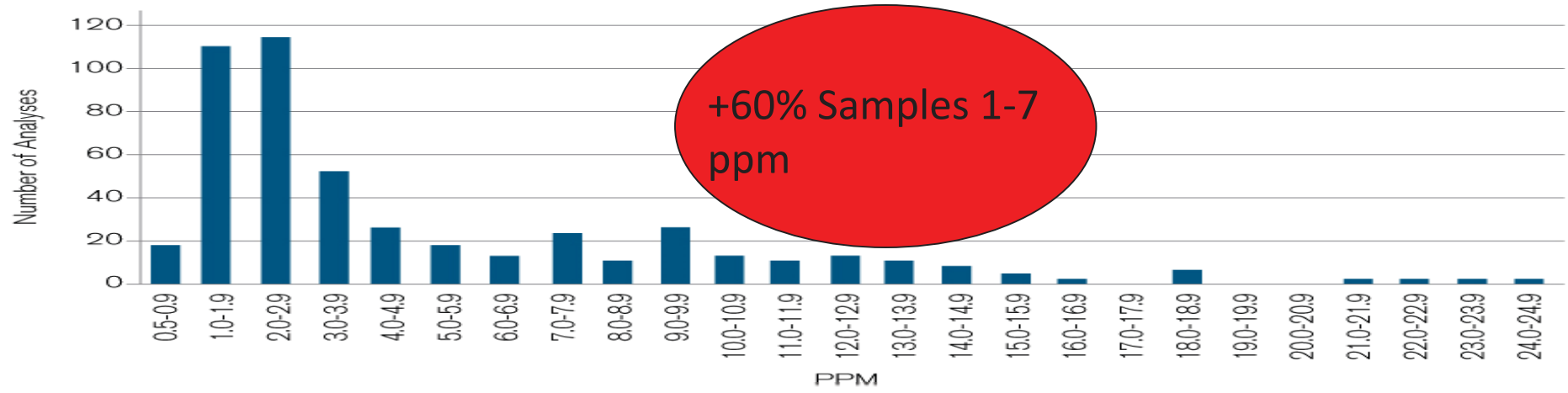
Environmental stresses

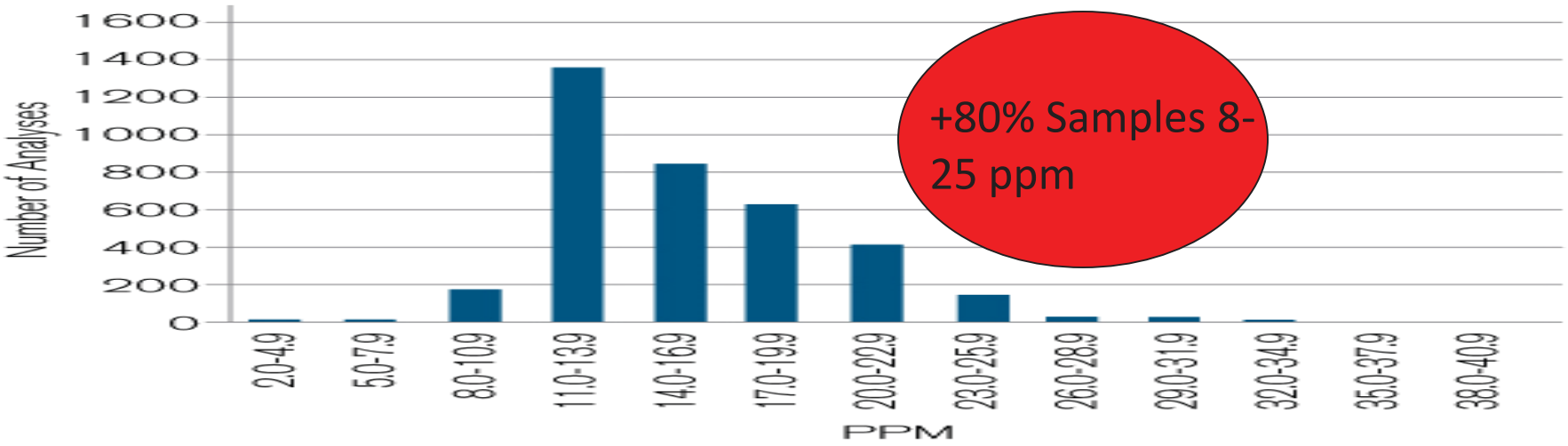
A Tale of 5 years





DON Results in 2009-2010 Ohio/Indiana Corn and DDGS

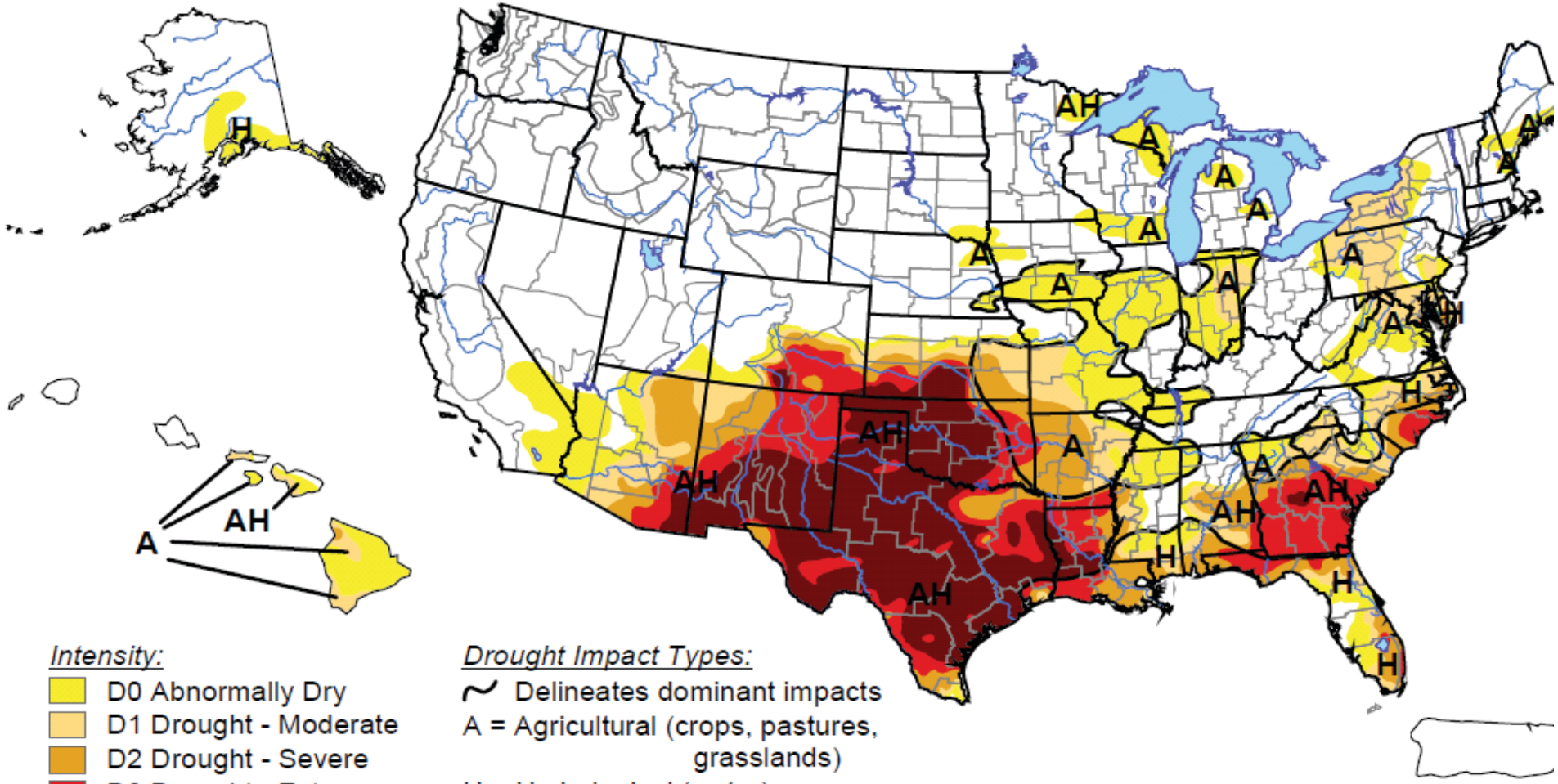




Courtesy of Trilogy Labs

U.S. Drought Monitor

August 2, 2011
Valid 7 a.m. EDT



Intensity:
- D0 Abnormally Dry
- D1 Drought - Moderate
- D2 Drought - Severe
- D3 Drought - Extreme
- D4 Drought - Exceptional

Drought Impact Types:
- ~ Delineates dominant impacts
- A = Agricultural (crops, pastures, grasslands)
- H = Hydrological (water)

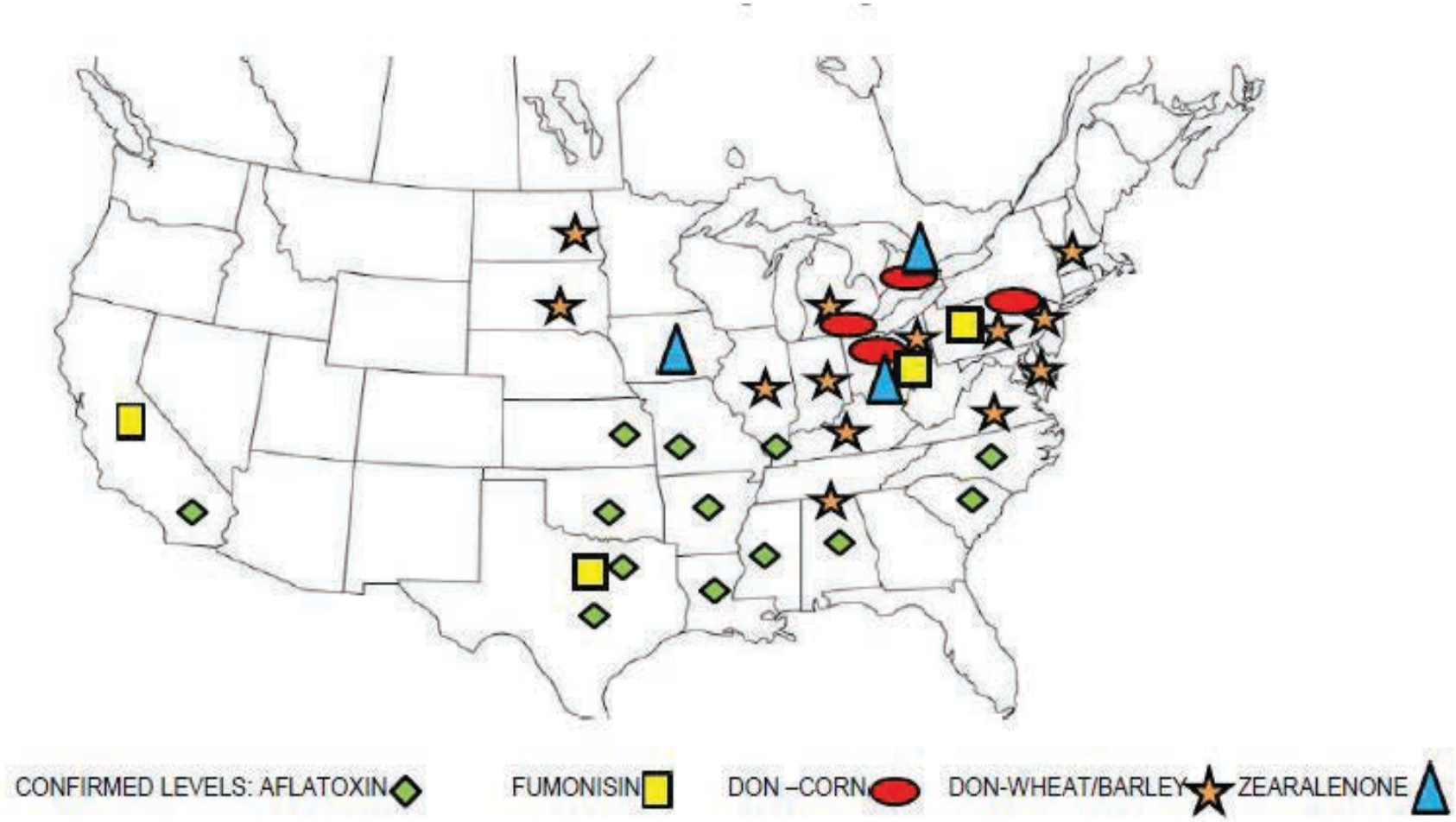
The Drought Monitor focuses on broad-scale conditions. Local conditions may vary. See accompanying text summary for forecast statements.

http://drought.unl.edu/dm

USDA National Drought Mitigation Center

Released Thursday, August 4, 2011

Author: Brad Rippey, U.S. Department of Agriculture

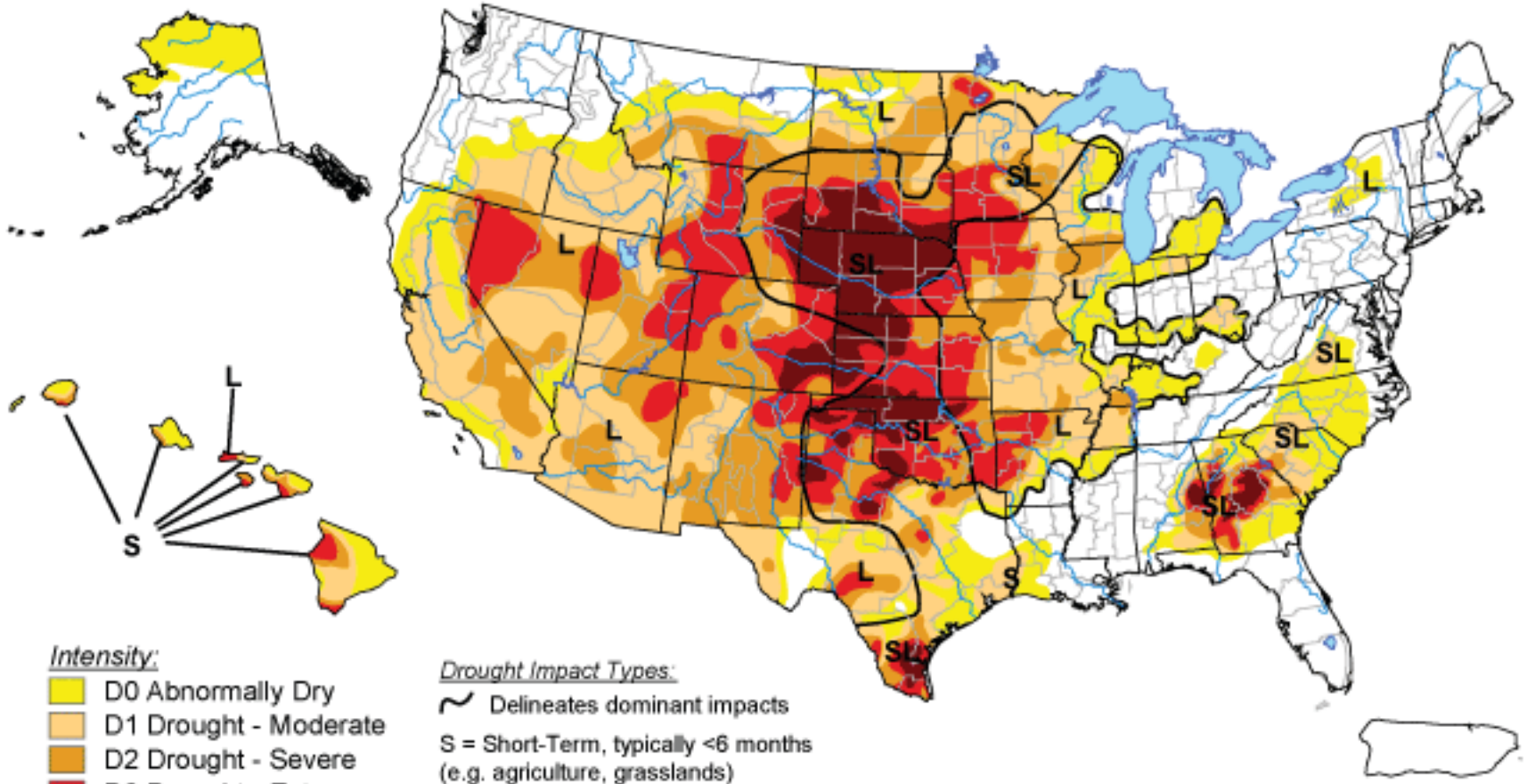


If you have questions please contact our office and speak with our staff. This report is brought to you by the Pet Food Department at Neogen. Reports are compiled from various sources and are subject to variability. For further details on the map or assistance with on-site mycotoxin monitoring please contact us at (800) 234-5333 or email us at foodsafety@neogen.Com or visit our website at www.Neogen.Com

NEOGEN® CORPORATION

U.S. Drought Monitor

November 13, 2012
Valid 7 a.m. EST



Intensity:
- D0 Abnormally Dry
- D1 Drought - Moderate
- D2 Drought - Severe
- D3 Drought - Extreme
- D4 Drought - Exceptional

Drought Impact Types:
~ Delineates dominant impacts

S = Short-Term, typically <6 months (e.g. agriculture, grasslands)

L = Long-Term, typically >6 months (e.g. hydrology, ecology)

The Drought Monitor focuses on broad-scale conditions. Local conditions may vary. See accompanying text summary for forecast statements.

http://droughtmonitor.unl.edu/

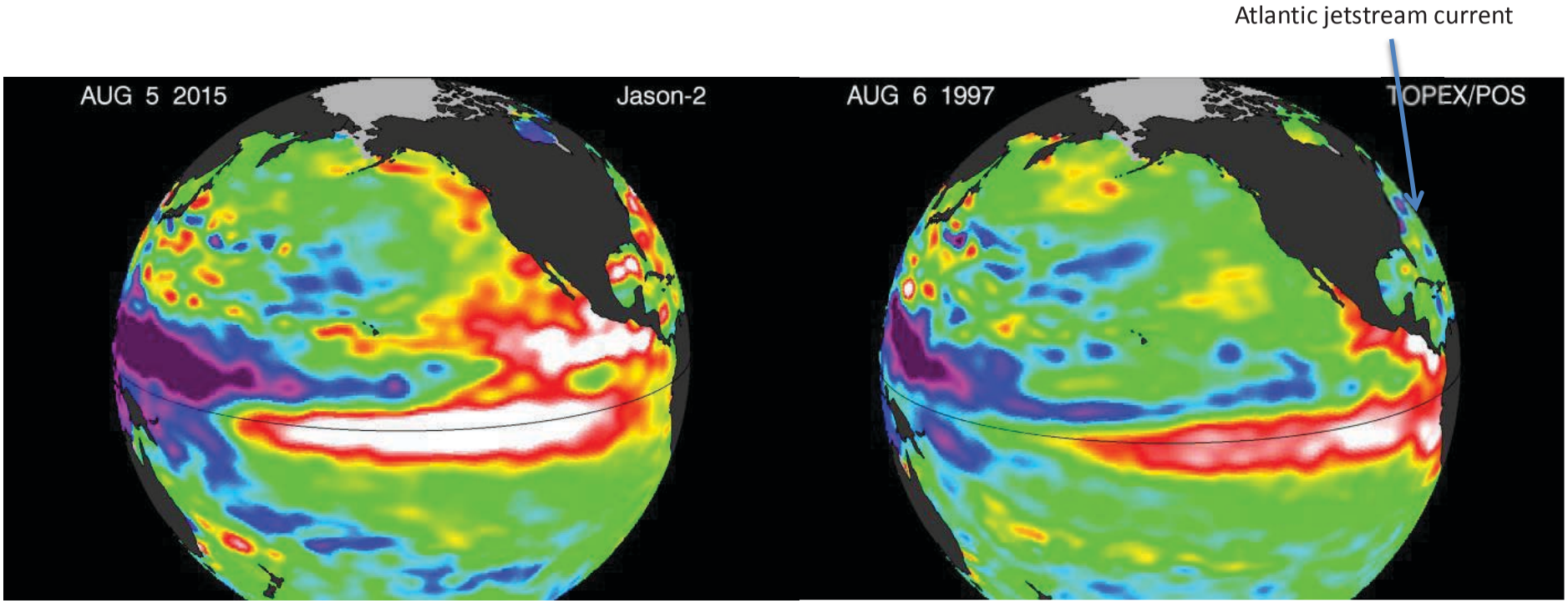
Released Thursday, November 15, 2012
Author: David Miskus, NOAA/NWS/NCEP/CPC

NEOGEN® CORPORATION

CONFIRMED LEVELS: Aflatoxin Fumonisin Zearalenone DON

CONFIRMED LEVELS OF **AFLATOXIN** IN NEW CROP CORN: Southern CA levels > 200 ppb; SC levels > 20 ppb; TX up to 1,000 ppb OK up to 200 ppb; KY > 20 ppb; AL up to 20 ppb; KS up to 80 ppb; MO up to 230 ppb ; IL up to 150 ppb; NE > 100 ppb, NC > 100 pp b; TN >30ppb; IA up to 100 ppb; IN over 150 ppb

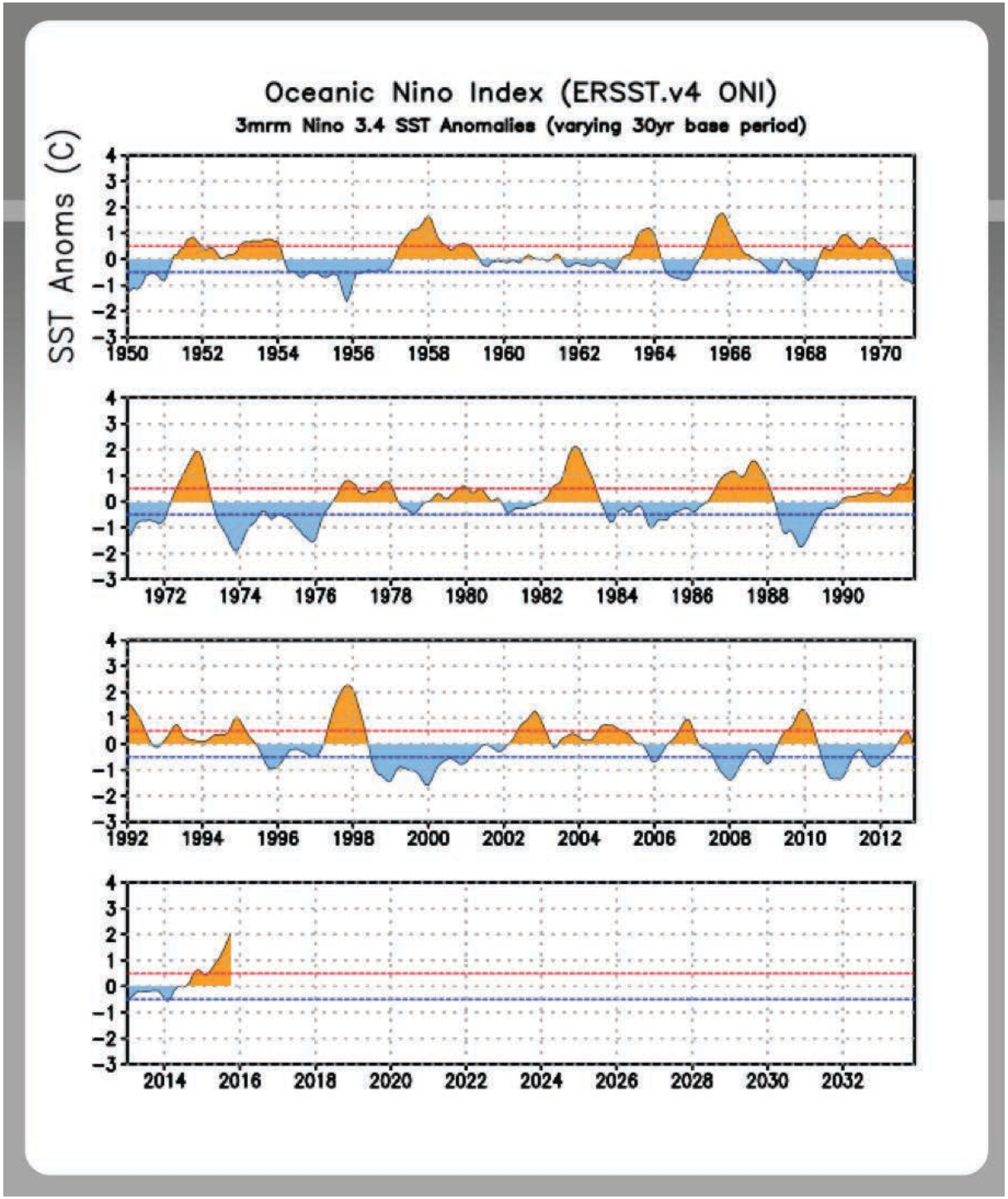
2015-2016 and Beyond



"The truth is we don't know what will happen. Will the two patterns reinforce each other? Will they cancel each other? Are they going to act in sequence? Are they going to be regional? We really don't know," said David Carlson, the director of the World Climate Research Programme.

El niño – characterized by a positive ONI greater than or equal to +0.5

La niña – characterized by a negative ONI greater than or equal to -0.5



Why have mycotoxin concerns increased?

- Better analytical methods
- Understanding of their occurrence and effects
- Increased incidence in some years
  - Environmental stresses
  - Agronomic practices

Impact of Agronomic Practices

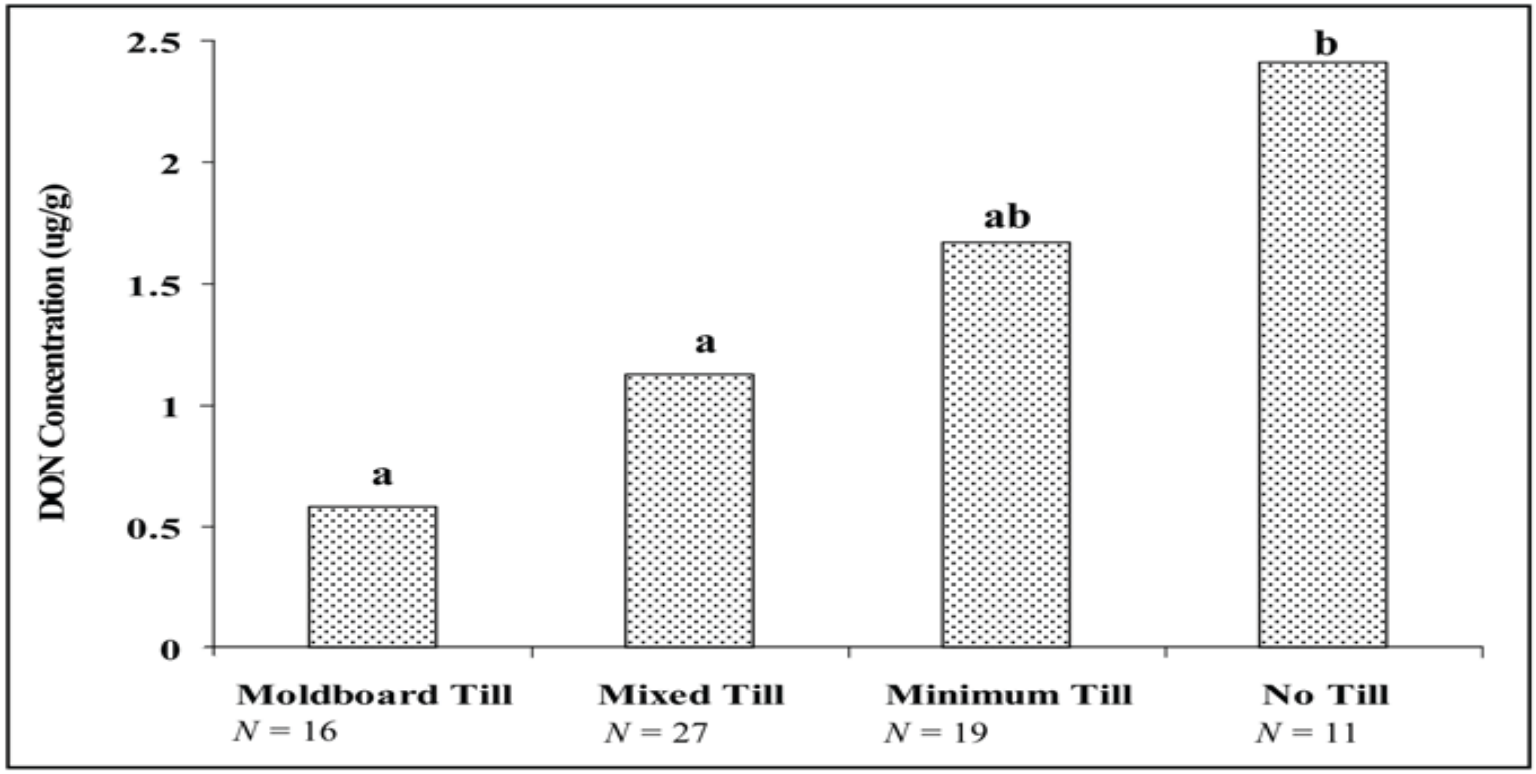


Fig. 6. Average deoxynivalenol (DON) concentration in 2001 and 2002 silage samples managed under different tillage systems. Mixed tillage refers to systems where more than one tillage type was used. Letters over bars indicate significant differences in DON concentration between tillage systems detected by the Tukey-Kramer test. Bars marked by the same letter are not significantly different.

Mansfield et. al. Plant Disease 2005

Why have mycotoxin concerns increased?

- Better analytical methods
- Understanding of their occurrence and effects
- Increased incidence in some years
- Higher production levels (animals)

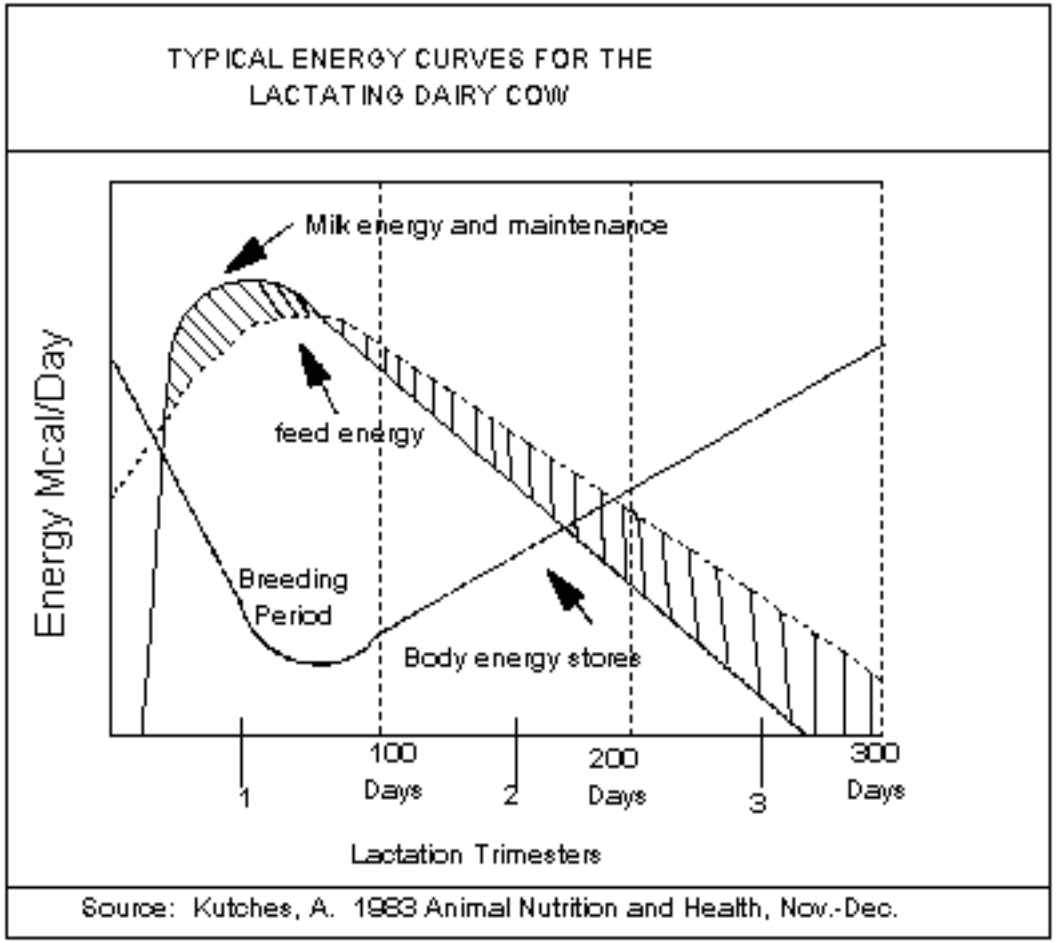
More general stress

Genetic vulnerability

Animal production changes/challenges

Animal Production Changes

	1960	2000
Piglets born / litter	9.8	10.9
Litter / sow / year	1.74	2.24
Pigs reared / per sow / year	13.3	21.9
FCR (kg/kg)	3.96	2.61
Overall herd feed requirements	6.18	3.81



Major mycotoxins in Dairy

Well known

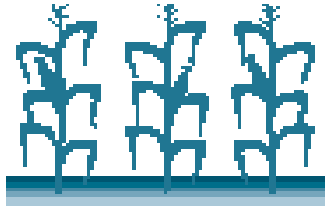
- Aflatoxin
- Zearalenone
- Fumonisins
- Tricothecenes (DON and T-2 toxin)

Least know

- Penicillium produced
  - Penicillic acid
  - Mycophenolic acid
  - Patuline
  - PR toxin
- Ochratoxin A

120

Factors affecting Mycotoxin occurrence in the food chain



Biological factors

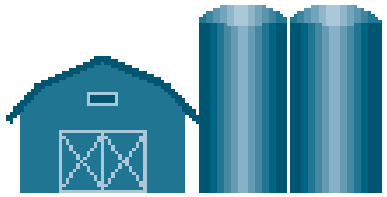
- Susceptible crop
- Compatible toxigenic fungus

Environmental factors

- Temperature
- Moisture
- Mechanical injury
- Insect/bird damage
- Fungus

Harvesting

- Crop maturity
- Temperature
- Moisture
- Detection/Diversion



Storage

- Temperature
- Moisture
- Detection/Diversion

Distribution - Processing

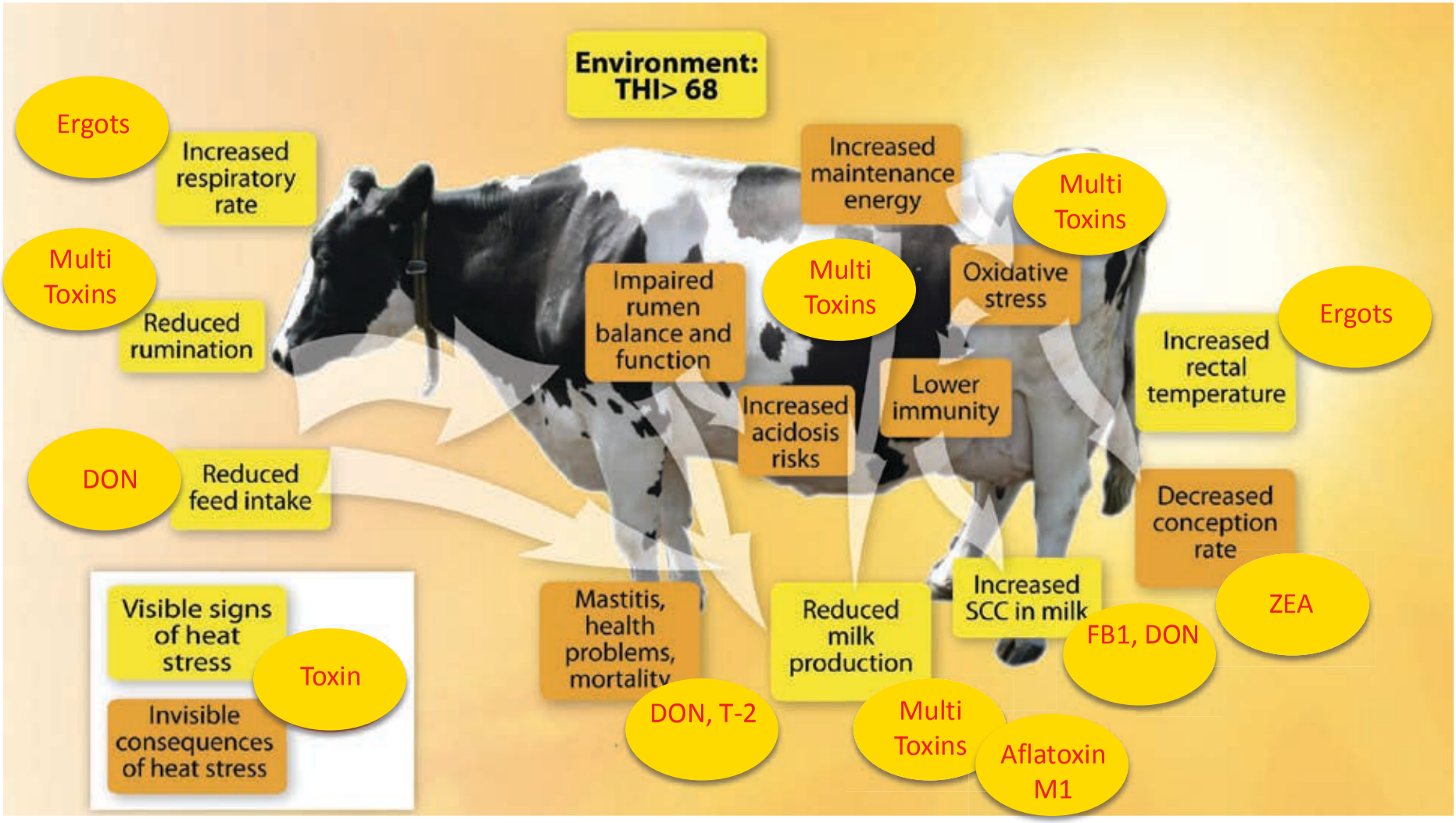
- Detection/Diversion

Humans

Animals

Animal products

New Area - Heat Stress - Mycotoxin



Control Strategies

Pre-harvest Management	- Insect Management - Management of crop residues - Irrigation and mineral nutrition - Crop rotation - Resistant Varieties - Bio-control	
Harvest Management	- Timelines - Cleanup - Drying	- Processing and manufacturing - Good manufacturing practices - Strict quality control
Post-harvest Management	Storage: Moisture and insect control	-Decontamination strategies: -Physical Separation -Physical decontamination -Biological decontamination -Chemical inactivation -Chemo-adsorption

Control Strategies

Bio-control / AF36

USDA ars Agricultural Research Service

Aflatoxin Reduction in Crops

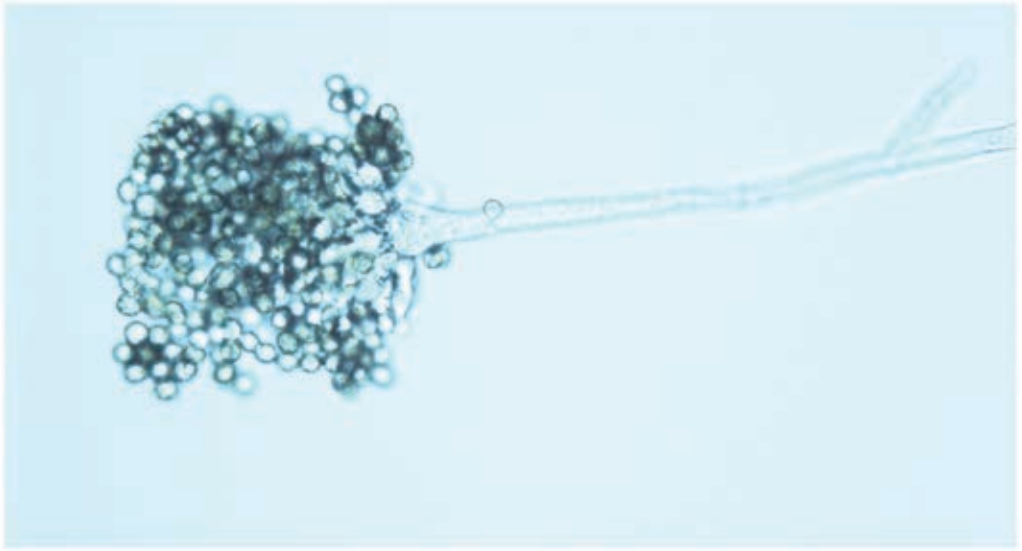
Aflatoxin Management from Etiology, Epidemiology, and Population Biology

Research Lab in Action Protocols Manufacturing Publications Partners People Contact

Biocontrol of Aspergillus Flavus

For the past decade, over 150,000 acres of crops have been treated with strains of A. flavus that do not produce aflatoxins.

