What are Prebiotics (Yeast Derived Carbohydrates) and how do They Promote Gut Health?

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The concept of prebiotics had its origin in monogastrics. Extrapolation to ruminant nutrition is logical and applicable. It requires thinking beyond the rumen, however, not to ignore what effect the rumen may have on the activity of prebiotics.

Prebiotics:

Prebiotics were first identified and named by Marcel Roberfroid in 1995. By definition, a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health (Roberfroid 2007)

The nature of prebiotic categorization can be related to their source and function. Categories of prebiotics include:

Fermentable/Digestible:

trans-galactooligosaccharide, inulin (Kleessen et.al. 2001),
fructooligosaccharide (FOS),
lactulose (Bouhnik et. al. 1999, Hughes and Rowland 2001).

Sub-categories:

- Short-chain" prebiotics, e.g. oligofructose, contain 2–8 links per saccharide molecule
- Longer-chain prebiotics, e.g. inulin, contain 9-64 links per saccharide molecule
- Full-spectrum prebiotics provide the full range of molecular link-lengths from 2-64 links/ molecule.

The length of molecule relates to the area of colonic fermentation: short chain fermenting more rapidly in the right side of the colon, whereas the long chain being fermented more slowly, nourishing bacteria predominantly in the left-side colon or full-spectrum providing nourishment throughout the colon. This category of prebiotic is typical derived from plant sources. Their role in ruminant diets may be questionable since they could be digested in the rumen, and are more intended for monogastrics to modify lower gut populations of Lactobacillus and Bifidobacterium.

Fermentation Resistant:

Immunosaccharides (Seifert and Watzl. 2007):
Mannan oligosaccharides (MOS) and beta glucans

These carbohydrate sources are typically not fermented in the rumen (deVaux et al 2002), and play a role in modifying the balance of lower gut microbial populations and serve as immune-modulators at the intestinal mucosal level. This category will be the focus of the perusing discussions.

General Aspects of Immune Function Modulation:

To understand how prebiotics function in mammalian systems, we must first revisit some of the basics of the immune system. A brief overview will be presented here to facilitate the discussion however, refer to Janeway et. al. (2005) for detailed information regarding the immune system.

There are two basic components of the mammalian immune system: innate and acquired (or adaptive).

Innate Immunity: The cells of the innate system recognize and respond to pathogens in a generic way and does not confer long-lasting or protective immunity to the host. Innate immune systems provide immediate defense against infection, and are found in all classes of plant and animal life. They include both humoral immunity and cell-mediated immunity components. Major functions of the vertebrate innate immune system include:

- Recruiting immune cells to sites of infection, through the production of chemical factors, including specialized chemical mediators, called cytokines
- Activation of the complement cascade to identify bacteria, activate cells, and promote clearance of antibody complexes or dead cells
- The identification and removal of foreign substances present in organs, tissues, the blood and lymph, by specialized white blood cells
- Activation of the acquired (adaptive) immune system through a process known as "antigen presentation"

- Acting as a physical and chemical barrier to infectious agents.

Leukocytes: All white blood cells (WBC) are known as leukocytes. Leukocytes are different from other cells of the body in that they are not tightly associated with a particular organ or tissue; thus, they function similar to independent, single-cell organisms. Leukocytes are able to move freely and interact with and capture cellular debris, foreign particles, or invading microorganisms. Leukocytes cannot divide or reproduce on their own, but are the products of multipotent hematopoietic stem cells present in the bone marrow.

The innate leukocytes include: Natural killer cells, mast cells, eosinophils, basophils; and the phagocytic cells including macrophages, neutrophils, and dendritic cells, and function within the immune system by identifying, presenting and eliminating pathogens that might cause infection.

Acquired immunity is triggered in vertebrates when a pathogen evades the innate immune system and (1) generates a threshold level of antigen and (2) generates "stranger" or "danger" signals activating dendritic cells.

The major functions of the acquired immune system include:
- Recognition of specific "non-self" antigens in the presence of "self", during the process of antigen presentation.
- Generation of responses that are designed to maximally eliminate specific pathogens or pathogen-infected cells.
- Development of immunological memory, in which pathogens are "remembered" through memory B cells and memory T cells.

Yeast Cell Wall Structure: MOS and Beta Glucan: Competitive Adhesion, Immune potential

Yeast cell walls are a rich source of MOS and beta glucan, therefore its interest as a prebiotic.
There is confusion associated with yeast cell wall products in relation to relative MOS concentration, exposed moieties and their potential bioactivities.
In the yeast cell wall, mannan oligosaccharides (MOS) are complex molecules that are linked to protein moieties. Sacarameyes cerevisiae cell wall represents 30% of the dry weight of the cell and is composed largely of polysaccharides (85%) and proteins (15%), (Lipke and Ovalle, 1998). Further analyses reveals that the polysaccharide fraction consists of glucose (80 to 90%), mannose residues (10 to 20%) and N-acetylglucosamine (GlcNAc, 1 to 2%). The MOS component can be attached to the cell wall proteins as part of –O and –N glycosyl groups and also constitute elements of large α-D-mannanose polysaccharides (α-D-Mannans), which are built of α-(1,2)- and α-(1,3)- D-mannose branches which are attached to long α-(1,6)-D-mannose chains (Vinogradov et. al. 1998, Lesage and Bussey, 2006). Therefore, although present, the physical/chemical orientation, and association with other molecules may render them non accessible.

Mannanoligosaccharides (MOS): a high affinity ligand providing competitive binding site options for gram negative bacteria, which possesses mannose-specific Type-1 fimbriae (Ofek et al 1977). The immediate benefits are associated with pathogen removal from the digestive system without intestinal attachment and colonization. This phenomenon elicits significant antigenic responses, thus enhancing humoral immunity against specific pathogens through presentation of the attenuated antigens to immune cells (Ballou,1970; Ferket, 2003; Spring et al., 2000).

In order for the pathogen to adhere to the mannose, the molecule must be physically exposed and accessible to the organism. Therefore, processing to expose the mannose moieties is critical and supersedes quantity. Singboottra (2005) evaluated the agglutination-inducing activity on E. coli cells of 6 yeast products containing different MOS levels (6-45% MOS). MOS molecular size and exposure of binding sites dictated how much pathogen was bond: percentage MOS in a product had little influence on pathogen agglutination. The method of processing the cell wall could dictate the degree and consistency of exposure associated with the various moieties. Enzymatic processing of yeast cell wall at an optimal temperature, time and pH yields a more consistent exposure of binding sites than chemical or mechanical fractionation (Balasundaram and Harrison, 2006; Pitarch et al., 2008). Therefore, comparative study evaluation in response to different yeast culture and/or cell wall preparations need clarification and greater definition to be meaningful.

N acetyl galactoseamine: Although mannose is an important high affinity cell wall ligand, as stated, other cell wall carbohydrates exist (N acetyl galactoseamine, d-galactoseamine, d-glucoseamine, d-glucose and d-galactose) and also possess other unique binding potential i.e. N acetyl galactoseamine with Cryptosporidium parvum (Hashim et. al., 2006).

B-Glucans: known as "biological response modifiers" because of their ability to activate the immune system (Miura et. al. 1996). Another predominant yeast cell wall component, beta-1,3/1,6-glucan (beta-glucan), has been shown to exhibit immuno-modulatory effects when used as a supplement in aquatic (Dalmo

and Bogwald, 2008), swine (Li, et al., 2007) and poultry (Lowry, et al., 2005) diets.

The most active forms of β-glucans are those comprising D-glucose units with (1,3) links and with side-chains of D-glucose attached at the (1,6) position. These are referred to as β-1,3/1,6 glucans. Some researchers (Miura et. al. 1996) have suggested that it is the frequency, location, and length of the side-chains rather than the backbone of β-glucans that determine their immune system activity. Another variable is the fact that some of these compounds exist as single strand chains, while the backbones of other β(1,3)-glucans exist as double or triple stranded helix chains. In some cases, proteins linked to the β(1,3)-glucan backbone may also be involved in providing therapeutic activity. There are differing opinions on which molecular weight, shape, structure, and source of β(1,3)-glucans which provide the greatest biological activity (Brown and Gordon (2001).

Yeast Cell Wall: The immune connection

Dendritic cells (DC) are phagocytic cells present in tissues that are in contact with the external environment, mainly the skin and the inner mucosal lining of the nose, lungs, stomach, and intestines (Janeway et al 2005). They are named for their resemblance to neuronal dendrites, but dendritic cells are not connected to the nervous system. Dendritic cells are very important in the process of antigen presentation, and serve as a link between the innate and acquired immune systems.

Dectin-1 is an intestinal cell receptor that will bind with beta glucan. From that, it can stimulate inflammation to get the body started in fighting the infection. It also prepares macrophages from engulfing pathogens to destroy them (Kankkunen et.al. 2010). Lastly, beta glucan binding to Dectin-1 produces cytokines which help the T and B cells produce antibodies for more targeted defense of the infection, supporting the acquired immune system (Kankkunen et.al. 2010).

Gut Health: The critical trilogy: Gut Microbiota, Gut Permiability and Mucosal Immunity

There is a complex and critical relationship among intestinal microbiota, gut permeability and mucosal immunity (Vaarala et al 2008). The intestinal epithelium is most critical component of the innate immune system. It is the primary surface physical barrier separating highly immunogenic luminal agents (pathogens, toxins, antigens) from a immune-reactive epithelial layer.

Commensal (Indigenous) Microbiota: The complexion of commensal macrobiotic is established early in life. In fact, the first week postpartum is a very dynamic developmental period in the bovine GIT with significant changes in both mucosal barrier and immune function (Griebel et. al. 2014). Correlation analyses of total bacterial numbers and specific families revealed significant associations between the commensal microbiome and the expression of genes involved in regulating both mucosal barrier and innate immune function. This microbiota is very critical in establishing the base community of resident microbiota. The stronger this establishment the more successful will be the competition for nutrients and attachment sites against aberrant microbes (pathogens). Although, not a preferred substrate, mannose can be utilized as a carbohydrate source by Lactobacillus (Lauret et.al. 1996) and Bifidobacteria (Lui et al 2011) which are predominate commensal strains.

Intestinal epithelium: Intestinal lining consists of intestinal epithelial cells with a primary function of intra cellular nutritive absorption, however, transduction of inflammatory signals from luminal microbes by way of toll-like receptors is critically important as well. The main controlling factor associated with inter (para)cellular transport is the bridging mechanism between cell bridges known as tight junctions (TJ) or zona occludens (Madara and Pappenheimer, 1987). The TJ complex consists of transmembrane proteins with proteins from the claudin and occludin groups which interact with the actin and myosin contractile elements to regulate para-cellular transport (Madara and Dharmsathaphorn 1985). The control of these "gatekeepers" is critical to para-cellular transport. Zonulin (pre-haptoglobulin 2) is a protein found in high levels with in rats prone to diabetes type 1 with increased intestinal permeability. Zonulin is the only physiological modulator of intercellular tight junctions described so far that is involved in routing macromolecules and therefore function in tolerance/immune response balance (Fasano 2011). Psychological stress and corticotrophin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism (Keita and Soderholm 2010). It could be speculated that stress therefore be linked to increased para-cellular intestinal permeability through a mast cell dependent release of zonulin. Para-cellular intestinal transport may be a critical route of antigen, toxin and pathogen entry during stressful episodes.

Critical Times of Heightened Susceptibility to Stress: The Prefect Storm

During the course of the cows life (neonatal calf: 1-35d) and production cycle (transition cow ,-21

to 35DIM, high producing cow, calving through 150DIM), there are key stress factors that contribute to her susceptibility to comprised gut health and overall health. These would include suboptimal nutritional (moldy feed, imbalanced diet ,fluctuations in DMI, poor bunk management, etc.) and/or environmental (general hygiene, calving area, stall beds, overcrowding, poor stall design and surface, poor birthing technique, etc.) management regimes and where heavy pathogen challenges may be present.

A good example to illustrate the potential for a compromised gut health scenario would be during the transition period (Figure 1). Even in a well-managed program, there is a dramatic change in the dietary regime during this period. In addition, DMI naturally declines and hours pre and post calving intake may become more restricted. These episodes can result in a change of ruminal environment which will have an effect on the lower gut resulting in alterations of commensal microbiota. These changes subsequently invoke environments which promote aberrant (pathogenic) populations. The stress component will advance triggers (acetylcholine, NGF, mast cells, zonulin, etc.) which will alter intra- and para-cellular transport of bacteria and antigens: "leaky gut". Both acute and chronic stress affects mucosal barrier dysfunction primarily through neuro-endochrinological factors (Keita and Soderman 2010). Bielke et.al. (2014) demonstrated feed restriction consistently resulted in increased tight junction leakage and bacterial translocation in poultry. The consequence of this cascade of events can lead to clinical or subclinical toxicosis. However, mounting an immune response to stress or infection can be energetically expensive (Demas et al., 2004), and prolongs negative energy balance at transition which further affects immunecompetence (Waldron et al., 2003; Goff, 2006) and predispose cows to infectious disease after calving.

Prebiotics play a role in supporting gut health:
Optimal management and stress reduction are critical factors in abating gut health problems. As mentioned previously, yeast cell wall carbohydrates can play a role in reducing the implications of stressful situations and aid in improving gut health:

- Mannose: used as a limited nutrient source for some commensal populations,
- MOS: competitive adhesion site for pathogens
- Beta glucans: Dectin-1 signaling of toll-like receptors and other signaling mechanisms of the innate immune system

Competitive agglutination assays, tissue adhesion determinations and clinical evaluations provide evidence yeast cell wall carbohydrates, as a prebiotic source, play a role in supporting gut health naturally.

Agglutination assays: used to characterize binding specificity and validate the bioactivity of the yeast cell wall products to bind gram negative bacteria which possess mannose-specific Type-1 fimbriae. Agglutination assays were used to demonstrate E.coli 2699 possesses adhesive sites in both the pili and outer cell wall suggesting a two-stage adhesion process to target cells (Eshdat et al 1981). Ganner et al (2013) quantitatively evaluated the capacity of different yeast derivatives to adhere E. coli F4 and Salmonella Typhimurium using a microbiological microplate based assay by measuring OD as the optical growth parameter. Different yeast derivatives showed different binding numbers, indicating differences in product quality. In addition, binding numbers were consistently higher for Salmonella than E.coli. Jalukar et al.2014 showed up to 98% agglutination of E.coli and salmonella with an enzymatically hydrolyzed yeast cell wall product. These agglutination procedures quantitate competitive adhesion, and are able to identify relative product efficacy differences.

Tissues adherence and tissue damage (cytotoxicity) assays: An digestive pathogenic challenge (either clinical or subclinical) typically manifests itself in epithelial damage. The degree and extent of tissue damage is critical to ascertain the relative impact on absorptive capacity. In vitro assays have been used to determine the degree of pathogen adherence to healthy excised tissue after exposure to differing doses of prebiotic (Baines et al 2008). In addition, monolayer cell lawn assays have been used to examine the cytotoxicity of feed extracts and purified mycotoxins in the presence or absence of prebiotics (Lowe et al 2009). The monolayers were enterocytes isolated from the jejunum and grown in culture. Pure mycotoxins or feed extracts were exposed to the monolayers of cells with or without prebiotic treatment. Lawns were stained with typan blue to assess the degree of cellular damage: 0= no blue, no damage, 1=faint blue, slight damage, 2= blue, moderate damage and 3= dark blue, high degree of epithelial cell damage. These assays allow quantitation of both the degree of pathogen adherence in the presence of competitive binding challenges and the intensity of damage associated with toxin exposure and are also able to identify relative product efficacy differences.

Clinical animal trials associated with prebiotics and toxin challenge:
Three Canadian trials were published that evaluated the ability of a prebiotic feed additive to modify the symptoms of jejunal hemorrhagic syndrome (JHS) and mycotoxicosis and the development of new cases.

Five dairy farms were experiencing weekly jejunal hemorrhagic syndrome JHS deaths (Baines et al

2011a). Dairy cattle developed JHS after consuming feed containing several types of mycotoxigenic fungi. Shiga toxin - producing E. coli (STEC) was colonized at the mucosa in the hemorrhaged tissues of the cattle and no other pathogens were identified. Feed extracts yielded cytotoxic scores of 3 when exposed to enterocytes. Celmanax™ (0.1% yeast cell wall prebiotic) treated cells showed a cytotoxicity score of 0. There was no effect of a probiotic (Dairyman's Choice™) on feed-extract activity in vitro. Celmanax™ also directly decreased E. coli O157:H7 colonization on mucosal explants in a dose-dependent manner. There was no effect of probiotic paste on E. coli O157:H7 colonization in vitro. The inclusion of the prebiotic in the feed was associated with a decline in disease.

Beef cattle developed JHS after consuming feed containing several types of mycotoxigenic fungi (Baines et al. 2011). Feed extracts containing mycotoxins were toxic to enterocytes. A dosage of 0.1% of a prebiotic, CelmanaxTM removed the cytotoxicity in vitro. The inclusion of this prebiotic in the therapy program for symptomatic beef calves was associated with 69% recovery.

Calves consuming 1–3 ppb aflatoxin and 50–350 ppb fumonisin in calf feed ration promoted STEC-associated hemorrhagic enteritis outbreaks (Baines et al 2013). Inclusion of 0.02 ppb aflatoxin in the growth media of STECs resulted in greater cytotoxic production and cytotoxicity in vitro supporting a role for mycotoxins in STEC pathogenesis. Application of a prebiotic and probiotic (CelmanaxTM/Dairyman's Choice™) to the calves eliminated STEC shedding and the morbidity/mortality losses. In addition, it was shown that different serotype of STEC possessed various threshold dosages that would result in cytotoxicity score of 3. (Figure 2). Furthermore, it was shown that the addition of aflatoxin reduced the threshold of each serotype from 40 (0177) to 600% (ExPEC). Thus the devastation caused by combining these 2 toxic agents is magnified compared to each separately.

A pathogenic challenge/stress: demonstrate efficacy

A prebiotic (P; Celmanax SCP, Vi-COR, Mason City, IA) was provided to turkeys throughout a 16-wk grow-out period to determine if it would prevent the effects of stress on production and pathogen colonization (Huff. Et al 2013). Prebiotic was provided either continuously at 100 g/t (P-CS) or intermittently during times of stress at 200 g/t (P-IS). Results showed transporting turkeys decreases performance and that P-IS may be more effective than P-CS for alleviating the effects of this stressor on feed efficiency.

Broilers were challenged with E. coli O78 was evaluated to determine the effect of CelmanaxTM liquid (CL) on performance, immune function and health (Adaiel et.al. 2011). Three hundred, one day old native chicks were assigned to the following treatments were 1) control, 2) CL 0.5 ml/L+ vaccination, 3) CL 0.5 ml/L + vaccination+ E. coli, and 4) control + Vaccination+ E. coli. Birds supplemented with CL showed significantly improved performance, cellular and humoral immunity and reduced morbidity and mortality in birds infected with E. coli O78.

Three scenarios were developed that covered a range of commercial environments involving growing male broilers: Best: new litter with clean water and coccidiostat (Salinomycin), Intermediate: used litter with dirty water and coccidiostat, or Worst: used litter with dirty water and no coccidiostat after16 d of age (Brake et al. 2015). Prebiotic (AviatorTM) was included in the starter, grower, and finisher feeds at either 0 or 50 g/MT in each of the three scenarios. Addition of AviatorTM to the Worst scenario improved FCR ($P<0.05$) to that observed in the Best scenario containing coccidiostat without affecting feed intake and BW. This demonstrated the capacity of a prebiotic to maintain feed efficiency in the absence of a coccidiostat in grower-finisher diets.

Animal production and health response:
There are few studies that have investigated the use of yeast cell wall components on immune function in dairy cattle. Seymour et al. (1995) reported decreased incidence of elevated body temperatures in calves when 1% brewer's yeast was supplemented to a calf starter. Franklin et al. (2005) supplemented dry cows with MOS and observed an enhancement of humoral immune response of cows to rotavirus and a tendency for enhanced transfer of rotavirus antibodies to calves. Supplementation of MOS in milk replacer improved fecal scores and reduced scours in calves to the same extent as antibiotics (Heinrich et al., 2003). Inclusion of enzymatically hydrolyzed yeast (EHY, CelmanaxTM, ViCor, Mason City , Iowa) demonstrated the ability to bind and prevent C. parvum from infecting bovine MDBK cells in in vitro experiments (Jalukar and Nocek. 2009). In addition, these researchers demonstrated with calves (<10d of age) diagnosed with a cryptosporidium infection, that supplementation with EHY reduced oocyte shedding 3-fold within 5 days after supplementation and fecal and dehydration scores were significantly ($P<.05$) less for supplemented calves.

Yeast culture and yeast culture with EHY, were fed to evaluate production performance and health in early lactation dairy cattle (Nocek et. al. 2011). Both yeast treatments yielded more milk than non -supple-

mented cows. Milk protein percentage and yields were elevated for EHY compared to Control. Somatic cell count was reduced with EHY during wk 8-14 postpartum. Although supplementation of early lactation cows with a yeast culture improved production performance, further performance and mammary gland health benefits were realized when cows were supplemented additionally with EHY, suggesting components of the cell wall possess certain immunosupportive attributes. Justification for a performance response (increased milk protein) associated with EHY could be derived from modification to enteric micro flora such that provisional nutrients are spared for host availability rather than bacterial utilization, thus more energy and amino acid substrate is available for protein synthesis as described by Ferket (2002) in turkeys.

Conclusions

- Yeast cell wall carbohydrates (mannan oligosaccharides (MOS) and beta glucans (BG)) play a key role in competitively binding gram negative pathogens and promoting immuno-competence.
- Preparation of yeast cell wall products (exposure of moieties), and not necessarily quantity significantly influence biological activity of carbohydrate, and thus competitive binding potential and performance.
- A complex and critical relationship exists among commensal intestinal microbiota, gut permeability and mucosal immunity that influences gut and overall health.
- There is a balance between competitive adhesion of a pathogen challenge (MOS) and continuously mounting a energetically demanding immune response (BG). Pathogen and stress load is important to consider in relation to dose of prebiotic.

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Nutrition Influences Health and Immunity – Critical for a Healthy and (Re)Productive Lactation

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Outline

- Immunological phenotype of transition cow
- What factors contribute to the increased susceptibility for infectious disease
  - Potential strategies to improve transition cow health
    - *Primarily focus on nutrition*

Physiological role

Disease Resistance

Microorganisms *Immune Defenses*

Transition cow immunology

•Transition - Diseases

- High incidence of both infectious and metabolic diseases
  - *Likely not mutually exclusive, but causative relationships not well understood*

Transition cow immunology

• Immune defenses

- (1) Primary protection = physical barriers (skin, closed teat end, teat canal keratin, mucosal barriers)
  - ***Analogy = A large fence protecting your property***
- (2) Secondary protection = anti-microbial secretions
  - ***Analogy = Rottweiler or Pitbull in your front yard***

Transition cow immunology

• Infection - Mastitis

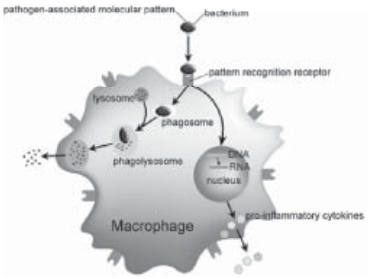
- Entry route for microorganisms
  - *Greatest rate of new intra-mammary infections occur during dry-off or colostrogenesis*
    - Excellent growth media
    - Pressure causes an open teat end
    - Leukocyte and lactoferrin concentrations low at dry off
  - *Estimated that 50 – 65% of coliform mastitis that occurred in early lactation came from infections during the dry period (Smith and Schoengerger, 1985; Green, 2000).*
    - Infection remains "quiescent"

Transition cow immunology

- Infection - Metritis and Endometritis
  - Entry route for microorganisms
    - *High rates of infection occur around calving or during early lactation*
    - *Any calving assistance increases risk of infection*
    - *Additional trauma during calving increases risk of infection*
  - Estimated that 80 – 100% of cows have microbial contamination of the uterus within 2 week of parturition (Sheldon et al., 2008)
  - Uterus has antimicrobial secretions, but not well understood if they change around parturition

Transition cow immunology

- Transition – Macrophage
  - Pro-inflammatory response



Transition cow immunology

- Transition – Disease
  - If a relatively small number of microorganisms infect a competent immune system can eliminate the threat without any disease.
  - For example: Coliform infections after peak lactation show minimal inflammatory symptoms.
    - *So what do the leukocyte phenotypes of cows in early lactation look like that increases the relative risk for developing infectious disease?*

Transition cow immunology

- Transition – Pro-inflammatory response
  - Increased inflammatory responsiveness to lipopolysaccharide
    - *Increased secretion of pro-inflammatory cytokine, TNF-α (Sordillo et al., 1995)*
    - *Local and systemic inflammation more pronounced in early lactation (Lehtolainen et al., 2003)*
- Working Hypothesis: Potentially an "un-coupling" of pro-inflammatory response and other innate immune defenses (Ballou, 2012)

Tertiary Immune Defenses

- Tertiary Protection
  - (1) Primary protection = physical barriers (skin, closed teat, mucosal barriers)
    - ***Analogy = A large gate protecting your property***
  - (2) Secondary protection = anti-microbial secretions
    - ***Analogy = Rottweiler or Pitbull in your front yard***
  - (3) Third layer of protection = Macrophage
    - ***Analogy = Alarm***

Tertiary Immune Defenses

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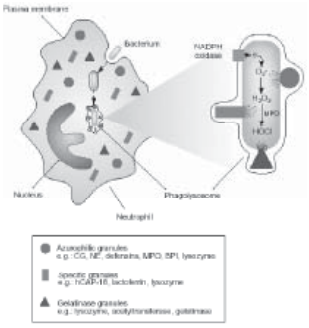
    - ***Analogy = Rottweiler or Pitbull in your front yard***
  - (3) Third layer of protection = Macrophage
    - ***Analogy = Alarm***
  - (4) Forth layer of protection = Neutrophil
    - ***Analogy = Law enforcement***

Transition cow immunology

•Transition – Neutrophil



Transition cow immunology

• Immune defenses – What about vaccines?

- Vaccines produce antibodies that either neutralize the microorganism or help the neutrophil recognize it as a potential pathogen more efficiently
  - ***Analogy = Fingerprint or DNA on File***

Transition cow immunology

•Transition – Neutrophil

- Well documented that many neutrophil responses are suppressed (Burvenich et al., 2003; Paape et al., 2003).
  - *Suppressed number of mature neutrophils*
  - *Suppressed chemotaxis*
  - *Suppressed phagocytic capacity*
  - *Suppressed oxidative burst & total killing capacity*

Transition cow immunology

• Immune defenses – What about vaccines?

- Lymphocyte responses suppressed during transition
- Antibody concentrations reduced in early lactation
  - *May further reduce function of neutrophils*
- However, implication to mastitis / uterine disease resistance not understood

Transition cow immunology

•Transition – Neutrophil

- Why would suppressed neutrophil responses increase disease?
  - *Neutrophils are like law-enforcement*
    - Keep the peace
    - Limit any uprising before they reach a critical threshold for a riot
- The degree of deficiencies have been linked to the severity of mastitis in early lactation (Heyneman et al., 1990; Shuster et al., 1996).

Conclusions & Implications

Peripartum cows high risk for infectious disease

- High infection rate
- Many soluble and neutrophil responses suppressed
- Coupled with a more robust pro-inflammatory response
- Suppressed lymphocyte responses

Transition cow immunology

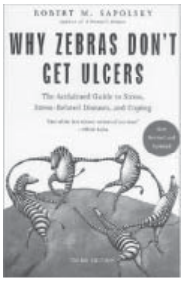
- Switch Gears – Etiology
  - Let's take a closer look at the mechanisms that contribute to a dysfunctional immunological phenotype during the peripartum period

Transition cow immunology

- Transition - Stress
  - Stress suppresses many immune responses
  - Some stressor are unavoidable
    - *Goal = Limit additional stressors*
      - Environment
        - Clean, dry, cool/warm
        - Not over-crowded
      - Management
        - Stocking density – 30 inches of bunk space / cow
        - Limit pen moves – weekly versus all-in-all out
        - Fresh, palatable feed
        - Keep fresh cows locked-up less than an hour
        - Cooling and ventilation
        - Separate heifers and cows

Transition cow immunology

- Transition – Stress
  - Switch from non-lactating to lactating is:
    - *Stressful*
      - Abrupt
      - Dramatic
  - Why are cows creatures of habit?
    - *Control*
    - *Less control = more stressful*
  - Laboratory Stress Model



Transition cow immunology

- Transition – Metabolic / Nutrition
  - What is the relative contribution of the metabolic demands of lactation?
    - *Novel model of mastecomized cows to differentiate between hormonal/stress responses of parturition with the metabolic demands of lactation (Kimura et al., 1999; 2002; Goff et al., 2002; Nonnecke et al., 2003).*

Transition cow immunology

- Transition - Stress
  - Stress suppresses neutrophil and lymphocyte functions
    - *Increases number of immature neutrophils*
    - *Decreases ability to get to the infection site*
    - *Decreases ability to recognize and kill the bacteria*
  - Goal is limit additional stressors

Transition cow immunology

- Transition – Metabolic / Nutrition
  - What is the relative contribution of the metabolic demands of lactation?
    - *Mastecomized = stress of parturition only; Intact cows = both parturition and metabolic demands of lactation*
      - Physiological impacts of high nutrient demand of the mammary gland contributed significantly to the suppressed lymphocyte and neutrophil functions
        - Generalization = approximately "2/3" of the suppression

Transition cow immunology

- Transition – Metabolic / Nutrition
  - Direct versus Indirect Effects?
    - *Direct would be considered nutrient demands of leukocyte responses are not being met or not prioritized*
    - *Indirect would be considered the switch to lactation is abrupt and stressful*

Transition cow immunology

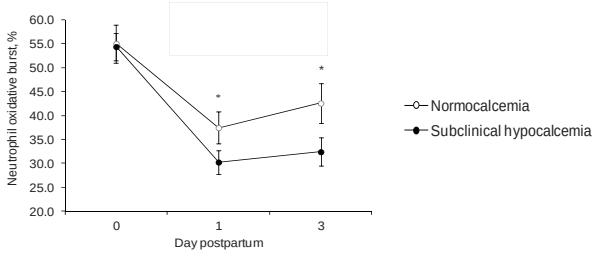
- Transition – Metabolic / Nutrition
  - Can we prepare the cow's metabolism for the nutrient demands of lactation during the dry and close-up periods on the peripartum immune responses (Graugnard et al., 2012)
  - **Energy –** Prevent excessive mobilization of NEFA elevated BHBA concentrations
    - ***Prevent freshening over conditioned cows ≤ 3.5***
      - Manage during late lactation not during the dry period
    - ***Controlled energy intake of dry cows (NE_L = 0.59 to 0.63 Mcal / lb DM)***
      - 10 to 12 pounds of NDF DM / d
      - Important to prevent sorting
      - Supply enough RUP to meet MP requirements