With domestic and international collaborators including farmers and industry organizations in Arizona, Texas, and California, we seek development of practical methods to utilize atoxigenic-strain technologies to reduce contamination. This includes development of grower-fun, commercial-scale atoxigenic strain manyfacturing facilities and area-wide alfatoxin management strategies with partner organizations.



Nutritional Toxicology in the Dairy Industry: Feed Quality and Mycotoxins

Duarte Diaz Ph.D.

Department of Animal and Comparative Biomedical Sciences

Occurrence of mycotoxins in California dairy feeds

Paige Gott PhD
Ruminant Technical Manager

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Mycotoxin Basics

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What are mycotoxins?

Fusarium spp.

Aspergillus spp.

Penicillium spp.

Toxic, secondary metabolites produced by fungi

- Produced on almost all agricultural commodities worldwide
- 300-400 mycotoxins identified (Bennett and Klich, 2003)
- High stability:
  - chemically and heat stable
  - persistent during storage
  - resistant to processing methods

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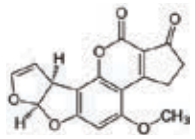
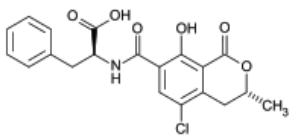
What are mycotoxins?



Aflatoxins

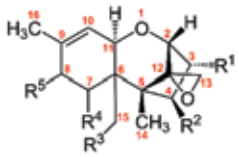
Aspergillus spp.



Ochratoxin A

Fusarium spp.

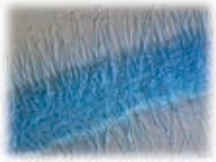
Penicillium spp.



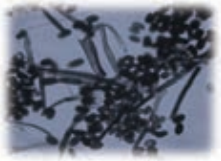
Trichothecenes

Fusarium spp.

Trichoderma spp.

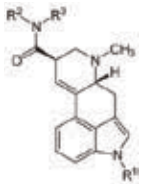


Acremonium spp.

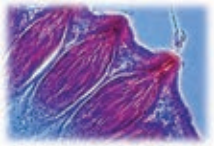


Stachybotrys spp.

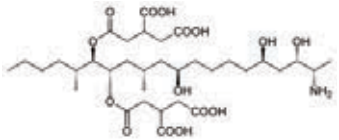
Myrothecium spp.



Ergot alkaloids



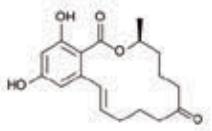
Claviceps spp.



Fumonisins

Fusarium spp.

Aspergillus spp.



Zearalenone

Fusarium spp.

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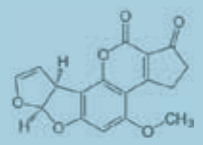
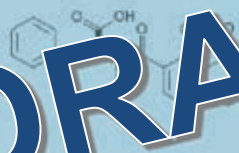
What are mycotoxins?

STORAGE



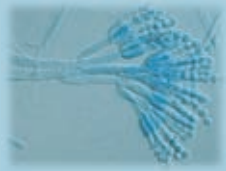
Aflatoxins

Aspergi… spp.



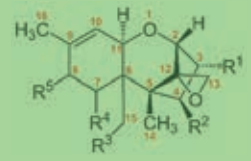
Ochratoxin A

Fusarium spp.



Penicillium spp.

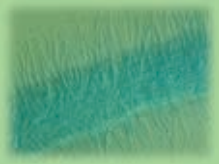
FIELD



Trichothecenes

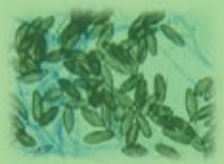
Fusarium spp.

Trichoderma spp.

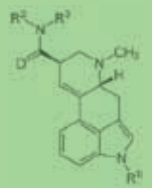


Acremonium spp.

Stachybotrys spp.

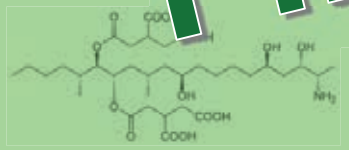


Myrothecium spp.



Ergot alkaloids

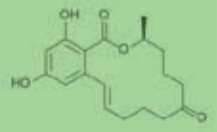
Claviceps spp.



Fumonisins

Fusarium spp.

Aspergillus spp.



Zearalenone

Fusarium spp.

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Mycotoxin production in the field

Production of mycotoxins by fungi in the field depends on:

- Temperature
- Relative humidity
- Insect attacks
- Bird/wildlife damage
- Stress conditions of the plants

Mitigation strategies: GMO corn, pest management strategies, Resistant plants, fungicide

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Storage

- Storage fungi
  - Moisture content
  - Storage humidity/temperature/location
  - Management
    - Packing density
    - Mold inhibitors, inoculants
    - Face management

“Emerging” & “Masked” Mycotoxins

Mycotoxin/Metabolite	+ve [n]	+ve [%]	Median [ppb]	Max [ppb]
Beauvericin	81	98	6.7	2326
Summe Enniatins	80	96	30.0	5441
Deoxynivalenol	74	89	122.0	25928
Emodin	74	89	9.8	1570
Equisetin	72	87	23.0	13680
Zearalenone	72	87	14.0	5326
Aurofusarin	70	84	85.0	17659
Alternariol methyl ether	68	82	1.4	733
Alternariol	66	80	2.8	221
Tentoxin	66	80	3.9	76
Moniliformin	63	76	45.0	12236
DON-3-Glucoside	62	75	15.0	7764
Culmorin	61	63	195.0	44616
Nivalenol	61	63	17.0	1760
Tryptophol	59	71	267.0	99040
Apicidin	55	66	1.9	160
Brevianamide F	54	65	69.0	2043
Tenuazonic acid	54	65	68.0	1983
15-Hydroxyculmorin	52	63	49.0	15620

IFA TULLN

Streit et al. 2013

Most often detected out of the 83 samples analyzed

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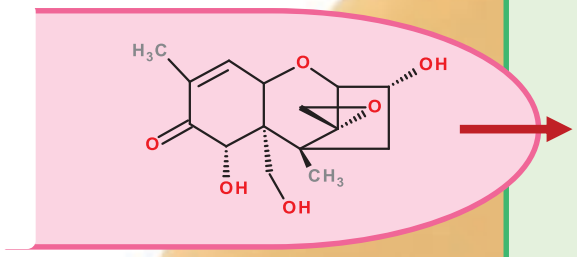
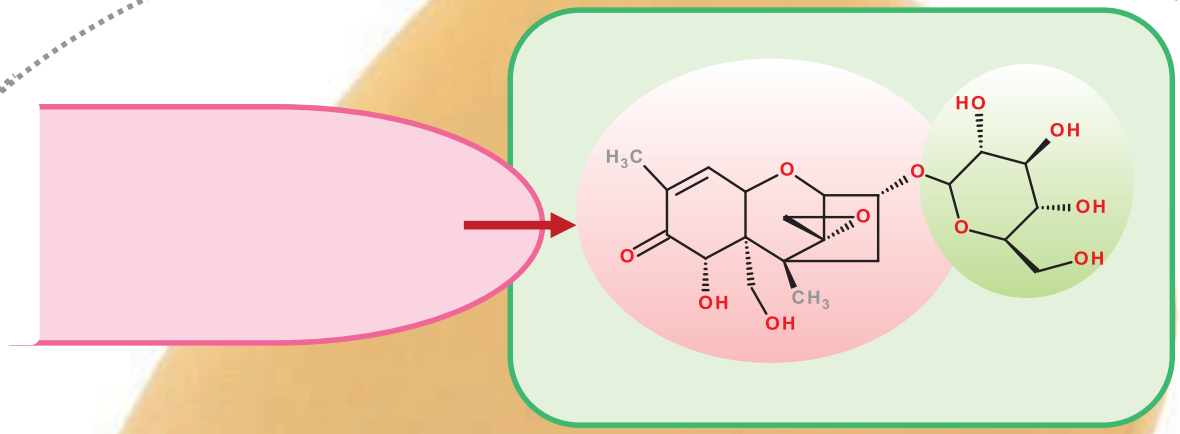
Masked mycotoxins

Fungus infects the plant



Fungus produces mycotoxins e.g. DON

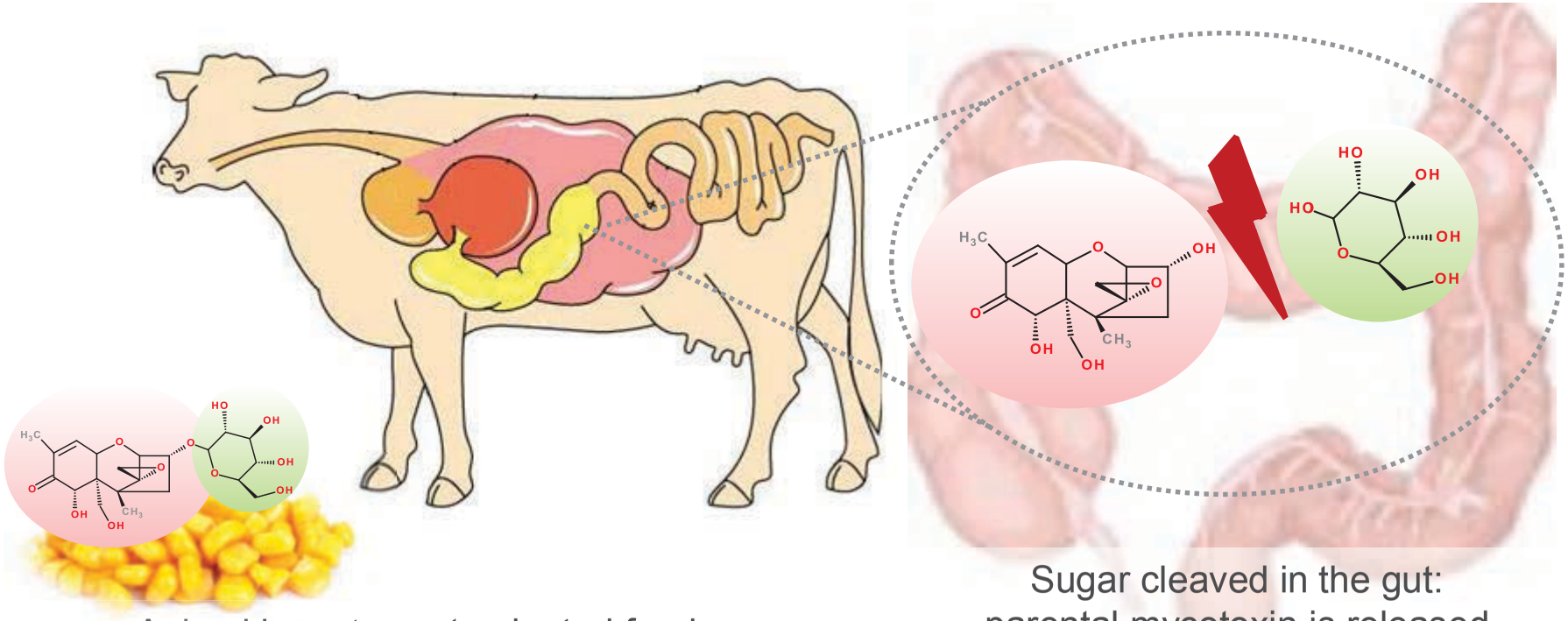
Masked mycotoxins



Defense mechanism of the plant: Conjugation of sugar to the mycotoxin = **masked mycotoxin**
e.g. DON-3-glucoside

Masked mycotoxins cannot be detected by conventional analytical methods!

Masked mycotoxins



Animal ingests contaminated feed containing masked mycotoxins

Sugar cleaved in the gut: parental mycotoxin is released
→ Increase in bioavailability

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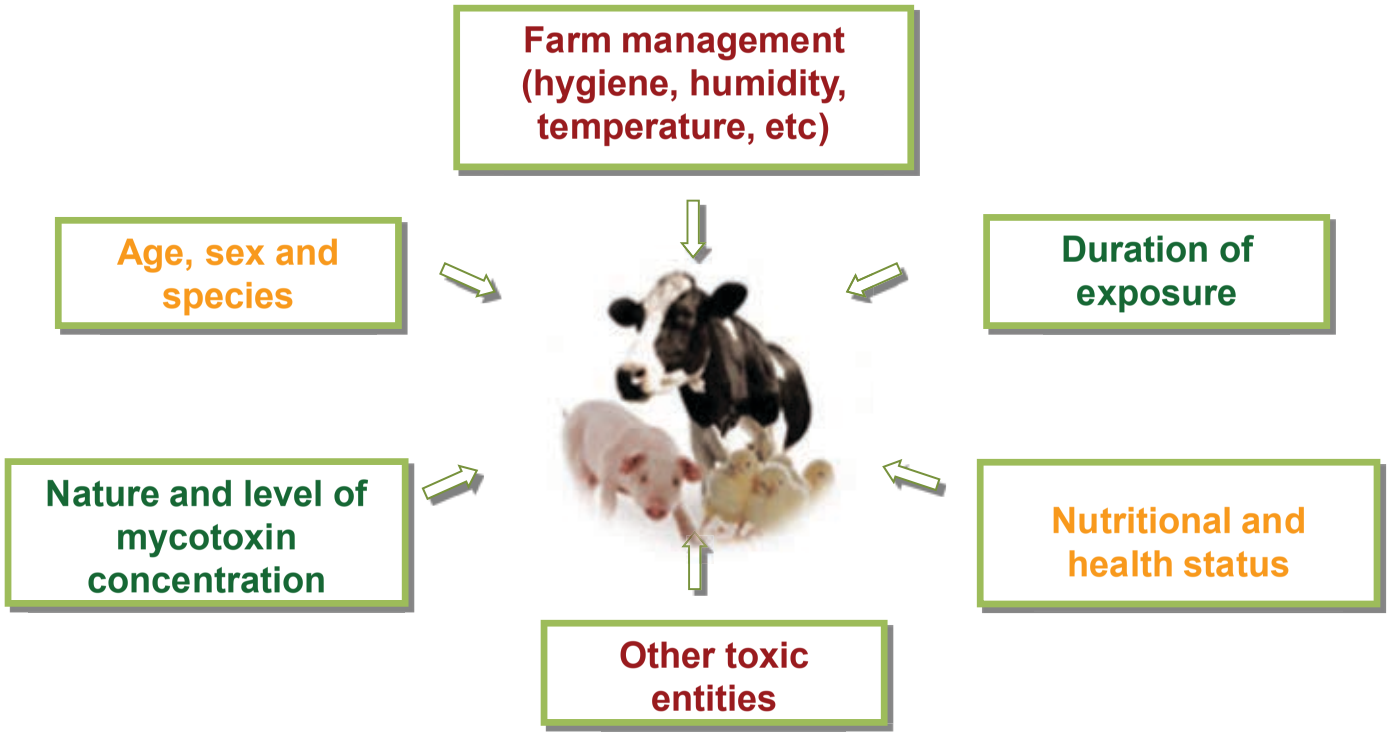
Mycotoxin effects

… **toxin**-, **animal**- and **environmental**-related factors:



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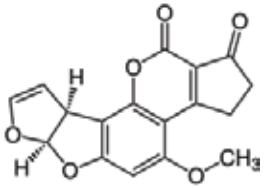
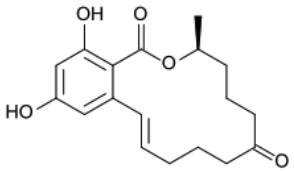
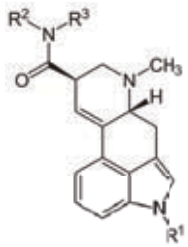
Mycotoxin effects



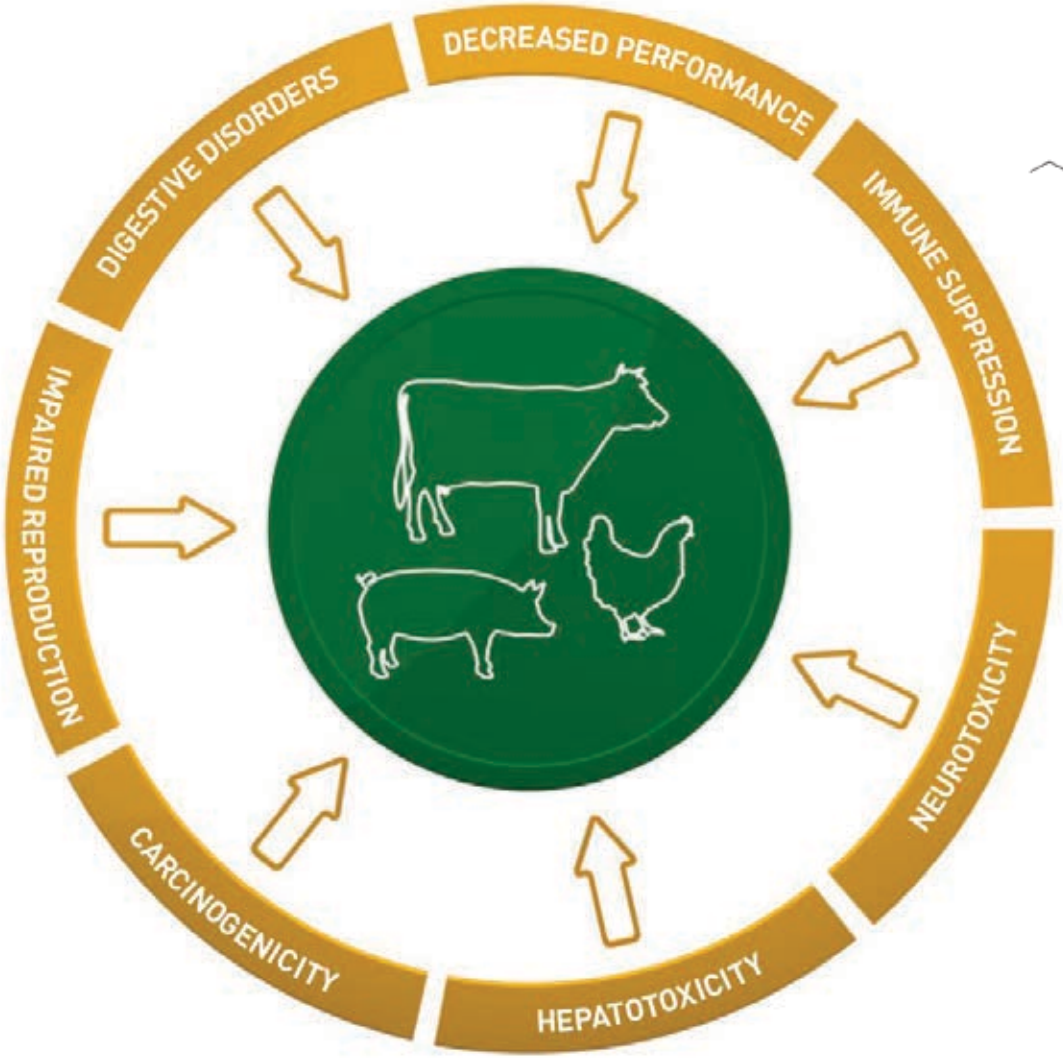
Aflatoxins

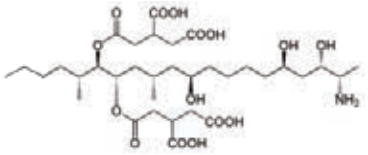


Zearalenone

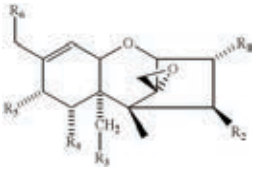


Ergot alkaloids

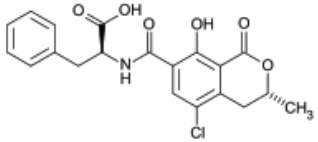




Fumonisins



Trichothecenes



Ochratoxin A

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Deoxynivalenol derivatives

DON can be modified by fungi, plant, animals and bacteria into several different metabolites - influencing its toxicity:

Metabolized by	DON-metabolites	Abbreviations
Fungi	3-acetyl-DON 15-acetyl DON	3AcDON 15AcDON
Plants	3-*O*-glucoside DON	D3G
Animals	DON-3-glucuronide DON-15-glucuronide	D3GA D15GA
Bacteria	De-epoxy-deoxynivalenol	DOM-1

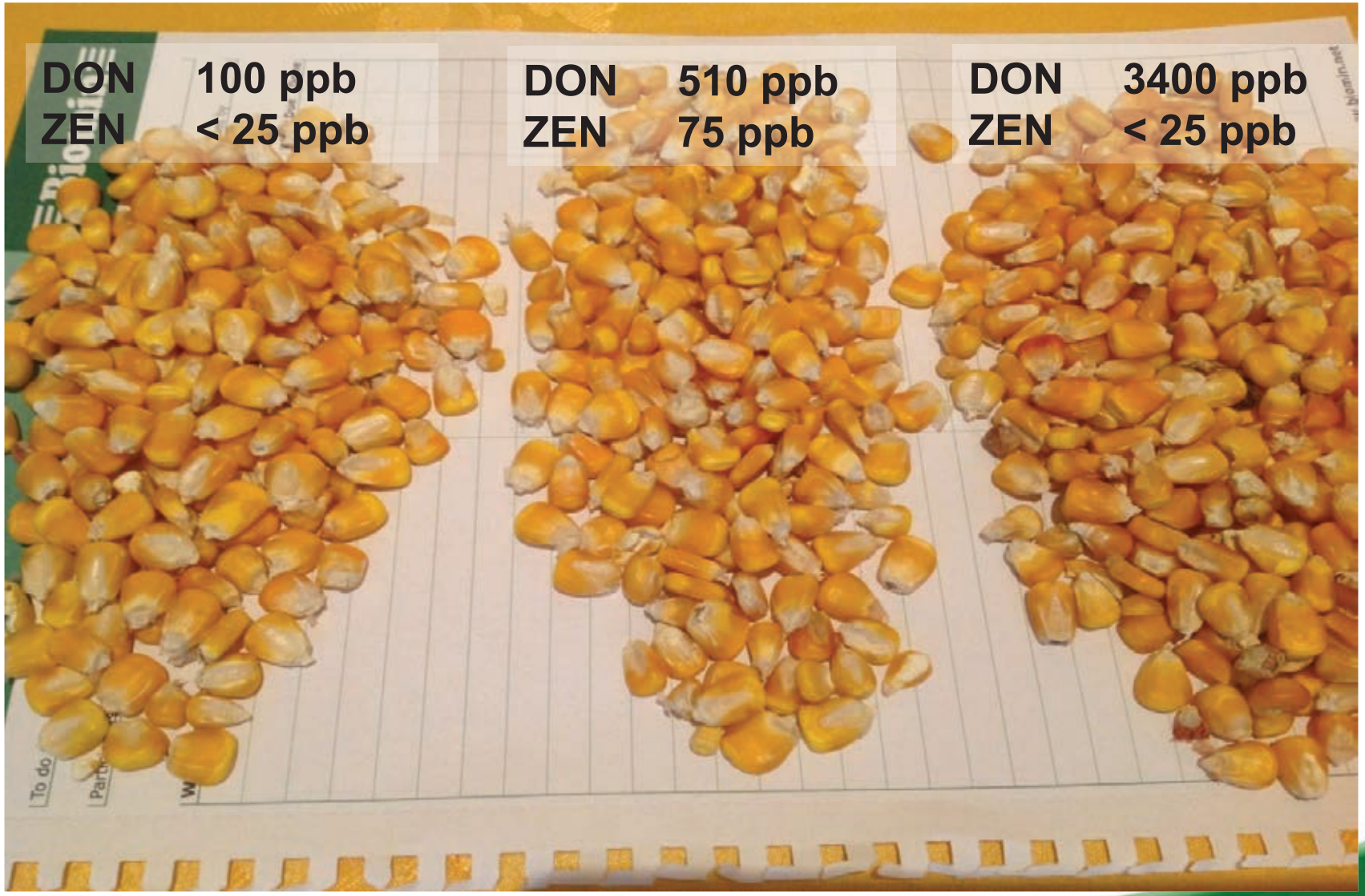
Deoxynivalenol derivatives

- 3/15AcDON and D3G, can account for an **additional up to 75 % of DON** contamination in feed.

Newly released wheat cultivars convert more efficiently DON to D3G

- **More resistant** towards DON producing *Fusarium graminearum,* but can contain **up to 10 times more D3G** than DON.

Mycotoxin detection



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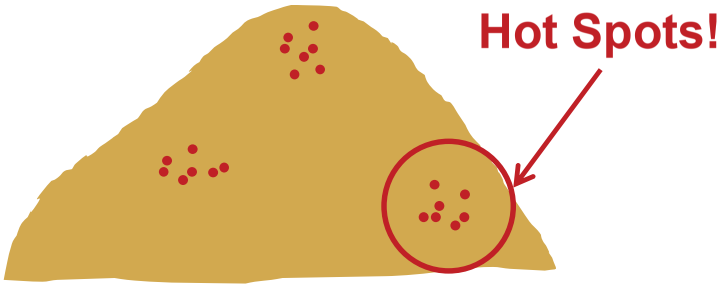
Mycotoxin detection

Uneven distribution of **MYCOTOXINS** in feeds:



Hot spots in silage:

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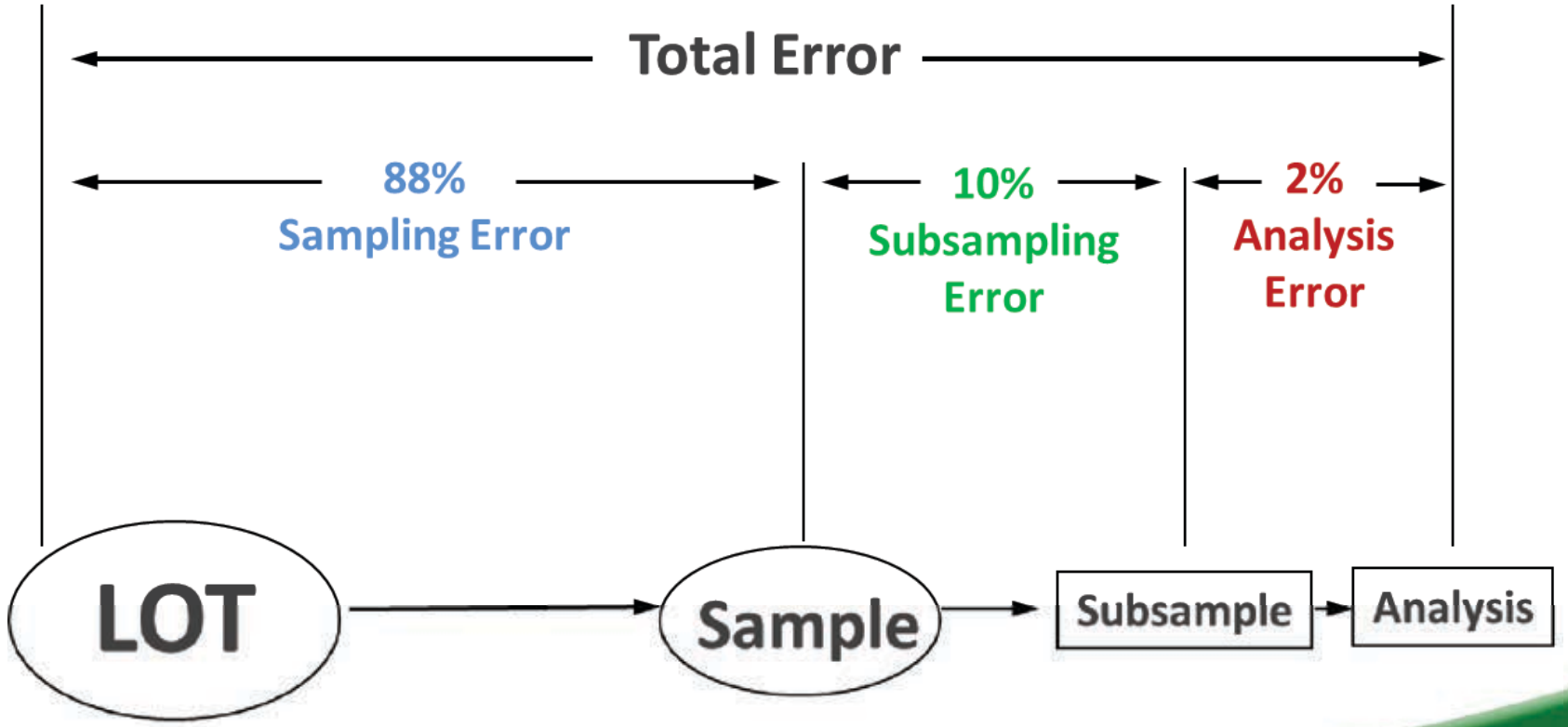
Mycotoxin sampling

Reliability of measured levels of mycotoxins is greatly affected by the collection of representative samples



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Mycotoxin analytical methods

ELISA
Quantification of specific mycotoxins in given matrices

+ Fast
+ Inexpensive
- Raw materials only

HPLC
Quantification of single toxins at low concentrations

+ Fullfils legal requirements
- More time consuming
- More expensive

LC-MS/MS:
Simultaneous detection of multiple toxins in a wide variety of commodities

+ Sensitive method
+ Suitable for various feed matrices
+ Detection of masked & emerging mycotoxins
- Highly qualified operator needed
- More expensive

BIOMIN Mycotoxin Surveys

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Occurrence of MTX in CA dairy feeds

- Samples collected from dairies in September 2016
- 100 samples total
  - 25 corn
  - 26 corn silage
  - 23 almond hulls
  - 26 cotton seed
- LC-MS/MS at Romer Labs, Inc.