Transition cow immunology

- Transition – Metabolic / Nutrition
  - Evidence for Direct Effects
  - Negative energy balance typically causes elevated NEFA and BHBA and decreased Glucose
    - *Elevated NEFA (as low as 0.125 mM) and BHBA (0.8 to 1.0 mM) in culture with leukocytes decrease function (Lacetera et al., 2004; Grinberg et al., 2008).*
    - *IV infusion of BHBA (1.7 mM) of cows in early lactation decreased neutrophil recruitment to the mammary gland following and intramammary LPS challenge (Zarrin et al., 2014).*
    - *NEB induced in mid-lactating cows decreased many inflammatory genes in mammary gland (Moyes et al., 2010)*

Transition cow immunology

- Transition – Metabolic / Nutrition
  - **Calcium Status**
    - *Cow's with sub-clinical hypocalcemia (serum Ca ≤ 8.59 md/dL) have reduced (P=0.07) neutrophil oxidative burst (Martinez et al., 2013; JDS)*



Transition cow immunology

- Transition – Metabolic / Nutrition
  - Evidence for Indirect Effects
  - Feed restriction (60% of calculated NE_L) in mid-lactating cows for 7 days (Moyes et al., 2009)
    - *Increased NEFA (~1.0 mM) and BHBA (~0.8 mM) concentrations*
    - *Minimally altered neutrophil function and response to an intra-mammary Strep. uberis challenge*
    - *Homeostatic and homeorhetic mechanisms during lactation may allow adaptation to support both the demands of lactation and host immunological defenses*

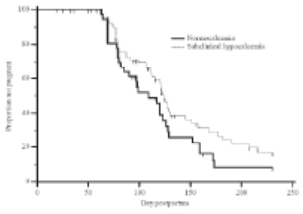
Transition cow immunology

- Transition – Metabolic / Nutrition
  - **Calcium Status**
    - *Cow's with sub-clinical hypocalcemia have increased (P<0.01) incidence of metritis (Martinez et al., 2013; JDS)*

	Incidence of Metritis				
Calcium Status	%	n	P	Adjusted Odds Ratio	95% Confidence Interval
Normocalcemia	15.8	(6/38)		Referent	
Sub-hypocalcemia	63.9	(46/72)	< 0.01	3.24	(1.51-6.95)

Transition cow immunology

- Transition – Metabolic / Nutrition
  - **Calcium Status**
    - *Cow's with sub-clinical hypocalcemia have increased (P<0.01) interval to pregnancy*
      - Median days to pregnancy (1) Normocalcemia = 109 d (95% CI = 82-126 d); (2) Sub-clinical hypocalcemia = 124 d (95% CI = 111-145 d)



Transition cow immunology

- Transition – Metabolic / Nutrition
  - Are NRC, 2001 levels sufficient?
  - Vitamin E (Increased significantly in 2001 because associated with improved neutrophil function and reduced mastitis)
    - 1,000 IU during the dry period and 500 IU during lactation
    - Some research has shown improvements with 2,000 – 4,000 IU during the transition period
  - Selenium – 0.3 ppm; organic sources increase plasma Se concentration more than selenite
  - Vitamin A = 80 KIU recommended, data do not support higher doses or that more bioavailable beta-carotene are beneficial

Transition cow immunology

- Transition – Metabolic / Nutrition
  - **Calcium Status –** Prevent subclinical hypocalcemia
    - *Estimated that up to 50% of cows have subclinical hypocalcemia when fed a non-acidogenic close-up diet*
    - *Distribution shifts when fed acidogenic diet – estimated only 15 - 25% of cows have subclinical hypocalcemia (Oetzel, 2004)*

Transition cow immunology

- Transition – Metabolic / Nutrition
  - Are NRC, 2001 levels sufficient?
  - Zinc – lactating cows require 45 – 65 ppm depending on milk yield
    - *Severe zinc deficiency reduces disease resistance*
    - *Increasing dietary zinc from 40 to 65 ppm reduced SCC and serum amyloid A (Cope et al., 2009)*
  - Copper – lactating cows require 10 – 20 ppm
    - *Marginal Cu deficiency reduces disease resistance*
    - *Iron (> 250 ppm), molybdenum (>5 ppm), S (> 0.25%), high zinc all reduce copper bioavailability*
    - *Most basal diets will contain ~ 10 ppm; therefore supplement additional 5 to 10 ppm depending on the concentrations of antagonists*
    - *Long-term supplementation of >25 ppm is not justified due to risk of toxicity*
    - *Heifers fed a low Cu diet (6.5 ppm) versus 20 ppm from dry to 42 DIM had reduced response to intramammary E. coli challenge (Scaletti et al., 2003).*

Transition cow immunology

- Transition – Metabolic / Nutrition
  - **Oxidative stress** – Balance between oxidants and anti-oxidants
    - *Production of reactive oxygen species is accelerated*
      - Can deplete antioxidant systems (Weiss et al., 1997)
    - *Expression of adhesion molecules and pro-inflammatory cytokines inversely related to antioxidant status (Aitken et al., 2009)*
    - *Suppresses other immunological defenses, including neutrophil functions (Spears and Weiss, 2008)*
  - Many vitamins and minerals are either directly anti-oxidants or participate in anti-oxidant systems
    - Vitamins A, C, and E
    - Selenium, Zinc, Copper, and Manganese

Transition cow immunology

- Transition – Metabolic / Nutrition
  - Are NRC, 2001 levels sufficient?
  - Manganese – NRC, 2001 recommendation was ~ 18 ppm
    - *More recent data suggest this is too low*
    - *Beef heifers fed 18 ppm resulted in clinical Mn deficiency (Hansen et al., 2006)*
    - *Recent digestibility trials suggest requirement might be closer to 30 to 50 ppm (Weiss and Socha, 2005)*
    - *Mn toxicity (> 1000 ppm) is not a major issue*

Conclusions & Implications

Etiology immune dysfunction

- Complicated and Multifactorial
- Unfortunately there is no “Silver Bullet”
- Must take a systematic approach
  - *Reduce additional stressors*
  - *Energy*
  - *Calcium*
  - *Antioxidants*

Questions / Comments

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On-Farm Applications of Yeast-Derived Carbohydrates

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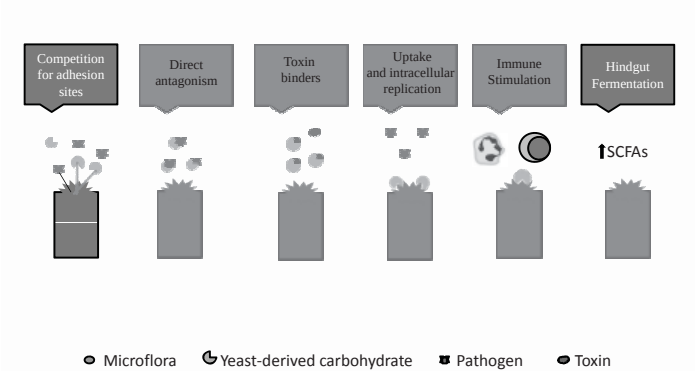
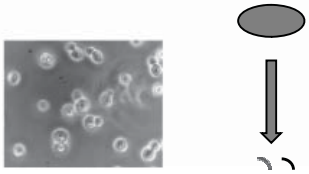
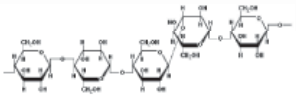
How do yeast-derived carbohydrates work?

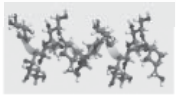


What are yeast-derived carbohydrates?



oligosaccharides
glucans





Why use yeast-derived carbohydrates?

New tools in your toolbox

How do yeast-derived carbohydrate products differ?

- Composition: oligosaccharides (MOS,GOS, FOS), glucans (beta), nutrients
- Quantity: How much? Is it really there?
- Quality: Standards for manufacturing the product?
- Formulation: stability?, consistency?

How are yeast-derived products applied?

Prophylactic – improve overall performance

Spot – recover symptomatic animals

Spot – recover milk production

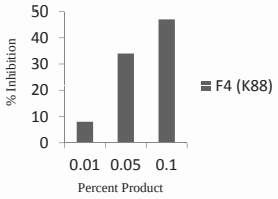
Application strategies span the entire production cycle

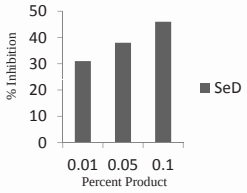
0-3 month calves	4 month to mature	fresh cow 21 days	early lactation 40-60 days	mid-lactation 80-200 days	late lactation	far away dry cow	close up dry cow

Critical for success

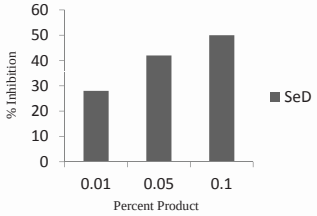
- developed application strategies for each stage with producers
- developed strategies for probiotic and yeast-derived carbohydrates
- probiotic, Dairyman's Choice™
- yeast-derived carbohydrate, Celmanax®
- developed a producer network to facilitate technology uptake

Adhesion





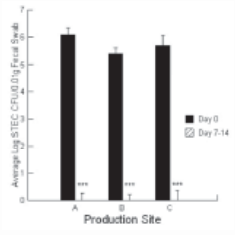
Intracellular Replication



Calves (0 - 3 months)

Scours

- > 80 production sites
- Clinical symptoms: scours, odd lop-sided bloat, bloat, weight loss, rough hair coats, lethargic, nutty
- Pathogens: *Escherichia coli*, *Salmonella enterica* etc.
- *Antibiotics not effective*
- Application Strategy: Spot or prophylactic
- Success is measured by cessation of scour or lower number of scour outbreaks



(Baines et al. 2013, Toxins 5(10): 1872-1895)

Older calves (3 - 12 months)

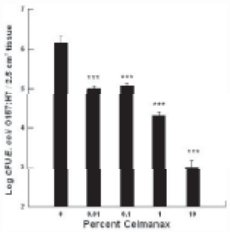
- Spot applications, drench "poor growth"
- Top-dress feed "off pens"
- Success is measured by growth

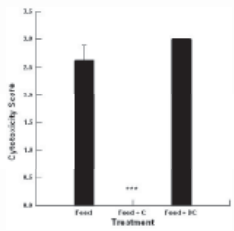
Application Rates

- Prophylactic rate to achieve 0.01 - 0.1 % yeast-derived carbohydrate per volume consumed per day per calf
- Best results for spot treatments when applied between feedings

Fresh Cows to 60 days

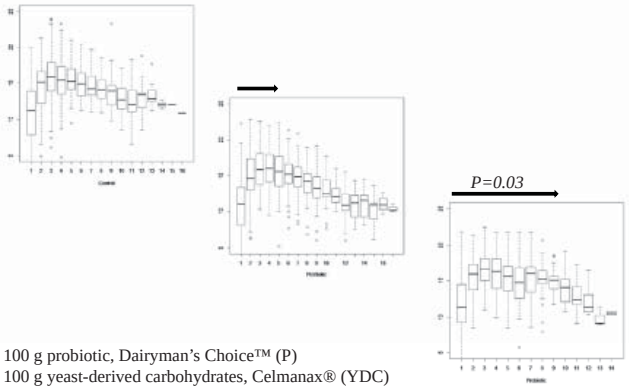
- production site in Alberta
- O145 STEC infections
- mycotoxins and mouldy feed
- JHS cases
- Spot YSB application for freshening cows
- Stopped JHS losses and development of disease





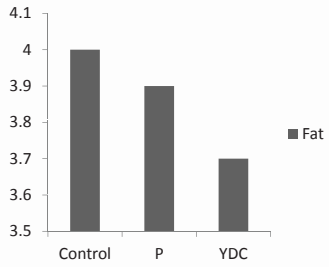
Baines et al. BMC Research Notes 2011, 4:110

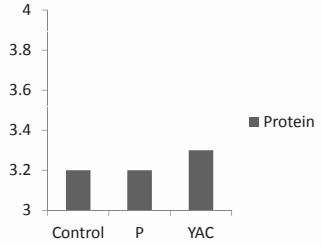
Milk production cycle – prophylactic application



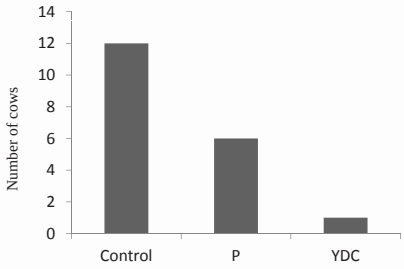
100 g probiotic, Dairyman's Choice™ (P)
100 g yeast-derived carbohydrates, Celmanax® (YDC)

Percent Fat and Protein



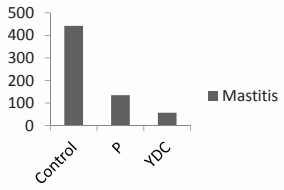


Cow Mortality Losses

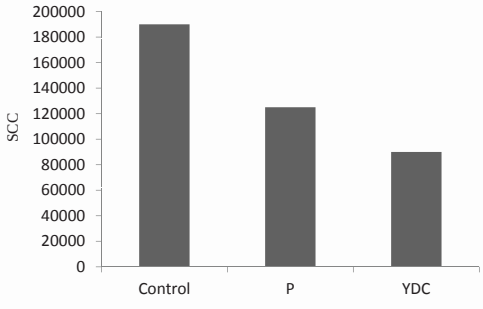


Impact on Production Costs

- Number of LDA surgery, abort, antiparasitic, reproductive treatments did not change (P > 0.200)
- Significant reduction in antibiotic applications and labor costs for P and YDC (P = 0.001)
- Significant reduction in hoof care

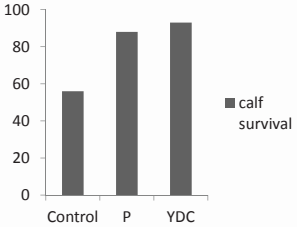


Somatic cell count (P = 0.001)



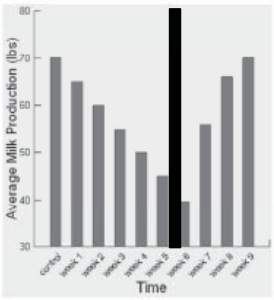
Other benefits

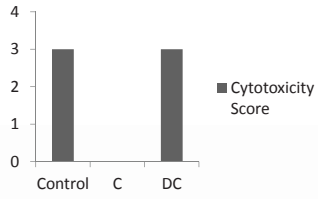
- Milk fever , no further treatments after addition of probiotic (P)
- Bloat, significantly reduced after addition of P, further reduced by the addition of YDC
- Ketosis, no further treatments after addition of P
- Calf survival, significantly improved after addition of P, further reduced by the addition of YDC (P = 0.003)



Other Applications

Spot application for milk production crashes





Clinical Symptoms

Skin nodules that ulcerated
Thickened lymph vessels
Enlargement of lymph nodes
Edema
Swollen hocks
Staggering
Hindlimb paralysis
Wasting
Grey-green runny feces
Runny nose

Management Strategy

Subclinical Mastitis	prophylactic application
Clinical Mastitis	spot application
Scours	spot application

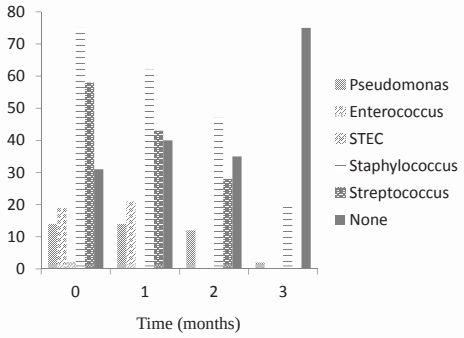
Mastitis

Inflammation of one or more quarters of the udder

Contagious Pathogens	Environmental Pathogens
"udder and teat skin"	"bedding etc"
Staphylococcus aureus *Streptococcus agalactiae etc*	*Streptococcus uberis* *Escherichia coli etc*
Clinical mastitis	Subclinical mastitis
mild (clots & flakes), moderate (milk & udder swelling), severe (milk, udder swelling & cow-systemically sick)	no symptoms

Cost = ~$200.00/cow

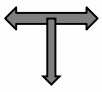
Impact of application on mastitis



Production Site 1 (PS1) and Production Site 2 (PS2)

- mortality of freshened cows, heifers, dry cows
- high mortality of calves (scours)
- increased mastitis
- increased disease (JHS)
- swollen hocks
- swollen lymphatic vessels
- erupting sores
- high SCC

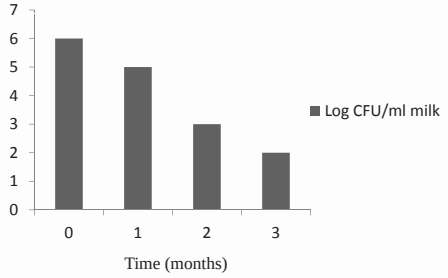
doubled herd size
mouldy barley silage



purchased corn (11 ppb AFL B1)
mouldy haylage, silage

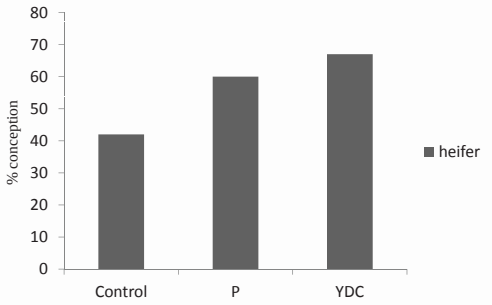
digestive stress
immune suppression
changes in microbiota
damage to the gut lining

Reduction in mastitis pathogens

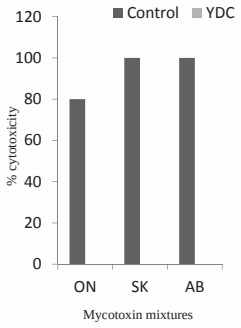


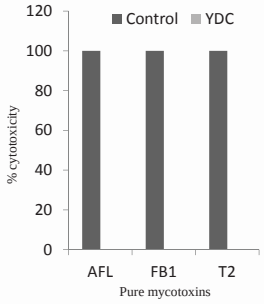
Dry cow programs

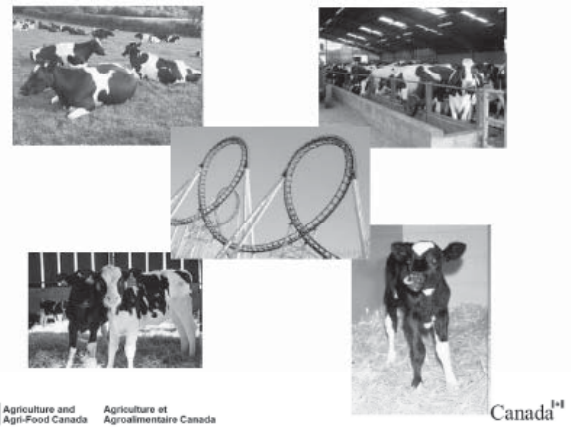
- Prophylactic applications for far-off and close-up dry cows
- Spot applications for close-up dry cows
- Success is measured as improved feed intake and good outcomes



Yeast-derived carbohydrates can be mycotoxin-binders







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Canada

Creating the Perfect Dining Experience: Integrating Cow Behavior, Housing, and Feeding Management

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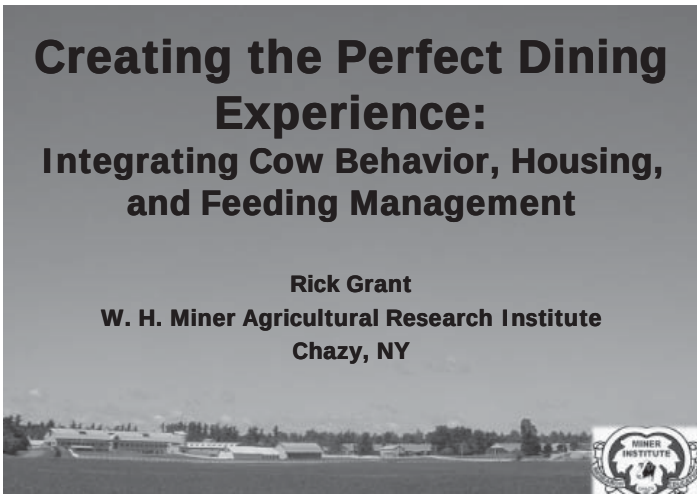
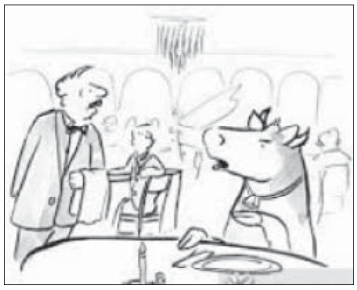


Miner Institute Dairy Herd

- ✓ 350 Holstein cows
- ✓ 3x, rbST
- ✓ 31,000 lb RHA, 4.2% fat, 3.2% protein

Creating the perfect dining experience …



- Well-formulated, palatable ration
- Feed available when cow wants to eat
- Competition doesn't limit feed access
- No restrictions on resting, ruminating
- Water availability…

Importance of management environment (Bach et al., 2008)

- 47 herds with similar genetics were fed same TMR
- Mean milk yield=65 lb/d
  - Range: 45 to 74 lb/d
- Non-dietary factors accounted for 56% of variation in milk yield

FEED AVAILABILITY

  - Stalls per cow

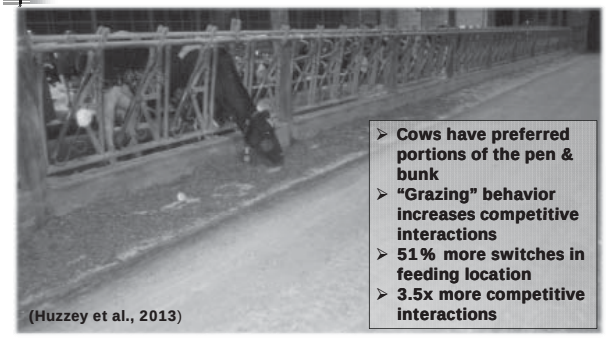
Know your customer…

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

Will this "dining experience" affect diet accessibility?

Management Environment: "The Big Picture"

Not even close to perfect: Non-uniformity of feed delivery



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"
  (Hansen and Pallesen, 1999)

Ruminating and resting enhance feed intake (Schirmann et al., 2012)

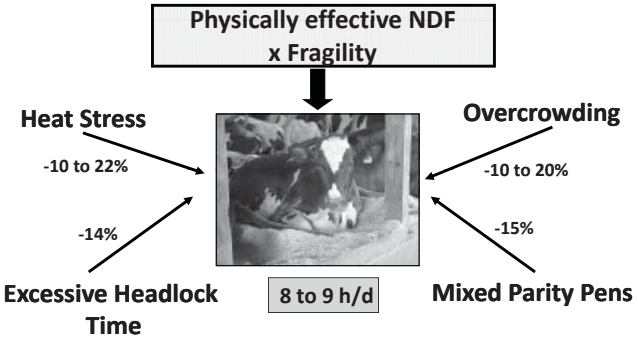
- Rumination and DMI are correlated positively.
  - Following periods of high feed intake, cows spend more time ruminating (Metz, 1975).
  - ±44 min/d in rumination ⟷ ±2.2 lb/d in DMI
- Cows prefer to ruminate while lying down (Cooper et al., 2007).
  - Rumination occurs in ~80% of resting bouts
- **Management that impairs resting and ruminating will reduce feeding activity.**

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

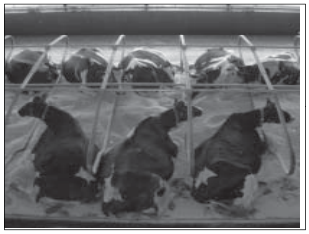
Item	1x/h	2x/h
DMI, lb/d	41.4	40.1
Milk, lb/d	61.3[b]	65.3[a]
Milk/DMI, lb/lb	1.48[b]	1.63[a]
Lying in stall, % of cows	45.3	43.8

Rumination and Management Environment



A well rested cow will eat more …

- <u>Lying time has priority over eating</u>
- Cows will sacrifice eating time to compensate for lost resting time
- With chronic rest deprivation
  - For every 3.5 min of lost rest, cows sacrifice 1 min of eating



(Metz, 1985; Hopster et al., 2002; Munsgaard et al., 2005; Cooper et al., 2007)

What Naturally Stimulates Feeding Behavior?

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

- **Biggest driver of feeding is <u>delivery of fresh feed</u>**

1x versus 2x TMR feeding
(Sova et al., 2013)

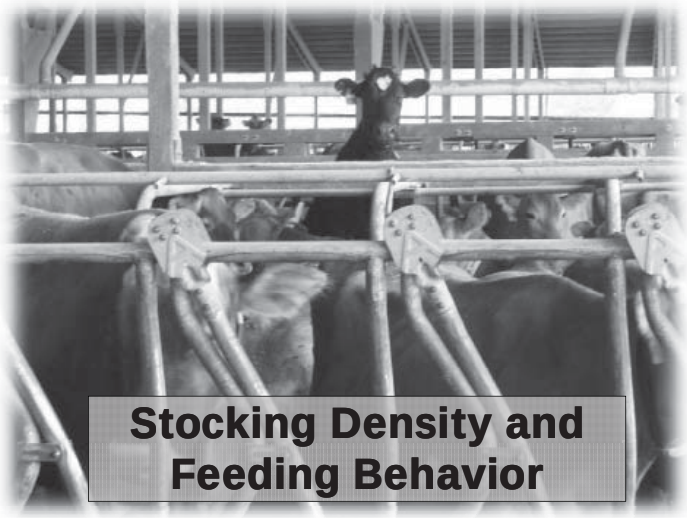
- **Twice versus once daily feeding:**
  - **More feed availability throughout day**
  - **Less sorting against long particles**
  - **Increased DMI by 3.1 lb/d, milk by 4.4 lb/d**
- **Overall improvement in efficiency**
- **Greater feeding frequency:**
  - **Improved rumen fermentation**
  - **Greater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

Circadian rhythms in feeding behavior **(Harvatine, 2012)**

- **With >4x/d feeding:**
  - **Decreased ruminating**
  - **Disruption of circadian lying pattern**
- **In particular, appears to be antagonism between resting and feeding at night.**

Feeding frequency greater than 2x/day?

Reference	FF /d	Eating time %	DMI %	Milk %	Rest %
DeVries et al. (2005)	1 vs 2x 2 vs 4x	+3.5 +4.6	-2.0 -3.0	NR NR	
Mantysaari et al. (2006)	1 vs 5x	+ 7.0	-4.8	-1.0	
Phillips and Rind (2001)	1 vs 4x	+11.0	-6.3	-4.7	
Nikkhah et al. (2011)	1 vs 4x	NS	-5.2	-2.5	



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Phillips and Rind (2001)	1 vs 4x	+11.0	-6.3	-4.7	-8.6
Nikkhah et al. (2011)	1 vs 4x	NS	-5.2	-2.5	NS

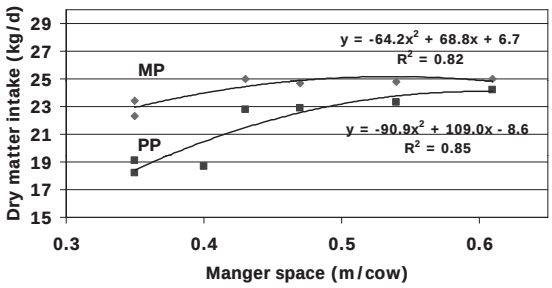
*Cows laid down more quickly with increased FF.

Increased TMR feeding frequency improves efficiency: Is it desirable long-term if it reduces resting time?

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**

Stocking density and DMI by parity in mixed groups



- Interaction between parity and stocking density

(Grant, 2010)

Are 24 in/cow enough?

- Cows cannot access feed all together
- Distribution of DMI changed – pushed to later hours of day
  - 3- versus 2-row pens
  - Is TMR of same quality?
- 24 vs 30 vs 36 in/cow
  - 10, 6, 3 displacements per cow/d
  - Greater feeding time with greater bunk space

Primi- versus multiparous cows and stocking density

(Hill et al., 2008)

	100%	113%	131%	142%
Multi - primi				
Milk, lb/d	+5.9	+13.8	+21.1	+14.9

- Milk losses reflect reductions in resting and rumination activity

What is optimal stocking density?

Close-up and fresh cows:
- ≤80% of bunk space (30 in/cow)
- Function of stall availability

Lactating cows
- 4-row barn: don't exceed 115-120% of stalls
  - mixed heifer & older cows: 100%
- 6-row barn: 100% of stalls?

Ensure access to feed, water, stalls

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

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…

Restricted feed access and overcrowding (Collings et al., 2011)

- **Restricted Feed** (10 h/d) x **Overcrowding** (1:1 or 2:1 cows:bin)
- ~3x displacements when restricted cows were overstocked
  - during 2 h after morning feeding and after afternoon milking
- 25% increase in feeding rate in first 2 h after feed delivery

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 pm to 6 am) reduced DMI by 3.5 lb/d (Collings et al., 2011)
  - 2x displacements at feeding

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% refusal target
- Bunk empty *no more than* 3 h/d (ideally never)

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
- Less restless

Using Rumination & Activity in Herd Management

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Pattison Dairy
PattisonGLee@gmail.com

Using Rumination & Activity in Herd Management

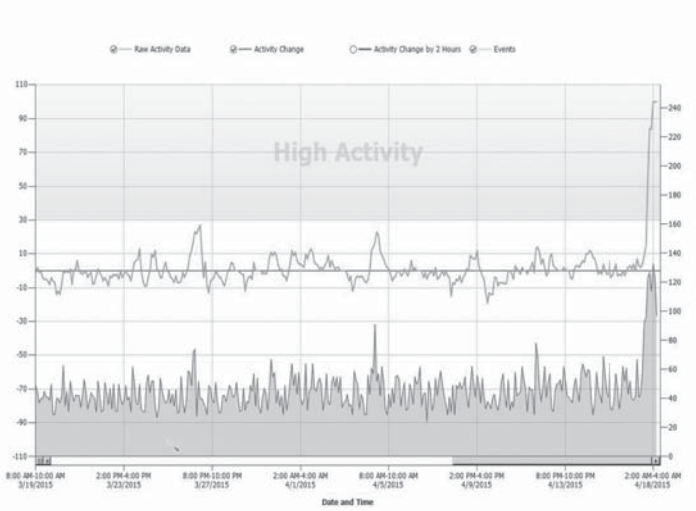
Lee Pattison
Pattison Dairy

Herd

- 700 cows
- 33,934 , 1,230 lbs fat, 1,029 lbs protein
- Raise all heifers – automatic calf feeders
- All AI done in house

Herd

- 390 collars
- On precalving 2-5 weeks
- Off preg check preg except twins
- Felt biggest payback is this period of precalving to preg.



Farm Manager/Herdsperson

- Advantages
  - Catch fresh cows challenges sooner
  - Increased heat detection and validation of heat
  - Minimum drop in heat detection during busy seasons
  - Move cows less after monitoring
- Disadvantages
  - Day of moving collars need extra help
  - Keeping collars working
  - Are we spending less time with cows? Good or bad?