BIOMIN mycotoxin panel

17 mycotoxins analyzed via LC-MS/MS method

- Type B trichothecenes
  - Deoxynivalenol
  - Nivalenol
  - Acetyl-DON
  - Fusarenon X
- Type A trichothecenes
  - T-2
  - HT-2
  - Diacetoxyscirpenol
  - Neosolaniol
- Aflatoxins
  - B_1, B_2, G_1, G_2
- Fumonisins
  - B_1, B_2, B_3
- Zearalenone
- Ochratoxin A

Occurrence of MTX in CA dairy feeds

Summary of 25 corn analyses

Parameters	B-Trich	FUM	ZEN	Afla	A-Trich	OTA
% positive	92	80	20	0	0	0
Average of positives [ppb]	461	635	414	0	0	0
Maximum contamination [ppb]	1054	1600	712	0	0	0

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Occurrence of MTX in CA dairy feeds

Summary of 26 corn silage analyses

Parameters	B-Trich	FUM	ZEN	Afla	A-Trich	OTA
% positive	0	23.1	0	0	0	0
Average of positives [ppb]	0	167	0	0	0	0
Maximum contamination [ppb]	0	300	0	0	0	0

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Occurrence of MTX in CA dairy feeds

Summary of 23 almond hulls analyses

Parameters	B-Trich	FUM	ZEN	Afla	A-Trich	OTA
% positive	4.3	0	0	0	0	4.3
Average of positives [ppb]	1841	0	0	0	0	6
Maximum contamination [ppb]	1841	0	0	0	0	6

Occurrence of MTX in CA dairy feeds

Summary of 26 cotton seed analyses

Parameters	B-Trich	FUM	ZEN	Afla	A-Trich	OTA
% positive	3.8	3.8	7.7	0	3.8	0
Average of positives [ppb]	105	200	128	0	477	0
Maximum contamination [ppb]	105	200	176	0	477	0

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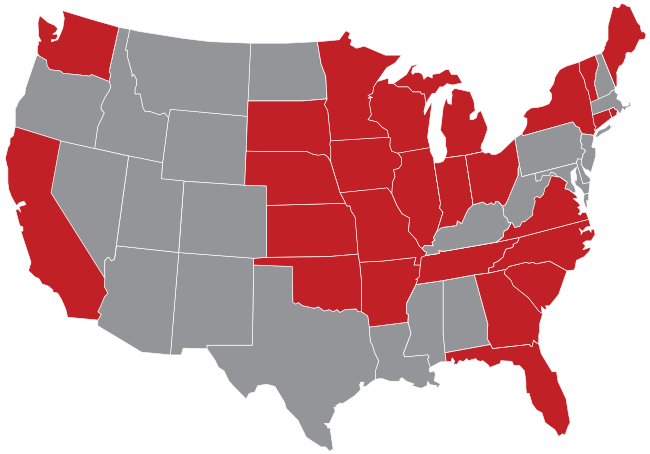
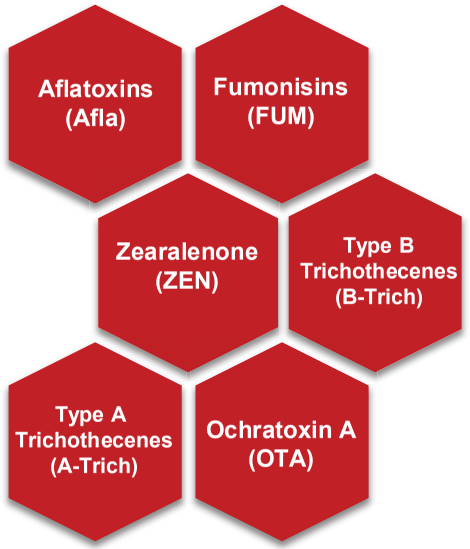
BIOMIN 2016 US Corn MTX Survey

387 corn samples



Collected from 26 states



6 major mycotoxins

LC-MS/MS

ROMER
Romer Labs®

IOWA STATE UNIVERSITY
Veterinary Diagnostic Laboratory

Actlabs

3 labs

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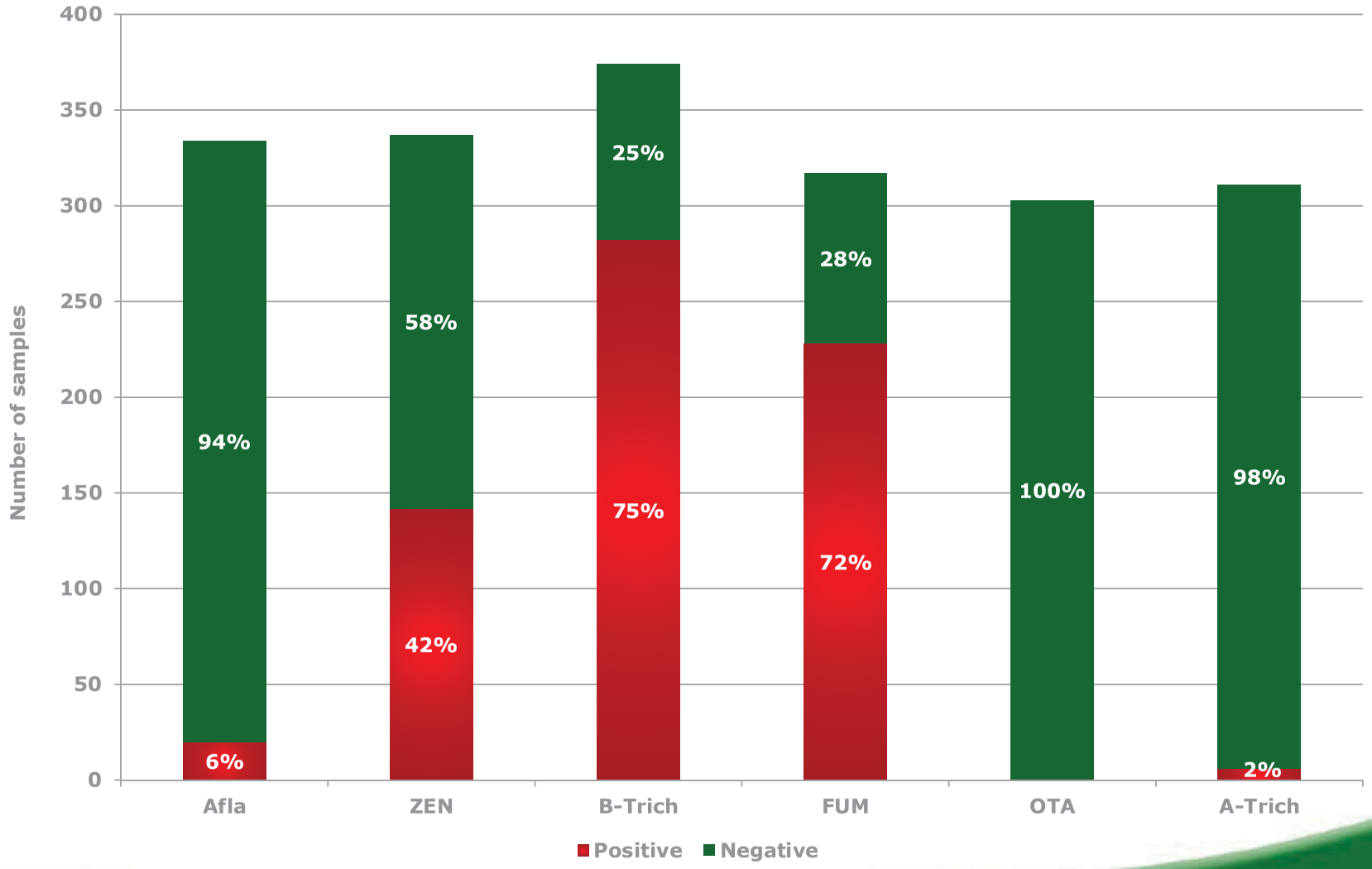
Sample Distribution – 2016 US Corn Crop



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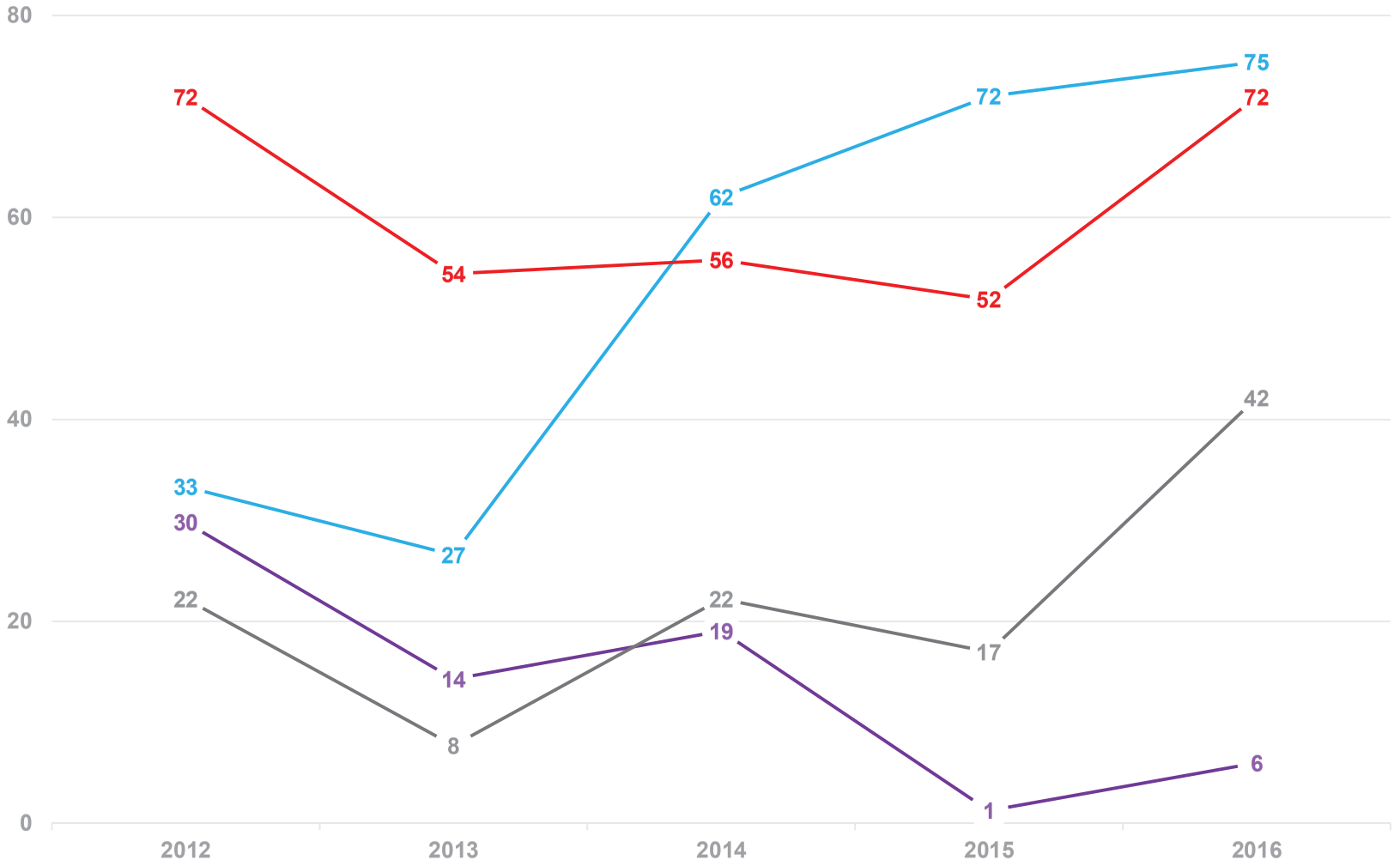
Prevalence – 2016 US Corn Crop



B-Trichothecenes include Deoxynivalenol. Nivalenol, Fusarenon-X, Acetyl-Deoxynivalenol
A-Trichothecenes and Ochratoxin are not presented due to small number of samples

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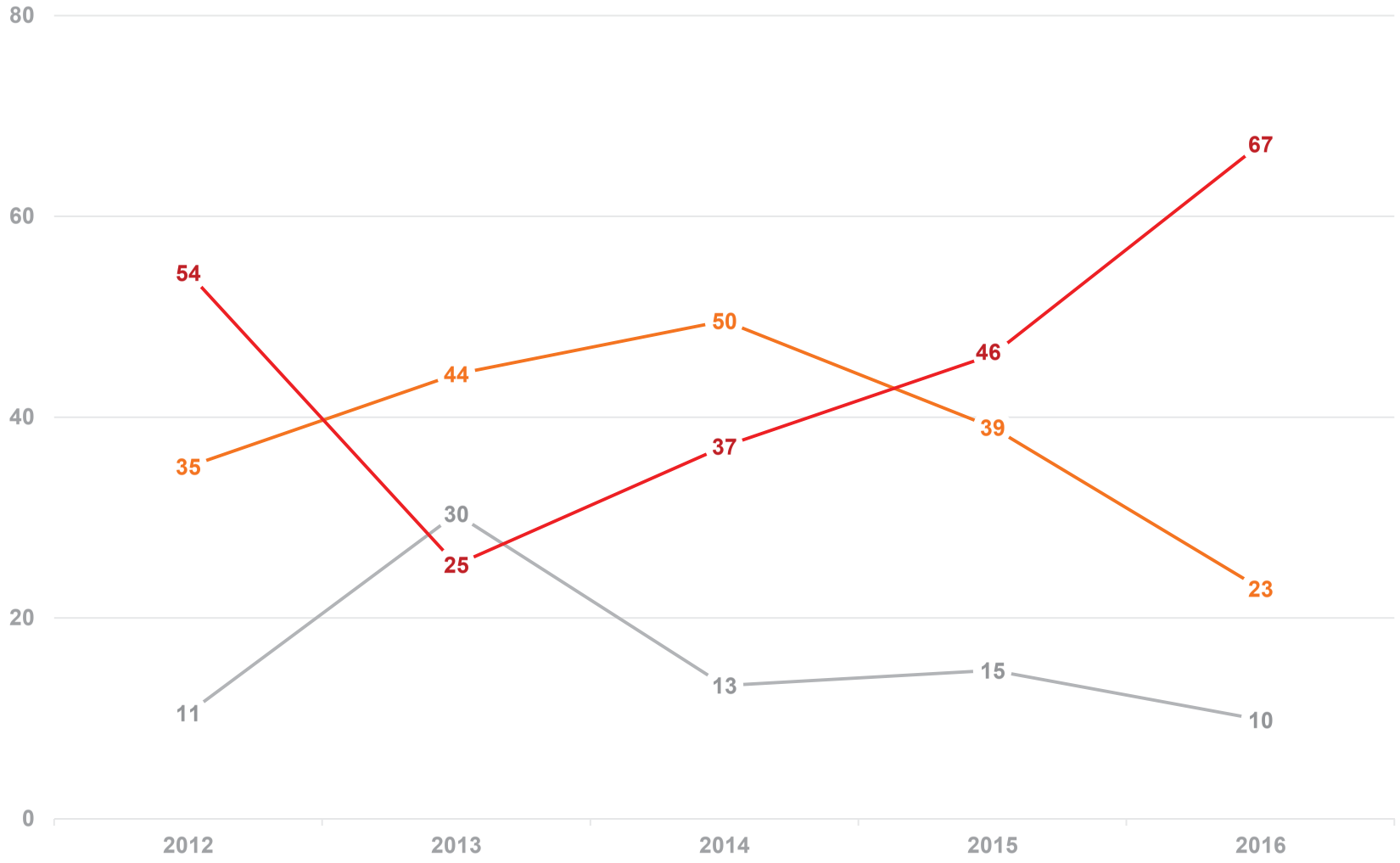
Co-Occurrence – 2016 US Corn Crop



A-Trichothecenes and Ochratoxin are not presented due to small number of samples

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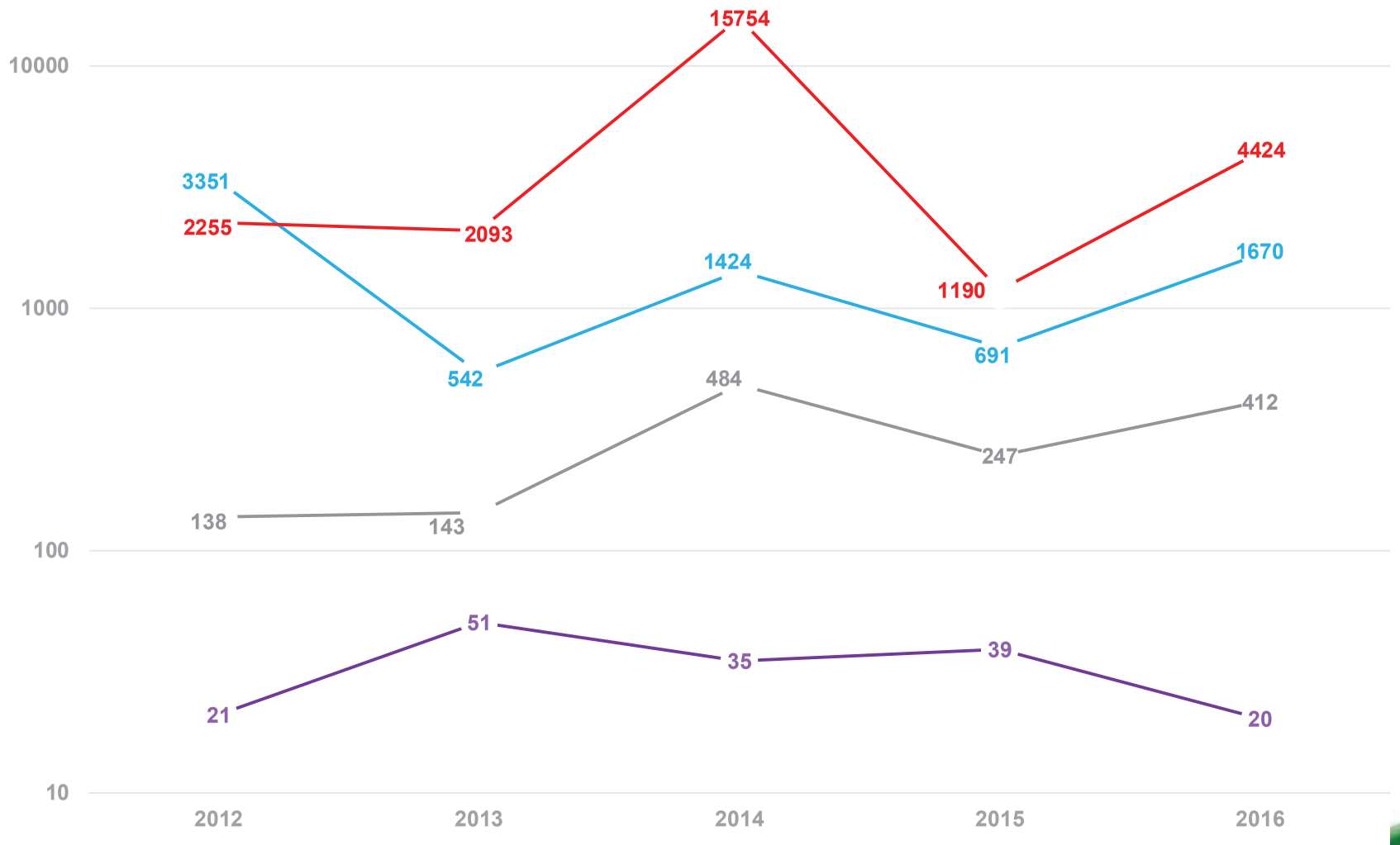
Mean of Positives – 2016 US Corn Crop



B-Trichothecenes include Deoxynivalenol. Nivalenol, Fusarenon-X, Acetyl-Deoxynivalenol
A-Trichothecenes and Ochratoxin are not presented due to small number of samples

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Summary

- Heterogeneous distribution of mycotoxins in feeds
- Corn common source of mycotoxin contamination
- Variety of mycotoxins
- Proper mycotoxin risk management is essential for the protection of animals

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Questions?

paige.gott@biomin.net

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WINNER OF THE 2017 CANC ANIMAL NUTRITION SCHOLARSHIP

ASHLEY NIESEN

The effects of blood composition and age on PBMC mitochondrial enzyme activity in pre-wean dairy calves

A.M. Niesen, H.A. Rossow
Department of Population Health and Reproduction, SVM VMTRC
University of California, Davis, CA

Mitochondria are central to metabolism, nutrition and health but many factors can influence their efficiency. The objective of this study was to determine if mitochondrial enzyme activity rates of peripheral blood mononuclear cells (PBMCs) were affected by changes in blood composition, aging or breed. Data from 23 Holstein and 23 Jersey heifer calves was collected from age 4 to 66 d. Blood samples were collected at 1, 2 and 9 wk of age and analyzed using a Hemavet 950FS Hematology Analyzer (Drew Scientific, Miami Lakes, FL) to determine levels of neutrophils (NE, K/ul, %), lymphocytes (LY, K/ul, %), monocytes (MO, K/ul, %), eosinophils (EO, K/ul, %), and red cell distribution width (RDW, %). Additional blood was used to determine plasma total protein (TP, g/dl) and obtain crude mitochondrial extracts from the PBMC fraction using a mitochondria isolation kit from Abcam (Cambrige, MA). Enzyme activities for citrate synthase (CS), Complex I (CI), Complex IV (CIV) and Complex V (CV) were all determined using kits from Abcam (Cambrige, MA). Activity rates were compared by time point, breed, and blood parameters and were analyzed using the MIXED procedure of SAS (v.9.4) with repeated measures, breed as a fixed effect and time point as a random effect. Breed was not significant for all comparisons. CI activity was not different between time points but was affected by RDW ($P < 0.02$). CIV ($P < 0.04$) and CV ($P < 0.01$) showed an increase in activity with time point while CS ($P < 0.01$) decreased. An increase in EO and MO immune cell production increased CIV activity ($P < 0.01$) and CV activity ($P < 0.01$) respectively. As white blood cell (WBC) differential fluctuated CV activity increased as NE% ($P < 0.01$) and MO% ($P < 0.01$) decreased and LY% ($P < 0.01$) and EO% ($P < 0.01$) increased. These findings suggest that changes in mitochondrial enzyme activity is impacted by fluctuations in NE, LY, MO, EO cell populations and changes as calves age.

Livestock's Contributions to Climate Change: Facts and Fiction

A white paper, defining the role animal agriculture and other sectors of society play in their respective contribution of greenhouse gases, as the societal concerns grow to seek a sustainable global future.

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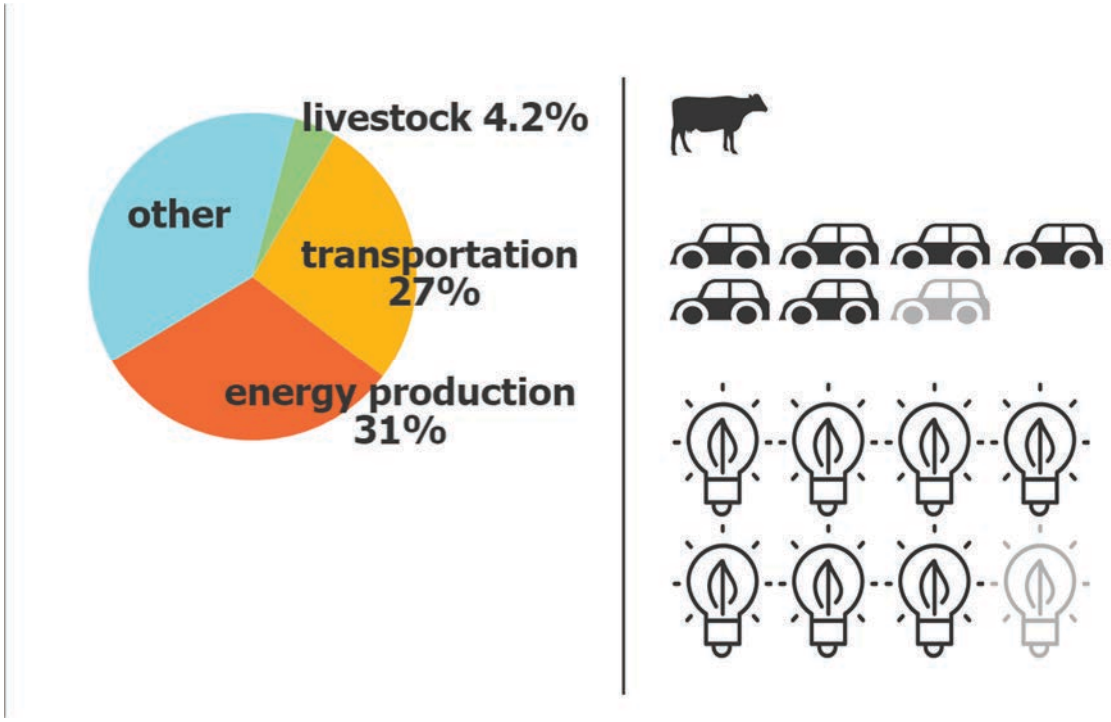
As the November 2015 Global Climate Change Conference COP21 concluded in Paris, 196 countries reached agreement on the reduction of fossil fuel use and emissions in the production and consumption of energy, even to the extent of potentially phasing out fossil fuels out entirely. Both globally and in the U.S., energy production and use, as well as the transportation sectors, are the largest anthropogenic contributors of greenhouse gasses (GHG), which are believed to drive climate change. While there is scientific consensus regarding the relative importance of fossil fuel use, anti-animal agriculture advocates, portray the idea that livestock is to blame for a lion share of the contributions to total GHG emissions.

One argument often made is U.S. livestock GHG emissions from cows, pigs, sheep and chickens are comparable to all transportation sectors from sources such as cars, trucks, planes, trains, etc. The argument suggests the solution of limiting meat consumption, starting with "Meatless Mondays," which will show a significant impact on total emissions.

When divorcing political fiction from scientific facts around the quantification of GHG from all sectors of society, one finds a different picture. Leading scientists throughout the U.S., as well as the U.S. Environmental Protection Agency (EPA[1]) have quantified the impacts of livestock production in the U.S., which accounts for 4.2%[2] of all GHG emissions, very far from the 18% to 51% range that advocates often cite. Comparing the 4.2% GHG contribution from livestock to the 27% from the transportation sector, or 31% from the energy sector in the U.S. brings all contributions to GHG into perspective. Rightfully so, the attention at COP21 was focused on the combined sectors consuming fossil fuels, as they contribute more than half of all GHG in the U.S.



[1] http://www3.epa.gov/climatechange/ghgemissions/sources/agriculture.html
[2] http://www3.epa.gov/climatechange/Downloads/ghgemissions/US-GHG-Inventory-2015-Main-Text.pdf

Breaking down the 4.2% EPA figure for livestock by animal species, shows the following contributors: beef cattle 2.2%, dairy cattle 1.37%, swine 0.47%, poultry 0.08%, sheep 0.03%, goats 0.01% and other (horses, etc.) 0.04%. It is sometimes difficult to put these percentages in perspective, however; if all U.S. Americans practiced Meatless Mondays, we would reduce the U.S. national GHG emissions by 0.6%. A beefless Monday per week would cut total emissions by 0.3% annually. One certainly cannot neglect emissions from the livestock sector but to compare them to the main emission sources would put us on a wrong path to solutions, namely to significantly reduce our anthropogenic carbon footprint to reduce climate change.

= 2x

U.S. Population Replace Incadescent with Energy Star bulbs – 1.2%

U.S. Population "Meatless Monday" = GHG Emission – 0.6%

In spite of the relatively low contributions to total GHG emissions, the U.S. livestock sector has shown considerable progress during the last six plus decades, and commitment into the future, to continually reduce its environmental footprint, while providing food security at home and abroad. These environmental advances have been the result of continued research and advances in animal genetics, precision nutrition, as well as animal care and health.

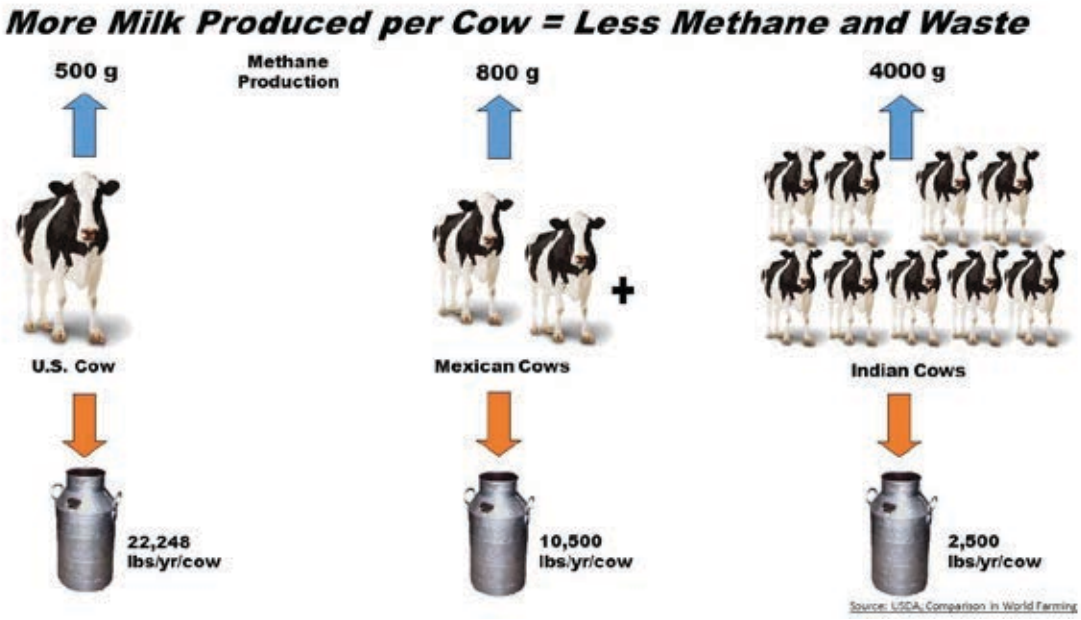
U.S. Dairy & Beef Production Continuous Improvement

	1950	**2015**
Total Dairy Cows:	22 million dairy cows	9 million dairy cows (-59%)
Milk Production:	117 million tons	209 million tons (+79%)
Carbon Footprint:		1/3 that of 1950

	1970	**2015**
Total Beef Cattle:	140 million head	90 million head (-36%)
Beef Production	24 million tons	24 million tons

Globally, the U.S. livestock sector is the country with the relatively lowest carbon footprint per unit of livestock product produced (i.e. meat, milk, or egg). The reason for this achievement largely lies in the production efficiencies of these commodities, whereby fewer animals are needed to produce a given quantity of animal protein food, as the following milk production example demonstrates: the average dairy cow in the U.S. produces 22,248 lbs. milk/cow/year. In comparison, the average dairy cow in Mexico produces 10,500 lbs. milk/cow/year, thus it requires 2-plus cows in

Mexico to produce the same amount of milk as one cow in the U.S. India's average milk production per cow is 2,500 lbs. milk/cow/year, increasing the methane and manure production by a factor of 9 times compared to the U.S. cow. As a result, the GHG production for that same amount of milk is much lower for the U.S. versus the Mexican or Indian cow. Production efficiency is a critical factor in sustainable animal protein production and it varies drastically by region.



Improvements in livestock production efficiencies are directly related to reductions of the environmental impact. Production efficiencies and GHG emissions are inversely related—when the one rises, the other falls.

The 2050 challenge to feeding the globe is real: throughout our lifetime, the global human population will have tripled from three to more than nine billion people without concurrent increases of natural resources to produce more food. Our natural resources of land, water and minerals (fertilizer) necessary for agricultural production, have not grown but in fact decreased. As a result, agriculture will have to become much more efficient worldwide and engage in an efficient path similar to the one it has traveled down in U.S. livestock production in recent decades.

How can emissions accurately and fairly be assessed to lay ground for a path for solutions?

In its quest to identify a sustainable, scientific path toward fulfilling the future global food demand, the Food and Agriculture Organization of the United Nations (FAO) has formed an international partnership project to develop and adopt a "gold standard" life cycle assessment (LCA) methodology for each livestock specie and the feed sector. The 'Livestock Environmental Assessment and Performance Partnership' (LEAP), engaged with more than 300 scientists from the world's most prestigious academic institutions in developing this unprecedented effort in developing a global benchmarking methodology. The first three-year phase project was finalized in December 2015 with six publically available LCA guidelines[3]. This globally harmonized quantification methodology will not only allow the accurate measurement by livestock species and production regions across the globe today, but will also identify opportunities for improvement and the ability to measure that progress in each region going forward.

[3] http://www.fao.org/partnerships/leap/en/

Summary

Addressing the 2050 challenge of supplying food to a drastically growing human population can sustainably be achieved through intensification of livestock production. Indeed, intensification provides large opportunities for climate change mitigation and can reduce associated land use changes such as deforestation. Production efficiencies reduce environmental pollution per unit of product.

The U.S. livestock, poultry and feed industries are one of the most efficient and lowest environmental impact systems in the world. The research, technologies and best practices that have been developed and implemented over time in the U.S. can also be shared with other production regions around the world. It is important to understand that all regions have unique demands and abilities, and thus require regional solutions. However, the advances in the U.S. agriculture and food system can be adapted within these regional solutions. These significant environmental advances and benefits are in addition to the well-documented human health and developmental value of incorporating animal protein in the diets of the growing population.

The livestock sector is committed to continuous improvement of their environmental impact in North America, and to doing its part in transferring knowledge, technologies and best practices to enhance global environmental livestock impact by region. Now is the time to end the rhetoric and separate facts from fiction around the numerous sectors that contribute emissions and to identify solutions for the global food supply that allow us to reduce our impact on the planet and its resources.

The California ARPAS Alfalfa Hay Project
California Chapter of ARPAS

Introduction

Analyses of feeds that do not allow for prediction of animal performance from inputs, and vice versa, lack utility. The first report of a system developed to predict input from output was in 1809 (Von Thaer, cited by Flatt et al., 1969); Von Thaer and associates compared body weight change of steers fed different feeds to a standard ("good meadow hay"). A simpler yet demonstrably less accurate scheme based on solubility of feeds in various dilute solutions was than employed, which led to another system and another system and so on. The history of feed analysis is replete with simple yet inaccurate feeding systems.

Energy based systems evaluate feeds and animal performance based on the conservation of energy and were developed in Europe; early feeding standards such as starch and barley equivalents, continued in use well past the middle of the last century. In the United States, however, the TDN system persisted until the second half of the last century. It is against this backdrop that Jim Meyer and Glen Lofgreen conducted studies to "establish regression equations which would be useful in predicting the total digestible nutrient (TDN) content from lignin or crude fiber analysis" in alfalfa hay (Meyer and Lofgreen, 1956). Correlation coefficients (R^2) for estimating TDN from either lignin or crude fiber were 0.77 and 0.74, respectively. Meyer and Lofgreen (1959) stated that the previous study was useful only in controlled nutritional investigations and had little practical application in the field. A subsequent study was undertaken to "develop a procedure by which chemical analyses could be used to reduce much of the uncertainty when hay is graded or evaluated for livestock feed". Included in the experimental work were:

Choice and simplification of analytical methods
Development of new regression equations
Development of tables so that monetary value of hay could be readily calculated

A modification of the crude fiber procedure was found to be superior in predicting TDN (R^2 = 0.79) when compared with predictions based on either lignin or crude fiber. These investigators also demonstrated that the relationship between NE/TDN and crude fiber was curvilinear. This observation is critical in evaluating predictive accuracy and model specification of current prediction equations, which are generally linear. Meyer and Lofgreen (1959) further suggested pricing energy and protein in alfalfa relative to other feeds, such as barley and cottonseed meal; a table was included in that paper. Since that time alfalfa hay has been traded in California on the basis of TDN content.

With the advent of detergent fiber analyses (Goering and Van Soest, 1970) studies were undertaken at UC Davis as part of a western regional project investigating the relationship between TDN and acid detergent fiber content of alfalfa hay. Our analysis of data from alfalfa digestion studies, using wether lambs (W.N. Garrett, unpublished data) indicated that the correlation between TDN and ADF was less (R^2 = 0.72)than that reported by Meyer and Lofgreen (1959) for modified crude fiber. Garrett also stated, and our analysis of those data bear this out, that digestible energy was more highly correlated (R^2 = 0.80) with ADF than was TDN. However, regardless of predictor (ADF, crude fiber or modified crude fiber) or response variable (TDN or DE) the response vector failed to mimic the observed vector (P < 0.05). Further analysis of these data indicated that estimating equations of the form:

TDN = a + b x fibrous entity (ADF, crude fiber or modified crude fiber)

when determined for lambs and steers were misspecified ($P < 0.05$). Misspecification of a linear model suggests the relationship between response and predictor variable is non-linear and other forms of the model should be investigated.

Consumption of ME provides fuel for processes associated with service and repair functions (maintenance), such as maintenance of ionic gradients, lipid and protein turnover or as Schiemann (1969) noted "The maintenance requirement is a requirement for ATP-equivalents". Metabolizable energy is also a property of substrates for synthesis of biomass, such meat, milk, fiber and products of conception (Baldwin, 1995 and Kennedy and Calvert, 2014).

Given the anachronism that is the TDN system and the lack of global fit to any of the current TDN estimating equations, the California chapter of ARPAS undertook this study to determine if ME content of pure stand alfalfa hay could be predicted from the NIR spectrum.

Materials and Methods

During the 2008 growing season pure stands of alfalfa hay were sampled throughout California and western Nevada. Sampling was done on roadsided stacks and barn-stored alfalfa; we sampled more than 200 lots of hay. Nine of the samples were chosen, based on analytical (Table 1) and environmental diversity (Table 2), for use in a lamb metabolism study conducted at the USDA Dairy Forage Research Center, Madison, WI. Lambs were fed hay cubes that had been reground and pelleted; each hay sample (n = 9) was fed to six lambs at two intakes, either maintenance (~2 percent of body weight) or ad libitum (~5 percent of body weight). Lambs were adapted for seven days followed by a seven day collection of urine and feces; intake energy, fecal energy and urinary energy were observed while gaseous energy was estimated from in vitro carbohydrate degradation.

There exist a number of prediction equations for estimating quality of alfalfa hay. We evaluated the current UC TDN equation (Putnam et al., 2007), relative feed value (Rohweder et al., 1978), relative feed quality (Moore and Undersander, 2002) and the NRC (2001) summative equation for model specification and internal validity using data developed from this study. In addition, a least squares estimating equation (**OLS**) of the form TDN = f(ADF) was developed for data from this study to further evaluate model structure and utility. Parametric stability and concordance of NDF degradability determined either in vivo, in vitro or by Bayesian inference (uninformed priors) for the NRC (2001) TDN equation were evaluated.

Rate and extent of *in vitro* degradation (120 hours) was evaluated using either a stochastic, heterogeneous rate model:

$$R_t = D \times (1 + \beta\ (t-\tau)^{-\alpha}) + I$$

where:

R_t = analyte residue at time t (h), R_0 = 1.00 (arbitrary unit)
α, β = shape and scale parameters of gamma distributions
t = time after inoculation of medium (h)
D = potentially degradable fraction
τ = time delay before losses begin or lag (h)

I = analyte incapable of being degraded

or a first order function:

$R_t = D \times e^{k\,(t-\tau)} + I$

where:

k = rate constant (h^{-1})

Metabolizable energy and TDN were estimated from the alfalfa hay NIR spectrum. For spectral analysis, all alfalfa hay samples were oven dried and ground before placement in a spectrophotometer. Reflectance values for λ were from 950 to 2,500 nm and were analyzed using the chemometric utility UNSCRAMBLER (CAMO Software, Norway) to develop NIR prediction models for ME or TDN at either restricted or ad libitum intake.

Results and Discussion

California estimating equation

The California TDN estimating equation: TDN = 82.38 – 0.7515 x ADF failed to predict TDN observed in this study (Lin's correlation coefficient (ρ_c) = 0.793 and root mean square error (**RMSE**) = 2.58. Lin's correlation coefficient measure the extent to which two vectors mimic each other; ρ_c = 1.0 indicates a perfect correspondence and ρ_c = 0 indicates that no relationship exists. The equation estimated for this study: TDN = 79.3 – 0.699 x ADF also failed to predict observed TDN (R^2 = 0.773, ρ_c = 0.856, RMSE = 1.99). Parametric instability was noted for the slope of the previous regression; a 95% confidence interval about the slope was from 0.594 to 0.804. Ordinary least squares regression, for both estimating equations, of vectors $\mathbf{TDN_{predicted}}$ and $\mathbf{TDN_{observed}}$ indicated that the vectors were different; the slope was not equal to one ($P < 0.05$) and the intercept was not equal to zero ($P < 0.05$). Figure 1 shows that, for TDN observed in this study, predicted TDN (UC equation) overestimates observed TDN; also shown is the prediction interval which averages ± 5.35 points of TDN (8.82%). A significant *F* ratio was noted for model misspecification; misspecification of a linear model indicates that the relationship between response ($TDN_{observed}$) and predictor variable(s) (ADF in this case) is non-linear and that other models and predictor variables should be evaluated. Residual heteroscedasticity (Figure 2) is further indication of inappropriate model specification. The uncertainty in alfalfa evaluation, of which Meyer and Lofgreen (1959) wrote, does not appear to have been resolved by the current estimating equation.

RFV and RFQ

Relative feed value (**RFV**) assumes that NDF is predictive of *ad libitum* dry matter intake (**DMI**) and that ADF is predictive of dry matter digestibility (Rohweder et al., 1978). Dry matter intake (*ad libitum*) was poorly predicted by NDF content of alfalfa hay. For the ordinary least squares relationship $DMI_{calculated} = f(DMI_{observed})$ the slope was not different from zero clearly indicating that, contrary to the assumption of Rohweder at al. (1976), no relationship exists between NDF content of alfalfa hay and dry matter intake. Lin's correlation coefficient (ρ_c = -0.159) also indicated that the vector $\mathbf{DMI_{calculated}}$ did not mimic the vector $\mathbf{DMI_{observed}}$. Studies by Sanson and Kercher (1996) and Hackman et al. (2008) indicated no relationship existed between dry matter intake and NDF content of alfalfa hay. Van Soest et al. (1978) noted "the common assumption that digestibility and intake are always

positively correlated is unsafe…" which is consistent with with our results and those of Sanson and Kercher (1996) as well as Hackman et al. (2008). Dry matter digestibility (**DDM**) was poorly predicted by ADF content; for the equation $DDM_{calculated} = f(DDM_{observed})$ the slope was different from one and the intercept different from zero ($P < 0.05$). Lin's correlation coefficient ($\rho_c = 0.402$), while greater than that calculated for the relationship between vectors $\mathbf{DMI_{calculated}}$ and $\mathbf{DMI_{observed}}$, ρ_c of 0.402 clearly indicates a lack of correlation, providing further evidence that dry matter digestibility was poorly predicted by ADF. Relative feed value, calculated as f(NDF & ADF) is not equal to observed RFV ($P < 0.05$) and Lin's correlation coefficient = 0.0525. Utility in the calculation of RFV does not reside in the prediction of dry matter intake nor dry matter digestibility nor in the prediction of observed RFV. Metabolizable energy is correlated ($R^2 \sim 0.88$) with both NDF and ADF; the use of either term should be preferred to RFV. Quality estimates, such as RFV, are often presented as fait accompli with little regard to predictive accuracy or model structure.