126 12 Month 21-Day Pregnancy Rate by Date

LEE AND SARA PATTISON - 42220361
Ref Date: 4/14/2015
AI Bred - No Exclusions
Lact>=1 L1VWP:71 L2+VWP:71 PgCk:32

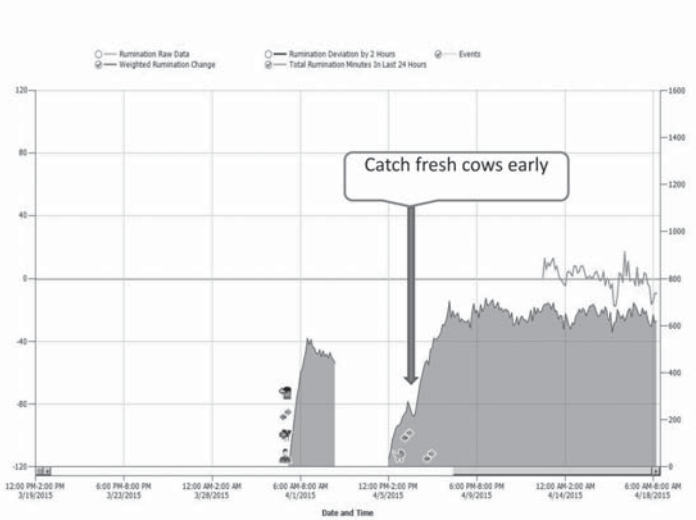
Last Date of 21 Days	Bred #Eligible	Bred #Reported	Bred Pct	Pregnancies #Eligible	Pregnancies #Reported	Pregnancies Rate	Pregnancies #Abort
5/13/2014	140	83	59	139	34	24	5
6/3/2014	148	85	57	147	31	21	2
6/24/2014	144	88	61	142	40	28	8
7/15/2014	134	74	55	133	28	21	3
8/5/2014	135	79	59	133	25	19	6
8/26/2014	147	82	56	145	30	21	1
9/16/2014	157	104	66	152	28	18	
10/7/2014	156	104	67		34	23	
10/28/2014	152	93	61		40	27	
11/18/2014	138	86	62		28	21	
12/9/2014	154	101	66		44	30	
12/30/2014	141	96	68		40	29	1
1/20/2015	132	87	66	129	35	27	2
2/10/2015	121	79	65	116	32	28	3
3/3/2015	121	74	61	118	30	25	0
3/24/2015	124	86	69	0	0		0
4/14/2015	101	87	86	0	0		0
Total	2345	1488	63	2074	499	24	55

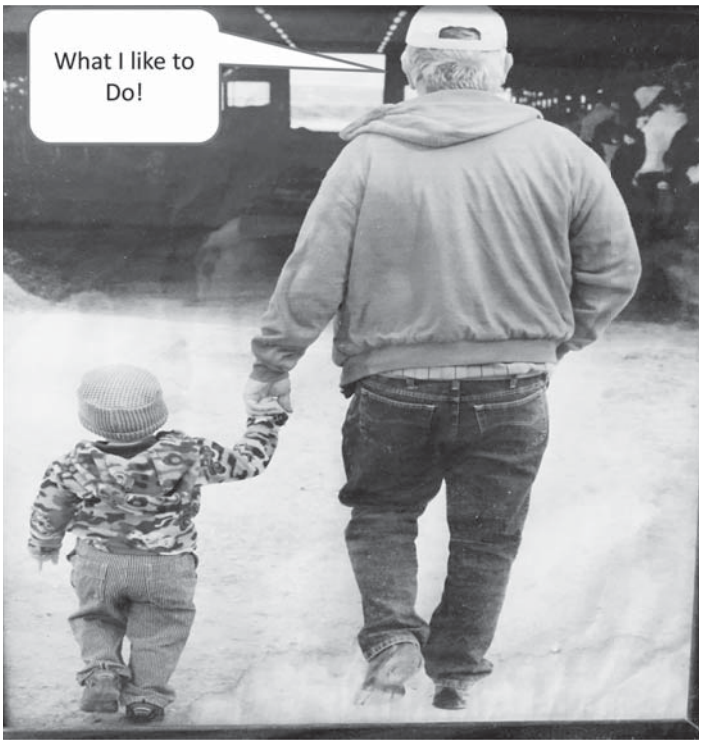
Increase insemination rate

Increase preg rate

Owner perspective

- Advantages
  - Keeps employees engaged
  - Employees spending more time with computer data
  - Increased reproductive performance
  - Health monitoring
  - Catch problem cows quicker
- Disadvantages
  - Cost – short life till potentially obsolete
  - Rely too much on equipment
  - Are we losing good husbandry skills?





Feeding the Dry Cow the "Goldilocks" Diet!

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Partner
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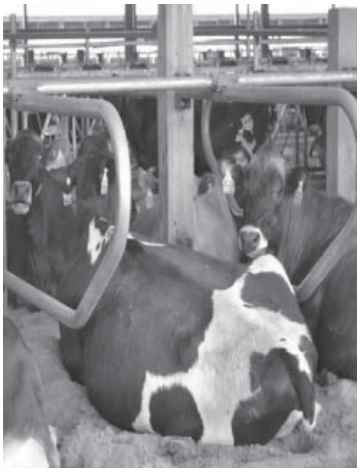
gordon.a.jones@att.net

Central Sands Dairy



Dr. Gordie Jones
Partner
Central Sands Dairy, LLC.

Feeding the Dry Cow The "goldilocks" diet!

Dr. Gordie Jones
Partner
Central Sands Dairy LLC

Dry Cow Programs, A new look at the old way!

In North America there has been a failure of the transition period

Too Little

- Body Condition
- Weight Loss in Dry Pen
- Time in the Dry Pen
- Selenium
- Cow Comfort
- DMI
- Fiber
- Protein
- Magnesium

Rules that still apply

- Nutrition
- Dry Cow program
- Cow Comfort
- Reproduction
- People get everything done above!

Too Much……Too Little

- Body Condition
- Weight Loss in Dry Pen
- Time in the Dry Pen
- Energy
- Too Many Lactations
- Twins / Triplets
- Grain
- Overcrowding
- Excess Soluble Protein

- Body Condition
- Weight Gain in Dry Pen
- Time in the Dry Pen
- Selenium
- Energy
- DMI
- Fiber
- Protein
- Magnesium

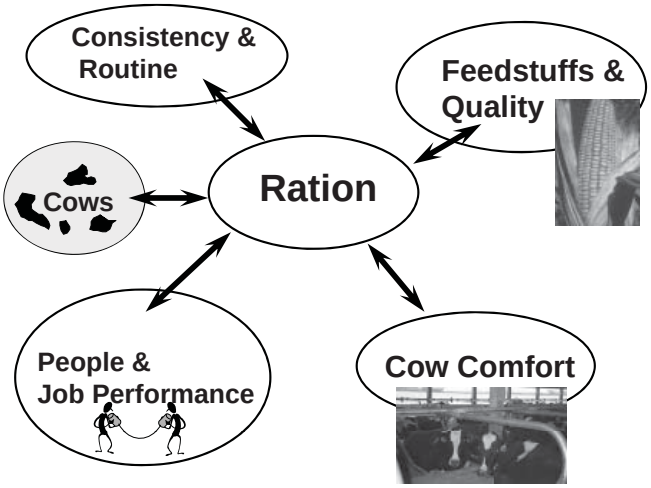
It's a Matter of Too Little or Too Much!

Or how do we get it just right?

Nutrition - Ration

Too Much

- Body Condition
- Weight Loss in Dry Pen
- Time in the Dry Pen
- Energy
- Too Many Lactations
- Twins / Triplets
- Grain
- Overcrowding
- Excess Soluble Protein



Rations that work best @ Central Sands

- > 50% forage
- No More than 6-8# total drymatter from feeds with 40% NDF that are not forage (by-product feeds)
- Butterfat's > 3.75 Holstein
- Butterfat's > 4.65 Jersey
- Rumensin @ 420mg/cow/day

Across the US there has been a failure of the transition period

So what have we tried?

Goldilocks Dry Cow Program

- Comfort
- Low Energy - High Fiber
- Refer to Jim Drackley's work

Dry - Fresh Cow Programs

- Close-up programs
- Steam-up programs
- "10-day" programs
- Drenching programs
- Short Dry Cow Period
- No Dry Cow Period
- Multiple Milkings

Displaced Abomasums

- US Dairy Industry
- Most Dairies have a goal of 4-6%
- Less than 1% is very achievable!

- Dairy Comp 305 ------------ Fair Oaks Dairy #3 ------------ Page {$PAGE}
- Command : EVENTS -
- Expanded : -
- -
- OAK30102 ---------- Dr. Gordie Jones ---------- 1/ 8/10 -

#	Event	Total	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	FRESH	3059	218	220	168	166	244	274	273	357	257	320	272	290
2	OK	836	20	2	4	6	7	18	22	89	188	224	114	142
3	RECK	79	3	2	12	13	7	13	8	1	0	10	5	5
4	HEAT	5787	256	363	351	384	369	526	623	736	724	420	623	412
5	BRED	8545	439	568	671	751	779	602	536	827	982	1020	687	683
6	PREG	1284	49	12	67	129	149	112	97	48	81	110	253	177
7	OPEN	3714	185	213	251	90	243	221	331	405	109	514	676	476
8	PREV	831	29	18	48	8	67	88	80	59	7	70	194	163
9	MOVE	4713	535	252	278	258	272	386	447	419	588	410	511	357
10	BULLPEN	2875	175	105	86	136	234	308	358	366	366	196	364	181
11	DRY	860	36	2	7	5	7	9	27	23	169	233	170	172
12	ABORT	215	1	8	13	27	16	21	32	26	18	18	13	22
14	SOLD	351	15	0	0	0	0	0	0	0	27	82	126	101
15	DIED	21	1	1	0	1	1	2	1	0	2	6	4	2
32	DA	11	0	0	1	1	1	2	0	1	2	1	1	1
36	LAME	33	1	1	0	0	0	0	0	0	0	11	3	17
37	MAST	771	101	70	68	75	94	43	69	33	22	79	91	26
38	METR	18	1	4	2	0	3	5	0	0	0	3	0	0
40	OFFEED	7	0	0	0	0	0	0	0	0	0	2	4	1
41	PNEU	1	0	0	0	0	0	0	0	0	0	0	0	1
43	USER	3849	357	156	120	104	151	221	357	427	327	451	518	660

Total cows listed : 4259

#NAME?	mp 305	----	-----	----	Cow a	nd He	ifer	File	-----	-----	#NAME?	ge {$	PAGE}
#NAME?	: EVE	NTS											-
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#NAME?	-----	-----	-----	-----	#NAME?	Gordi	e Jon	es --	-----	-----	----	1-Oct	/02 -

# Event	Total	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1 FRES	3228	223	145	172	270	269	312	455	347	368	227	241	199
2 OK	1	0	0	0	0	0	0	0	0	0	0	1	0
3 RECK	54	4	9	7	8	1	2	7	3	2	2	4	5
4 HEAT	3059	201	175	142	136	235	200	317	432	380	399	244	198
5 BRED	9085	658	599	628	585	571	552	601	1048	1311	1045	779	708
6 PREG	3478	209	206	320	474	345	396	436	266	334	101	184	207
7 OPEN	2892	245	82	204	252	265	260	288	229	144	247	388	288
8 PREV	1069	174	7	123	82	97	90	45	66	23	13	191	158
9 MOVE	5813	336	304	246	338	714	784	980	388	678	498	241	306
10 BUL	3442	221	171	261	378	318	338	467	287	367	267	199	168
11 DRY	1597	7	57	58	196	351	239	243	262	180	0	1	3
12 ABO	221	28	26	12	29	26	43	7	11	6	3	12	18
14 SOL	336	0	0	0	0	0	69	82	76	108	1	0	0
15 DIE	79	5	4	6	4	6	8	18	12	9	1	2	4
32 DA	15	2	0	0	0	3	1	2	2	1	0	4	0

15 DA's

# Event	Total
1 FRESH	3228
2 OK	1
3 RECK	54
4 HEAT	3059
5 BRED	9085
6 PREG	3478
7 OPEN	2892
8 PREV	1069
9 MOVE	5813
10 BULLPEI	3442
11 DRY	1597
12 ABORT	221
14 SOLD	336
15 DIED	79
32 DA	15
36 LAME	19
37 MAST	1922
38 METR	30

========	=====
1 FRESH	3228
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11 DRY	1597
12 ABORT	221
14 SOLD	336
15 DIED	79
32 DA	15
36 LAME	19
37 MAST	1922
38 METR	30
40 OFFEED	9

# Event	Total
========	=====
1 FRESH	3228
2 OK	1
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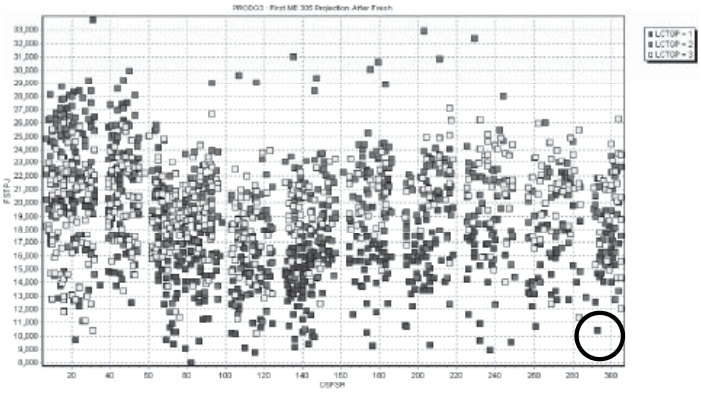
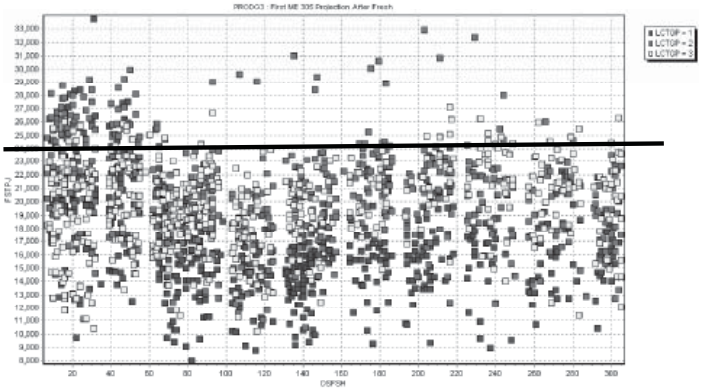
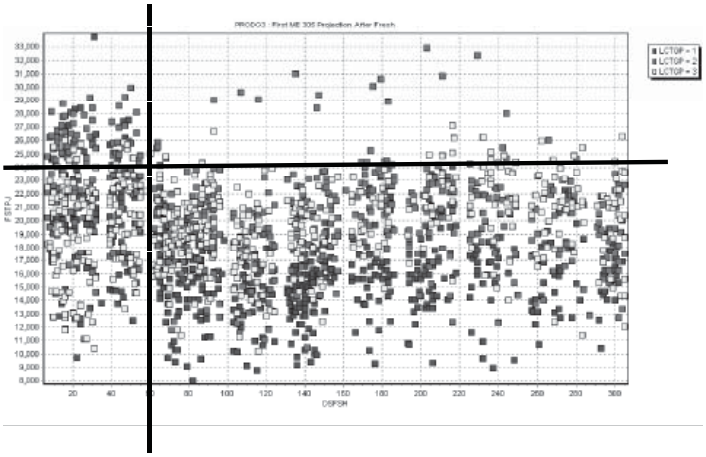
9 MOVE	5813
10 BULLPEI	3442
11 DRY	1597
12 ABORT	221
14 SOLD	336
15 DIED	79
32 DA	15
36 LAME	19
37 MAST	1922
38 METR	30

15 /3228 =.0046
00.4% DA's per Year

Fresh Cow Starts?