Relative feed quality (**RFQ**) differs from RFV in that TDN replaces ADF in the equation. According to Moore and Undersander (2002) RFQ is preferred because the estimating equation is improved. Really. When observed TDN is used in the RFQ estimating equation (DMI estimated as f(NDF)) $RFQ_{calculated} \neq RFQ_{observed}$ ($P < 0.05$), $\rho_c = 0.0500$. Replacing observed TDN with TDN calculated from the UC TDN equation did not improve the fit; $\rho_c = 0.0424$. This analysis indicates that RFQ, when estimated as described by Moore and Undersander (2002), is as predictive of $RFQ_{observed}$ as estimates of RFV are of $RFV_{observed}$.

Summative estimates of TDN

The classical definition of TDN: (% digestible crude protein + % (digestible ether extract x 2.25) + % digestible crude fiber + % digestible nitrogen free extract) is a summative equation. The NRC (2001) publication "Nutrient Requirements of Dairy Cattle" suggests the following digestion coefficients: crude protein – 93%, ether extract – 97%, nitrogen free extract – 98% and an additive constant of -7; the additive constant is inconsistent with the classical definition of TDN. In vitro degradation of NDF (48 hours) replaces NDF degradation observed in a metabolism study. Total digestible nutrients, estimated using the NRC (2001) summative equation, failed to predict observed TDN; the slope of the OLS regression $TDN_{predicted} = f(TDN_{observed})$ was different from one ($P < 0.05$) and the intercept was different from zero ($P < 0.05$). Further evidence of the lack of correspondence of estimated TDN with observed TDN is provided by Lin's correlation coefficient ($\rho_c = 0.760$). Digestion coefficients found in the NRC (2001) summative equation are different ($P < 0.001$) than those observed in this study. *In vitro* degradation of NDF (48h) was different from observed *in vivo* ($P < 0.001$) These observations are consistent with the statement by Hungate (1966) "*In vitro* experiments as usually conducted do not provide reliable estimates of the rates at which the phenomena under study occur in the rumen". Digestion coefficients for unpublished data from W.N. Garrett (late professor emeritus, The University of California, Davis) for cattle fed alfalfa at maintenance were different ($P < 0.001$) as well. The digestion coefficient for ether extract was 0.0059 (Garrett data) and a 95% confidence about that value included zero. It appears that the estimate of ether extract digestibility found in the NRC (2001) estimating equation may be different from the true parameter for alfalfa hay.

Parameter estimates (presumed digestion coefficients) for the OLS (zero intercept) regression TDN = f(CP, EE, NDF and NSC) for data from the California ARPAS study were different from observed digestion coefficients ($P < 0.05$), however, $\rho_c = 0.912$ a value greater than that for other TDN estimating equations. Ninety five percent

confidence intervals about digestion coefficients for crude protein and ether extract included 1.00 indicating an infeasible solution. Increasing the number of predictor variables may improve predictive accuracy but the model is wrong. When an additive constant was included, only the parameter estimate for NDF was not different from zero (-0.603). Inappropriate sign and magnitude of parameter estimates (an additive constant different from zero and digestion coefficients less than zero) are clear indicators of a misspecified equation. This observation lends further credence to the observation that OLS equations poorly predict TDN for California alfalfa hay. Confidence intervals (95%) for standardized regression (means = 0, standard deviations = 1) coefficients for crude protein, ether extract and nonstructural carbohydrates were not different from zero while that for NDF was -0.704 and a 95% confidence interval was (-1.24 to -0.172). The additive constant for such equations is necessarily zero and lack of stability in all parameter estimates provides further evidence of model misspecification. The sign and magnitude of the coefficient for NDF does indicate variability in NDF is responsible for more variability in TDN than the other predictor variables (CP, EE and NSC).

Comparisons of NDF degradability observed *in vivo*, *in vitro* and estimated using the NRC (2001) TDN equation (Bayesian inference) were different ($P < 0.05$) with the exception of the Siskiyou sample. Degradability of NDF, for the Siskiyou sample (0.357) in vivo (lambs fed *ad libitum)*, was not different from the Bayesian estimate (95% credible interval = 0.338 to 0.358) of NDF degradability in the NRC (2001) summative equation. Degradability of NDF (in vitro) observed at either 30 or 48 hours was different from that estimated using Bayesian inference ($P < 0.05$) indicating that the NRC (2001) TDN equation may be inappropriate for use with pure stand alfalfa hay from California. The more complex summative equation (NRC, 2001) fails to predict TDN with the accuracy of a single variable OLS equation, the latter is preferred.

Near infrared estimates of TDN and MEI

Both TDN and ME, for lambs fed *ad libitum*, were well predicted from the NIR spectrum; R^2 for TDN was 0.90 and the standard error of calibration (**SEC**) was 1.3. When TDN was estimated from ADF the RMSE (RMSE = SEC) was 1.99; error in the estimate was reduced. For ME predicted from the NIR spectrum R^2 was 0.92 and the standard error of calibration was 0.07. Average estimated ME was 2.19 Mcal/kg and the range was from 1.82 Mcal/kg (Imperial #2) to 2.57 Mcal/kg (Stanislaus). Estimates of alfalfa quality, determined by NIR spectroscopy, are more accurate than those determined using the current system; further testing is required to make the system functional.

Acknowledgements

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Table 1. Composition of samples

Source[1]	DM (%)[2]	OM (%)[2]	aNDF (%)[2,3]	ADF (%)[2]	Lignin (%)[2]	CP (%)[2]	IE (Mcal/kg)[2]
Imperial #1	87.7±0.250	90.6±0.032	40.1±0.199	33.0±0.342	6.10±0.108	20.3±0.036	4.40±0.009
Imperial #2	88.9±0.227	89.2±0.064	41.9±0.222	34.9±0.163	6.32±0.105	18.0±0.118	4.31±0.007
Kern #1	90.0±0.183	89.2±0.043	31.4±0.273	23.5±0.104	4.90±0.064	20.3±0.173	4.33±0.011
Kern #2	87.6±0.192	89.1±0.066	30.7±0.187	24.6±0.185	4.56±0.058	24.2±0.154	4.34±0.005
Merced	87.8±0.205	89.2±0.034	37.0±0.186	29.3±0.318	5.38±0.097	20.1±0.203	4.26±0.008
Stanislaus	86.7±0.187	91.1±0.043	24.7±0.229	19.1±0.185	3.47±0.055	31.8±0.124	4.44±0.011
San Joaquin	87.1±0.099	89.4±0.052	35.5±0.198	29.2±0.177	5.48±0.049	19.7±0.154	4.29±0.005
Siskiyou	85.7±0.283	87.3±0.042	25.6±0.182	20.2±0.127	3.39±0.020	25.1±0.132	4.25±0.007
Mason Valley	87.4±0.179	88.1±0.086	33.8±0.216	26.9±0.153	4.88±0.063	20.6±0.153	4.22±0.004

[1] California county of origin; Mason Valley is in Lyon County, Nevada.
[2] Means are shown ± SEM.
[3] Amylase treated NDF (Mertens, 2002)

Table 2. Cutting times, average temperature minima and maxima

Source[1,2]	Month cut	Minimum temperature, °F	Maximum temperature, °F
Imperial #1	July	80	106
Imperial #2	July	80	106
Kern #1	May (early)	44	78
Kern #2	May (early)	44	78
Merced	July	63	96
Stanislaus	March (mid)	44	65
San Joaquin	September	59	94
Siskiyou	May	23	70
Mason Valley	August	53	95

[1] California county of origin; Mason Valley is in Lyon County, Nevada.
[2] All cuttings were in 2008.

Figure 1. Relationship between observed TDN and TDN predicted as 82.38 - 0.7515 x ADF

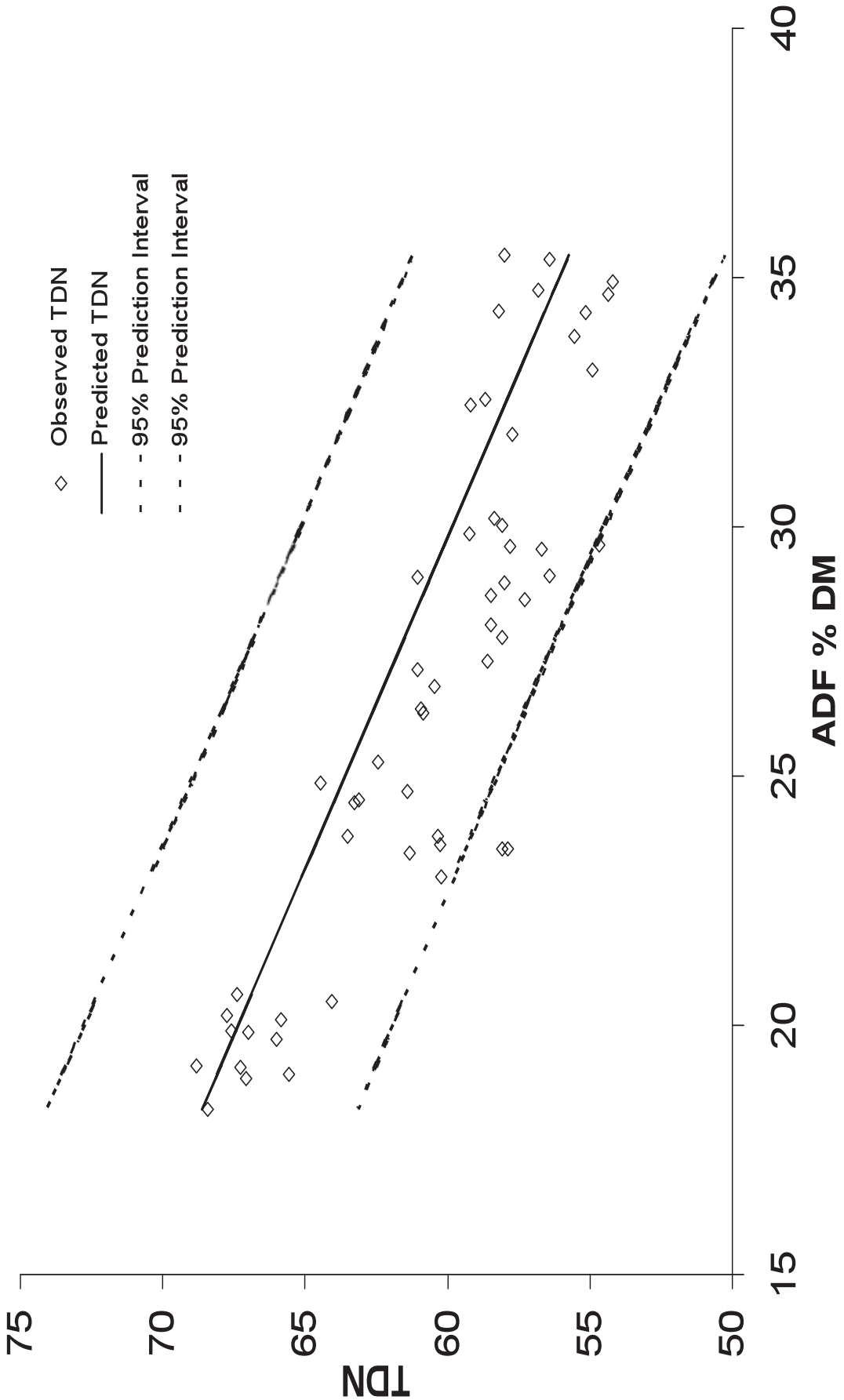
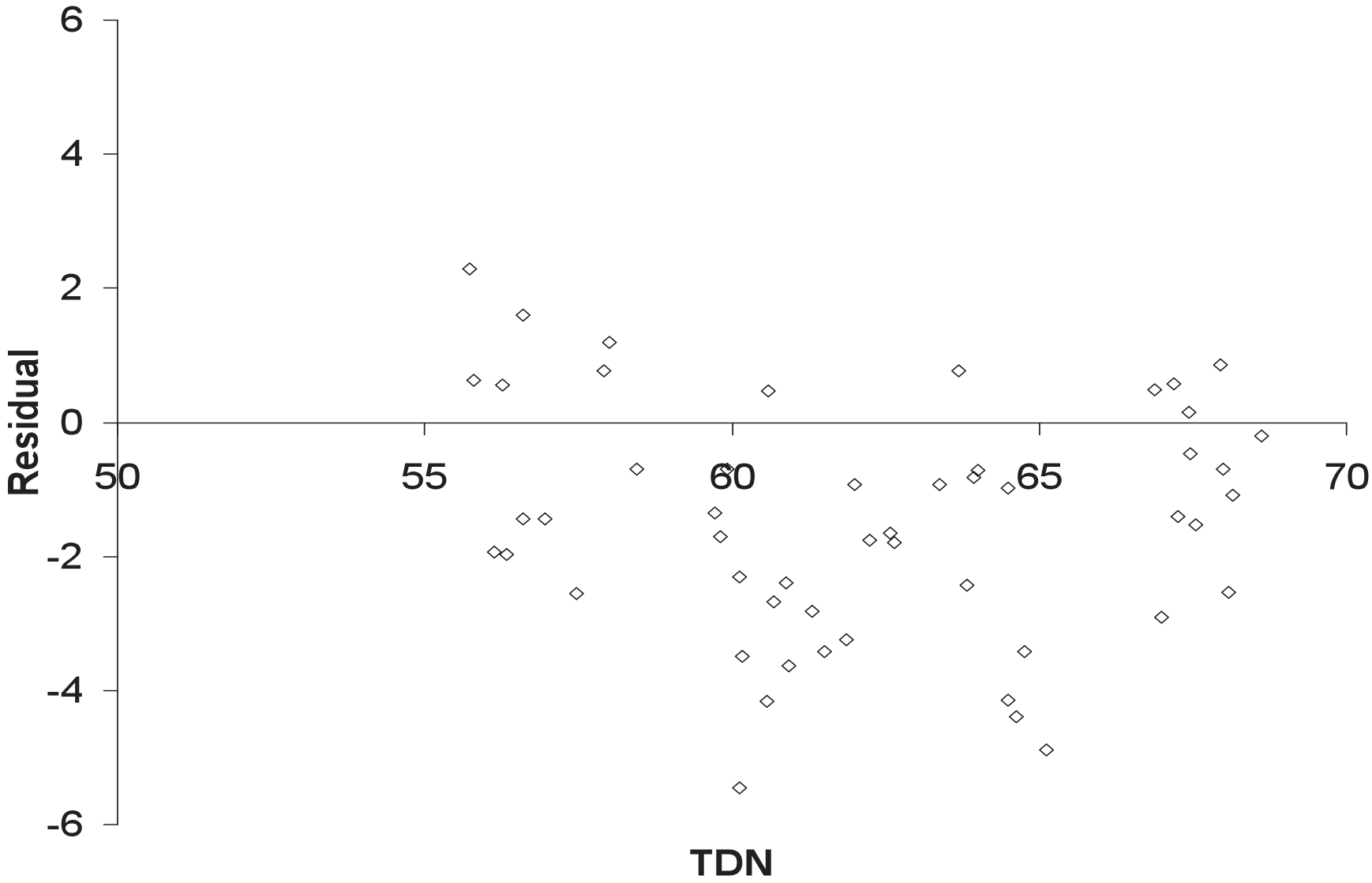

Observed TDN
Predicted TDN
95% Prediction Interval
95% Prediction Interval
TDN
ADF % DM
75
70
65
60
55
50
15
20
25
30
35
40

Figure 2. Plot of residuals for TDN observed - TDN predicted as calculated by the current UC estimating equation; TDN = 82.38 – 0.7515 x ADF


Residual
6
4
2
0
-2
-4
-6
50
55
60
65
70
TDN

Applications of uNDF in Ration Formulation and Feedbunk Management

Rick Grant
William H. Miner Agricultural Research Institute
Chazy, NY

What fiber digestibility analyses do you use? …what do they mean to the cow?

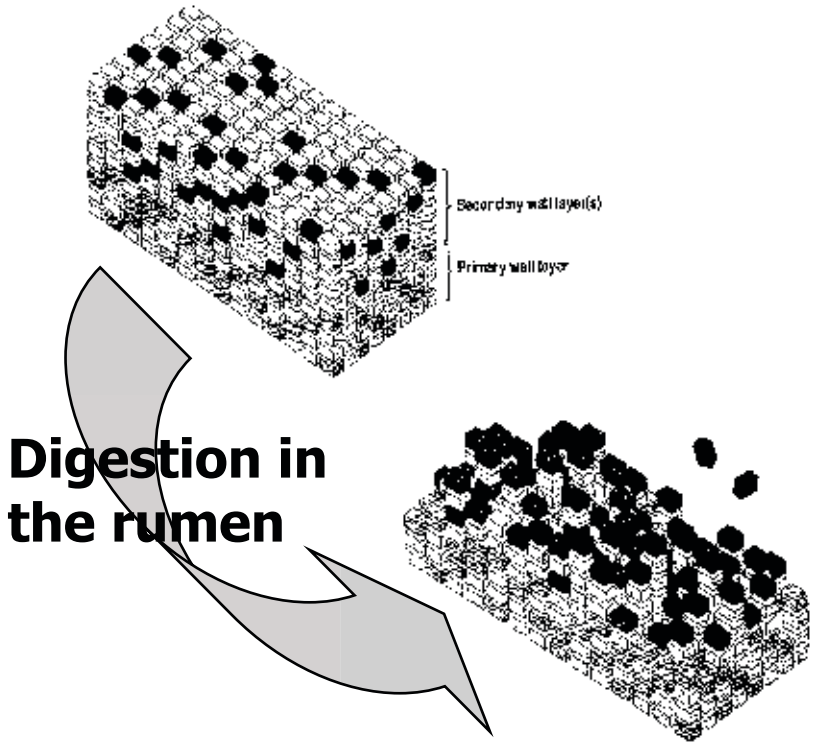
Commercial lab assays for NDF digestibility…

- Lignin, lignin/NDF or ADF
- NDF digestibility (or uNDF) in vitro, in situ
  - 12, 24, 30, 48, 72, 120, 240 h
- Apparent total tract digestion of NDF, TMR-D
  - $uNDF_{120}$ or $uNDF_{240}$ as marker
- TTNDFD
- Fermentrics – gas production systems
  - CHO digestion rates and microbial biomass production

The list continues…

Can we simply use lignin or L/NDF ratios?

- Alfalfa
  - Range: 11-20%
  - Goal: <15%
- Corn silage
  - Range: 3-9%
  - Goal: <6%
- Grass silage
  - Goal: <9%



Lignin = "plant plastic"

Measured NDFD or Estimation from Lignin?

NDF, %	Lignin, %	30-h NDFD
45.0	3.52	?
45.0	3.26	?
45.0	3.32	?
45.1	3.18	?
45.0	3.43	?

- Corn silage data set from Van Amburgh (2005)
- Similar relationships from 36.5 to 51.8% NDF

Measured NDFD or Estimation from Lignin?

NDF, %	Lignin, %	30-h NDFD
45.0	3.52	46.0
45.0	3.26	48.4
45.0	3.32	54.4
45.1	3.18	55.0
45.0	3.43	67.3

- Corn silage data set from Van Amburgh (2005)
- Similar relationships from 36.5 to 51.8% NDF

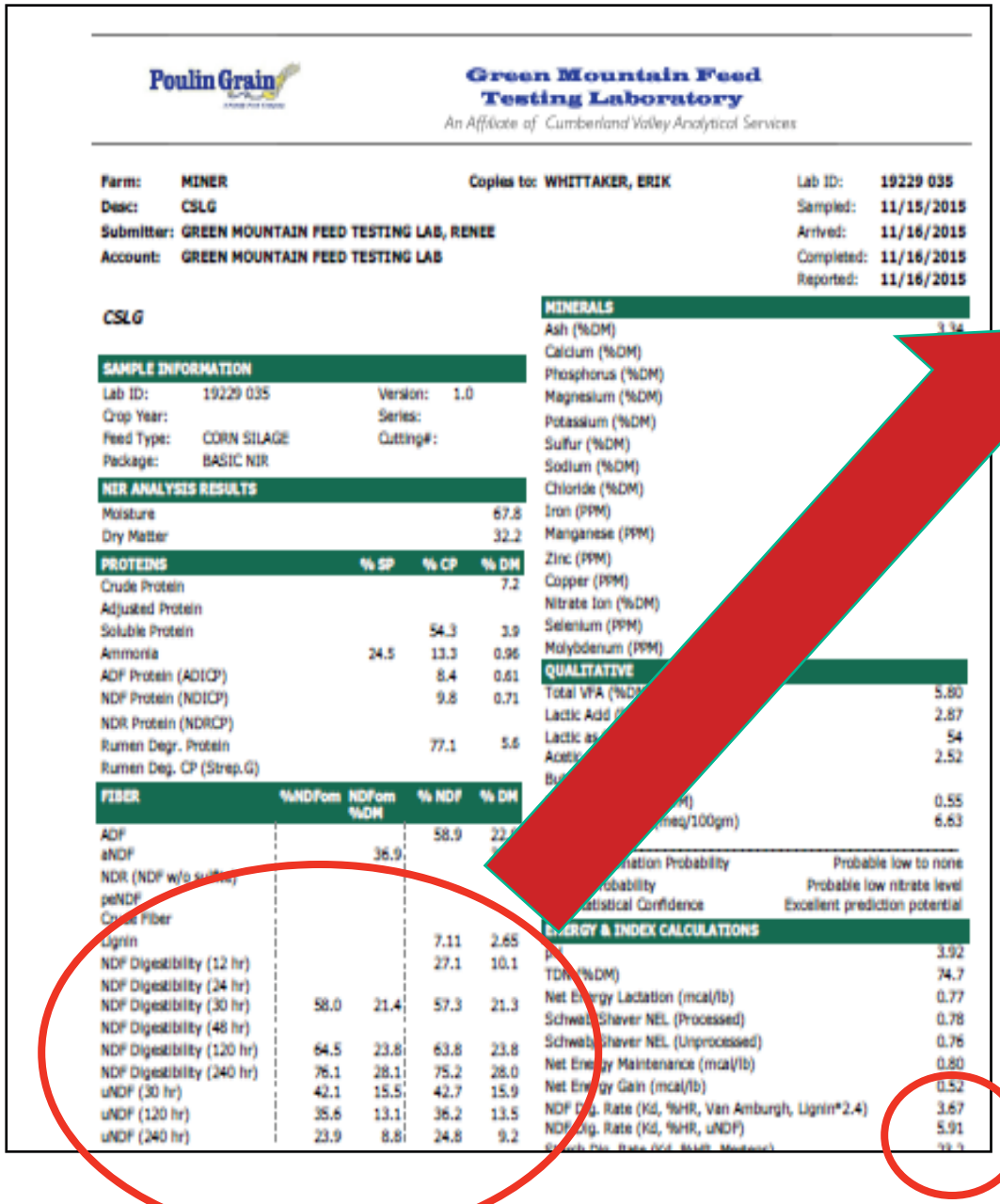
Future for lignin and ADF?

- ✓ Will they become extinct?
  - ✓ Measure digestibility directly.
    - ✓ Measure <u>indigestibility.</u>
    - ✓ ADF still useful to assess grass proportion in sample.

uNDF: why is it important?

- Not a new concept;
  New perspective
- Inverse of digestible NDF
  - 100% - digNDF% = uNDF%
- Undigested NDF measured at 12, 24, 30, 48, 72, 120, 240 h
- uNDF related to DMI, milk components, rumen health
  - Rumen mat, chew factor, and fill factor

Tilley-Terry "artificial rumen" batch in vitro system



Poulin Grain

Green Mountain Feed Testing Laboratory

An Affiliate of Cumberland Valley Analytical Services

Farm: MINER — Copies to: WHITTAKER, ERIK — Lab ID: 19229 035
Desc: CSLG — Sampled: 11/15/2015
Submitter: GREEN MOUNTAIN FEED TESTING LAB, RENEE — Arrived: 11/16/2015
Account: GREEN MOUNTAIN FEED TESTING LAB — Completed: 11/16/2015
Reported: 11/16/2015

CSLG

SAMPLE INFORMATION

Lab ID:	19229 035	Version:	1.0
Crop Year:		Series:	
Feed Type:	CORN SILAGE	Cutting#:	
Package:	BASIC NIR		

NIR ANALYSIS RESULTS

Moisture	67.8
Dry Matter	32.2

PROTEINS	% SP	% CP	% DM
Crude Protein			7.2
Adjusted Protein			
Soluble Protein		54.3	3.9
Ammonia	24.5	13.3	0.96
ADF Protein (ADICP)		8.4	0.61
NDF Protein (NDICP)		9.8	0.71
NDR Protein (NDRCP)			
Rumen Degr. Protein		77.1	5.6
Rumen Deg. CP (Strep.G)			

FIBER	%NDFom	NDFom %DM	% NDF	% DM
ADF			58.9	22.[illegible]
aNDF		36.9		
NDR (NDF w/o sulfite)				
peNDF				
Crude Fiber				
Lignin			7.11	2.65
NDF Digestibility (12 hr)			27.1	10.1
NDF Digestibility (24 hr)				
NDF Digestibility (30 hr)	58.0	21.4	57.3	21.3
NDF Digestibility (48 hr)				
NDF Digestibility (120 hr)	64.5	23.8	63.8	23.8
NDF Digestibility (240 hr)	76.1	28.1	75.2	28.0
uNDF (30 hr)	42.1	15.5	42.7	15.9
uNDF (120 hr)	35.6	13.1	36.2	13.5
uNDF (240 hr)	23.9	8.8	24.8	9.2

MINERALS

Ash (%DM)	3.34
Calcium (%DM)	
Phosphorus (%DM)	
Magnesium (%DM)	
Potassium (%DM)	
Sulfur (%DM)	
Sodium (%DM)	
Chloride (%DM)	
Iron (PPM)	
Manganese (PPM)	
Zinc (PPM)	
Copper (PPM)	
Nitrate Ion (%DM)	
Selenium (PPM)	
Molybdenum (PPM)	

QUALITATIVE

Total VFA (%DM)	5.80
Lactic Acid (%DM)	2.87
Lactic as[illegible]	54
Acetic [illegible]	2.52
Bu[illegible]	
[illegible] PM)	0.55
[illegible] meq/100gm)	6.63
[illegible]nation Probability	Probable low to none
[illegible]obability	Probable low nitrate level
[illegible]tatistical Confidence	Excellent prediction potential

ENERGY & INDEX CALCULATIONS

[illegible]	3.92
TDN (%DM)	74.7
Net Energy Lactation (mcal/lb)	0.77
Schwab Shaver NEL (Processed)	0.78
Schwab Shaver NEL (Unprocessed)	0.76
Net Energy Maintenance (mcal/lb)	0.80
Net Energy Gain (mcal/lb)	0.52
NDF Dig. Rate (Kd, %HR, Van Amburgh, Lignin*2.4)	3.67
NDF Dig. Rate (Kd, %HR, uNDF)	5.91
[illegible]	[illegible]

- Lignin = 2.65% of DM
- aNDF = 36.9% of DM
- uNDF (30 h) = 15.5%
- uNDF (120 h) = 13.1%
- uNDF (240 h) = 8.8%

- NDF digestion rate
  - Lignin*2.4 = 3.67%/h
  - uNDF240 = 5.91%/h

Should you focus on these new numbers?

Why the focus on uNDF?

1. uNDF240 is more sensitive to growing environment, genetics, and maturity than L/NDF or ADL x 2.4.
   - ✓ Forage quality assessment
   - ✓ Benchmarking: (+) feedback from field
2. Need accurate measure of indigestible NDF to calculate pdNDF and NDF rate of digestion.
   - ✓ More accurate rates = more accurate milk predictions.

Why the focus on uNDF?

3) Biological reality: uNDF, fast & slow NDF
   - ✓ 0, 30, 120, and 240 h for forages
   - ✓ 0, 12, 72, and 120 h for NFFS
   - ✓ Best benchmark: 30 or 240 hour?

4) DMI prediction
   - ✓ uNDF in rumen is 1.6x uNDF in TMR
   - ✓ uNDF in TMR equals uNDF in feces
   - ✓ 1% increase in uNDF ~ 1% decrease in DDMI

7) Forage energy value – NRC equation
   - ✓ Replace ADL/NDF$^{0.67}$ with analytical value

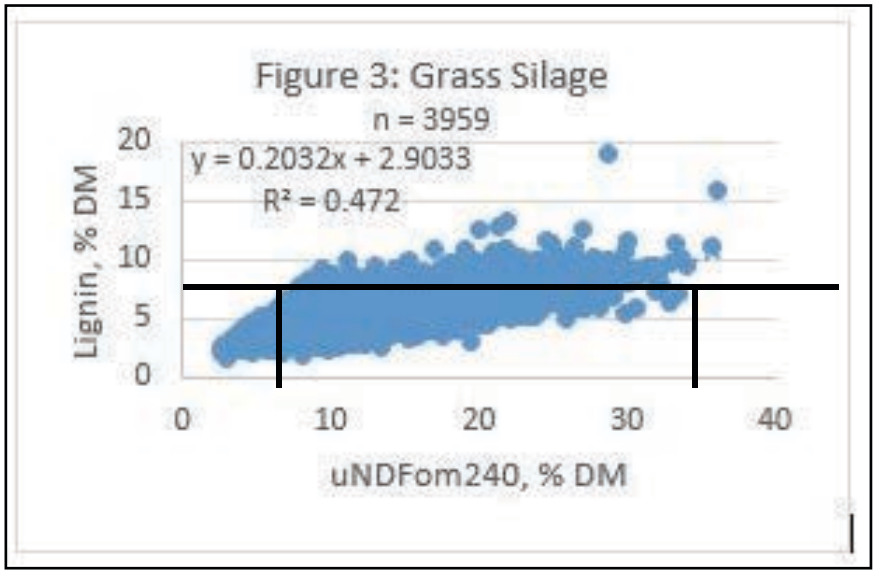
Measured ranges in $uNDF_{240}$

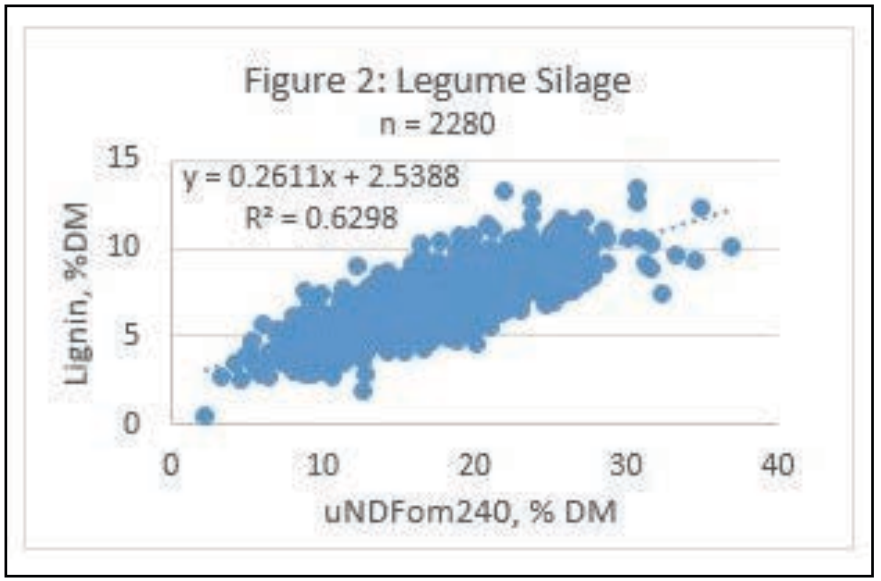
(source: Dairy One, May 2015 newsletter)

- **Corn silage**
  - 8.7% of DM
  - Range: 2.0 to 25.5%
- **Legume silage**
  - 17.6% of DM
  - Range: 5.5 to 31.7%
- **Grass silage**
  - 15.5% of DM
  - Range: 2.3 to 44.8%

Tremendous variation in uNDF that we need to capture when formulating diets and predicting cow response!