Too Much

- Body Condition
- Weight Gain in Dry Pen
- Time in the Dry Pen
- Energy & Grain
- One Lactation to many
- Twins / Triplets
- Overcrowding
- Excess Soluble Protein
- Potassium
- Molds & Mycotoxins

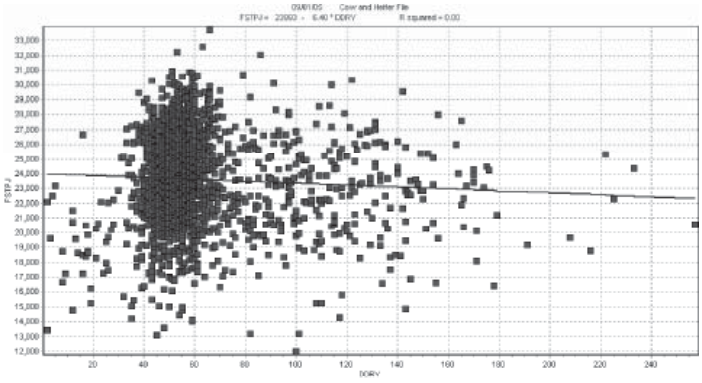
Time in the Dry Cow Pen?

Dry Period Guidelines

- At least 6 weeks
  - shorter periods will decrease profits!
- TWO or ONE group
  - Far off
  - Close Up
    - Separate pen for
    - 3 wks before calving

INTAKE, INTAKE, INTAKE!

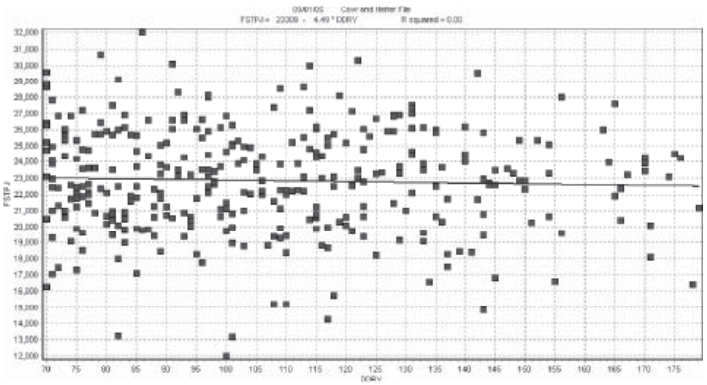
First projection by days dry



General Dry Cow Ration Guidelines

- No more then 8# DM (3.6Kg) of Corn Silage
- 4-6# (2Kg -3.5) dry straw (high quality, low energy) MUST be CHOPPED short
- 2-3# total grain (all will come from C/S)
- No sorting!!
- When it fails…..LOWER the energy!!

First projection by days dry for ddry=70-180



Body Condition

- Fat
- Thin
- Weight Gain
- Weight Loss
- Avoid Weight gain in last 4-6 wks

DMI On Ration Changes from Dry to Milk Cow Ration

- Dry Cow 50%NDF ~100% Forage
- Dry Cow 26# DMI = 13# NDF
- Dry Cow .60 NeL *24# = 14.4 Mcals (NRC)
- Milk Cow 50# DMI * 26% NDF-F = 13# NDF

DMI with Low Energy, High Fiber, Dry Cow Diets

- Far Off Cows 28-32# DMI
- Close-Up Cows 25-29# DMI
- Dry Cows .60mcal x 28 # = 16.8 Mega Cal
- Well above NRC of 14.5 Mega Cal

DMI On Ration Changes from Milk to Dry Cow Ration

- Far Off 50%NDF ~100% Forage
- Far Off 26# DMI @ 50% NDF = 13#NDF
- Far Off .60 NeL *26# = 15.6 Mcals
- Milk Cow 50#DMI 26% NDF-f = 13#NDF
- Milk Cow 50#DMI .80 NeL = 40 Mcals

Dry Cow - SPECIFICATIONS

- **DMI** **26-32 lb/day**
- **CP** **13.5-14.5%**
- **Protein @ least** **1000 g of MP**
- **Ne L** **.58-.62 Mcal/lb**
- **NDF** **40-50%**
- **NDF forage, min.** **40-44%**
- **NFC** **>26%**

NDF Forage (same as milk cow!) 12-13#

Dry Cow - SPECIFICATIONS

DMI	**26-32 lb/day**
DMI	**11-13 Kg/day**
Phos	**40g**
Ca	**125-150g**
Mg	**>.36%**
K	**As low as Poss**
Mg/K	**1/4**

NDF Forage (same as milk cow!) 13#
1000 g of MP

Close-Up Feeding Troubleshooting

Goals
Feed Bulky Forages, Adequate Pe-NDF
Exercise the Cows
Cow Comfort - Well Bedded Pack or Stalls
Adequate Quality Water
Bunk Space ~ 2 Feet Per Head

Close-up Management Troubleshooting

Acidosis Prone Ration
Low Protein & Protein Quality
Excess Soluble Protein
Low Quality Protein
Low Magnesium Levels – High K
Added Phos
Too Much Energy!

Close-Up Management Troubleshooting

Common Pitfalls
Sorting !!!! # 1 problem !!!
Poor Quality Forages are Fed
Mold & Mycotoxins A Problem
Excess Potassium,
No Forage Wet Chem Mineral Analysis
Slug Feeding/No TMR Delivery System
Over Crowding

Rumensin all rations at 320mg
to the lowest intake cow

ELANCO
ANIMAL HEALTH

3 thinks a Cows Should Do!

- Stand to EAT & DRINK
- Stand to MILK
- LAY DOWN

Questions? Thoughts?

Questions? Thoughts?

Dr. Gordie Jones
Partner
Central Sands Dairy, LLC.

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Factors Associated with Pregnancy-Associated Glycoprotein Levels in Plasma and Milk of Holstein Cows during Early Pregnancy and Their Impact on the Accuracy of Pregnancy Diagnosis

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INTRODUCTION

Identification of nonpregnant dairy cows early after AI improves reproductive efficiency and the 21-day pregnancy rate by decreasing the interval between AI services thereby increasing the AI service rate (Fricke, 2002). Thus, new technologies to identify nonpregnant dairy cows early after AI may play a key role in management strategies to improve reproductive efficiency and profitability on dairy farms. Assays for detecting pregnancy-associated glycoprotein (PAG) levels in maternal circulation originating from mononucleated and binucleated cells of the embryonic trophoblast have been developed and commercialized to determine pregnancy status in cattle (Sasser et al., 1986; Zoli et al., 1992; Green et al., 2000).

Pregnancy-associated glycoproteins belong to a large family of inactive aspartic proteinases expressed by the placenta of domestic ruminants including cows, ewes, and goats (Haugejorden et al., 2006). In cattle, the PAG gene family comprises at least 22 transcribed genes as well as some variants (Prakash et al., 2009). Mean PAG concentrations in cattle increase from 15 to 35 d in gestation; however, variation in plasma PAG levels among cows precludes PAG testing as a reliable indicator of pregnancy until about 26 to 30 d after AI (Zoli et al., 1992; Humblot, 2001). Assessment of pregnancy status through detection of placental PAG levels in maternal blood (Sasser et al.,1986; Zoli et al 1992; Green et al 2005) is now used to evaluate pregnancy status within the context of a reproductive management scheme on commercial dairies (Silva et al., 2007, 2009; Sinedino et al., 2014). A commercial test for detecting PAG levels in milk (The IDEXX Milk Pregnancy Test, IDEXX Laboratories, Westbrook, ME) has been developed and marketed to the dairy industry and is now being assessed in field trials (LeBlanc, 2013).

Few studies have compared factors associated with PAG levels in blood and milk of dairy cows early in gestation and the impact these factors may have on the accuracy of pregnancy diagnosis. This paper overviews results from an experiment conducted to assess factors associated with PAG levels in plasma and milk during early gestation in Holstein cows and to determine the accuracy of pregnancy outcomes based on PAG levels in plasma and milk compared to pregnancy outcomes based on transrectal ultrasonography (Ricci et al., 2015).

MATERIALS AND METHODS

Lactating Holstein cows (n = 141) were synchronized for first timed artificial insemination (TAI) using a Double Ovsynch protocol (Souza et al., 2008). Pregnancy diagnosis was initially performed 32 d after TAI for all cows using transrectal ultrasonography. Pregnant cows diagnosed with singletons (n = 48) based on transrectal ultrasonography 32 d after TAI continued the experiment in which pregnancy status was assessed weekly using transrectal ultrasonography from 39 to 102 d after TAI. Blood and milk samples were collected weekly from 25 to 102 d after TAI. From 32 to 102 d after TAI, blood and milk samples were collected from cows on the same day that pregnancy status was assessed using transrectal ultrasonography once a week.

After completion of sample collection at the end of the experiment, frozen plasma samples were shipped overnight in a cooled container by courier from the University of Wisconsin to IDEXX laboratories for analysis of plasma PAG levels using a commercial ELISA kit (the IDEXX Bovine Pregnancy Test, IDEXX Laboratories, Westbrook, ME). Milk samples were delivered weekly to AgSource headquarters (Verona, WI) on the day of collection throughout the experiment and then to AgSource Laboratories (Menomonie, WI) for analysis of milk PAG levels using

a commercial ELISA kit (The IDEXX Milk Pregnancy Test, IDEXX Laboratories, Westbrook, ME). Results were calculated from the optical density (OD) of the sample (corrected by subtraction of the reference wavelength OD of the sample (S) minus the OD of the negative control (N) at 450 nm (with both values corrected by subtraction of the reference wavelength OD of the negative control), which resulted in an S-N value. Each microplate included negative and positive controls.

Pregnancy outcomes were determined based on cutoff values determined by the PAG ELISA manufacturer. For the plasma PAG ELISA, when the S-N value was < 0.300, the cow was classified "not pregnant"; when the S-N value was > 0.300 to < 1.000, the cow was classified "recheck"; and when the S-N value was ≥ 1.000, the cow was classified "pregnant." For the milk PAG ELISA, when the S-N value was < 0.100, the cow was classified "not pregnant"; when the S-N value was > 0.100 to < 0.250, the cow was classified as "recheck"; and when the S-N value was ≥ 0.250, the cow was classified "pregnant."

RESULTS AND DISCUSSION

Plasma and Milk PAG Profiles

Overall, the weekly PAG profile in both plasma and milk from 25 to 102 d after TAI for pregnant cows was similar (Figure 1); however, plasma PAG levels were approximately 2-fold greater compared to milk PAG levels. Temporal PAG profiles from the present study are similar to other studies reporting PAG profiles in serum. In the first study to evaluate PAG-1 concentrations throughout gestation in Holstein cows (Sasser et al., 1986), serum PAG-1 concentrations were detectable in some but not all cows 15 d after AI, increased to about 40 d after AI and stayed constant until about 70 d, then steadily increased until the end of gestation. A study that evaluated the same commercial PAG ELISA test kits evaluated in the present experiment reported similar relative PAG profiles (S-N values) in both plasma and milk (Lawson et al., 2014).

Plasma and milk PAG levels were affected by both week after TAI and parity (Figure 1). When all cows that maintained pregnancy from 25 to 102 d after TAI were analyzed, plasma and milk PAG levels increased from 25 d after TAI to an early peak 32 d after TAI. Plasma and milk PAG levels then decreased from 32 d after TAI to a nadir from 53 to 60 d after TAI for the plasma PAG ELISA and from 46 to 67 d after TAI for the milk PAG ELISA followed by a gradual increase in PAG levels from 74 to 102 d after TAI. Primiparous cows had greater plasma and milk PAG levels compared to multiparous cows.

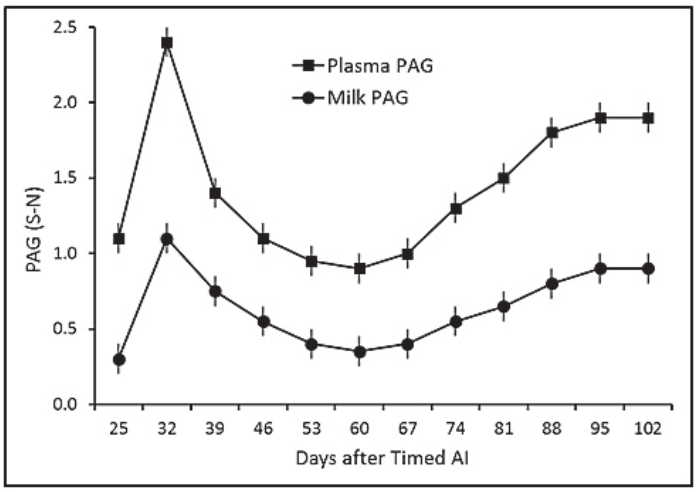

Figure 1. Plasma and milk pregnancy-associated glycoprotein (PAG) profiles for Holstein cows (n = 48) that maintained pregnancy from 25 to 102 d after *AI. ELISA outcomes were calculated from the optical density (OD) of the sample (corrected by subtraction of the reference wavelength OD of the sample (S) minus the OD of the negative control (N) at 450 nm with both values corrected by subtraction of the reference wavelength OD of the negative control), which resulted in an S-N value. Plasma and milk PAG levels were affected by week after AI (P < 0.01). Adapted from Ricci et al., 2015. 1Proportion of cows* [1]diagnosed pregnant using the PAG ELISA that truly were pregnant.

Accuracy of Pregnancy Outcomes 32 d after TAI

To evaluate pregnancy outcomes from the plasma and milk PAG ELISA tests in cows of unknown pregnancy status, 2 × 2 contingency tables were constructed to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the pregnancy outcomes for the plasma and milk PAG ELISA tests 32 d after TAI, and these outcomes were compared to those based on transrectal ultrasonography 32 d after TAI (Table 1).

Sensitivity for both the plasma and milk PAG ELISA tests in the present experiment was high (100% and 98%, respectively), compared to specificity (87% and 83%, respectively). As a result, the NPV for the plasma and milk PAG ELISA tests in the present experiment was high (100% and 99%, respectively) compared to the PPV of both tests (84% and 79%, respectively). The overall accuracy of the plasma and milk PAG ELISA tests 32 d after TAI was 92% and 89%, respectively. Results from this sensitivity analysis support that the accuracy of using plasma or milk PAG levels as an indicator of pregnancy status in dairy cows 32 d after AI is high, and our results agree with others who have conducted similar analyses from 27 to 39 d in gestation when PAG levels in both plasma and milk are at early peak levels (Silva et al., 2007; Lawson et al., 2014; Sinedino et al., 2014).

Table 1. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of plasma and milk pregnancy-associated glycoprotein (PAG) ELISA tests for determination of pregnancy status 32 d after AI. Adapted from Ricci et al., 2005.

PAG ELISA	PPV[1] % (no./no.)	NPV[2] % (no./no.)	Sensitivity[3] % (no./no.)	Specificity[4] % (no./no.)	Accuracy[5] % (no./no.)
Plasma	84 (57/68)	100 (73/73)	100 (57/57)	87 (73/84)	92 (130/141)
Milk	79 (52/66)	99 (68/69)	98 (52/53)	83 (68/82)	89 (120/135)

[2]Proportion of cows diagnosed as not-pregnant using the PAG ELISA that truly were not-pregnant.
[3]Proportion of pregnant cows with a positive PAG ELISA outcome.
[4]Proportion of not-pregnant cows with a negative PAG ELISA outcome.
[5]Proportion of pregnancy status outcomes, pregnant and not-pregnant, that were correctly classified by the PAG ELISA.