Variation in lignin and $uNDF_{240}$





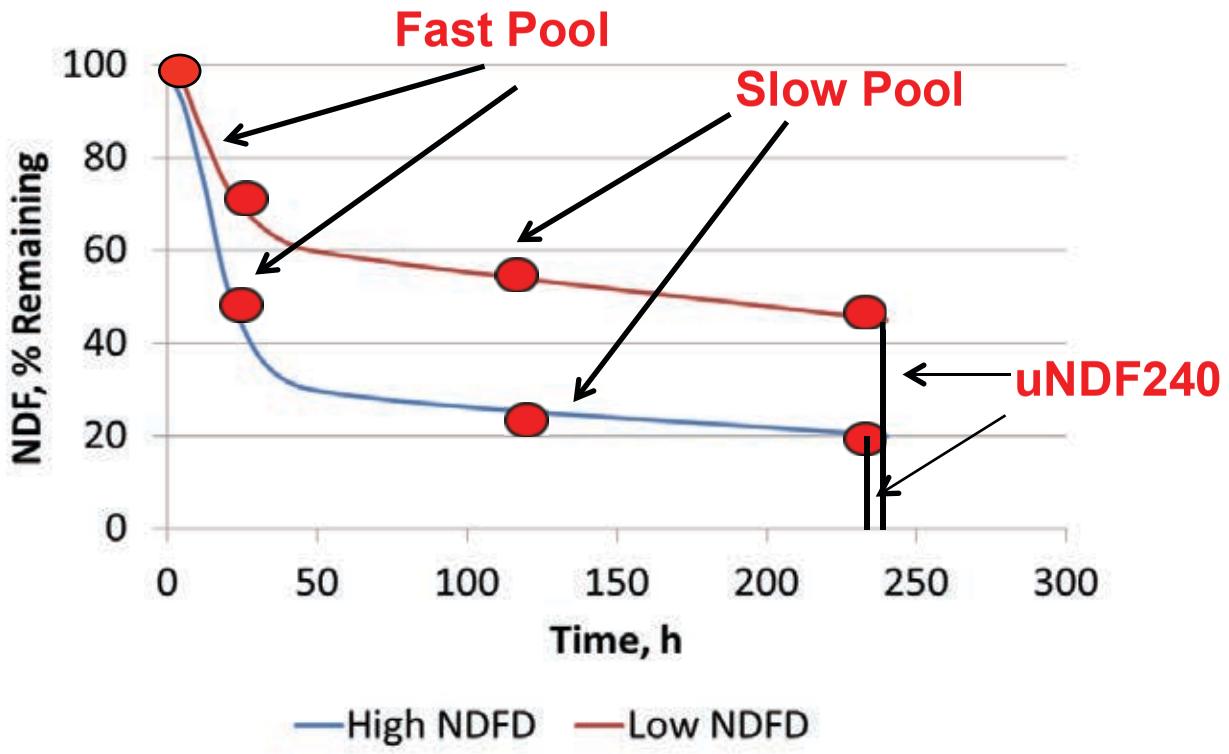
Differing genetics, maturity, and environment –
Differing crosslinking between lignin and CHO

(Dairy One, April 2016 Newsletter, S. Flis)

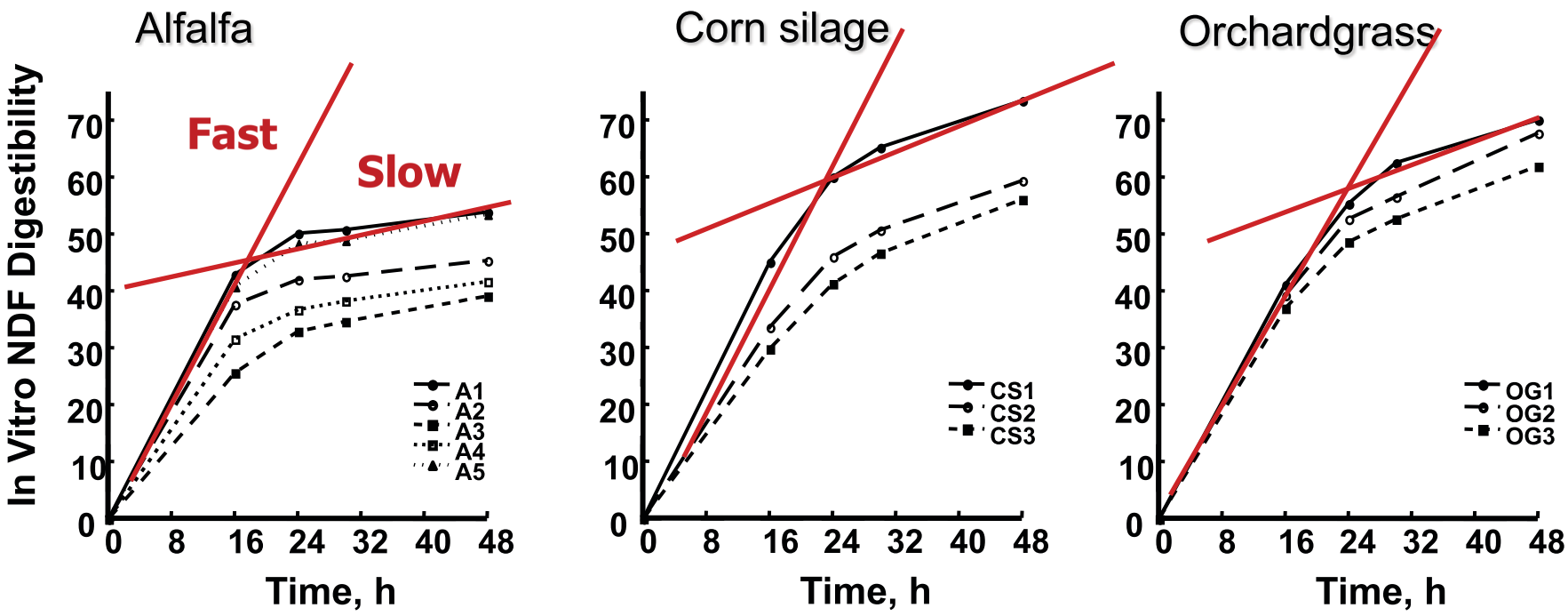
Biological reality and kinetics of uNDF

uNDF is needed to measure fast and slow NDF

- uNDF measured at 240 h of in vitro fermentation
- pdNDF = NDF - uNDF
- pdNDF has 2 fractions: fast-NDF and slow-NDF



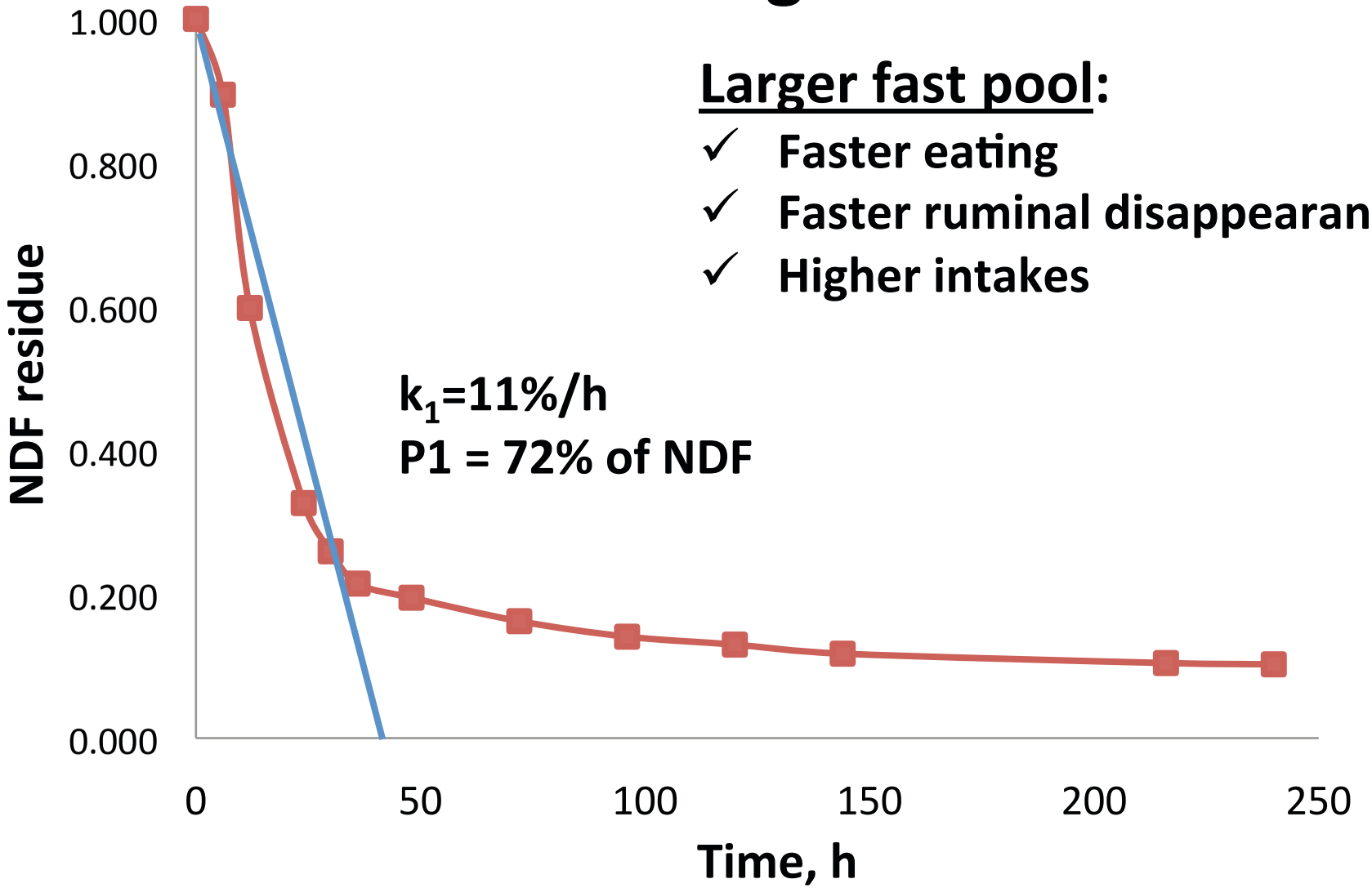
Fast and slow NDF exists in all forage types (Allen, 2005, unpublished)



Slide courtesy of Dr. Mike Allen

Nutritional Implications of 3-Pool Model of NDF Digestion?

Corn Silage Example: 3-Pool NDF Digestion



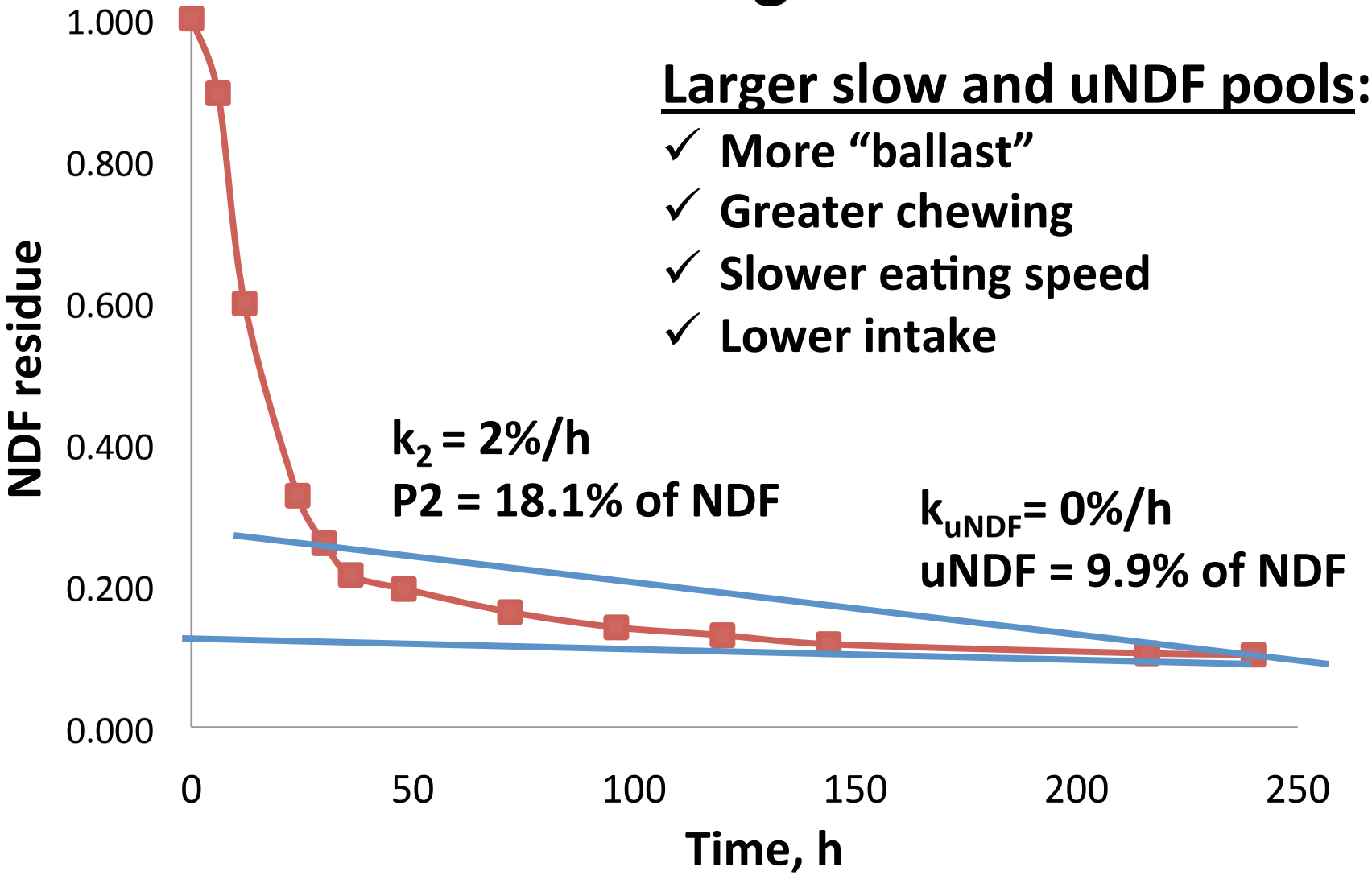
Larger fast pool:

- ✓ Faster eating
- ✓ Faster ruminal disappearance
- ✓ Higher intakes

k_1=11%/h

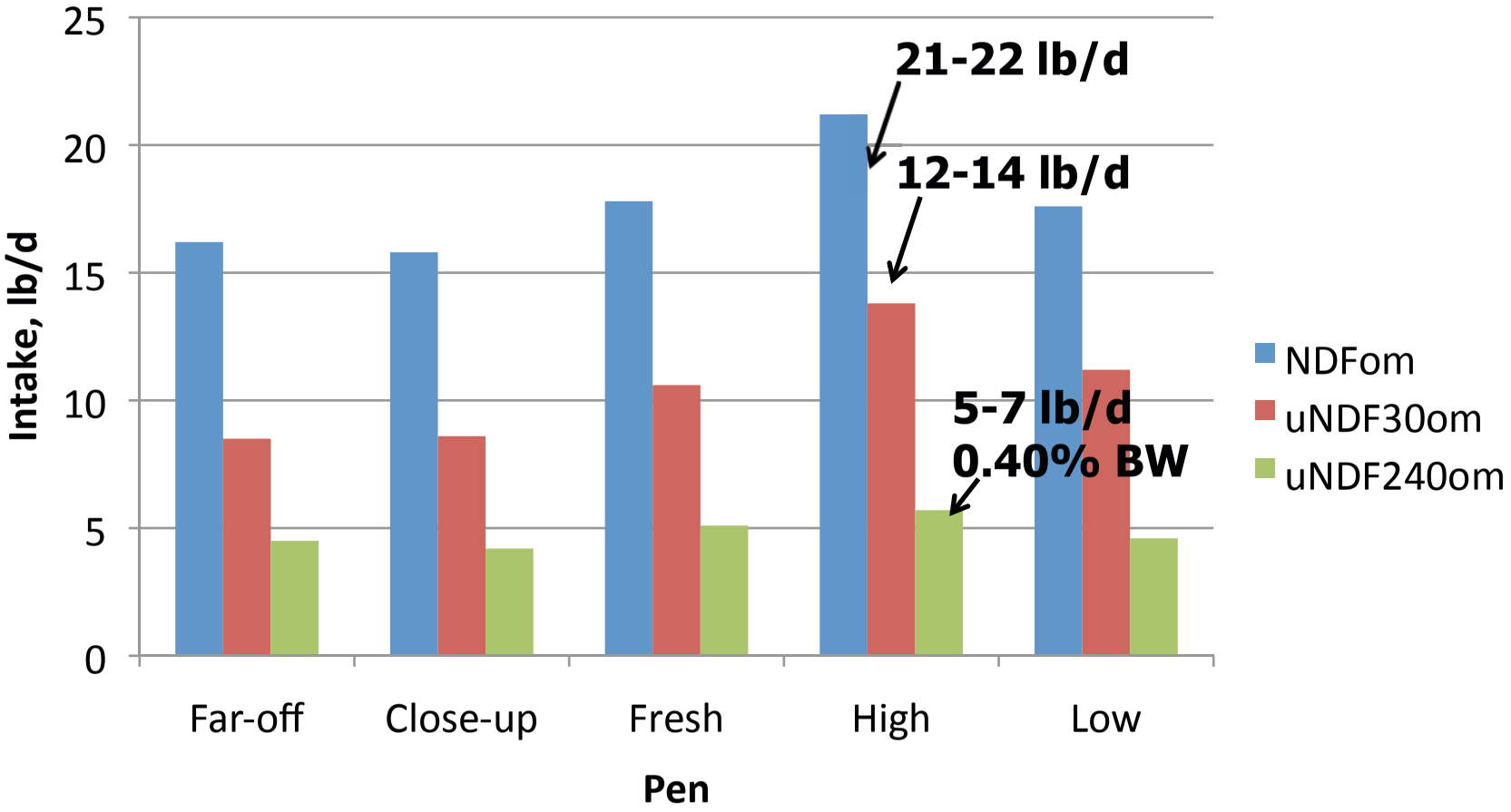
P1 = 72% of NDF

Corn Silage Example: 3-Pool NDF Digestion

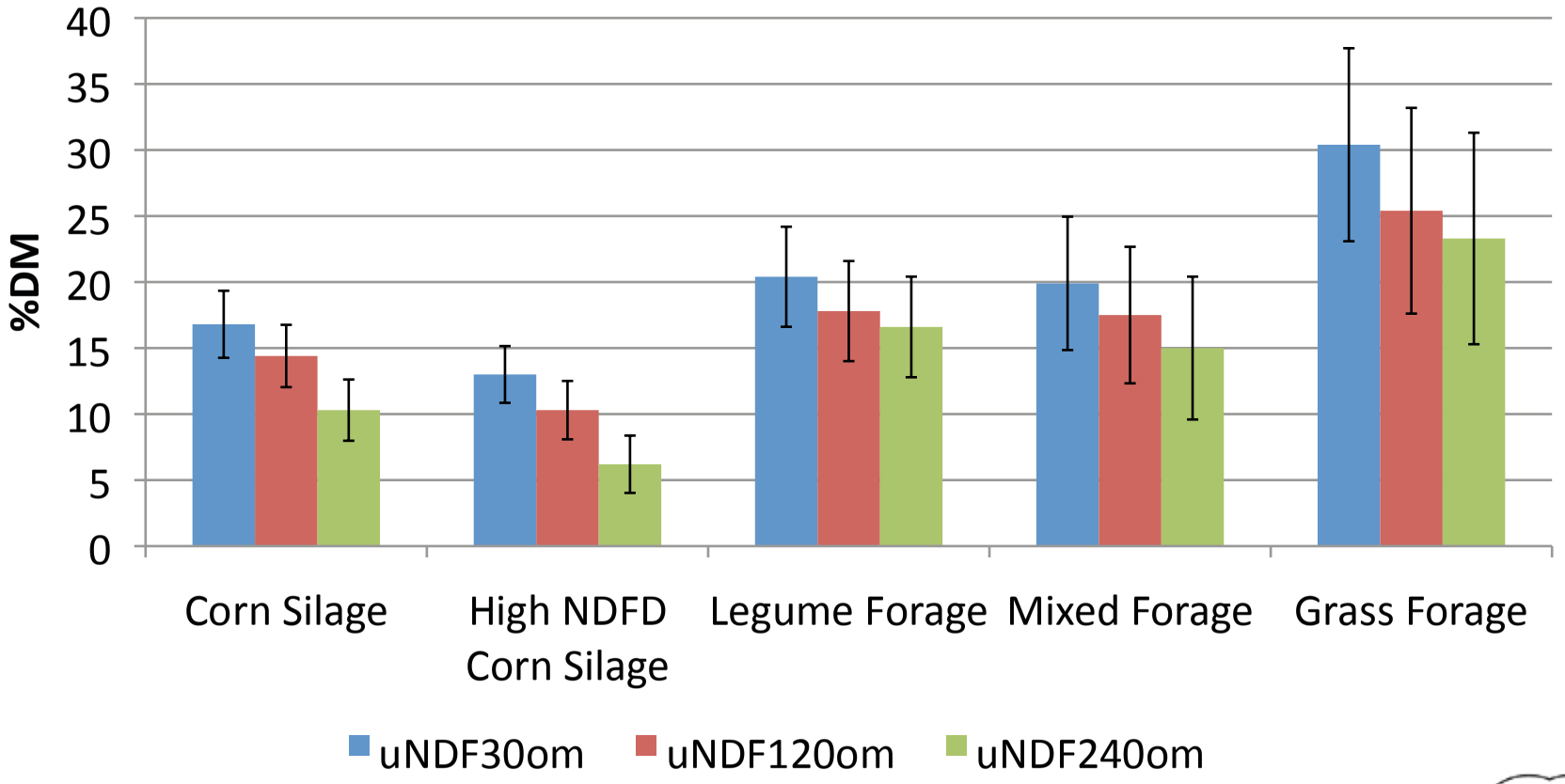


Forage and TMR uNDF benchmarks: how good is my forage?

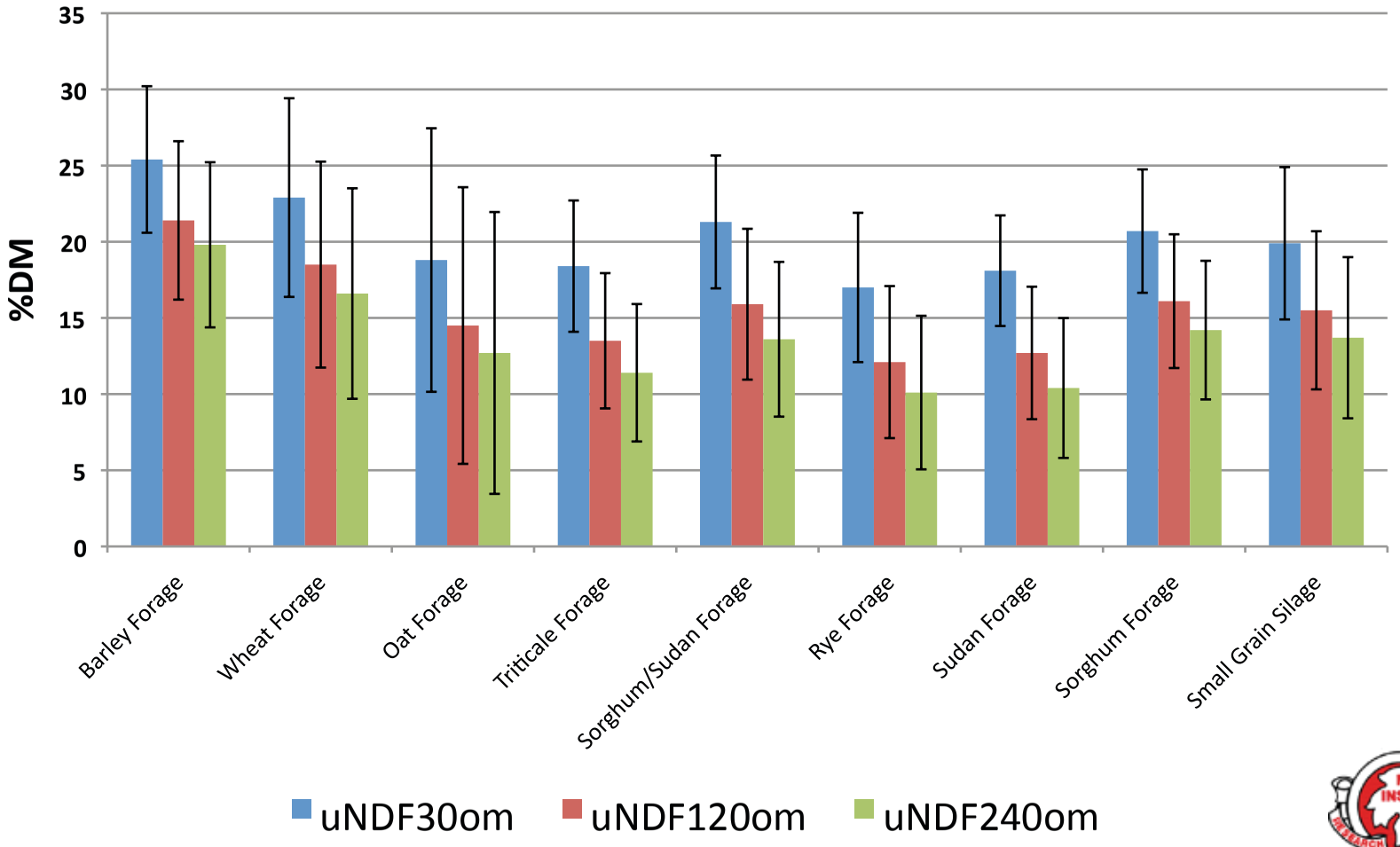
Miner Herd Dietary NDF and uNDFom Intake: Targets



uNDFom profile: 30, 120, and 240 h (CVAS 2015-2016)



uNDFom profile: 30, 120, and 240 h (CVAS 2015-2016)



What do we know about uNDF intake, ruminal fill, and turnover?

Perspectives from Miner Studies…

- Substantial range in diet forage base
  - 36-55% corn silage
  - 39 to 68% total forage
  - Conventional vs BMR (±10%-units NDFD)
  - Added straw (up to 10%)
  - High forage vs NFFS diets
- Cows responded predictably to NDF, NDFD
- High-performance cows across the studies
  - ~61 lb/d DMI
  - ~100 lb/d SCM

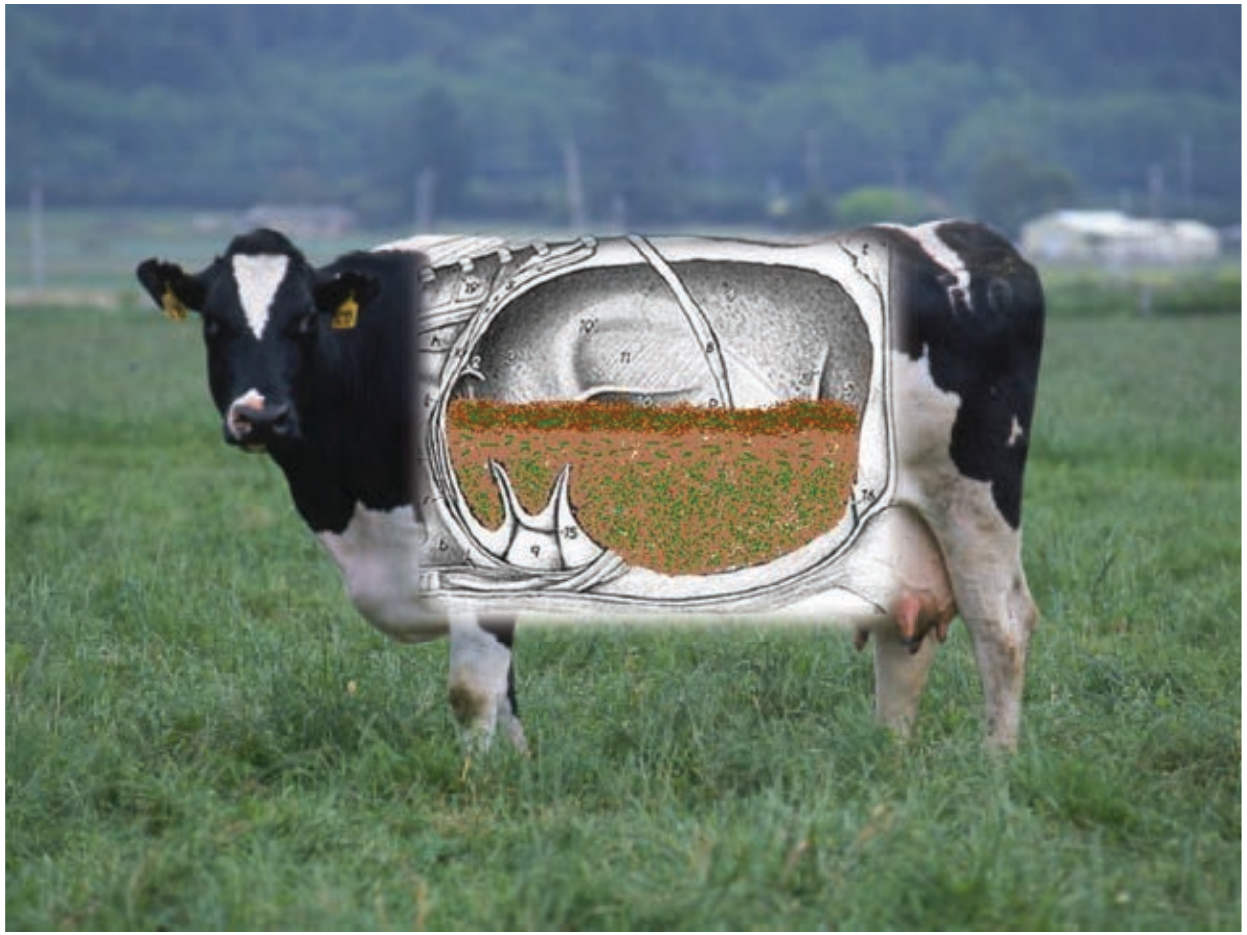
Benchmarks from Miner Studies…String Theory?

- Maximum aNDFom intake is ~1.47% of BW
- Maximum rumen aNDFom is 8.5 kg (1.28% of BW)
- Range in uNDF intake is 0.30 - 0.45% of BW
- Range in uNDF mass in rumen is 0.48 - 0.62% of BW
- uNDF in diet equals uNDF in feces
- Ratio of intake uNDF/rumen uNDF is approximately 0.63 regardless of diet…
  - Equates to rumen passage rate of 2.6%/h for uNDF (0.63/24)
  - Agrees with recent measures of rumen MRT for marked NDF particles from HCS and CS (30-40 hours)

Range in TMR uNDF240 (% of BW)

- Is there a max and min uNDF240 for high-performing cows?
- Suggest:
  - (0.25-0.30) to (0.40-0.45)% of BW
  - Below this range, inadequate rumen fiber
  - Above this range, fill constraint
  - Work in progress…

Rumen Fill Dynamics:
uNDF, Fast and Slow NDF



High quality forage in the future…

- More "fast NDF"
- Less "slow NDF"
- Less "$uNDF_{240}$"
- Decreased eating and ruminating time per unit of NDF consumed
- Increased rumen turnover – can feed more forage
- Makes space for greater dry matter intake

Interaction of Management and Forage Fiber

Forage NDF and time spent eating…

Item	Low CCS	High CCS	Low BMR	High BMR
	53% forage 40%CS:13% HCS	67% forage 54%CS:13% HCS	49% forage 36%BMR:13%HCS	64% forage 51%BMR:13%HCS
TMR NDF, % of DM	32.1	35.6	31.5	35.1
TMR 24-h NDFD, %	56.3	54.0	62.0	60.3
Eating Behavior				
Eating, h/d	4.6^{ab}	5.1^{a}	4.1^{b}	4.6^{b}
% of TCT	34.7	35.7	35.1	33.8

[abc] Least squares means within a row without a common superscript differ ($P \leq 0.05$).

- Higher forage diets with slower fermenting forage-NDF take longer to process.
- Time budget challenge especially when overstocked at feed bunk or mixed parity pens.

Particle size of ingested feed
(Schadt et al., 2011)

	%NDF	Feed, mm		Bolus, mm		Chews /g NDF
		LSM	SEM	LSM	SEM	Avg
Long rye grass hay	57.1	…	…	10.3^{c}	0.4	2.6
50-mm rye "hay"	58.6	42.2^{a}	2.7	9.9^{c}	0.4	3.5
19-mm PSPS hay	57.9	43.5^{a}	1.3	10.7^{bc}	0.4	2.2
8-mm PSPS hay	59.1	25.1^{b}	0.2	10.8^{bc}	0.4	1.7
1.18 PSPS hay	54.2	9.7^{f}	0.2	8.1^{d}	0.4	1.9
Grass silage	53.1	13.8^{c}	0.3	11.6^{ab}	0.4	0.4
Corn silage	48.1	12.0^{e}	0.3	11.2^{bc}	0.4	0.7
TMR	37.7	13.1^{d}	0.2	12.5^{a}	0.4	0.6

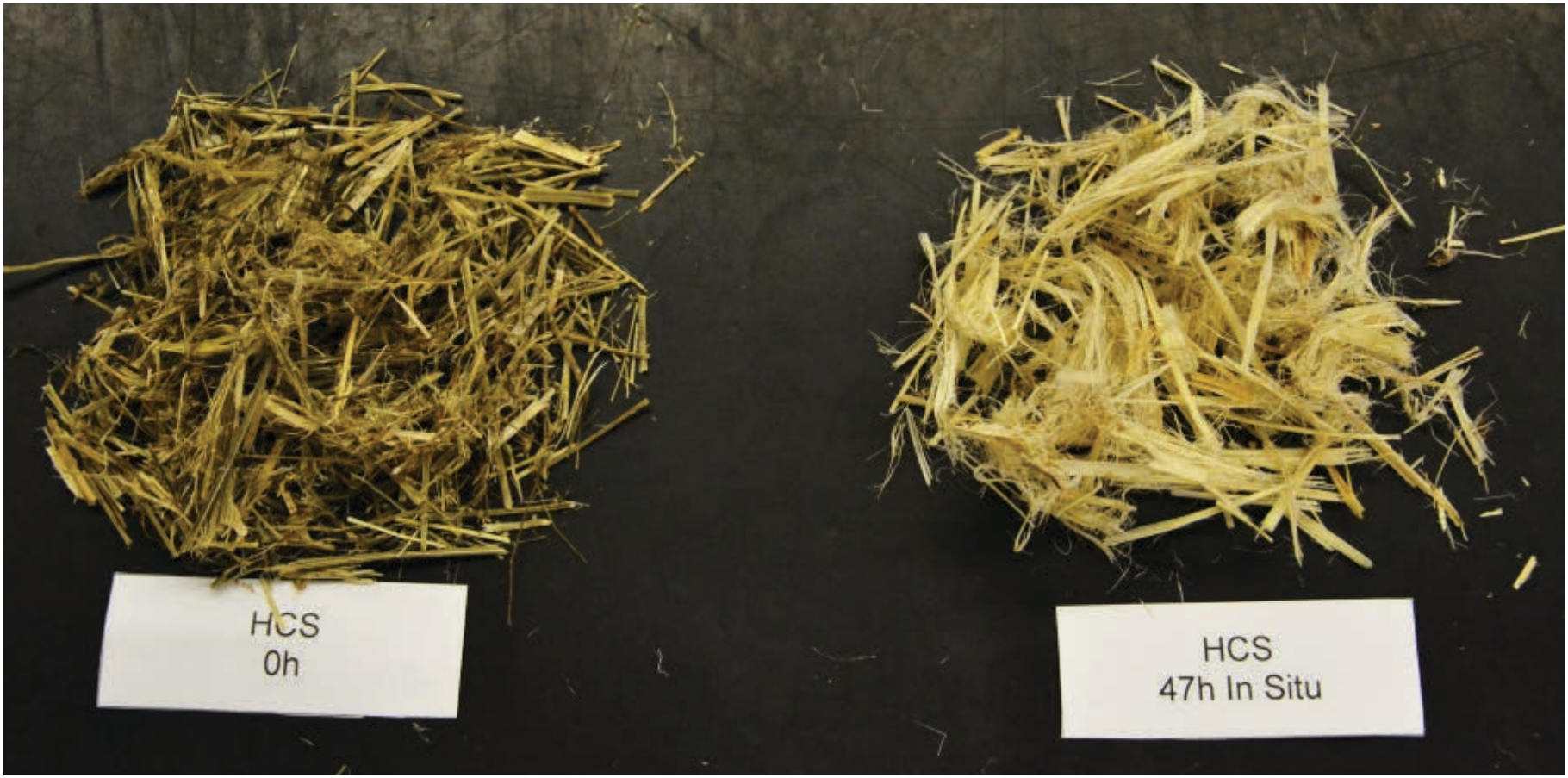
Suggested PSPS targets: Miner Institute (2017)

	Sieve mm	PSPS 2013 %	Miner 2017 %	Comments
Top	19	2-8	0-5	Sortable material, too long, increases time needed for eating; especially if >10%
Mid 1	8	30-50	>50	Still long and functional pef, more so than 4 mm material. Maximize amount on this sieve 50 -60%
Mid 2	4	10-20	10-20	Functions as pef sieve, no recommendation for amount to retain here other than total on the top 3 sieves = pef
Pan	---	30-40	25-30	40-50% grain diet results in at least 25-30% in the pan

Food for thought: have we undervalued rumen digestion and its role in passage and DMI?

Importance of rumen digestion: corn silage NDF (47-h in situ)

Grass silage NDF
(47-h in situ)



Wheat straw NDF residue
(47-h in situ)

Microbial degradation

- Opening of cells
- Leaching of chlorophyll and pigments
- Fraying of fiber strands
- Fragmentation, size reduction
- Buoyancy changes
- Faster passage, greater intake

Perspectives - 1

- Understanding role of NDF **digestibility** and **indigestibility** is critical for predicting cow response to forage-NDF.
- Moving to 3-pool NDF digestion model; better measure of reality
  - uNDF240om
  - Fast NDF
  - Slow NDF

You can formulate a better ration…

Perspectives - 2

- If cow eats more uNDF, there is more in the rumen, up to a maximum amount
- 0.30 to 0.45% of BW, max and min for uNDF240?
- NDF digestibility (indigestibility) affects:
  - Rumen fill and DMI
  - Time budgeting x feeding management
  - Chewing response to peNDF and rumen pH

Bottom Line …

- We are *close* to being able to better model effects of rumen NDF digestibility and indigestibility.
- Exciting time to be feeding forages to dairy cattle.
- Version 7 of CNCPS
- Other models?
- Stay tuned…

Thank you!

www.whminer.org

Carbohydrates: Measuring Them and Managing Them in Dairy Cattle Rations

Mary Beth Hall, Ph.D.
U. S. Dairy Forage Research Center, USDA-Agricultural Research Center, Madison, WI

If you can't measure it, you can't manage it. That saying applies to many things including feed analyses and ration formulation. It's only been in the last 10 or 20 years that we've gotten practical analyses that let us break out feed carbohydrates into fractions that are more nutritionally relevant. And we probably are not done, yet. For the moment, understanding what we can measure and measure accurately, and how the fractions may affect performance is a good start.

Carbohydrate Analyses

Nonfiber carbohydrate (NFC) were intended to describe the most digestible carbohydrates. Calculated by difference as 100 – crude protein – neutral detergent fiber – ether extract – ash, NFC has been in use in some form since the late 1800's. Where NFC fails is that it treats all of the carbohydrates as though they were nutritionally equivalent, and all the errors and variability in the fractions subtracted go into NFC. One of the errors is the degree to which nitrogen content x 6.25 accurately reflects the mass accounted for by protein; it varies by feedstuff (Jones, 1931). Any feed component not measured by the protein, fiber, fat, or ash analyses is included in NFC, even if they are not carbohydrates: ethanol, organic acids, browning reaction products, neutral detergent-soluble gunk (technical term), etc. How inaccurate NFC is for approximating non-NDF carbohydrates varies by feedstuff. In a case such as molasses that can contain approximately 10% browning reaction products (Binkley and Wolfram, 1953), NFC will be overestimated by at least that amount. Ultimately, use of NFC is not recommended if we can do a better job with more nutritionally accurate fractions.

Current schemes for feed analysis splits carbohydrates into water-soluble carbohydrates (WSC), starch, neutral detergent-soluble fiber, and neutral and acid detergent fibers (Figure 1). This fairly

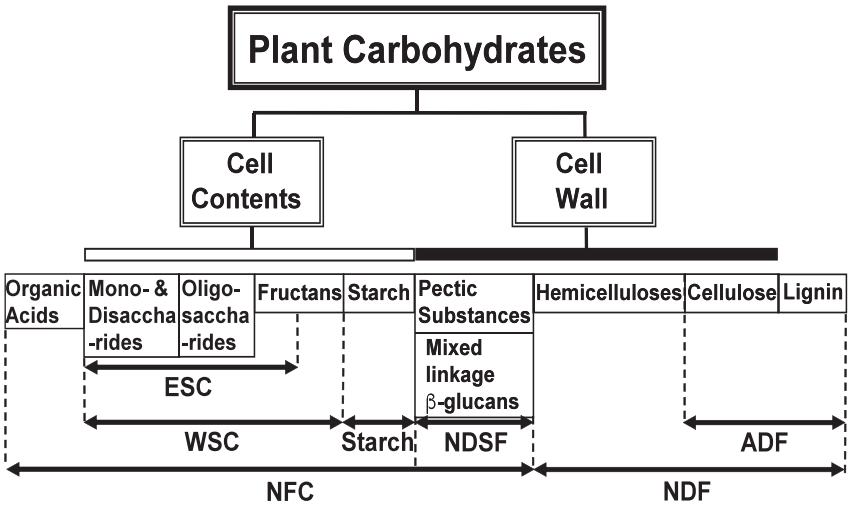


Figure 1. Carbohydrate analyses. ESC: 80% ethanol-soluble carbohydrates, WSC: water-soluble carbohydrates, NDSF: neutral detergent-soluble fiber, NFC: nonfiber carbohydrates, ADF & NDF: acid and neutral detergent fibers.