From an economic perspective, the sensitivity of an early nonpregnancy test (i.e., correct identification of pregnant cows) is more important than the specificity (i.e., correct identification of nonpregnant cows) based on two economic simulations (Galligan, 2011; Giordano et al., 2013). Further, to obtain a positive economic value for an early chemical nonpregnancy test, the sensitivity had to be greater than 96% when the test is used 31 d and greater than 94% when used 24 d after AI (Giordano et al., 2013). The sensitivity of both the plasma and the milk PAG ELISA tests evaluated in the present study (Table 1) as well as the sensitivity reported by others (Silva et al., 2007; Romano and Larson, 2010) exceed those criteria and support that use of these commercial tests to diagnose pregnancy status 32 d after AI would economically benefit a dairy farm.

Results from the present study support use of plasma PAG testing around 32 d after TAI and milk PAG testing 32 to 39 d after TAI when PAG levels in pregnant cows are at an early peak and pregnancy outcomes for pregnant cows approach 100% accuracy. By contrast, the advantages of the plasma and milk PAG ELISA tests are diminished when conducted during the temporal nadir in plasma and milk PAG levels from 46 to 74 d after TAI due to an increase in pregnant cows with outcomes of not pregnant or recheck. Pregnant cows incorrectly diagnosed not pregnant ultimately may undergo iatrogenic pregnancy loss if they continue the resynchronization protocol and are treated with PGF2α thereby resulting in an economic loss (Galligan, 2009; Giordano et al., 2013).

Accuracy of Pregnancy Outcomes during the First Trimester of Gestation

To determine the accuracy of plasma and milk PAG ELISA outcomes during the first trimester of gestation, pregnancy outcomes from cows that maintained a singleton pregnancy from 25 to 102 d after TAI (n = 48) were analyzed. Cows diagnosed pregnant 32 d after TAI based on transrectal ultrasonography continued the experiment in which pregnancy outcomes based on PAG levels in plasma and milk were classified based on cutoff levels specified by the manufacturer. Overall, pregnancy outcomes for all pregnant cows based on both plasma and milk PAG ELISA tests were a reflection of PAG levels in plasma and milk (Figure 1). Plasma and milk PAG ELISA outcomes of "not pregnant" and "recheck" occurred 25 d after TAI for pregnant cows. Plasma PAG ELISA outcomes for pregnant cows, however, were 100% pregnant 32 d after TAI, whereas the milk PAG ELISA exceeded 98% pregnant outcomes 32 d and 39 d after TAI. Plasma and milk PAG ELISA outcomes of "not pregnant" and "recheck" increased concomitant to the temporal decrease in plasma and milk PAG levels during the nadir and then decreased as plasma and milk PAG levels increased as gestation ensued.

In a study to assess aggressive early nonpregnancy diagnosis with a strategy for resynchronization of ovulation, pregnancy status of cows initiating the first GnRH injection of an Ovsynch protocol 25 d after TAI was determined 27 d after TAI by using a PAG ELISA test (Silva et al., 2009). Cows diagnosed not

pregnant continued the Resynch protocol by receiving an injection of PGF2α 7 d after the initial GnRH injection and a second GnRH injection 54 h after the PGF2α injection. Cows received TAI approximately 16 h after the second GnRH injection 35 d after AI. The authors concluded that earlier detection of nonpregnant cows using the PAG ELISA in conjunction with a protocol for resynchronization of ovulation and TAI increased the rate at which cows became pregnant in a dairy herd compared with transrectal ultrasonography conducted at a later stage after TAI. This agrees with an economic simulation of use of chemical tests for identification of nonpregnant cows early after AI in conjunction with a protocol for resynchronization of ovulation and TAI which concluded that the major economic advantage of using a chemical test was to decrease the interbreeding interval (Giordano et al., 2013).

Pregnancy Loss

The incidence of pregnancy loss in the present study for cows diagnosed with singleton pregnancies 32 d after TAI during the experiment was 13% (7/55) which agrees with the 13% loss reported to occur from 27 to 31 and 38 to 50 d of gestation based on transrectal ultrasonography in a summary of 14 studies (Santos et al., 2004). For the plasma PAG ELISA, all but one cow that underwent pregnancy loss tested positive, whereas all cows undergoing pregnancy loss tested positive at one or more time points for the milk PAG test. Similarly, 5 of 7 cows tested recheck based on the plasma PAG test before the loss occurred compared to 3 of 7 cows based on the milk PAG test. Thus, PAG levels detected by these ELISA tests in the present study have a half-life in maternal circulation resulting in a 7 to 14 d delay in identification of cows undergoing pregnancy loss based on plasma or milk PAG levels compared to transrectal ultrasonography. Because PAG levels are high during late gestation, it takes up to 60 d for residual PAG to be cleared from maternal circulation after parturition in cows (Sasser et al., 1986; Zoli et al., 1992) and other ruminants (Haugejorden et al., 2006). Because of the PAG half-life in circulation, cows submitted for a pregnancy diagnosis before 60 d postpartum can test positive due to residual PAG levels from the previous pregnancy (Giordano et al., 2012), and the manufacturer of the plasma and milk PAG ELISA tests evaluated in this experiment recommends that cows be > 60 d after parturition when tested.

Based on serum samples assayed using the same PAG ELISA test evaluated in the present experiment to determine how rapidly PAG concentrations decrease after an induced pregnancy loss in dairy cows at 39 d in gestation (Giordano et al., 2012), approximately 5 to 7 d elapsed before PAG levels returned to basal levels when luteal regression was induced with PGF2α or when the embryo died. Thus, most cows undergoing pregnancy loss will test pregnant or recheck at an early pregnancy diagnosis conducted using either the plasma or the milk PAG ELISA test. Because it is impossible to distinguish between the pregnancy outcomes of cows undergoing pregnancy loss and those of pregnant cows that test as “recheck” or “not pregnant” during the temporal PAG nadir, it is important that all cows with “pregnant” or “recheck” outcomes at an early test be retested at a later time. Based on temporal PAG profiles in the present study, the best time to conduct a first pregnancy test is around 32 d after TAI with all pregnant cows submitted for a pregnancy recheck 74 d after AI or later when PAG levels in plasma and milk of pregnant cows are rebounding from their nadir.

Effect of Milk Production on Plasma and Milk PAG Levels

Plasma PAG levels in pregnant cows were negatively correlated with milk production for both primiparous ($P = 0.002$; $R2 = 0.05$) and multiparous ($P < 0.01$; $R2 = 0.18$) cows. Similarly, milk PAG levels in pregnant cows were negatively correlated with milk production for both primiparous ($P < 0.01$; $R2 = 0.14$) and multiparous ($P < 0.01$; $R2 = 0.23$) cows. López-Gatius et al (2007) first reported a negative association between plasma PAG levels and milk production in dairy cows. Because relative PAG concentrations decreased in both plasma and milk with increasing milk production, the negative association between PAG levels and milk production is not a result of dilution of PAG levels in milk with increasing production. One possible explanation not tested in this experiment is that PAG production by the conceptus decreases with increasing milk production. If PAG production by the conceptus is a proxy for embryonic growth and development during early pregnancy, the decrease in plasma and milk PAG levels with increasing milk production might suggest that cows with greater milk production may have had slower growing embryos during early development. Further experiments are needed to fully understand the relationship between increased milk production and decreased PAG levels in plasma and milk and what, if any, implications this may have on the health of the developing embryo.

Which pregnancy test is Better - Blood or Milk?

Based on the sensitivity analysis in this experiment (Table 1), both the plasma and milk PAG ELISA tests are accurate for pregnancy diagnosis when conducted 32 d after AI based on the temporal plasma and milk PAG profiles (Figure 1). Further, several economic analyses support the use of early nonpregnancy tests for improving reproduction within a dairy

herd (Galligan et al., 2009; Giordano et al., 2013). Thus, the choice of whether to use the blood or the milk test to diagnose pregnancy is determined by the availability of the test, and the ability to collect the samples.

From a practical perspective, neither the plasma nor the milk PAG tests are cow-side or on-farm tests. Cows must be identified and restrained to collect a blood or a milk sample, and the samples must be sent to an off-farm laboratory that can run the ELISA test. Within several days and after receiving the pregnancy outcome, cows diagnosed not pregnant must again be identified and restrained to submit them to a strategy for rapidly returning them to AI. This is best achieved as part of an aggressive resynchronization strategy for nonpregnant cows as we have described in a number of experiments (Fricke et al., 2003; Sterry et al., 2006; Silva et al., 2009; Bilby et al., 2013; Lopes et al., 2013). It is important to note that no matter what method of pregnancy testing you use (i.e., transrectal palpation, transrectal ultrasonography, or chemical testing) that there are three possible outcomes: 1) pregnant; 2) not pregnant; and 3) recheck. For the plasma and milk PAG tests evaluated in this experiment, the proportion of recheck outcomes is highly dependent on when after AI blood or milk samples are collected (Figure 1); however, a few cows will test recheck even at 32 d after AI due to the occurrence of pregnancy loss and the variation in PAG levels among pregnant cows.

Depending on the farm, milk samples may be easier to collect than blood samples. The only commercially available milk PAG ELISA (IDEXX Laboratories, Westbrook, ME) is marketed through regional DHIA testing centers throughout the United States making the test widely accessible to most farms. A pregnancy diagnosis can be easily conducted on the same milk samples sent for DHIA testing on a monthly basis; however, monthly pregnancy examinations are not frequent enough to drive the reproductive program on a dairy farm. This makes it necessary to conduct additional tests on a weekly or bi-weekly basis. By contrast, many farms can easily collect blood samples, and three commercial blood pregnancy tests are available in North America (BioPRYN, BioTracking, LLC, Moscow, ID; DG29, Conception Animal Reproduction Technologies, Beaumont, QC; IDEXX Bovine Pregnancy Test, IDEXX Laboratories, Inc, Westbrook, ME). The blood ELISA tests are run in regional laboratories located around North America and should be accessible to most farms. Care should be taken, however, to make sure samples are labeled correctly.

CONCLUSIONS

The experiment described herein (Ricci et al., 2015) is one of the first studies to directly compare factors associated with plasma and milk PAG levels during the first trimester of gestation in Holstein cows. Stage of gestation, parity, pregnancy loss, and milk production were associated with relative PAG levels in both plasma and milk in a similar manner; however, milk PAG levels were about 2-fold lower than plasma PAG levels. Based on PAG profiles in plasma and milk samples collected weekly, the optimal time to conduct a first pregnancy diagnosis is around 32 d after TAI when plasma and milk PAG levels are at an early peak, whereas conducting either the plasma or milk PAG test during the temporal nadir in plasma and milk PAG levels would result in poor overall accuracy. Because of the occurrence of pregnancy loss, all pregnant cows should be submitted for a pregnancy recheck 74 d or later after AI when relative PAG levels in plasma and milk of pregnant cows have rebounded from their nadir.

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Making Milk with Forage: Understanding Rumen Fiber Dynamics

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Summary

To optimize milk component production from forages we must understand rumen fiber digestion and passage. Digestion characteristics of neutral detergent fiber (NDF) influence feeding and rumination, rate of particle breakdown, rumen turnover and fill, dry matter intake, and overall rumen and productive efficiency. Traditionally, nutritionists have focused primarily on measures of NDF digestibility, but recently the focus has included undigested NDF as well because of the recognition of its importance in setting the extent and influencing the rates of rumen fiber fermentation. Grasses, legumes, and grain-forages such as corn silage behave differently in the rumen and we must understand their unique digestion and passage characteristics. Legumes such as alfalfa have more fragile NDF than grasses and their forage particle size decreases more rapidly with rumination. Across a wide range of forage types, we have observed a positive relationship between 24-hour NDF digestibility and forage fragility measured as rate of particle reduction. Grasses tend to increase the rumen pool size of large fiber particles compared with legumes thereby retaining more small fiber particles and contributing to a slower passage rate from the rumen (i.e. selective retention) thus increasing rumen fill and mass of physically effective NDF. In addition to increasing rumen fill, higher forage diets with slower fermenting forage-fiber require substantially longer to process by the cow (eating and ruminating) which can pose an often overlooked time budgeting constraint, especially with overstocked feed bunks. In contrast, diets containing highly fermentable forage-fiber that is highly fragile can result in lower chewing, rumen pH, fat output, and efficiency of solids-corrected milk production, but this lower rumen and productive efficiency can be corrected by addition of forages that elicit greater chewing per unit of NDF such as straws or grass hays. High-producing cows with their greater intake and appetite will be more quickly limited by rumen fill with average quality grasses versus legumes. The typical NDF digestion curves for legume and grass forages show that legumes such as alfalfa have a15-20% faster initial rate of NDF digestion versus grasses, but the extent of NDF digestion is 30-40% greater for grasses reflecting 30-40% less lignin. For average grasses and legumes, the digestion curve lines cross at approximately 24-30 hours. Beyond this point, the greater extent of grass NDF digestion will be an advantage. Recent research indicates that the mean rumen retention time for haycrop silage and corn silage NDF particles is approximately 40-45 hours for cows consuming 20 kg/d of dry matter and producing 45 kg/d of milk. These data indicate that highly productive cows can effectively utilize grass forage as a source of fermentable NDF. A critical management goal is to shorten the fermentation time needed for the two forage digestion curves to cross. The normal range in 30-hour NDF digestibility for grass silage is about 55 to 70%. We need to manage grass for harvesting at the upper end of this quality range.

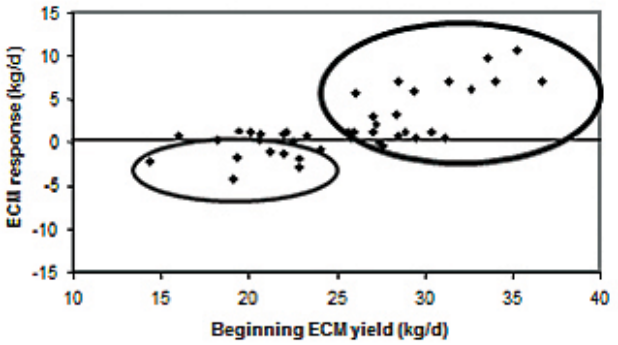
Introduction – Importance of Forage Digestibility

When dairy cows consume high quality forage, we typically observe higher milk component output, fewer metabolic disorders, healthier feet, greater longevity, less purchased grain, and overall greater income-over-feed-cost (Chase, 2012).There is a well-known relationship between forage NDF digestibility and dairy cow performance. For each one percentage-unit increase in NDF digestibility, we see 0.18 kg/d more dry matter intake (DMI) and 0.25 kg/d more 4% fat-corrected milk (FCM; Oba and Allen, 1999). More recently, Jung et al. (2010) evaluated diets containing >40% corn silage and found that each one-percentage unit increase in NDF digestibility was associated with 0.12 kg/d greater DMI and 0.14 kg/d more 3.5% FCM.

In addition to these average responses to forage-NDF digestibility, we also must understand that the relative response to NDF digestibility is a function of the individual cow's milk production level and stage of lactation. Figure 1 shows the response to higher corn silage NDF digestibility when cows were fed total mixed rations (TMR) containing each hybrid. Overall, cows responded modestly to higher NDF digestibility, as expected, but higher producing cows responded much more positively whereas lower producing cows either did not respond, or else they even responded negatively to greater forage-NDF digestibility (Ivan et al., 2004). The bottom line is that feeding dairy cows higher quality forage (i.e. higher fiber digestibility)

typically enhances intake and milk production, but we must consider the milk production level of the cow to most efficiently feed these forages.