Water-soluble carbohydrates (WSC). These are very literally the carbohydrates soluble in water and then measured by a broad spectrum carbohydrate analysis (the phenol-sulfuric acid assay). The soluble categorizes the carbohydrates by digestibility characteristics and how rumen microbes utilize them, though there are some differences by carbohydrate type which are also mostly aligned with feed source. carbohydrates have been referred to as "sugars", but the fraction can contain a great variety of carbohydrates, depending on the feed. The group includes simple sugars (glucose, fructose), disaccharides (sucrose, lactose), short chain carbohydrates ("oligosaccharides" like stachyose and raffinose which are in soybeans), and short and long chain fructans which are found primarily in cool season grasses. Typically, sucrose is used as the sugar standard in the analysis, because it's the predominant WSC in most of our feeds. But, we need to keep in mind that the phenol-sulfuric acid assay gives different responses for different sugars. So, if another carbohydrate is known to predominate (like lactose in whey permeate), you will get a more accurate WSC analysis if you use that carbohydrate as the standard for that feed.

Besides WSC, 80% ethanol-soluble carbohydrates (ESC) has been used to measure soluble carbohydrate. However, ESC does not include the long chain fructans or lactose, both of which are insoluble in 80% ethanol. At the end of the day, WSC appears to be a better assay to use than ESC because it gives a more complete value for this group of carbohydrates.

"Total sugars as invert" is a value given for molasses. This is not WSC per se, but is a good value for sum of sucrose, glucose, and fructose in molasses. If whey was added to the molasses to help it flow, the value may or may not include lactose, or may count only half of the lactose, depending on the analysis used.

Starch. This polysaccharide made entirely from glucose is commonly analyzed using enzymes (heat-stable, α-amylase and amyloglucosidase) that specifically hydrolyze the α-1,4 and α-1,6 linkages between the glucoses. The enzymatically-released glucose x 0.9 gives the amount of starch. The 0.9 is the mathematical approach to accounting for the molecule of water that is lost from each glucose molecule when the glucoses bond to each other. Any free glucose in the sample should not be counted as starch; it needs to be extracted away or measured in an assay using no enzymes and then subtracted from the glucose measured after digestion with enzymes.

Starch values may be lower than they should be if the assay is run at neutral or close to neutral pH (should be slightly acidic; Dias and Panchal, 1987), if the sample is not finely ground (1 mm abrasion mill, or 0.5 mm cutting mill), or if the sample is not completely gelatinized so that the enzymes can attack the chemical bonds. Starch values will be inflated if free glucose isn't subtracted, or if the enzymes or run conditions release glucose from carbohydrates other than starch (sucrose can be a problem). Analytical labs should get dry matter basis values of approximately (+/- 2% of dry matter) 100% for corn starch, 90% for glucose, and 0% for sucrose on control samples if the assay is working well.

Neutral Detergent-Soluble Fiber (NDSF). Soluble fiber includes polysaccharides such as pectin, mixed linkage beta-glucans and gums that are soluble in neutral detergent and are not digestible by mammalian enzymes. Present assays that specifically analyze for pectins and gums are tedious and expensive and not typically practical to be run on feeds. The NDSF is a by-difference approach to give an approximation of this group of carbohydrates. The soluble carbohydrates are extracted away, starch is measured, and the difference between the 80% ethanol extracted residue and the neutral detergent residue, both corrected for crude protein and ash, minus starch gives NDSF. This assay has the same issues that any by-difference assay has: the errors and variability in the component assays. Large fructans will be found in both WSC and NDSF, but this should only affect cool season grasses.

Fiber. Analysis of neutral detergent fiber (NDF) varies in whether heat-stable, α-amylase or sodium sulfite is added. Amylase removes starch and sulfite breaks disulfide linkages to remove protein. Their combined use gives NDF values that are more hemicellulose, cellulose, and lignin, and less contaminating material. The only advisable reason not to use sodium sulfite is to produce samples for determining neutral detergent insoluble nitrogen (NDIN or NDFCP both expressed as crude protein with N x 6.25). Subtraction of NDFCP from the NDF value is only appropriate when both assays are run using the same reagents.

Acid detergent fiber (ADF) is comprised of cellulose, lignin, and acid detergent insoluble nitrogen (ADIN or ADFCP both expressed as crude protein with N x 6.25). ADIN has been used to estimate undigestible or heat-damaged protein in feeds. However, at least some portion of heat-damaged proteins may be digestible and utilized by the animal (Machacek and Kononoff, 2009).

Ash is the mineral in feedstuffs that remains after incinerating a sample. Ash is important to the discussion of fiber because of its potential to inflate values with a contaminant that is not carbohydrate and has no potential to be fermented by gut microbes. Common methods for analyzing NDF and ADF report results on a with-ash or ash-free basis. Ash in feed samples comes from minerals in plant cells, added minerals, from soil contamination, or from biogenic silica that plants, commonly grasses, naturally incorporate into their structure. "Ash-free" requires that the residue remaining after extraction with neutral or acid detergents be incinerated and the residual ash subtracted so that it is not counted as part of the fiber. "With-ash" leaves that mineral as part of the fiber and so inflates the fiber value and the estimate of potentially fermentable cell wall. Most commercial analyses have given fiber values on a with-ash basis; it improves turnaround time on samples and the contaminating mineral is often a low value with NDF. Soil contamination inflates both NDF and ADF analyses because neither completely solubilizes it. A feed sample heavily contaminated with soil will also show unusually high ash values (approximately 5% or more of dry matter than average values for a feed). If the high ash content is specific to the subsample, the best option is to take and analyze a different, uncontaminated sample. If the feed source itself is high in ash, running the fiber measures on an ash-free basis is the best way to determine the actual carbohydrate content. Because biogenic silica is soluble in neutral detergent but is quantitatively recovered in the residue with acid detergent (Van Soest, 1994), to get an accurate assessment of carbohydrate in ADF, particularly with grasses,

ADF should be determined on an ash-free basis, or by running a sample sequentially through the NDF and then ADF analyses.

Managing Carbohydrates In Rations

One of the challenges we have in coming up with firm recommendations for carbohydrate feeding in dairy cattle diets is that "it depends", and we do not always know on what, and we continue to learn more about how the different fractions function in the cow. How do these things affect the outcome: the amount, particle size, fragility, and fermentability of the fiber sources? Fermentability of the starch? Total protein or ruminally degradable protein (RDP) content of the diet? Types of RDP? And how do all these pieces fit together and interact to determine nutrient supply and animal performance? Here, we will focus on the non-NDF carbohydrates. There is relatively little information available on NDSF and fructans.

Current TMR formulations in the upper Midwest and Northeast use from 3 to 7% WSC, 20 to 30% starch, 7 to 12% NDSF, and 26 to 32% NDF (~75% forage NDF/total NDF). The 2001 Dairy NRC (Table 4-3) had NFC decreasing and total NDF increasing as forage NDF decreased; the NFC in their example would have mainly been starch. Feeding studies have shown that starch isn't required to provide energy, though it is a convenient and readily available form here in the U.S. A survey of rations that maintained good health and performance in commercial herds was updated with 3 studies that varied starch vs. other NFC and maintained milk production of 75 to 88 lb of milk (Figure 2). Two of the studies only reported starch composition of the diets. The starch, ESC (proxy for WSC), and NDSF in the original survey showed starch increasing, ESC decreasing, and NDSF little changed as forage percentage of the rations increased. Those changes did not necessarily indicate what the ideal diets were, as much as what worked with feeds that were available in given areas. It did agree with our concept that you need to make sure that there is adequate chewable fiber from forage in the ration to maintain ruminal pH when much fermentable starch is fed. However, with the research studies added, the picture is not as clear (gray symbols in graph). The starch, ESC and NDSF contents of the research diets differed a lot from the pattern that showed up on the commercial farms,

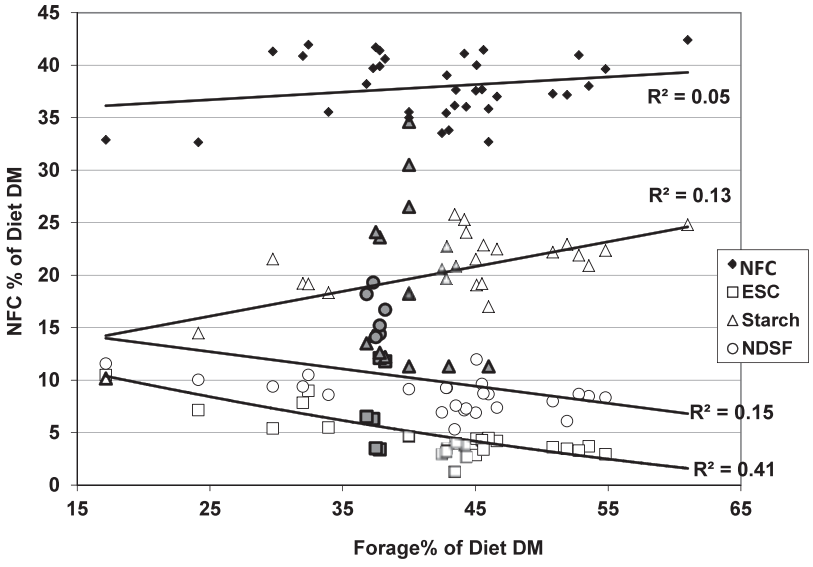


Figure 2. Relationship of ESC (proxy for WSC), starch, NDSF and their sum to dietary forage in healthy, high producing herds, and in research studies (gray-shaded symbols). (Hall and Van Horn, 2001; Voelker and Allen, 2003; Hall et al., 2010; Hall and Chase, 2014).

but cow performance was still good. Starch (triangles) was higher (35%)or lower (11%), ESC (squares) was higher (12%), and NDSF (circles) was higher (15-19%) on some of the experimental diets. The research rations were in a far narrower range of forage inclusion than were the commercial herd diets. What is interesting is that NFC or the sum of ESC, starch, and NDSF was relatively flat across the herds and studies, averaging 38% of ration dry matter, and ranging primarily from 35 to 42% of dry matter. A side note: diets up to approximately 20% sugars from sugar cane have been fed to Nellore steers at intakes of ~12 lb (Sousa et al., 2014).

Lacking clear targets for how much of the individual carbohydrate types to feed, understanding how they behave in the rumen and can affect animal performance can help us to figure out how to work with them.

Rumen Function

Rumen microbes convert feed carbohydrates into a number of products including organic acids (lactate, acetate, propionate, butyrate, valerate), gases (carbon dioxide and methane), microbial cells, and glycogen (Figure 3). Glycogen is a polysaccharide with a structure very similar to starch that is made and stored internally by both protozoa and bacteria. It may be fermented later by the microbes, or pass from the rumen. There can be a significant flow of glycogen (with potential to digest like starch) to the small intestine even on all forage rations (Branco et al., 1999). So, glycogen production essentially slows down fermentation and acid production, relative to the readily available carbohydrate from which it was formed. A hidden cost of glycogen accumulation: each glucose stored in glycogen has a cost equivalent to 1 ATP to add it to the polysaccharide chain (Ball and Morell, 2003). So, if rumen microbes obtain 3 to 4 ATP from fermenting a carbohydrate (Russell and Wallace, 1988), storing it as glycogen effectively decreases the ATP yield by 25 to 33%, reducing the amount of energy available to drive microbial cell production. Factors affecting glycogen production:

- More microbial glycogen is made when greater amounts of rapidly available carbohydrate are present (Prins and Van Hoven, 1977), particularly if there is more relative to their ability to process it and use the energy; this may be an alternative to energy spilling.
- More available RDP can decrease glycogen production (McAllan and

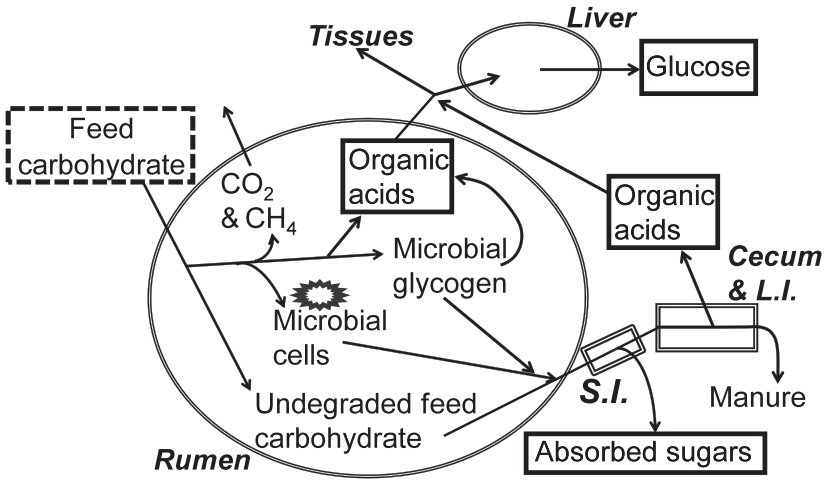


Figure 3. Fates of carbohydrates. SI = small intestine, LI = large intestine.

Smith, 1974) and

increase the flux of carbohydrate through fermentation, which can also increase lactate production (Counotte and Prins, 1981; Malestein et al., 1984).

Some general observations about the effects of carbohydrates, but that are not always observed:
Increased milkfat production with feeding more sugars (Broderick and Radloff, 2004; Broderick et al., 2008; Penner and Oba, 2009).
Starch gives more milk protein than other NFC (Broderick and Radloff, 2004; Sannes et al. 2002; Hall et al., 2010).

What we know (the most information is available on starch and sugars…):

- Sugars can be very rapidly utilized in the rumen for fermentation but also can be used by microbes to produce substantial amounts of glycogen which slows fermentation and acid production (Thomas, 1960).
- Lactose and orchardgrass fructans are taken up more slowly by microbes and produce less glycogen than does glucose (Figure 4; Hall 2016; Hall and Weimer, 2016). Fructans appear to ferment more rapidly than lactose.
- Starch can also be converted to microbial glycogen.
- Sucrose has been reported to yield less microbial protein than starch (Hall and Herejk, 2001; Sannes et al., 2002) even when rumen pH was not an issue. The reduction in protein with sucrose may be related to greater glycogen production and less energy available for microbial cell growth.
- Given glucose as a substrate, ruminal microbes prefer to use amino nitrogen (amino acids, peptides) rather than ammonia or urea (Hristov et al. 2005; Hall, 2017).

❖ For the milk protein picture, it is possible that this is related to relatively more glycogen and less microbial protein being produced with sucrose and glucose than with starch. There's also the possibility that the type of RDP available in the rumen will modify the results. As for lactose, reports from the field suggest that this more slowly fermenting sugar can be substituted for corn grain.

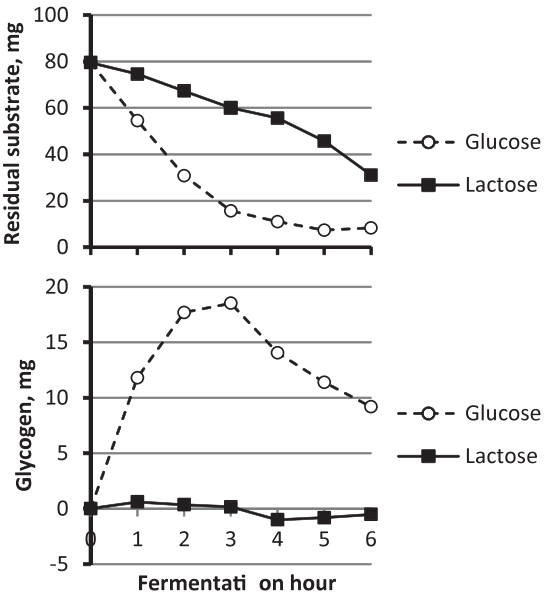


Figure 4. When fermented with ruminal microbes in vitro, lactose disappears more slowly than glucose, and the microbes make more glycogen from glucose. (Hall, 2016).

• Fermentation of sugars tends to produce more butyrate than does starch, and may yield more lactate (Strobel and Russell, 1986).
• Some species of glucose-utilizing microbes perform biohydrogenation on fatty acids in the rumen (*Butyrivibrio fibrosolvens*; McKain et al., 2010).

❖Milkfat production increases associated with sugar feeding may be related to increased biohydrogenation of fatty acids to forms that will not cause milkfat depression, or the increased butyrate influences milkfat production. Occasionally, modifying dietary protein can increase milkfat. Is this due to increasing the fermentation and growth of the microbes that biohydrogenate or make butyrate?

Additional note on sugars vs. starch: In a ruminal acidosis induction study, crushed wheat caused more severe ruminal lesions than did molasses. With crushed wheat, the ruminal pH continued to fall over 120 hours reaching 3.93, whereas with molasses, it fell to 4.76 by 24 hours and then started to rise (Randhawa et al., 1982). This may be due to the molasses moving with liquid vs. starch being a solid that would not as readily pass from the rumen. And possibly due to formation of glycogen with the more readily available sugars.

Additional note on starch: We think of starch as a major source of propionate, but that may only be true when substantial amounts of starch or fermented feeds with lactate are fed (Murphy et al., 1982). The increase in ruminal propionate concentrations commonly associated with increased starch feeding may be a function of a change in the microbial population and pH rather than a characteristic of starch itself (Baldwin et al., 1962; Mackie and Gilchrist, 1979).

Influence of Protein on Ruminal Carbohydrate Use

• Increasing availability of RDP increases the yield of microbial protein per amount of carbohydrate (Figure 5; Argyle and Baldwin, 1989). Possibly by decreasing glycogen production and providing a needed nutrient for growth.
• Increasing RDP may increase ruminal organic acid concentration and decrease pH (Aldrich et al., 1993), and increase ruminal lactic acid concentration (Hall, 2013). Again, this may be due to decreasing glycogen production and increasing the flux of carbohydrate through fermentation.
• Increasing RDP can remove the depressing effect of nonfiber carbohydrates on fiber fermentation (Heldt et al., 1999).

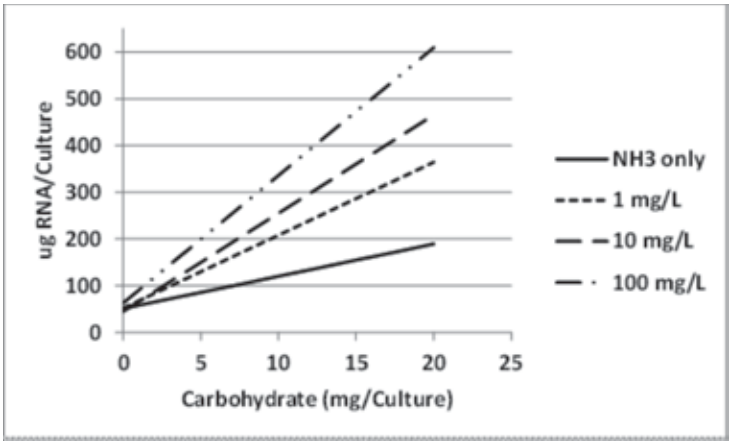


Figure 5. Microbial yields (as micrograms of RNA/mg of carbohydrate added) for mixed ruminal microbes after 6 hours of fermentation. The "mg/L" values are for the amount of amino acids + peptides added; media for all treatments contained 3.6 millimolar ammonia. (Argyle and Baldwin, 1989).

Thoughts on Applications

The different fractions of carbohydrate have distinctly different fermentation and digestion characteristics that may have benefits or drawbacks under different scenarios. A key seems to be providing enough readily digestible carbohydrate of whatever types without creating problems in the rumen (acidosis) and the rest of the digestive tract (enteritis). This means paying attention to adequacy of the physical form of the ration and how that fits with the different carbohydrate components. That requires observing the cows for signs that they are functioning well with their ration. That protein supply can modify how rumen microbes process carbohydrate tosses in another variable that we have not explored well. Use RDP without overfeeding as a modifier of microbial growth or acid production in the rumen? Potentially. We still have substantial gaps in our knowledge regarding how best to utilize different carbohydrate sources in dairy cattle rations.

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Biology, Behavior, and Management of Flies associated with Animal Agriculture

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FLY IMPACTS:

Of the many thousands of species of true flies (order Diptera), only a few have a close association with animals. Of particular significance are those fly species that complete their immature development in animal feces and are therefore called "filth flies" (Table 1). Wet feces are an excellent developmental environment for these filth fly species, while also supporting the survival of many disease-causing pathogens. In addition to contact with feces during immature development, adult flies will visit feces to feed and to deposit eggs. To acquire nutrients needed for adult survival and egg development, some of these fly species also feed readily on available animal feed, as well as on sweat or other animal body secretions.

Two species (stable fly and horn fly) feed on animal blood as adult flies, and their painful bites result in reductions in animal weight gains and milk yields due to animal discomfort. These production impacts are largely the result of reduced feed and water consumption coupled with increased animal activity and animal heat stress as harassed animals exhibit a number of defensive behaviors to avoid the painful bites. One of these defensive behaviors, cattle bunching, is often observed when biting stable flies become numerous (Weiman et al. 1992). Overall losses to the animal industry in the United States from these blood-feeding filth flies are estimated at over $2 billion annually (Drummond 1987, Taylor et al. 2012).

Filth flies, particularly house flies and flies in the blow fly family (Calliphoridae), have been implicated in the transmission of a phylogenetically diverse group of human and animal pathogens associated with feces and which cause enteric illness (Greenberg 1973, Sasaki et al. 2000, Chakrabarti et al. 2007, Ahmed et al. 2007). While the extent to which filth flies are responsible for the transmission of pathogens to animals is unclear, there is little doubt that filth flies are responsible for the dispersal and transmission of numerous enteric pathogens among animals, and may also move these enteric pathogens to nearby pre-harvest human food plants (Talley et al. 2012).

Table 1: Common filth fly species in the U.S. and their developmental habitat

Filth Fly Species	**Immature Habitat**
House fly (*Musca domestica)*	Feces, food waste, sewage sludge
Face fly (*Musca autumnalis)*	Fresh cattle feces
Little house fly (*Fannia canicularis)*	Poultry feces
Stable fly (*Stomoxys calcitrans)*	Cattle feces, hay waste, green waste, sewage sludge
Horn fly (*Haematobia irritans)*	Fresh cattle feces
Blow flies (Family: Calliphoridae)	Food waste, carrion, feces

FLY LIFE CYCLE:

Filth flies undergo complete metamorphosis with egg, larva, pupa, and adult stages in their development (Fig. 1). Female flies deposit eggs in animal waste or other moist organic material where the larvae complete their development feeding on the bacteria or organic material associated with the developmental site. Immature filth flies pass through three larval instars (stages), growing larger with each successive instar. At the end of the third instar stage, the larvae typically enter a "wandering stage", where they leave their development sites in search of a dry and protected location to pupate. The rate of fly development from egg to adult, as with all insects, is dependent upon temperature. Under summertime conditions throughout the United States, many filth flies can complete development from egg-to-adult in as little as 6-8 days (Moon 2009).

Figure 1: Life cycle of the house fly. Images by Alec Gerry and Kim Hung, UC Riverside.

FLY MANAGEMENT

Although adult flies are the cause of nuisance and the carriers of pathogens, the larval stages should be the prime target for control efforts. Because elimination of suitable larval habitat will prevent subsequent production of adult flies, sanitation is the first line of defense against filth flies. At animal production facilities, filth flies are best managed through practices designed to reduce the attractiveness of manure to egg-laying female flies, as well as practices designed to reduce the quantity and quality of manure that might be suitable for larval development. Frequent collection and removal of wet animal manure to offsite sanitary landfills can be a suitable strategy, but is often cost prohibitive for a large animal facility. More typical is the frequent collection of manure by scraping animal housing areas or the capture of manure solids in wash water followed by composting of manure in piles or windrows to reduce the surface area of the manure available to developing flies. In cases where frequent manure removal is not an option, every effort should be made to keep the manure as dry as possible by good ventilation and the prompt repair of water leaks that wet the collected manure and result in the creation of larval development "hot spots".

Many natural enemies of flies are generally present wherever these flies occur. Fly eggs and larvae are eaten by predators, fly pupae are killed by parasitic wasps, and adult flies have viral and fungal diseases that shorten their lifespan and reduce their egg output (Geden 2006). These natural enemies provide "free" fly control and can be encouraged by avoiding broadcast insecticide applications and by efforts to keep manure accumulations as dry as possible.

Chemical control methods may be needed on animal facilities when sanitation measures fail and fly surveillance shows rapidly increasing numbers of adult flies exceeding a treatment threshold (Gerry 2011). Adults of all filth fly species may be reduced using insecticides applied as liquid sprays or fogs to locations such as animal housing or shade structures where the adults of these fly species tend to rest. Application of insecticides as sprays or liquid pour-ons directly applied to animals, or in insecticide impregnated ear tags can be used to reduce blood-feeding flies (Gerry et al. 2007). Flies exposed to the same chemical class continuously will surely and rapidly become resistant to products in the same chemical class. Consultation with local extension staff or other knowledgeable personnel can help identify which insecticides are still effective for these pests in a particular geographic area.

Recent efforts to reducing fly biting pressure on cattle have focused on the application of low-toxicity repellents applied directly to animals. Some plant essential oils may even prove suitable for use on organic facilities. Two low-toxicity repellents, geraniol and a mixture of short chain fatty acids (C8-C9-C10) have shown considerable promise in this regard (Mullens et al. 2009, Zhu et al. 2014). Additional studies with these repellents that were recently conducted in California and North Carolina will be discussed.

Given that this presentation on fly management will be given to researchers and others interested in animal nutrition, I would be remiss if I did not mention the "feed-through" products that are registered for use to control flies that develop in animal feces. These products pass through the animal digestive system and into the animal feces where they are present in sufficient concentrations to prevent fly development in the feces. A search of the on-line VetPestX pesticide registration database for "feed-through" products to control flies on cattle

yields eight different products with only two different active ingredients (diflubenzuron and tetrachlorvinphos) representing two different chemical classes. This searchable database of insecticides registered for use on animals was recently produced by my laboratory and is available to search for free by visiting the Insect Pests of Animals website at http://veterinaryentomology.ucr.edu/ and then selecting the VetPestX tab. Recently, a newer benzoylphenyl urea insecticide (Novaluron) has also showed promise as a feed-through for control of biting flies by disrupting insect development in feces of treated animals (Lohmeyer et al. 2014). I will discuss the availability and use of feed-through insecticides during the presentation.

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Creating the Perfect Dining Experience:

Integrating Cow Behavior, Housing, and Feeding Management

Rick Grant

W. H. Miner Agricultural Research Institute

Chazy, NY

Do you provide the perfect dining experience?

- Well-formulated, palatable ration
- Feed available 24/7
- Competition doesn't limit feed access
- Water availability
- No restrictions on resting or ruminating activity

Know your customer…

- **Natural feeding behavior of dairy cows:**
  - **Crepuscular**
  - **Allelomimetic**
  - **Competitive**
- ***Does your "dining" environment accommodate or frustrate these basic feeding drives?***

Focus on diet formulation and feeding environment

Stocking Density, Dietary Fiber, and Feed Access: Focus on Rumen pH

(Campbell, 2016)

Ingredient composition
(% of ration DM)

Item	No Straw (NS)	Straw (S)
Corn silage	39.7	39.7
Hay crop silage	6.9	2.3
Wheat straw, chopped	…	3.5
Citrus pulp, dry	4.8	4.8
Whole cottonseed, fuzzy	3.5	3.5
Soybean meal, 47.5% solvent	…	1.1
Molasses	3.2	3.2
Concentrate mix	41.9	41.9

Chemical composition
(% of ration DM)

Item	NS	S
CP	15.0	15.1
NDF	30.8	30.1
Starch	25.0	25.5
Sugar	7.4	8.1
Ether Extract	5.9	5.7
7-h starch digestibility, % of starch	73.3	74.3
$peNDF_{1.18\ mm}$	18.8	20.1
30-h uNDFom	13.1	14.9
240-h uNDFom	8.5	9.7

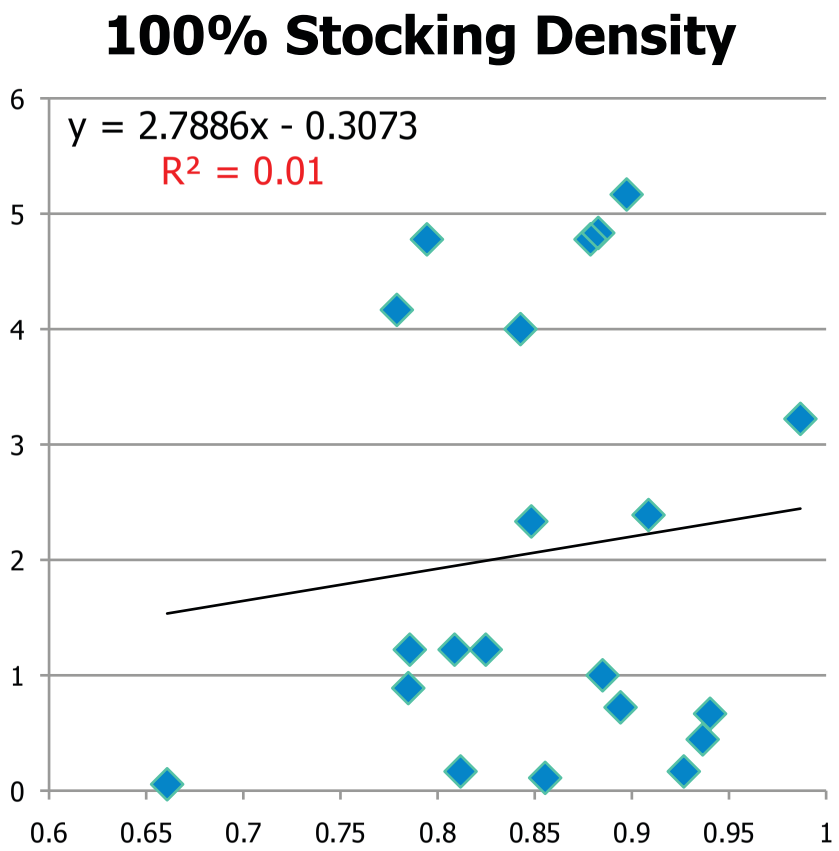
Stocking density, dietary fiber, and rumen pH

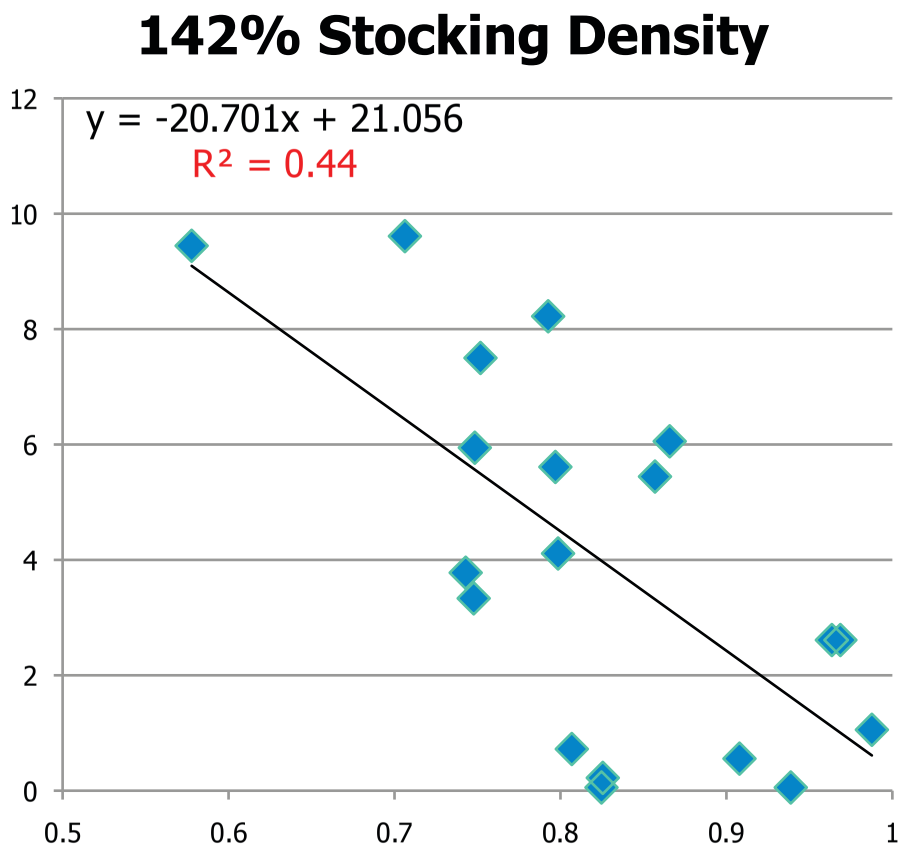
	100%		142%		SD	Diet
	S	NS	S	NS		
pH<5.8, h/d*	1.90	2.29	2.77	4.12	<0.01	0.01
AUC<5.8, pH units x h/d*	0.19	0.38	0.34	0.58	0.06	0.03

*Interaction ($P < 0.09$).

- ✓ Associated with 6% less lying time (~50 min/d) and 7% less recumbent rumination (~30 min/d).

Rumination in stalls and ruminal pH (h/d < pH 5.8)



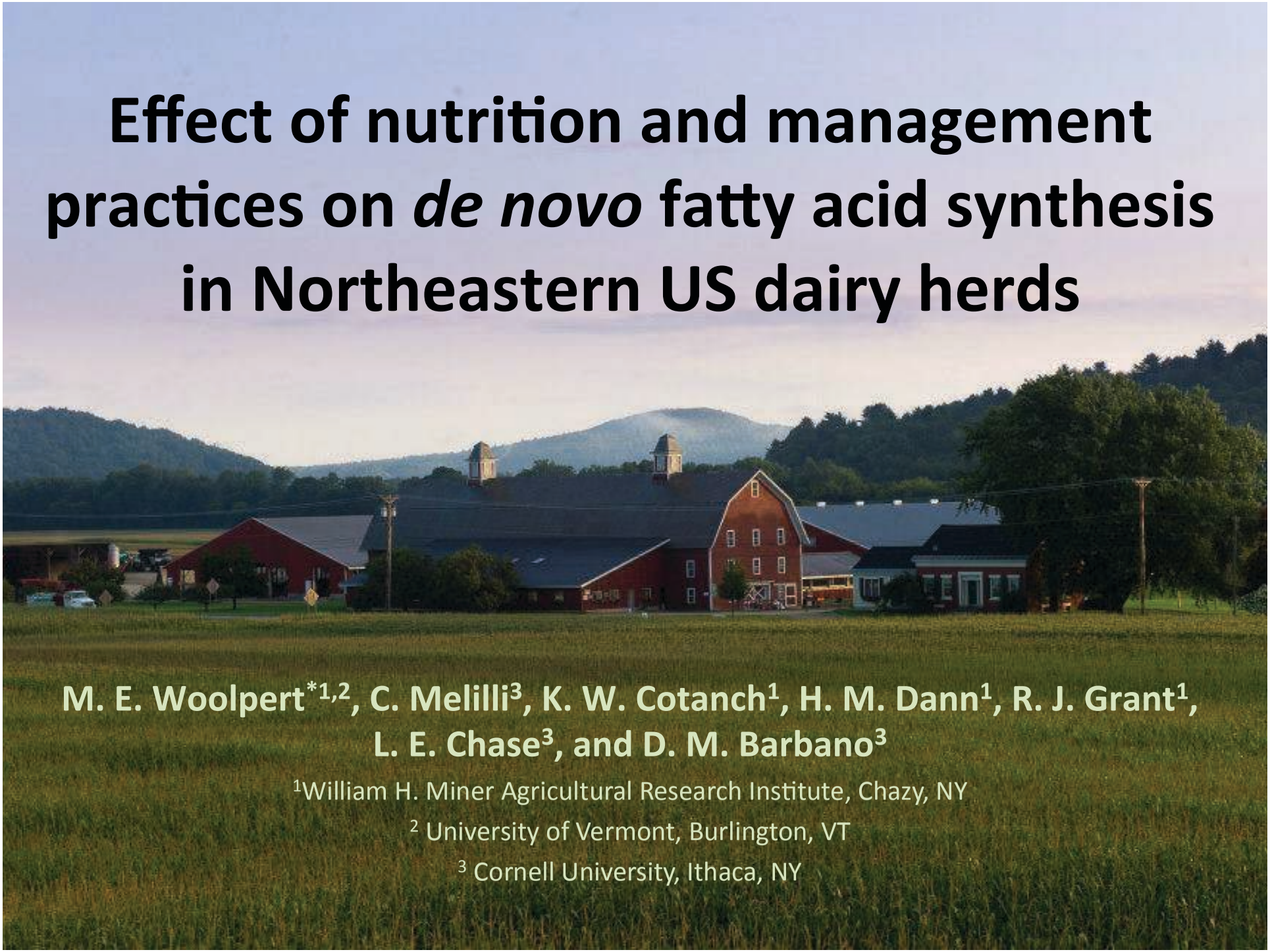


Feed access and stocking density: rumen pH (Campbell et al., 2016)

	100%		142%		
Variable	NR	R	NR	R	SEM
Time pH < 5.8, h/d	6.62	5.23	6.78	8.77	1.27
AUC < 5.8 pH, pH x unit	1.66	1.24	1.73	2.55	0.63

- ✓ Feed restriction: 5-h without access to TMR mimics "slick bunk" management.
- ✓ Feed restriction is more negative when cows are overcrowded.

Effect of nutrition and management practices on *de novo* fatty acid synthesis in Northeastern US dairy herds



M. E. Woolpert[*1,2], C. Melilli[3], K. W. Cotanch[1], H. M. Dann[1], R. J. Grant[1], L. E. Chase[3], and D. M. Barbano[3]

[1]William H. Miner Agricultural Research Institute, Chazy, NY

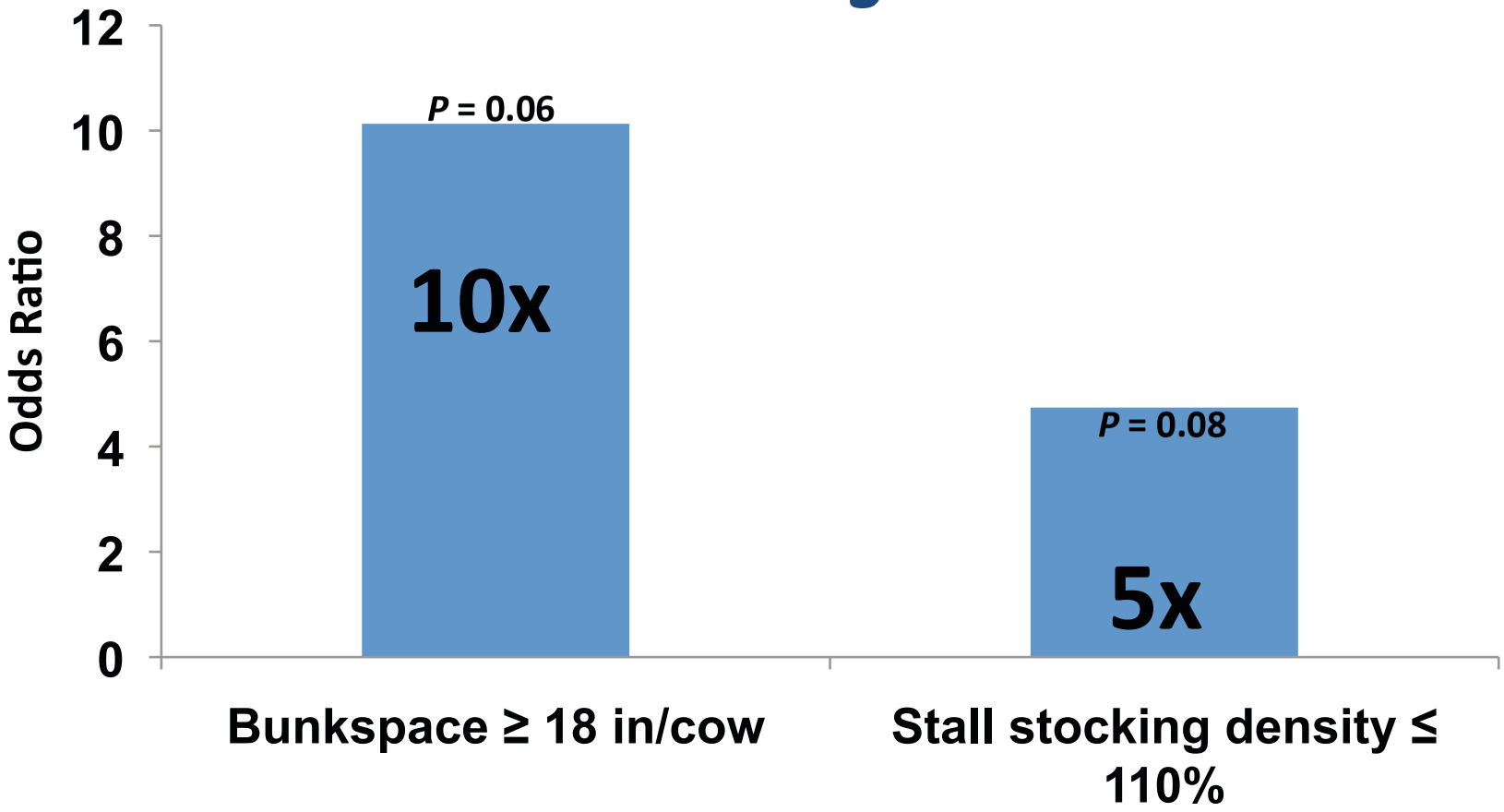
[2] University of Vermont, Burlington, VT

[3] Cornell University, Ithaca, NY

Why the interest in de novo fatty acids? (Woolpert et al., 2015)

- De novo FA synthesized from rumen fermentation products acetate and butyrate (i.e. fiber fermentation).
- ***De novo* FA synthesis is a reflection of rumen health…**
- Greater de novo FA mean more milk fat and protein.
- Milk cooperatives are beginning to use routine milk FA analysis to monitor feeding and management on-farm.

HDN herds have lower stocking density



	HDN	LDN
Mean	19.7 in	15.7 in

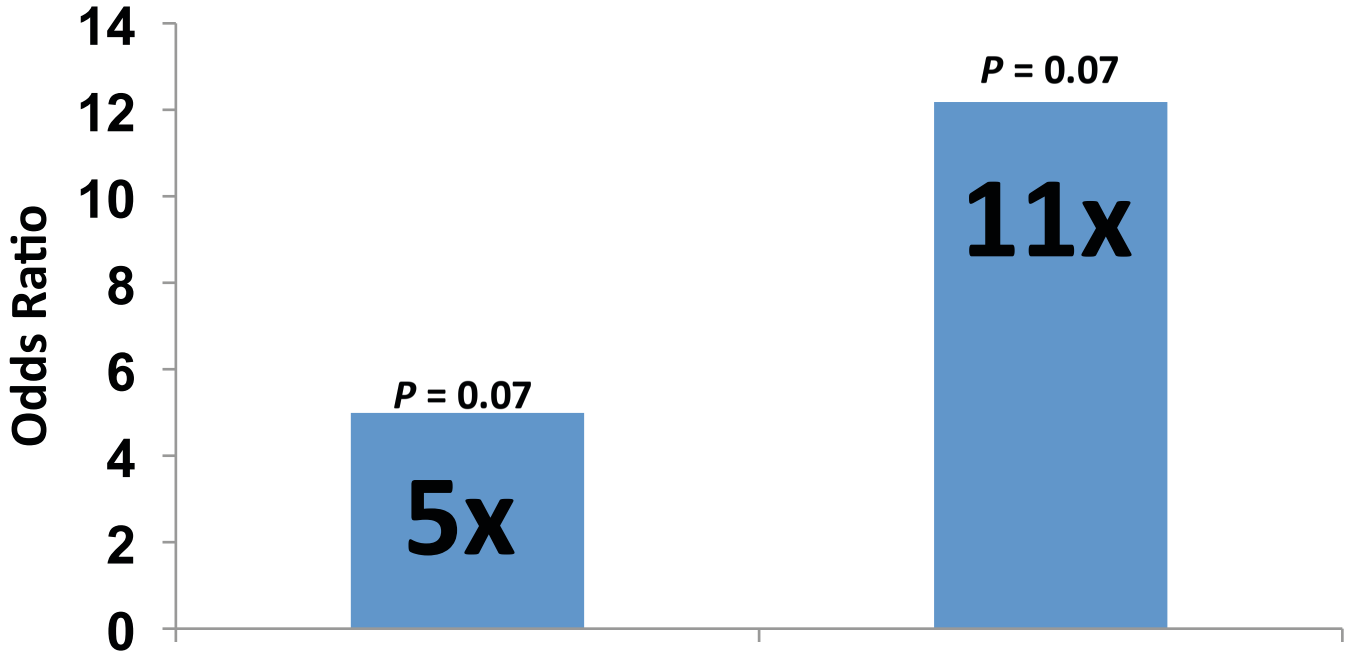
	HDN	LDN
Mean	111%	116%

Management and Milk Components

- Higher de novo milk fatty acid synthesis
  - 65% of variation explained by bunk space
  - De novo, relative % = 20.12 + 0.09 x bunk space, cm;

    P < 0.002

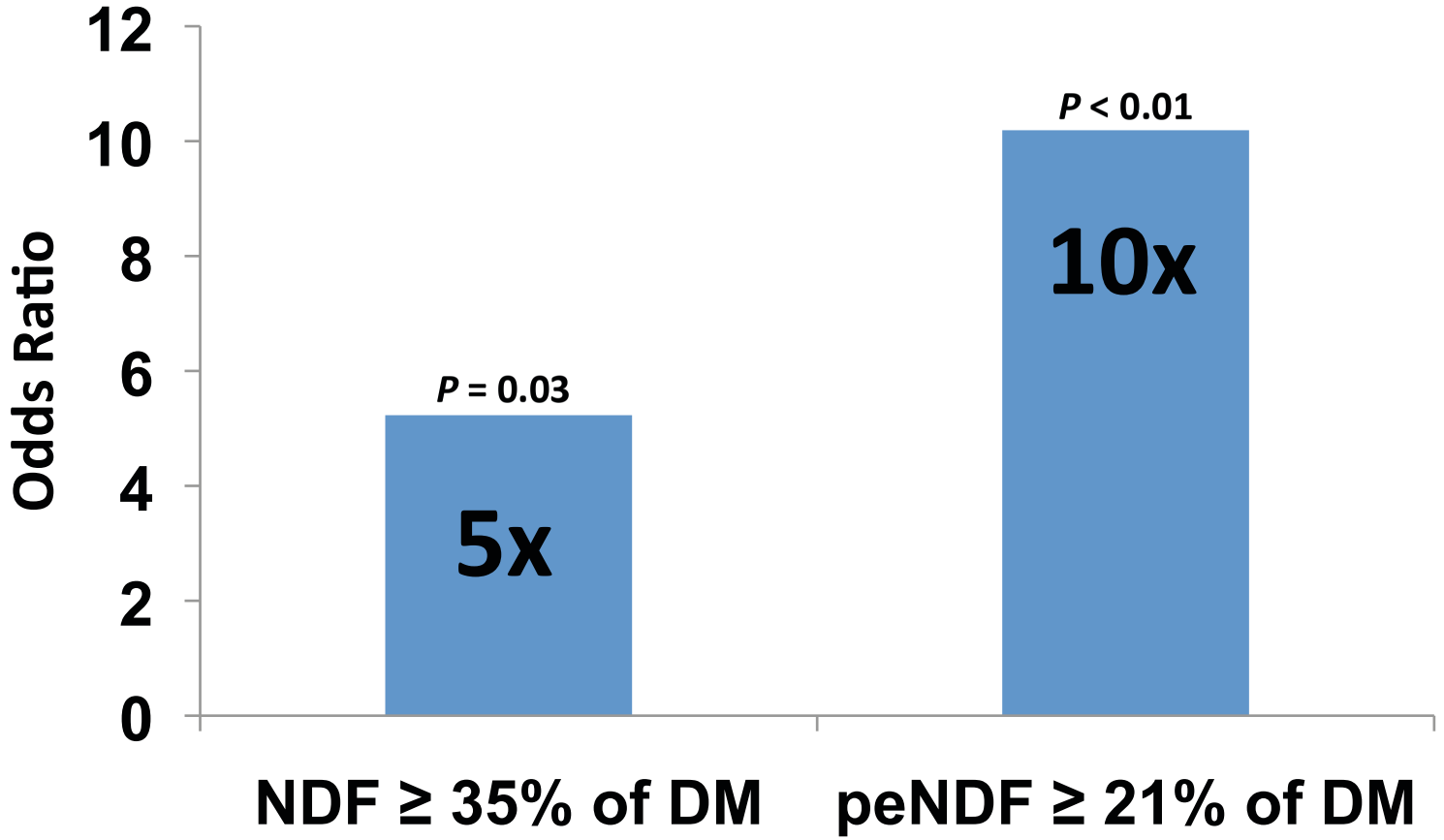
HDN herds feed more frequently



	HDN	LDN
Mean	1.7	1.4

	HDN	LDN
Mean	4.6	2.9

HDN herds feed more physically effective fiber



	HDN	LDN
Mean	37.3	34.6

	HDN	LDN
Mean	26.8	21.4

Cows naturally have aggressive feeding drive …

- Cows willingly exert **>500-lb** pressure against feed barrier while eating
  - **225 lb** causes tissue damage
- Defines **"aggressive feeding drive"**
- We can train cows to become less aggressive eaters!

(Hansen and Pallesen, 1999)

Feed push-up (Armstrong et al., 2008)

- 1 to 2 hours post-feeding is most competitive; most displacements
- Push-up each ½ hour for first 2 hours versus once per hour
  - Fed 3x/day

Item	1x/h	2x/h
DMI, lb/d	41.4	40.1
Milk, lb/d	61.3[b]	65.3[a]
Milk/DMI, lb/lb	1.48[b]	1.63[a]
Lying in stall, % of cows	45.3	43.8

What Naturally Stimulates Feeding Behavior?

- Delivery of fresh feed
- Feed push-up
  - More important during the day rather than at night (DeVries et al., 2005)
- Milking

- **Biggest driver of feeding is delivery of fresh feed**

1x versus 2x TMR feeding

(Sova et al., 2013)

- **Twice versus once daily feeding:**
  - **More feed availability throughout day**
  - **Less sorting against long particles**
  - **Increased DMI by 3.1 lb/d, milk by 4.4 lb/d**
- **Overall improvement in efficiency**
- **Greater feeding frequency:**
  - **Improved rumen fermentation**
  - **Greater rumination**
  - **Greater eating time**

Refusal amount and sorting …

Individually fed cows:

Sorting occurs over day, but by 24 h cows consume ration similar to that offered (Maulfair and Heinrichs, 2013).

Competitive feeding situation:

- Each 2%-unit increase in refusals associated with 1.3% increase in sorting (Sova et al., 2013).
  - Milk/DMI decreases 3% for each 1% increase in sorting.

Two percent feed refusals: What it looks like…

How long can the feed bunk be empty?

- **Cow's motivation to eat increases markedly after 3 hours** (Schutz et al., 2006)
  - 0, 3, 6, 9 h/d feed restriction
  - Linear increase in motivation to eat

- **Restricted feed access time by 10 h/d**
  (8:00 pm to 6:00 am; Collings et al., 2011)
  - 2x displacements at feeding
  - DMI reduced by 3.5 lb/cow per day

Case Study: Effect of empty -bunk time (Matzke and Grant, 2003)

Compared 0 vs 6 h/d functionally empty bunk (midnight to 6:00 am)

- +7.9 lb/d milk yield
- 1.8x greater lying in stalls
- 2x greater feeding at bunk
- Cows less restless

Stocking Density and Feeding Behavior

No fun being the cow in the middle …

- As stocking density increases:
  - Greater aggression and displacements
  - Time of eating shifted
  - Fewer meals
  - Eating rate increased
  - Greater potential for sorting
  - Largest effect on subordinate cows
- **Within limits, cows can adjust feeding behavior in response to variable SR**

Table for one?

(Rioja-Lang et al., 2012)

- Compared 30, 24, 18, and 12 in of bunk space and preference for:
  - low-palatability feed alone
  - high-palatability feed next to a dominant cow
- Y-maze testing to offer choices

Space (in)	HPF Dominant	Equal choice	LPF Alone	*P*
12	0	1	11	<0.001
18	1	3	8	<0.05
24	3	4	5	>0.05
30	5	2	5	>0.05

Are 24 in/cow enough?

- Cows cannot access feed all together
- Distribution of DMI changed – pushed to later hours of day
- 24 vs 30 vs 36 in/cow
  - 10, 6, 3 displacements per cow/d
  - Greater feeding time
- If you ask the cow, the answer is no.

Guarding the water trough: water and milk production

Milk yield increases by 2.1 lb/d for every 1 in/cow of water trough space within an observed range of 1.5 to 5 in /cow (Sova et al., 2013).

The Perfect Dining Experience? Recommended Feeding Management

- Management that enhances rest and rumination
- Feed available on demand
- Consistent feed quality/quantity along the bunk
- Bunk stocking density ≤100% (≥24 in/cow)
- TMR fed 2x/day
- Push-ups focused on 2 hours post-feeding
- ~3% feed refusal target
- Bunk empty no more than 3 h/d (ideally never)

Thank you!

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TECHNICAL SYMPOSIUM SPEAKERS

Tanya Gressley, Ph.D. is an Associate Professor in the Department of Animal and Food Sciences at the University of Delaware. Tanya received her BS and MS degrees in Animal Science from the University of Maryland and a Ph.D. in Dairy Science from the University of Wisconsin. Tanya's research program focuses on nutrition and nutritional immunology. Her primary area of interest is the impact of intestinal digestion on dairy cattle health and digestion. Recent studies have used abomasal infusions of fermentable carbohydrates to mimic excessive hindgut fermentation that accompanies rumen acidosis. Current work is focusing on quantifying relationships among intestinal leukocyte populations, intestinal inflammation, and mucosal micrabiome. A secondary area of work is the use of dietary supplements to improve animal health and performance. Supplements evaluated in recent projects include egg yolk antibodies, rumen protected trace minerals, and rumen protected amino acids. Tanya's primary teaching responsibilities are junior/senior level undergraduate Dairy Production and Lactational Physiology courses. She additionally teaches a freshman animal science lab, an honors freshman animal science course, and a freshman animal handling course.

Nicholas Gable, Ph.D. obtained his Bachelors of Agricultural Science from La Trobe University, Melbourne, Australia. In 2005, he received his Ph.D. degree in Animal nutrition and physiology also from La Trobe University. Upon completion of his Ph.D., he conducted postdoctoral research in the USA at both Purdue and Iowa State Universities. Here he worked on evaluating sources of n-3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid) in nursery-finisher pig production and using the pig as a biomedical model. In 2008 he joined the Animal Science Department at ISU as an assistant professor in fundamental swine nutrition and metabolism. Presently, Dr. Gabler has an active and diverse research program that focuses on understanding and improving swine feed efficiency and intestinal physiology at the basic and applied, cellular and whole animal levels. His research program can be divided into four areas: (1) Understanding the physiology and molecular pathways that define feed efficiency differences in swine; (2) Gastrointestinal physiology (integrity and function) of swine; (3) Using the pig as a biomedical model or dual purpose research (livestock and human application); and (4) Understanding the impact of disease and poor health on metabolism, nutrient requirements and tissue accretion. This later research has been using PRRS and PED virus challenge models to study how health challenges alter pig productivity and nutrition. Altogether, these research areas are developing into a highly productive, successful, integrated, hypothesis driven programs. Over the last six years, Dr. Gabler has graduated

four M.S. students and three Ph.D. students from his program. He has been active as an author and co-author and has published over 45 peer-reviewed journal articles, a review paper and a book chapter since August 2008. His research is making important scientific contributions to swine production, nutrition and health.

Duarte E. Díaz, Ph.D. holds a M.S. and a Ph.D. in nutrition from North Carolina State University. His research for the past 20 years has focused on the effects of mycotoxins on agriculture. Dr. Diaz has given over 40 invited presentations around the world and has published over 70 articles in scientific journals, proceedings and popular press magazines. In 2005 Dr. Diaz served as editor of a publication that focused on the applied impact of mycotoxins on agriculture titled "The Mycotoxin Blue Book" (Nottingham University Press). The book has sold over 5,000 copies and is widely considered an important reference on the subject. Dr. Diaz has worked in Academia at several institutions including Utah State University, University of Bologna and the Catholic University of the Sacred Heart in Italy. In 2015, after several years working in the private sectors, Dr. Diaz joined the faculty at the University of Arizona as an Associate Professor and Dairy Extension Specialist.

Paige Gott, Ph.D. is a Ruminant Technical Manager for BIOMIN America, Inc. She received both her B.S. and M.S. degrees in Animal Sciences as well as her Ph.D. in the College of Veterinary Medicine from The Ohio State University. Paige focused on udder health and transition cow management during her graduate programs. Dr. Gott is a Ruminant Technical support manager for BIOMIN and is specializing in mycotoxin risk management. Paige resides in Coshocton, Ohio with her fiancé Chip.

CANC SPEAKERS

Frank Mitloehner, Ph.D. - Keynote Speaker - is a Professor and Air Quality Specialist in Cooperative Extension in the Department of Animal Science at the University of California, Davis. He received his MS degree in Animal Science and Agricultural Engineering from the University of Leipzig, Germany, and his PhD degree in Animal Science from Texas Technical University. Dr. Mitloehner is an expert for agricultural air quality, livestock housing and husbandry. Overall, he conducts research that is directly relevant to understanding and mitigating of air emissions from livestock operations, as well as the implications of these emissions for the health and safety of farm workers and neighboring communities. Dr. Mitloehner has served as chairman of a global United Nations Food and Agriculture Organization (FAO) partnership project to benchmark the environmental footprint of livestock production. He served as workgroup member on the President's Council of Advisors on Science and Technology (PCAST) and as member on the National Academies of Science Institute of Medicine (IOM) committee on "A Framework for Assessing the Health, Environmental, and Social Effects of the Food System".

Carl Old, Ph.D., received a B.S. and Ph.D. from the University of California, Davis. For the past 35 years he has worked as a ruminant nutritionist. Old resides near LeGrand, California.

Ralph Ward is the founder and owner of Cumberland Valley Analytical Services (CVAS), one of the largest forage testing labs in the United States. A graduate of Virginia Polytechnic Institute and State University, Mr. Ward was a dairy herdsman and then spent a number of years involved in on-farm dairy nutrition work. He started CVAS in 1992 as he saw a need for more extensive forage diagnostic services for dairy nutritionists and their clients. With a focus on chemistry analysis and application of non-traditional forage evaluation techniques, CVAS has grown to over 100 employees in three U.S. locations. Mr. Ward was the first to commercialize the use of the "fermentation analysis" in the U.S. and is one of the leaders in the development of in vitro fiber and starch digestibility services. With one of the largest and most comprehensive sets of forage NIR equations and supporting information management technology, Mr. Ward is focused on the establishment of a global NIR forage and feed evaluation network. As part of this network CVAS supports multiple U.S. lab operations as well as operations in Italy, Chile, Argentina, Uruguay, Mexico, British Columbia, Ontario, Quebec, Australia, Japan, China, and South Africa with labs soon to come on line in the U.K. and Germany.

Rick Grant, **Ph.D.** was raised on a dairy farm in northern New York State. He received a B.S. in Animal Science from Cornell University, a Ph.D. from Purdue University in ruminant nutrition, and held a post-doctoral position in forage research at the University of Wisconsin-Madison from 1989 to 1990. From 1990 to 2003, Rick was a professor and extension dairy specialist in the Department of Animal Science at the University of Nebraska in Lincoln. Since February of 2003, he has been President of the William H. Miner Agricultural Research Institute in Chazy, NY, a privately funded educational and research institute focused on dairy cattle, equine, and crop management. Rick's research interests focus on forages, dairy cattle nutrition, and cow behavior. He has been the recipient of the Pioneer Hi-Bred International Forage Award in 2010 and the Nutrition Professionals Applied Dairy Nutrition Award in 2015.

Mary Beth Hall, Ph.D. is a research animal scientist at the U.S. Dairy Forage Research Center part of the USDA - Agricultural Research Service in Madison, WI. The main areas of her work are feed carbohydrates, their analysis, digestion and use by dairy cattle and their microbes, with special attention paid to carbohydrates in forages and by-product feeds. Over the years, she has worked on dairy farms, as a sales representative for a feed company in the northeastern United States, and as a county agent for Cooperative Extension working on nutrition and management with commercial dairy farms in New York State. She was on faculty working in dairy nutrition extension and research at the University of Florida for 8 years. She received her Animal Science degrees from Cornell University (BS and PhD) and Virginia Tech (MS). She presently lives in rural Wisconsin with her husband (Stu), and a varied pack of dogs.

Alec C. Gerry, Ph.D., Professor and Cooperative Extension Specialist, Department of Entomology, University of California at Riverside. Dr. Gerry received his B.A. Biology, UC Berkeley (1990) and Ph.D. Medical & Veterinary Entomology, UC Riverside (1999). Dr. Gerry has been with the University of California at Riverside since February of 2003. Prior to joining the faculty at UC Riverside, Dr. Gerry was a Senior Public Health Biologist for the California Department of Public Health where he was responsible for protecting Californians from pathogens transmitted by insects and other vectors. Dr. Gerry additionally served as a medical entomologist in the United States Army Reserve, with over 26 years of active and reserve service prior to his retirement from the military in 2015. Research and extension efforts in his laboratory at UCR are focused on the biology, ecology, and integrated management of pest arthropods and disease vectors associated with animals. Recent research has included studying the dispersal and control of filth flies, investigating the role of biting flies in the transmission of disease agents, and evaluating integrated pest management techniques for control of nuisance and disease-transmitting insects.

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Louis Armentano, Ph.D., graduated from Cornell University in 1975, earning a B. S. with distinction in Animal Science. He went to North Carolina State to study the use of by-products as feeds for dairy cattle and received an M.S. in Animal Nutrition. His Ph.D. was from Iowa State where the relationship between rumen carbohydrate fermentation and the metabolic processes of cattle was examined at the whole animal level. After a brief research appointment at Virginia Tech working with protein nutrition of dairy cows, Lou joined the Department of Dairy Science at Madison in 1983 as an Assistant Professor with teaching and research responsibilities. In addition to a program studying basic liver metabolism in cattle, Lou has maintained a program addressing use of by-product feedstuffs and their role in providing energy, fiber, and protein to dairy cows. His most recent research efforts have been to explain the effects of dietary fat on milk fat secretion, and resulted from examining the effects of the sometimes high levels of corn oil found in distillers grains. In addition to serving as a professor for 33 years at the University of Wisconsin-Madison, Lou had the pleasure of chairing the department for 8 busy and exciting years. Lou recently moved his appointment from Full Professor to Professor Emeritus, but is still actively involved in dairy research and outreach. He currently serves on the National Research Council dairy nutrition guidelines writing committee and is president of the American Dairy Science Association.

California Animal Nutrition Conference
2017 Steering Committee

Chairperson: Phillip Jardon, DVM, MPVM, is a Technical Consultant for Elanco Animal Health. He earned his DVM from Iowa State University and MPVM from UC Davis. Dr. Jardon has worked in the dairy industry for 26 years. He has a wide range of experience in research, dairy veterinary practice, nutritional consulting, and in technical service.

Vice Chairperson: Jason Brixey, M.S., P.A.S. - Consulting Animal Nutritionist
Jason D. Brixey was born and raised in Crescent City, CA. He graduated in 2001 from Cal Poly San Luis Obispo with a Dairy Science and Ag Business Undergraduate Degree. He attended the University of Idaho in Moscow under Dr. Mark McGuire, graduated with a Masters of Science in 2003. He started his animal nutrition career with West Milling LLC in Phoenix, AZ as the Nutrition Technical Representative. In April of 2005, Jason was hired by Pine Creek Nutrition Service Inc. out of Denair, CA to work on dairy farms as a Consulting Animal Nutritionist. He became partner at Pine Creek Nutrition Service in January of 2008. Married to my lovely wife Jodie that I share in the duties of raising three wonderful children; Jack (7), Kaydee (4 ½), and Will (2).

Ex Officio: Ben Tarr, Adisseo USA Inc. Ben Tarr is a native Californian who grew up on his family's cattle ranch in the foothills of the Sierra Nevada near Oakhurst, California. He holds a Bachelors of Science in Animal Science and a Masters of Agriculture in Animal Science Business from Texas A&M University. His career experiences have spanned from working in the commodity sector for Cargill Investment Services and Integrated Grain and Milling to livestock production with Cactus Feeders in Amarillo, Texas and Harris Ranch in Selma, California. Ben worked for Adisseo as their U.S. Ruminant Sales Manager prior to taking a position with Global Animal Products in Texas.

Committee Members:

Anthony Allen, Biomin USA Inc., Ruminant Key Account Manager for Western U.S. with Biomin since since May of 2010. He has worked for Nutrius, Foster Farms Commodities Division, and PM Ag Products on the dairy direct side before joining Biomin on the supplier side. Anthony received his B.S., Animal Science degree from Fresno State in 1995 and he is a Certified Dale Carnegie Trainer and teaches a couple of classes each year. He is a lifelong Fresno resident. Anthony and his wife Stephanie have three boys, Nathan, Garrett, and Sam.

Marc Etchebarne, Michel A. Etchebarne, Ph.D. Inc., Independent Dairy Nutritionist since 2010. USMC 2003-2008, Sergeant. Colorado State University graduate emphasis on Sheep, Feedlot, and Dairy Systems in 2010. ARPAS member, PAS.

Jennifer Heguy, is a native of California's San Joaquin Valley. She received her B.S. in Animal Science, with an emphasis in Livestock and Dairy, at the University of Caifornia, Davis. In 2006, she received her M.S. degree at UC Davis, focusing on dairy cattle nutrition. Jennifer currently serves as the University of California Dairy Farm Advisor in Merced, Stanislaus and San Joaquin Counties where milk is a major agricultural commodity. Jennifer's major program focus is improving silage and feeding management practices on California dairies.

David Ledgerwood graduated in 2004 with a BS degree in Animal Science focusing on livestock and dairy cattle from the University of California Davis. Upon graduating he worked in the university ruminant nutrition lab with Dr. Ed DePeters as a lab technician performing feed nutrient and milk analysis while assisting graduate students run various ruminant nutrition focused research trials. In 2007 he graduated with a MS degree in Animal Biology focusing on ruminant nutrition working with Dr. DePeters. After graduation in 2007 he worked as a lab and research program manager in the field of animal behavior/welfare on the UC Davis campus performing various research trials focused on improving cow comfort. In January of 2014 he accepted a job with the Veterinary Medicine Teaching and Research Center in Tulare as a research program manager for the clinical department performing various research projects covering cow behavior, calf health, and nutrition. In April of 2011 he was offered a position with Western Milling LLC working as a dairy nutritionist and currently works one on one with dairymen to find the nutrition program that works for their facility and cows. He also takes part in the quality control program at the Goshen mill and works with Quality Assurance to write and edit programs to ensure they produce quality products.

Heidi Rossow, Ph.D., is an Assistant Professor of Ruminant Nutrition with UC Davis Veterinary School located at the Veterinary Medicine Teaching and Research Center (VMTRC) in Tulare, California. Her areas of research are computer modeling of nutrient metabolism and systems analysis of dairy and beef production. She has authored over 20 publications and created several computer programs that evaluate rations and production for dairy and beef cattle, estimate body weight changes based on dietary information for humans, predict liver and adipose nutrient metabolism and track nitrogen and phosphorus balance for a dairy farm. Dr. Rossow is currently developing a nutrition program in conjunction with several farms in the central valley to teach veterinary students the role of nutrition in dairy management and disease.

Honorary Member:

Kyle Thompson, Ph.D. received his B.S. degree in animal science from Fresno State (2006) and his master's and Ph.D. degrees in animal science from Oklahoma State (2011/2015). He joined the Fresno State staff in the fall of 2016 after taking classes and teaching at Oklahoma State from January 2007-June 2016 and serving as the graduate student assistant manager of the campus dairy cattle center. His research included dairy nutrition research trials and lactating cow probiotics. He also assisted in research for bovine respiratory disease, rumen temperature bolus, milk production by weigh-suckle-weigh and swine antimicrobial replacements. He also assisted in 4-H and FFA Field Day dairy judging competitions. While in Stillwater, OK, he owned and operated Wild Acre Farms and Exotics, which raised ewes, game birds, free range hens and other fowl/animals, and produced grasses and winter wheat for grazing and hay production. As a Fresno State student, he worked in the sheep unit three years, served as a campus farm tour guide, and dairy unit herdsman and feed/hospital technician. He also worked as an exotic animal nutrition intern (2009) and global nutrition fellow at the San Diego Zoo (2013).

CALIFORNIA ANIMAL NUTRITION CONFERENCE HISTORY

YEAR	CHAIRPERSON	COMPANY AFFILIATION
2016	Dr. Phillip Jardon, DVM, MPVM	Elanco Animal Health
2015	Mr. Ben Tarr	Adisseo USA Inc.
2014	Dr. Jeffrey M. DeFrain	Zinpro Performance Minerals
2013	Mr. Doug DeGroff	Diversified Dairy Solutions, LLC
2012	Mr. Eduardo Galo	Novus International, Inc.
2011	Dr. Michael A. DeGroot	DeGroot Dairy Consulting
2010	Dr. Jim Tully	Pine Creek Nutrition Service, Inc.
2009	Mr. Michael Braun	Phibro Animal Health
2008	Dr. Luis Rodriguez	Zinpro Corporation
2007	Dr. Marit Arana	A.L. Gilbert Company
2006	Mr. Dennis Ervin PAS	Prince Agri Products, Inc.
2005	Dr. Lawson Spicer	Nutri Management Inc.
2004	Dr. Luis Solorzano	Purina Mills, Inc.
2003	Dr. Alfonso Mireles, Jr.	Foster Farms
2002	Mr. Edmund Vieira	Pine Creek Nutrition Service, Inc.
2001	Dr. Melinda Burrill	California State Polytechnic University - Pomona
2000	Mr. Dave Fischer	Foster Farms
1999	Dr. M. Steven Daugherty	California State Polytechnic University - SLO
1998	Dr. Doug Dildey	Alltech, Inc.
1997	Ms. Carla Price	Nutritionist
1996	Dr. H.John Kuhl, Jr.	Nest Egg Nutrition
1995	Mr. Dennis Ralston	M. Rinus Boer Co., Inc.
1994	Dr. Doug Dildey	Alltech, Inc.
1993	Dr. Mark Aseltine	Consulting Animal Nutritionist
1992	Dr. Carl Old	MacGowan-Smith Ltd.
1991	Mr. Nick Ohanesian	Ohanesian & Associates
1990	Mr. Rod Johnson	M. Rinus Boer Co., Inc.
1989	Mr. Timothy Riordan	Nutri-Systems, Inc.
1988	Dr. Russ W. Van Hellen	Great West Analytical
1987	Dr. John E. Trei	California State Polytechnic University, Pomona
1986	Dr. A.A. Jimenez	Ancon, Inc.
1985	Dr. Wm. A. Dudley-Cash	Foster Farms
1984	Dr. Joel Kemper	Penny-Newman Co.
1983	Dr. Alex J. Kutches	O.H. Kruse Grain & Milling Co.
1982	Dr. Howard Waterhouse	Bell Grain & Milling
1981	Mr. Don Ulrich	Diamond Shamrock Chemical Co.
1980	Mr. Tom Geary	PMS-West, Inc.
1979	Dr. Frank Parks	Kemlin Industries
1978	Mr. Fred Pfaff	Zacky Farms
1977	Mr. Rene Lastreto	Diamond Shamrock Chemical Co.
1976	Mr. Rene Lastreto	Diamond Shamrock Chemical Co.
1975	Dr. R.D. Hendershott	Nulaid Foods
1974	Dr. R.D. Hendershott	Nulaid Foods
1973	Dr. Leland Larsen	Nutri-Systems, Inc.
1972	Dr. Leland Larsen	Nutri-Systems, Inc.
1971	Mr. Rene Lastreto	Diamond Shamrock Chemical Co.

CALIFORNIA ANIMAL NUTRITION CONFERENCE HISTORY- Continued

YEAR	CHAIRPERSON	COMPANY AFFILIATION
1970	Mr. Fred Pfaff	Balfour Guthrie
1969	Mr. Fred Pfaff	Balfour Guthrie
1968	Mr. Fred Pfaff	Balfour Guthrie
1967*	Mr. Gary L. Frame	J.G. Boswell Co.
1966*	Mr. Gary L. Frame	J.G. Boswell Co.
1965*	Mr. Arne Jalonen	Topper Feed Mills
1964*	Mr. Arne Jalonen	Topper Feed Mills
1963*	Dr. W.P. Lehrer	Albers Milling Co.
1962*	Dr. H.J. Almquist	The Grange Co.
1961*	Dr. H.S. Wilgus	The Ray Ewing Co.
1960*	Mr. Bert Maxwell	Nulaid Foods
1959*	Mr. Bert Maxwell	Nulaid Foods
1958*	Mr. Robert Caldwell	Anderson Smith Milling Co.
1957*	Mr. Emery Johnson	P.C.A., Los Angeles
1956*	Mr. Emery Johnson	P.C.A., Los Angeles
1955*	Dr. H.J. Almquist	The Grange Co.
1954*	Dr. H.J. Almquist	The Grange Co.
1953*	Mr. Clifford Capps	California Milling Co.
1951*	Mr. Dolph Hill	Golden Eagle Milling Co.
1950*	Dr. H.J. Almquist	The Grange Co.
1949*	Dr. H.J. Almquist	The Grange Co.
1948*	Dr. H.J. Almquist	The Grange Co.

* California Animal Industry Conference

History of the
California Animal Nutrition Conference

The California Animal Nutrition Conference (CANC) originated in the 1940's as the California Animal Industry Conference, sponsored by the California Grain & Feed Association (CGFA). CGFA wanted to expand the continuing education program into a forum encompassing animal health, nutrition and management. The expectations were that communications between (nutritionists) industry, educational institutions and regulatory agencies would be improved. In 1972, CGFA discontinued sponsoring the Animal Industry Conference.

After the conference was discontinued, a small group of nutritionists began meeting annually in Fresno. Two or three invited speakers from industry or the universities presented information on nutrition, especially poultry.

In 1975 a set of organizational bylaws were developed by the steering committee. CANC was established and was provided support by CGFA. The CGFA Board of Directors appointed a chairperson annually and approved the steering committee. In 1978, Dr. Frank Parks, the Chairperson, requested that CANC be granted independent status and be established as a self-governing committee of CGFA. This request was granted.

For a few years, meetings were held in Fresno and Corona, California. For a couple of years starting in 1978, CANC published "Nutri-Facts", a "newsletter" consisting of articles in animal production.

In 1979, donations were requested from industry companies to help keep registration fees low. During the 1980's and through the 1990's the attendance at CANC continued to grow as the quality of the conference improved and the conference became known nationwide. In the 1990's a pre-symposium was added. The pre-symposium is sponsored by a company selected by the CANC Steering Committee. This process allows the selected company to showcase its research and products. In the year 2000, posters on research by students were included.

Attendance at the conference has grown from 50 in the 1970's to over 300 attendees. To encourage attendance, different activities have been tried such as keynote speakers, skiing expeditions and a very successful barbeque dinner put on by the Animal Science Department at Fresno State University.

The California Grain & Feed Association has supported and allowed CANC to work and grow. The premise of the CGFA and CANC relationship is to work together to educate the feed industry with information for problem solving and to disseminate valuable research information. CANC is not an industry, university, or government entity, but a committee collectively working together for the good of agriculture in California.

STUDENT ABSTRACTS FOR

POSTER PRESENTATION

AT THE

CALIFORNIA ANIMAL

NUTRITION CONFERENCE

MAY 10 & 11, 2017

Estimation of the requirement for water and ecosystem benefits of cow-calf production on California rangelands[1]

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ABSTRACT

Among other agricultural sectors, beef production is accused of using large amounts of water, and in an effort to reduce water use, some studies recommend decreasing or halting meat consumption. Beef production water footprints vary and some do not consider the tradeoffs associated with ecosystem benefits provided by cattle on rangeland. A static model depicting water use for cow-calf production on California rangeland was developed on an Excel spreadsheet. Range water use for beef production was modeled at two UC Agriculture and Natural Resources (ANR) Research and Extension Centers: Hopland (HREC) and Sierra Foothill (SFREC), and at the USDA Forest Service San Joaquin Experimental Range (SJREC). These three locations were chosen based on evapotranspiration (ET) zones and differences in forage production and rainfall. The model accounted for green water (i.e., water used for range forage production), and blue water (i.e., drinking water, and water used to grow alfalfa and irrigated pasture). As liters per kg of live weight, green water consumption was estimated to be 42,492 for HREC, 28,106 for SFREC, and 22,102 for SJREC. Blue water consumption as liters per kg of live weight was estimated to be 4,631 for HREC, 12,784 for SFREC, and 9,140 for SJREC. The model was sensitive to changes in range forage production and irrigated pasture use. Green water usage appears large; however, cattle consume less than 18% of the total water range forage plants use to grow. Given that green water is sourced from rainfall and is not designated for another use, it is misleading to associate negative environmental impacts to rangeland beef production based on these numbers. It is important to consider the water use associated with beef production in the context of ecosystem services cattle provide to rangelands, such as preventing grasslands from being converted to shrub lands, woodlands, or even forests, and the role grazing cattle play in managing and improving rangeland.

KEY WORDS: Water use, beef cattle, rangeland, ecosystem benefits

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Description and evaluation of the AusBeef model of beef production

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As demand for animal products such as meat and milk increases, and concern over environmental impact grows, mechanistic models can be useful tools to better represent and understand ruminant systems and evaluate mitigation options to reduce greenhouse gas emissions without compromising productivity. AusBeef is a whole-animal, dynamic, mechanistic model of beef production that calculates methane emissions from net ruminal hydrogen balance. AusBeef incorporates a unique fermentation stoichiometry that represents four different microbial groups, as well as the effects of ruminal pH on microbial degradation of feed. The objectives of this study were to evaluate the performance of the AusBeef model of beef production with regard to predicting daily methane production (DMP, g/d), dry matter intake (DMI, kg/d), gross energy intake (GEI, MJ/d) and methane yield (MY, %GEI), using independent data derived from the literature. AusBeef predictions were compared for the full dataset (n=37) as well as for high-forage diets (n=21) and mixed diets (n=16) using a root mean square predicted error expressed as a percentage of the observed mean (RMSPE%). AusBeef predicted DMP with RMSPE% of 26.6, 30.1, and 21.3% for the full dataset, high-forage, and mixed diets, respectively. AusBeef predicted MY, DMI, and GEI with a RMSPE% of 38.5, 8.91, and 9.86% for the full dataset, respectively. There were prediction differences between forage and mixed diets with a RMSPE% of 9.32 and 8.43% for DMI; 6.38 and 11.1% for GEI and 41.7 and 28.4% for MY. AusBeef prediction errors for DMI ranged from -18 to +42%, with AusBeef underpredicting DMI 76% of the time. AusBeef underpredicted methane emissions 65% of the time, with prediction error ranging from -51 to +59%, and underpredicted GEI 90% of the time, with prediction error ranging from -1 to +30%. Further studies are required to improve the prediction of methane on forage only diets.

Keywords: Modeling, beef cattle, sustainable agriculture, methane, greenhouse gases

Predicting *in sacco* rumen undegraded crude protein and rumen degraded neutral detergent fiber in canola meal

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In sacco values for rumen undegraded crude protein (RUP) and rumen degraded neutral detergent fiber ($dNDF_{30}$) are often estimated by incubating samples in the rumen of cows for 16 or 30 hours respectively. This utility of the method in real time, however, is limiting due to the time and expense involved in processing samples. Therefore, the ability to predict *in sacco* RUP or $dNDF_{30}$ values based on more easily measured analytes would be useful to quickly estimate $dNDF_{30}$ or RUP of a feed sample, as well as identify potential outliers. Our objective was to determine the predictive power of dry matter (DM), crude protein (CP), soluble crude protein (SolCP), acid detergent fiber (ADF), neutral detergent fiber (aNDF), starch, lignin and ether extract (EE) on measured RUP and $dNDF_{30}$ values of canola meal (Table). Samples of canola meal (n=24) were collected at 2 week intervals from 9 commercial California dairy farms in the San Joaquin Valley, and analyzed for nutrient composition. Linear models were fit to the data using R (2016), with DM, CP, SolCP, ADF, starch, lignin and EE as fixed effects. Of the variables investigated, variations in RUP can be best (albeit not too well) predicted by DM and SolCP (each r^2=0.37; Figure), and variation in $dNDF_{30}$ is reasonably well predicted by aNDF (r^2=0.67; Figure). Use of these more readily determined nutrient components as predictors of rumen degradability of CP and NDF

offer a potential alternative to the *in sacco* digestion procedure to provide a rapid estimate of the RUP and $dNDF_{30}$ in canola meal.

Figure. Relationships of DM and SolCP with RUP and of aNDF with $dNDF_{30}$.

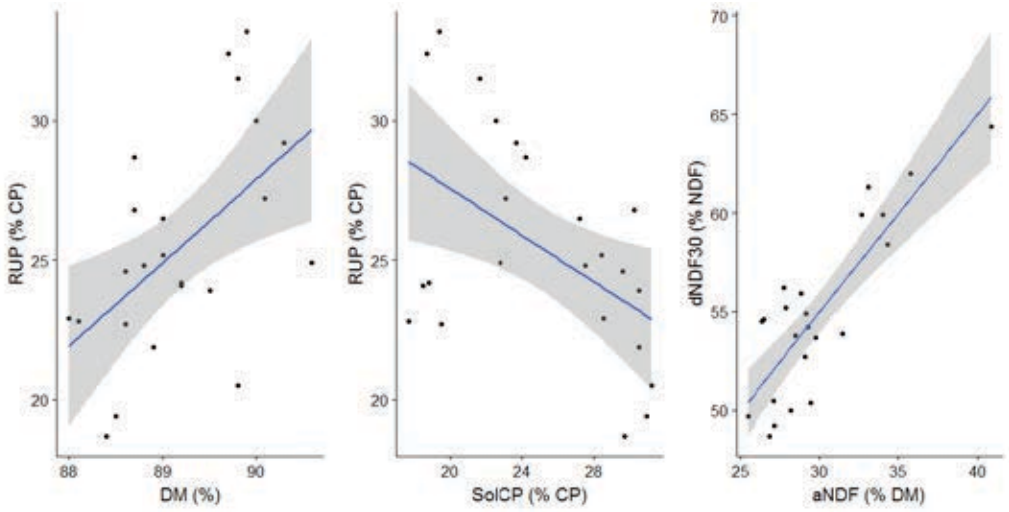

Table. Average values and standard deviations of several analytes of the canola meal samples.

Analyte	Average	Standard Deviation
DM (%)	89.2	0.72
CP (% DM)	44.2	0.70
SolCP (% CP)	25.0	4.78
RUP (% CP)	25.5	3.95
aNDF (% DM)	30.0	3.67
$dNDF_{30}$ (% NDF)	55.0	4.36
ADF (% DM)	23.9	1.81
Lignin(sa) (% DM)	9.1	1.16
Starch (% DM)	0.9	0.21
EE (% DM)	3.3	0.72
NE_L (Mcal/kg DM)	1.67	0.04

Impact of monobutyrin supplementation in liquid diet on growth, health and intestinal development of preweaning calves

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Butyric acid, a fermentation product in forestomach of ruminant, is naturally present in cow milk. Dietary supplementation of butyrate has a broad beneficial effect on growth and digestibility in weanling piglets. However, its effect in preweaning dairy calves is controversial. In this study, we hypothesized that supplementing butyric acid in form of its glycerol ester in milk may enhance its intestinal delivery and stimulate epithelial development in preweaning calves. Twenty-two Holstein bull calves (< 4 d old) were stratified by arriving BW and serum total protein and randomly assigned to treatments. Calves were fed milk replacer that supplemented with 0 (CON), 0.4 (LOW) or 0.8% (HIGH) of monobutyrin (MB, solid basis of milk replacer) until 8 wk of age. Milk replacer containing 27.6% CP and 14.4% crude fat (DM basis) were fed twice daily at 1.5% (solid basis) of BW which was updated weekly. Starter grain and water were provided for ad libitum consumption during the study. Scores for health (respiration, diarrhea, and alertness) were assigned once daily. The appearance and respiration were based on a scale from 1 to 5, where 1 was alert, bright and clear eyes, ears up and/or normal breathing and 5 was flat on side with severe depression and/or chronic dry cough weak or rapid breathing. Fecal and nasal score was given on a 1-4 scale where 1 was normal moist nose and/or firm and well-formed but not hard faeces, and 5 was copious, bilateral mucopurulent discharge and/or liquid faeces. BW was measured at arrival and weekly, and body frame parameters were measured at arrival and on wk 4, 6 and 8. Calves were weaned and euthanized on wk 8. Tissue, mucosa and digesta samples from GIT were collected. The jejunum epithelial permeability was measured through ussing chamber immediately after collection. The data was subject to analysis of variance using mixed procedure of SAS. The categorical data of health status was analyzed using Chi-square test. Our results showed that body weight was not significantly different among treatment groups, whereas wither height and body length were significantly greater ($P < 0.05$) in calves from the LOW group than those from the CON group on wk 6 and 8. Supplementation of MB tended to increase ($P = 0.08$) hip height. Calves from the LOW group had the highest intake of starter grain from wk 4 to 6 ($P < 0.01$) followed by calves from the CON and HIGH groups. The risk of diarrhea (fecal score > 2; 4-scale score, 1=firm, 4=watery) was not affected by MB supplementation. However, calves of LOW groups had the lowest risk of respiratory distress (respiratory score > 2; 5-scale score, 1=normal, 5=chronic dry cough and rapid breathing) than that of the other two groups ($P=0.024$). Despite lack of significance ($P = 0.17$), villus height of jejunum epithelium was the highest in LOW followed by HIGH and CON. The crypt depth was not affected by treatment. However, the ratio of villus height to crypt depth was significantly higher ($P = 0.04$) in LOW than that in CON. MB increased ($P < 0.05$) mRNA of tight junction proteins (*CLDN1* and *OCLN*) in jejunal mucosa. Para- and transcellular permeability were not affected by treatment. In conclusion, low dose of MB supplementation in milk replacer moderately improved growth performance and jejunal epithelial development in preweaning calves.

Keywords: monobutyrin calf preweaning

Effect of phytogenic feed supplements added to starter grain on weight gain and rumen development in Holstein calves

HA Rossow, KE Mitchell, A Johnson, B Miller (Biomin America Inc.)

The goal of pre wean calf operations is to maximize rumen development and weight gain. Feed supplements that increase starter intake should also encourage rumen development and increase weight gain. The objective of this study was to compare rumen development and body weight gain in pre wean calves given two different starter supplements, phytogenic blend A (A) or phytogenic blend B (B) (Biomin, San Antonio, TX) or no supplement (Control) at a commercial calf ranch. One hundred and twenty four holstein calves were randomly assigned to 1 of three treatments, Control, A or B, at 1 d of age. Control (nothing added), A or B were added to individual feed buckets at each feeding at the rate of 0.25g / kg starter at AM and PM feedings. Both starter intake and milk intakes were assessed daily. Calves were weighed at enrollment and at weaning, and blood samples were collected from a subset of 38 calves and analyzed for glucose (Glu, mg/dl) and β-hydroxybutyrate levels (BHBA, mmol/L) with Precision Extra blood meters (Abbott Diabetes Care, Inc., Alameda, CA) to assess rumen development. Weekly average DMI, milk intake, Glu and BHBA were analyzed using the Mixed procedure of SAS (v. 9.4) with repeated measures by calf, fixed effects treatments and random effect week. Weekly average DMI (P <0.01), BHBA (P < 0.01) and Glu (P < 0.01) were different by week but not by treatment. However, weekly milk intake was less for group D (P < 0.05). Total DMI, initial bodyweight, final body weight and gain were analyzed using the Mixed procedure of SAS with repeated measures by calf, fixed effects hutch, gender, birthdate. There were no differences in initial bodyweight and effects of hutch, birthdate or gender among treatments. Product B group was numerically greater in total DMI, gain, ADG and had faster rumen development indicated by overall higher BHBA values but differences were not significant among treatments. Therefore Supplementation with B decreased milk intake but calves maintained similar starter DMI, gain and ADG.

Effects of dietary β-glucan on systemic immunity of weanling pigs experimentally infected with a pathogenic *E. coli*

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ProGlucan™ is 100% dried algae, *Euglena gracillus*, which contains approximately 54% β-1,3-glucan. The objective of this experiment was to investigate the influence of dietary supplementation of ProGlucan on systemic immunity of weaned pigs experimentally infected with a pathogenic F-18 *E. coli*. Weaned pigs (n = 36, 7.69 ± 0.77 kg BW) were individually housed in disease containment rooms and randomly allotted to one of three dietary treatments with 12 replicate pigs per treatment. The three diets were a nursery basal diet (control), and 2 additional diets containing with either 100 or 200 mg/kg of ProGlucan added to the basal diet. The experiment lasted 17 d [5 d before and 12 d after the first inoculation (d 0)]. The inoculum used in this experiment was F-18 *E. coli*, containing LT, STb, and SLT-2 toxins. The inoculation doses were 10^{10} cfu/3 mL oral dose daily for 3 days. Blood samples were collected right before *E. coli* challenge, and on d 2, 5, 8, and 12 post-inoculation (PI). Total and differential blood cell count were analyzed by CBC test. The concentration of CD4+ T cells, CD8+ T cells, and B cells were analyzed by flow cytometry. The concentrations of cytokines (TNF-α, IL-6, and IL-10), cortisol, and haptoglobin in serum samples were analyzed by enzyme-linked immunosorbent assay. All data were analyzed by ANOVA using the PROC MIXED of SAS in a randomized complete block design and repeat measurement by time. Pigs fed with 100 mg/kg of ProGlucan had less ($P < 0.05$) total white blood cells and neutrophils on d 8 PI, had greater ($P < 0.05$) CD4+ T cells on d 2 PI, and had less ($P < 0.05$) serum haptoglobin and cortisol on d 5 and 12 PI, compared with pigs fed the control diet. Supplementation of 200 mg/kg of ProGlucan increased ($P < 0.05$) the percentage of CD8+ T cells on d 5 PI, but reduced ($P < 0.05$) the percentage of CD8+ T cells on d 12 PI, compared with the control diet. Inclusion of 200 mg/kg of ProGlucan also decreased ($P < 0.05$) serum haptoglobin on d 2 and 5 PI, reduced ($P < 0.05$) TNF-α concentration on d 5 PI, and reduced ($P < 0.05$) serum cortisol on d 5, 8, and 12 PI. Results indicate that supplementation of ProGlucan may regulate systemic immunity and reduce systemic inflammation of weaned pigs caused by *E. coli* infection.

Key words: ProGlucan, systemic immunity, weaned pigs

Colostrum mineral concentrations and their association with calcemic status at calving in Jersey cows

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The aim of the present study was to evaluate the association of postpartum calcemic status and colostrum concentration of Ca, P, Mg, K, Na, Fe, Zn and Cu on 131 multiparous Jersey cows. Colostrum samples were harvested at 9 h 36 min (± 3 h 36 min) after calving and analyzed for mineral concentration by Inductively Coupled Plasma – Optical Emission Spectrometry. Final colostrum weigh was recorded at milking. Blood samples for serum Ca analyses were collected from the coccygeal vessels within 6 h after calving. Based on serum Ca concentration, cows were classified as hypocalcemic (SHC; Ca ≤ 8.5 mg/dL; n = 103) and normocalcemic (NC; Ca > 8.5 mg/dL; n = 28). Despcriptive statistics, including first (Q_1), second (Q_2) and third (Q_3) quartiles of colostrum mineral concentrations based on calcemic status at calving are shown in the Table 1. Associations among calcemic status were analyzed using mixed models with MIXED procedures of SAS. There was a tendency ($P = 0.07$) for higher colostrum weigh on SCH cows (4.2 kg) than NC cows (3.2 kg).

Cows with SHC had higher colostrum P concentration (1400.13 vs. 1140.43 mg/kg; $P < 0.01$) Mg (338.88 vs. 299.52. mg/kg; $P < 0.05$), K (1494.87 vs.1302.73 mg/kg; $P < 0.01$) and Zn (18.54 vs.15.25 mg/kg; $P < 0.05$) than NC cows, but lower Na (822.19 vs. 1003.73 mg/kg; $P < 0.05$).

Cows with SHC had higher colostrum excretion P ($P < 0.05$) and Mg ($P < 0.05$) than NC cows.

Our results show that calcemic status tends to affect colostrum yield and is associated with mineral concentration at calving.

Table 1. Colostrum mineral concentrations at first milking

SHC	**Ca** (mg/kg)	**P** (mg/kg)	**Mg** (mg/kg)	**K** (mg/kg)	**Na** (mg/kg)	**Fe** (mg/kg)	**Zn**(mg/kg)	**Cu**(mg/kg)
Q_1	2000	1100	280	1287	619	0.51	13	0.16
Q_2	2200	1400	330	1428	759	0.64	18	0.21
Q_3	2600	1600	380	1677	980	0.79	23	0.26
NC								
Q_1	1600	730	230	936	763	0.66	8.4	0.18
Q_2	2100	1100	280	1248	913	0.73	12	0.21
Q_3	2500	1500	340	1638	1276	0.87	22	0.30

KEYWORDS: colostrum minerals, hypocalcemia, Jersey cow

Monitoring ketosis in a commercial Holstein and Jersey herd

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Subclinical ketosis, a common metabolic issue in the transition period, is estimated to cost $78 per case due to decreased milk production, reduced fertility, displaced abomasum, and other health issues (Geishauser et al., 2001). Considering the potential loss of profit to a producer, this study focused on identifying the most effective monitoring program to identify at risk cows. Holstein (n=54) and Jersey (n=52) multiparous cows at a commercial dairy were enrolled during the prepartum period and then followed to approximately 21 DIM. Weekly blood samples were analyzed for Glucose (Glu mg/dl) and β-hydroxybutyric acid (BHBA, mmol/L) using Nova Max® (Nova Diabetes Care, Inc., Billerica, MA). Weekly milk tests were taken in first 21 DIM and compared to BHBA and Glu values recorded that same week. Both Jerseys and Holsteins both experience a decrease in Glu and an increase in BHBAs from prepartum to postpartum ($P<0.0001$). However, Jerseys were lower than Holsteins for Glu and BHBA levels during early lactation with values of 2.45 mg/dl and 0.13 mmol/L, respectively ($P=0.061$, $P<0.0001$). Due to this difference, the rest of analysis were run with breeds separated. Blood categories were assigned as 1 (less than) or 2 (greater than) for multiple thresholds for both BHBA and Glu levels. Initially, blood parameters were compared to health issue risk and milk production level individually, but neither alone yielded any significant results. Due to the lower incidence of hyperketonemia, Jerseys did not have enough samples available for analysis; only 5 cows had >1.0 mmol/L BHBA versus 25 Holstein cows. For Holsteins, using both Glu and BHBA blood levels as markers were more accurate for identifying suppressed milk production. They had increased risk of health issues and a decrease of 5.44 kg/d of milk when BHBA > 1.0 mmol/L and Glu<50 mg/dl ($P=0.099$). The highest incidence of ketosis for both breeds was in the first week of production. Therefore a monitoring program within the first week of lactation would be more beneficial for Holsteins. Based on these results, both BHBA and Glu testing is recommended. Since Glu strips are less expensive, Glu can be used to prescreen for hypoglycemia first, and then BHBA can be used to confirm the diagnosis if Glu values are low.

The effects of different feeding practices on heifer growth at five California dairies

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The objective of this study was to determine the effect of diet and age on Holstein heifer growth at five California dairies. This is noteworthy, as growth was found to differ between dairies within the same age groups. Feed samples for each heifer diet were collected weekly for four weeks at each dairy from September to December, 2016. Samples were analyzed by Analab (Fulton IL) for nutrient composition. Body weight and hip height were measured using a weigh tape (Nasco, Fort Atkinson, WI) and hip stick (Valley Vet Supply, Marysville, KS) on approximately 10% of the heifers in each pen from ages 3 to 32 mo (n=1720). Weigh tape was used in place of a scale as it is cheap, simple to use, and allows dairymen to easily compare their herd to this data using the same methods. All heifers were measured only once. Weight and hip height were regressed on age and pen nested within dairy to compare differences in heifer age and growth among dairies. Heifer weight and hip height were regressed on age and diet to compare the effects of diet on heifer growth among dairies. Regressions were performed using General Linear Models procedure of SAS (v. 9.4, 2014). Differences were observed in weight ($p<0.0001$; $R^2 = 0.94$) and hip height ($p<0.0001$; $R^2 = 0.91$) across dairies within the same age groups. Diets varied among dairies which affected weight ($p<0.0001$; $R^2=0.93$) and hip height ($p<0.0001$; $R^2 = 0.89$). Therefore, differences in growth between dairies within age groups is due to each dairy having a unique set of heifer diets. Differences among diets reflects differences in heifer feeding programs between dairies. It has been demonstrated that heifer growth correlates with reproduction. Thus, altering heifer diet programs to maximize growth may allow producers to improve heifer reproduction and therefore improve herd productivity.

Characterization of California Corn Silage Piles

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Ensiling is a popular practice to preserve nutrients in fresh chop plants as it enables farmers to provide adequate forage for livestock throughout the year. Among the many forages used worldwide, corn silage among the most important, especially by dairy farmers in California's Great Central Valley (GCV).

The process of making silage is facilitated by bacteria that produce lactic acid by degrading sugars in fresh forages in anaerobic conditions. During this fermentation, rapid change occurs in pH (down) and temperature (up) and these measures are often used as indicators of silage quality after the silage fermentation has stabilized. Factors such as pack density and crop moisture are considered to affect the silage fermentation process. Even though many guidelines exist relative to optimal silage making and management of the large piles common in the GCV, the complex biological processes of ensiling are not fully understood – which can lead to unexpected undesirable silage outcomes, especially immediately under the cover plastic.

We measured density, pH, temperature, and dry matter (DM) of 14 wedge-type corn silage piles at 12 commercial dairy farms in the GCV with the aim of characterizing surface silage (*i.e.* to 20 in of depth) before pile opening. Silage samples were collected from each pile at 6 locations (*i.e.* 2 on the flat top, 2 about 6 feet above the base on each side, 2 ~½ way up each side) in a single line at about mid-pile. Each coring event consisted of 2 depths from each location (*i.e.* outer - 0 to 10 in: inner - 10 to 20 in).

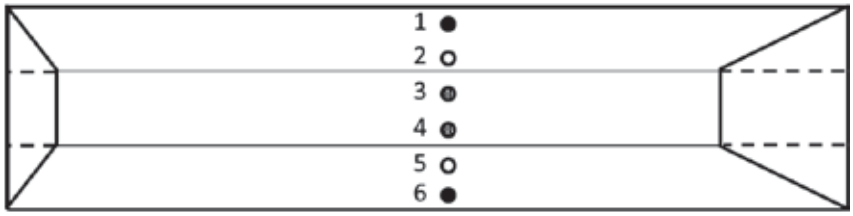


***Figure 1**. Aerial view of a silage pile. (Dots indicate the 6 coring locations)*

There was a wide range in wet weight density of inner cores among piles (10 to 18 lb/ft^3), which were much higher than the outer cores (19 to 34 lb/ft^3). Cores from the flat top of the piles had higher density than those from the sides. These results were consistent with DM which tended to be higher in inner *versus* outer cores and top *versus* side cores. Another finding in this study is that silage samples from inner cores had lower and consistent pH among piles (*i.e.* 3.52 to 4.23), while outer cores had higher and much more variable pH (*i.e.* 3.52 to 5.00), as shown in Figure 2. The lack of a correlation

between pH and temperature (Figure 3) is perhaps surprising as it suggests that silage temperature is a poor predictor of silage pH.

Table. Some characteristics of 12 corn silage piles at different locations and depths.

Location	Depth	Density (lb/ft^3)	pH	Temperature (F°)	DM (%)
Pile top	Outer	18.7 (3.33)	3.70 (0.14)	77.9 (9.62)	31.2 (3.13)
	Inner	39.3 (6.39)	3.71 (0.15)	85.5 (7.59)	33.0 (2.59)
Pile side	Outer	11.8 (2.62)	3.96 (0.29)	78.5 (10.90)	29.5 (3.76)
	Inner	23.5 (3.61)	3.77 (0.14)	85.3 (8.13)	32.3 (1.96)

Average (SD)

Lower density in outer core samples may be due to lower DM which is caused by moisture loss immediately under the underlay cover. As outer cores are closer to the underlay covers they tend to lose more moisture due to solar radiation and very slow movement of air over the surface under the plastic covers. Higher silage density in the flat top of the piles, *versus* the sides, indicates that silage on the side of wedge type piles is not packed as tightly as silage at the top due to the way piles are built. Low and consistent pH of inner cores, compared to outer, suggests the possibility that surface spoilage had already occurred in some piles prior to opening, probably due to outer core silage being exposed to some oxygen between the underlay plastic and silage.

Our follow-up study is characterizing the fermentation profile and mold/yeast counts near the exposed face of 10 corn silage piles during feedout in order to better understand peripheral face spoilage, which is common in corn silage piles.

Figure 2. Boxplots of pH from outer (0-10 inch) and inner (10-20 inch) cores.

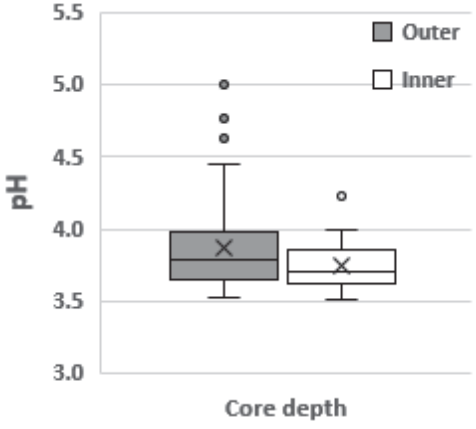
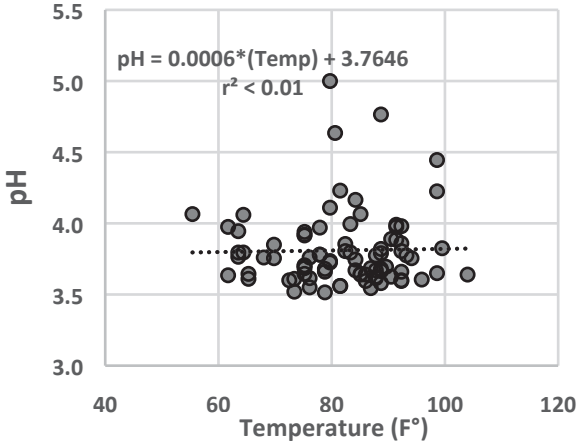


Figure 3. Lack of a temperature/pH relationship in cores.



Effects of β-mannanase (CTCZYME) supplementation of feed efficiency and lactation persistency in Holstein dairy cows

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Exogenous enzymes are used in livestock production systems to increase feed conversion efficiency. Although responses to the use of exogenous enzymes in non-ruminants are well documented there are limited studies in ruminants. These studies report variable responses to use of exogenous enzyme supplementation in ruminants. However, high feed cost and limited agronomic attempts to improve plant cell wall digestibility have required development enzymes that help break down complex plant compounds. Mannans are one of the polysaccharides present in the hemicellulose fraction of plant cell wall. Mannan polymers comprise glucose residues and are associated with decreases on cellulase activity. β-mannanase is a critical enzyme in the degradation of multiple types of mannan polysaccharides. The objective of this experiment was to evaluate the effect of β-mannanase (CTCZYME®) supplementation on milk yield and composition in lactating dairy cows. Twenty lactating Holstein cows blocked by parity, selected by previous milk production were randomly assigned 14 days after calving to two diets: control diet and control supplemented with β-mannanase (CTCZYME®) at 0.1% of the total of concentrate. The enzyme was offered twice daily with a total mixed ration (TMR). The TMR included alfalfa (25.5%), corn silage (24.5%), steam-flaked corn (10.6%), soybean hulls (9.4%), distillers grain (7.6%), soybean meal (8.3%) and rolled barley (6.9%); DM basis. Cows were milked (0700 h and 1900 h) and fed using Calan gates (American Calan Inc.) (0800 h and 2000 h) twice per day. Lactating animals were housed for 175 days in a covered free stall barn with ad libitum access to water and feed. Milk samples were taken once per week starting at the end of adaptation period (25 ± 1.4 d in milk) for 24 weeks. The analyses were carried out using a linear mixed effect model including cow as a random effect. No effects of enzyme supplementation were detected on milk components concentrations and yields of milk fat, protein, lactose, MUN and SNF, feed efficiency, BW, BW change and dry matter intake. Cows fed the enzyme supplemented diet had higher excretion of MUN (P=0.163) and higher proportion of MUN in milk (P=0.354). The results indicate that supplementation of dairy cows with β-mannanase may have improved N utilization and could offer a way to reduce crude protein concentration in diet without compromising milk yield.

Key words: fibrolytic enzymes, Mannan, dairy cattle.

Abbreviation key: MUN = milk urea N, **SNF** = solids non-fat, **ECM** = energy corrected milk

Immunological and metabolic responses of lactating dairy cows fed diets supplemented with exogenous β-mannanase enzyme (CTCzyme)

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Hemicellulose plays an important role in maintaining cell wall structure and accounts for a quarter of total plant biomass, thus making it a considerable anti-nutritive factor in livestock diets. Exogenous fibrolytic enzymes have been used to improve feed efficiency by releasing nutrients bound in complex feed matrices such as hemicellulose. β-mannanase is an exogenous fibrolytic enzyme that is known to hydrolyze mannan structures found in hemicellulose matrices. β-mannanase has been suggested to act in three main ways; 1) reduction of feed viscosity, 2) improvement of energy metabolism, and 3) decreased immune stimulation. The objective of this study was to determine the effects of β-mannanase supplementation on immunological and metabolic responses in lactating Holstein dairy cows. Two weeks after calving, twenty Holstein cows (milk yield = 43 ± 10 kg/d), blocked by parity, were assigned to one of two diets for approximately 182 days. All cows were housed in the same environment and fed the same basal diet. The basal diet of the treatment group was supplemented with β-mannanase (CTCzyme) at 0.1% of concentrate DM. Haptoglobin, Immunoglobulin G (IgG) and somatic cell counts (SCC) were analyzed as a proxy for immune responses and non-esterified fatty acids (NEFA) were analyzed to explore metabolic responses. Blood samples were taken weekly and were analyzed for immune and metabolic markers. Milk samples were collected twice daily and were analyzed separately for SCC. Cows fed β-mannanase showed tendencies for reduced Haptoglobin levels (P=0.06), regardless of parity. Specifically, there was as significant reduction in blood Haptoglobin levels (P=0.01) in supplemented multiparous cows, compared to control multiparous cows, indicating that β-mannanase was associated with decreased systemic inflammation. Furthermore, NEFA levels tended to be lower in cows fed β-mannanase (P=0.08), regardless of parity, suggesting that β-mannanase was associated with improved energy balance during early to mid-lactation. β-mannanase supplemented cows, regardless of parity, displayed slight indications of lowered SCC, numerically, however was not statistically different (P=0.18). There was no differences in IgG response were recorded between treatment and control cows, regardless of parity.

Keywords: β-mannanase, fibrolytic enzymes, immune response, lactating cows

Effect of prophylactic oral calcium supplementation on postpartum mineral and metabolic status on multiparous Jersey cows

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ABSTRACT

The effects of prophylactic oral Ca supplementation on blood mineral and metabolic status, subclinical ketosis, and clinical endometritis prevalence were evaluated on 205 multiparous Jersey cows housed in a commercial dairy. After calving, cows were randomly assigned to receive no oral Ca supplementation (control; n = 105) or 2 doses of oral Ca each containing approximately 50 g of Ca (CaOS; n = 100; QuadricalMINI Ca boluses; Bio-Vet, Barneveld, WI) at 0 and 1 days in milk (DIM). Blood samples for analyses of serum minerals (Ca, P, Mg, K, Na, Fe, Zn, and Cu) were collected before and 1 h after treatment administration at 0 and 1 DIM and also at 2 DIM. A subset of 74 cows was evaluated for plasma glucose and fatty acids concentrations at 0 and 1 DIM before treatment administration and at 2 DIM. Urine pH was measured immediately before, and 1 h after the administration of each oral Ca dose. Blood β-hydroxybutyrate (BHB) concentration was evaluated at 5, 8, and 11 DIM. Clinical endometritis

was assessed once between 21 and 40 DIM. Cows were classified according to their initial calcemic status (Ca-status) as normocalcemic (NC; Ca >8.5 mg/dL) or subclinically hypocalcemic (SHC; Ca ≤8.5 mg/dL). After treatment, serum Ca concentration was higher for CaOS than control cows (8.53 vs. 8.24 mg/dL). Initial calcemic status had a significant effect on treatment response; SHC showed a greater increase in Ca levels than NC after oral Ca dose administration. Oral Ca supplementation reduced the prevalence of SHC (Ca ≤8.5 mg/dL) 1 h after treatment administration at 0 DIM (32 vs. 71%) and at 1 DIM (41 vs. 64%) for CaOS and control cows, respectively. However, at 2 DIM the prevalence of SCH tended to be higher for CaOS than control cows (70 vs. 44%). Serum Mg concentration was higher for control-SCH cows. Regardless of Ca-status, serum K concentration was higher in CaOS than control cows (4.68 vs. 4.53 mEq/L). Plasma glucose concentration tended to be lower for CaOS than control cows (53.1 vs. 55.4 mg/dL). Higher plasma fatty acids concentration was observed for CaOS compared to control cows at 2 DIM (0.43 vs. 0.35 mEq/dL). Urine pH was lower for CaOS than control cows (6.10 vs. 7.04). No treatment effect was observed on subclinical ketosis or clinical endometritis prevalence. These results suggest that postpartum Ca levels can be increased with oral Ca supplementation, but the response to treatment varies with initial calcemic status.

Keywords: oral calcium supplementation, subclinical hypocalcemia, dairy cow.

Effects of an immunomodulatory feed additive on peripheral blood neutrophil function of transition Holstein cows

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Transition cow typically experience immunosuppression with dysregulated neutrophil functions (e.g. compromised phagocytosis), which is causally associated with increased risk of infections. To enhance neutrophil immune competence has significant bearing with wellbeing of transition dairy cattle. In current study, we investigated the effect of OmniGen-AF® (OG; Phibro Animal Health, Quincy, IL, USA) in modulation of neutrophil function of transition cows. Forty-eight multiparous cows were stratified by parity, somatic cell count (SCC) and expected calving date and randomly assigned to 3 treatments that OG was fed at 0 g/head/day (CON), 60 g/head/day (OG60; recommended value), or 90 g/head/day (OG90, 1.5× recommended value). The OG was added from 60 d before parturition to 28 DIM, and removed from all treatment groups during 29-35 DIM. Blood samples were collected (at 0800 h) on d -60, -28, -14, -7, 1, 7, 14, 28, 32, 35 for analysis of PMN phagocytosis and gene expression. A mixed-effects model with repeated measures and generalized linear model that included the effect of treatment at each sampling point were used for the data analysis. The results showed that neutrophil phagocytosis of *S. aureus* and *E. coli* was enhanced and tended to be enhanced by OG ($P < 0.05$ and $P = 0.086$, respectively) from 28 d before parturition to 28 DIM. Cows in OG60 had higher neutrophil phagocytosis of *S. aureus* and *E. coli* compared with that of cows in CON group from 28 d before parturition to 28 DIM ($P < 0.05$). Neutrophil phagocytosis of *S. aureus* and *E. coli* was higher and tended to be higher for OG60 than that of CON on 35 DIM ($P < 0.05$ and $P = 0.094$, respectively). The relative gene expressions of IL-8 and L-selectin was up-regulated and tended to be up-regulated by OG ($P < 0.05$ and $P = 0.095$, respectively) from 60 d before parturition to 28 DIM such that cows in OG60 had higher L-selectin and IL-8 gene expression than that of CON ($P < 0.05$ and $P < 0.01$, respectively). L-selectin gene expression of OG60 was greater than that of OG90 ($P < 0.05$), and the relative expression of IL-8 gene tended to be higher for OG60 compared with that of CON ($P = 0.067$) on 35 DIM. In conclusion, feeding OG at 60 g/head/d (recommended value) from dry-off period was effective in maintaining peripheral blood neutrophil function in transition dairy cows, and it is not necessary to feed OG beyond the recommended value.

Key words: OmniGen-AF, neutrophil phagocytosis, IL-8, L-selectin