Figure 1. Difference in energy-corrected milk (ECM) response for cows fed high versus low NDF digestibility corn silage hybrids as it varies with milk production level (Ivan et al., 2004). Circles indicate that higher producing cows respond positively to higher NDF digestibility whereas lower producing cows do not respond, or respond negatively, to higher corn silage NDF digestibility.

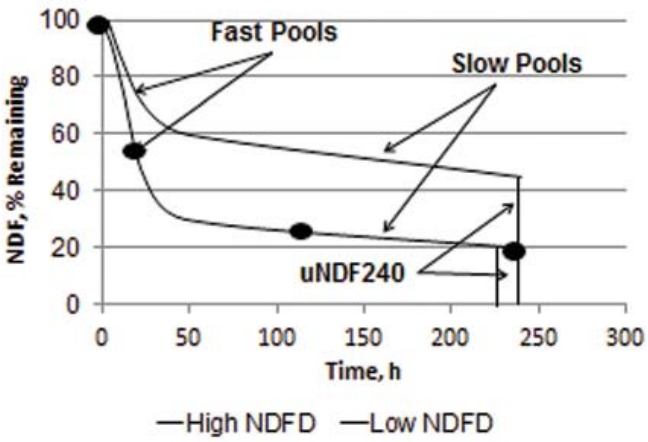


Optimizing Cow Response to Forages – Understanding Fiber Digestibility and Indigestibility

Fiber digestibility and indigestibility are critical factors when assessing forage quality and formulating diets. Digestion characteristics of NDF influence feeding and rumination behavior, rate of particle breakdown, ruminal turnover and fill, dry matter intake, and overall efficiency of milk component output. Traditionally, nutritionists have focused primarily on measures of fiber digestibility, but recently the focus has included indigestible fiber as well because of the recognition of its importance in setting the extent and influencing the rate(s) of fiber fermentation in the rumen. For purposes of nutritional modeling, indigestible NDF is required as the end point for fermentation to allow accurate estimation of the potentially digestible NDF fraction and its rate(s) of digestion. Mertens (2013) coined the term "undigested NDF (uNDF)" as the laboratory measure (typically in vitro or in situ) of indigestible NDF at a specified fermentation time. The method recommended by the Cornell group requires 240 hours of in vitro fermentation using a Tilley-Terry system with modifications described by Raffrenato and Van Amburgh (2010).

To-date, we have relied on a 2-pool model of ruminal NDF digestion (Waldo et al., 1972): 1) potentially digestible NDF, and 2) indigestible NDF. With the advent of the 3-pool model for NDF digestion we are entering a new era in terms of our ability to accurately formulate diets and predict cow response to forage – whether it is a high-forage diet or strategic use of smaller amounts of forage. With this approach, the three pools are: 1) fast-digesting NDF, 2) slow-digesting NDF, and 3) undigested NDF (uNDF) measured at 240 h of in vitro fermentation. Potentially digestible NDF is NDF minus uNDF. Figure 2 illustrates these three NDF fractions for a typical high and low NDF digestibility forage. Fast and slow NDF exists in all forages: legumes, grasses, corn silage and other grain-containing forages.

Figure 2. NDF fermentation curves illustrating time points currently recommended for estimating fast, slow, and undigested NDF for an example high and low NDF digestibility forage.



High NDF digestibility forages are associated with: 1) more fast-pool NDF, 2) less slow-pool NDF, and 3) less uNDF. Higher forage NDF digestibility decreases eating and ruminating time per kilogram of NDF consumed and increases ruminal turnover.

Biological Importance of uNDF

Determination of uNDF should be included in routine forage and feed analysis because indigestible NDF is a uniform feed fraction with a predictable digestibility (i.e. zero). By contrast, NDF is a non-uniform feed fraction; it contains multiple pools that digest predictably as a function primarily of lignification (Van Soest, 1994).

Undigested NDF is the functional fiber fraction that influences physical effectiveness, gut fill, and digestion/passage dynamics of forages. Undigested NDF is important biologically because:

- it can be used to estimate potentially digestible NDF (NDF - uNDF),
- the uNDF fraction together with earlier time points of fermentation can be used to estimate the fast and slow pools of NDF digestion and their digestion rates (Raffrenato and Van Amburgh, 2010),

- measures of NDF pools and rates of digestion based on uNDF can help explain feeding and ruminating behavior, especially when chemical composition (i.e. ADL, NDF, ADF) are similar,
- chewing response to peNDF is likely influenced by forage uNDF,
- estimates of the slow pool of NDF and its rate of digestion plus the uNDF are related to dry matter intake and passage from the rumen,
- uNDF plays a critical role in maintaining the ruminal digesta load, and
- uNDF predicts forage quality because of the relationship between uNDF and OM digestibility (Nousiainen et al., 2003).

At any given time, rumen fiber fill is a function of dietary uNDF, slowly fermenting NDF, and undigested fast-pool NDF. The rumen space resulting from turnover of the fast fiber together with the slow fiber and uNDF allows for more dry matter intake. The more rapidly rumen space is made available (i.e. the greater the turnover), the higher the intake that can be attained. The total mass of uNDF within the rumen can be thought of as a "baseline" of fill which constrains the possible NDF flux. We propose that there is a maximum and minimum amount of ruminal uNDF to avoid limits on feed intake and to maintain proper ruminal health, respectively. Undigested NDF can improve the precision of estimating dry matter intake by telling us how much high-uNDF forage a cow can eat before filling her rumen, and how much low-uNDF forage must be fed to maintain rumen fill and digestive efficiency.

The total mass of uNDF within the rumen can be thought of as a "baseline" of fill which constrains the possible NDF flux. We propose that there is a maximum and minimum amount of ruminal uNDF to avoid limits on feed intake and to maintain proper ruminal health, respectively. Undigested NDF can improve the precision of estimating dry matter intake by telling us, for example, how much uNDF in a TMR that a cow can consume before filling her rumen, and conversely, how much uNDF must be consumed to maintain rumen fill and digestive efficiency.

In fact, there may be an optimal mass of digesting NDF within the rumen; above this amount, fill limits intake while below this amount, intake could increase further although possibly at the expense of feed efficiency (Weakley, 2011). Although the effect on dry matter intake of adjusting dietary NDF is 2 to 3 times greater than changing the NDF digestibility (Mertens, 2009), in many practical feeding situations where dietary NDF has reached the maximum fill potential in high-producing cows, then NDF digestibility (or indigestibility) becomes most important (Weakley, 2011). We believe that uNDF measured at 240 hours of in vitro fermentation (uNDF240) is a forage fraction that accurately assesses the indigestible component of NDF.

How Much NDF Can the Dairy Cow Consume – and How Long Does It Take?

NDF Intake System and Optimal NDF Intake. Consumption of NDF by dairy cows is related to rumen fill and intake potential of a forage or ration. Dr. Dave Mertens developed the NDF Intake System to account for both forage quality and cow productivity, and it determines the maximum proportion of forage in the ration that does not limit intake or performance of the cow. Optimal NDF intake occurs at the point of maximum milk production and is ordinarily about 1.25 ± 0.10 % of body weight per day (mean ± standard deviation). It is important to understand that the optimal NDF intake is not the maximum NDF intake, but instead it is the NDF intake that maximizes milk production. Mertens (2009) provides a complete discussion of the NDF Intake System, its assumptions, and applications in ration formulation for dairy cows.

NDF Intake Targets and Time Budgets. The target of 1.25% of body weight applies to cow in mid to late lactation. This target NDF intake varies with parity and stage of lactation. For example, first-lactation cows have a smaller ability to process NDF than mature cows. We also know that the NDF amount and digestibility will influence NDF intake for cows at a given stage of lactation.

In Table 1, we have some data from a study conducted at Miner Institute in which we compared lower forage diets (49 to 53% of ration DM) to higher forage diets (64 to 67% of ration DM). Within each forage level, we compared conventional (CS) to brown midrib (BMR) corn silage. All diets contained 13% haycrop silage (HCS). So, we had four diets that tested the effect of NDF amount and NDF digestibility on cow responses. It is important to understand the impact that forage-NDF characteristics have on eating and ruminating time. NDF intake varied predictably with dietary NDF content and digestibility and reached its highest amount when cows were fed a higher forage, high NDF digestibility diet. Note that there was an hour per day difference in eating time between cows fed the lower forage, high NDF digestibility diet versus the higher forage, lower NDF digestibility diet. There was a half-hour difference for cows fed the higher forage diet, but receiving either conventional or BMR corn silage.

The bottom line is that higher forage diets with slower fermenting forage-NDF take longer to process. There are important time budget challenges when

cows are overstocked at the feed bunk, especially for younger cows that cannot process forage fiber as effectively as mature cows. Be sure that feed bunk and pen management provide sufficient time for the cows to eat and effectively ruminate the forage in the diet as NDF quality and amount vary.

Table 1. Forage NDF, NDF digestibility, NDF intake, and time spent eating. Do cows have sufficient time to process the quality of forage being fed?

	Low CS	High CS	Low BMR	High BMR	SEM	*P*
Eating behavior Eating, min/d % of total chewing time	273[ab] 34.7	301[a] 35.7	250[b] 35.1	273[ab] 32.8	14	<0.01
Ruminating behavior Ruminating, min/d % of total chewing time	514[ab] 65.3	543[a] 64.3	463[b] 64.9	536[a] 66.2	17	<0.01

Current Insights into NDF and uNDF Intake Targets

All of the details on diet ingredients and nutrient composition and cow responses can be found in the 2014 and 2012 Cornell Nutrition Conference proceedings (Cotanch et al., 2014; Grant and Cotanch, 2012). At Miner Institute, we have evaluated diets with a wide range in corn silage source and amount. Diets have ranged between 36 and 55% corn silage (DM basis), have contained conventional versus brown midrib corn silage that varied by 10%-units in NDF digestibility, and some diets have contained up to 10% added chopped straw to maintain chewing activity as forage percentage was reduced from 52 to 39% (DM basis). Overall, diets contained between 39 and 68% total forage. In all studies, cows responded predictably to dietary NDF and NDF digestibility and were uniformly high-performing, averaging 27 kg/d dry matter intake and 45 kg/d solids-corrected milk production.

Figure 3 summarizes the dietary composition, intake of uNDFom240 and the rumen amount of uNDFom240 (% of BW). As expected, the uNDF varied by diet and reflected the amount and digestibility of forage-NDF. Interestingly, across this range of diets, we observed that the ratio of intake uNDF to rumen uNDF was virtually the same at 0.632. This ratio equates to a rumen passage rate of approximately 2.63%/h for uNDF. We have measured similar ratios of intake:rumen uNDF across several studies with very different forage bases. Although we still need to fully understand the nutritional meaning of this apparently constant relationship for uNDF, it is interesting to note that this passage of uNDF matches well with the passage and mean retention times we have measured using marked forage-NDF particles.

Figure 3. Intake of uNDFom, rumen uNDFom, and the ratio of rumen:intake uNDFom for cows fed diets differing in amount and digestibility of forage-NDF.

Project	Diets				
% Forage		53% 40%CS:13%	67% 54%CS:13%	49% 36%BMR:13%	64% 51%BMR:13%
2011	`Intake	0.36	0.39	0.30	0.33
	Rumen	0.57	0.62	0.48	052
	Rumen: Intake	1.60	1.58	1.58	1.57

Perspectives from these studies. This is still very much an active area of research, but here are the conclusions we have drawn so far regarding uNDFom240 and ration modeling. Based on recent research conducted at Miner Institute and the University of Bologna, here are some potential targets and ranges for NDF intake that are applicable to highly productive dairy cows (25-27 kg/d dry matter intake, and 41-45 kg/d milk production) fed diets based on corn silage, haycrop silage, and chopped dry alfalfa hay. Note that all NDF values are expressed as amylase-modified, sodium sulfite-treated, and ash-corrected NDF (organic matter basis) abbreviated as aNDFom:

- Maximum NDFom intake is ~1.47% of BW (range of 1.26-1.47)
- Maximum rumen NDFom is about 1.28% of BW
- Range in uNDFom240 intake is 0.30 - 0.48% of BW
- Range in uNDFom240 mass in rumen is 0.48 - 0.62 % of BW
- Ratio of rumen uNDFom240/intake uNDFom240 is approximately 1.60 regardless of diet…
- Equates to rumen passage rate of 2.6%/h for uNDFom240
- Agrees with recent measures of rumen mean retention time

Cows respond predictably to NDF and NDF digestibility, and we are learning that the ratio of undigested NDF in the TMR and the rumen appears to be constant over a fairly wide range of diets. We understand the cow's intake response to NDF, how it varies with NDF digestibility, and we must better appreciate the impact of NDF amount and digestibility (or indigestibility) on the length of time it takes for a cow to process her daily allotment of forage-NDF.

Rumen Fiber Dynamics: Grasses versus Legumes

To optimize milk component production from forages we need to understand rumen fiber dynamics. Digestion characteristics of forage fiber influence eating and rumination behavior, rate of particle breakdown, rumen turnover and fill, dry matter intake, and rumen efficiency. Grasses, legumes, and grain-containing forages such as corn silage all behave differently in the rumen.

For example, legumes typically have more fragile NDF than grasses and their particle size decreases more rapidly with chewing. Across a wide range of forages we have observed a positive relationship between NDF digestibility and fragility measured as rate of particle size reduction. Highly lignified, low digestibility straw is often the least fragile forage-fiber and stimulates 1.5+ times the chewing per kilogram than higher quality legumes or grasses. In contrast, low-lignin, highly digestible forages such as brown midrib corn silage or early maturity haycrop silages are highly fragile and require relatively less chewing.

Grasses tend to increase the rumen pool size of long particles versus legumes (Kammes and Allen, 2012). Grasses naturally break into long and slender pieces when chewed compared with most legumes. The net effect of the longer forage particles with grass-based diets is slower passage rate of smaller particles from the rumen (i.e. selective retention), greater rumen fill, and mass of physically effective NDF. In essence, longer forage particles in the rumen act as a filter and modulate passage of particles that are otherwise sufficiently small and dense to escape.

Grass versus Legume Digestion Kinetics

High producing cows that have greater appetites and higher dry matter intake will be more quickly limited by rumen fill when consuming average or low quality grasses compared with legumes. Figure 4 illustrates typical rumen NDF digestion profiles for both legume and grass forages. The figure shows that legumes ordinarily have a 15-20% greater initial rate of NDF digestion versus grasses, but the extent of NDF digestion is 30-40% greater for grasses reflecting 30-40% less lignin.

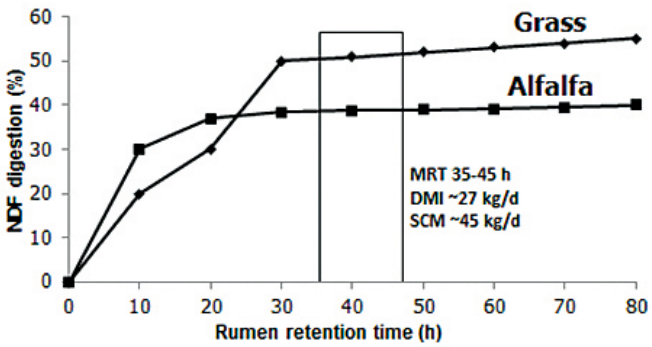
Table 2 summarizes the average measured rate of NDF digestion, digestible NDF fraction, and lignin content (extracted from a detailed review of grass dynamics written by Mertens and Huhtanen, 2007). Averaged across maturity, grasses contain less lignin than legumes and so have a greater extent of NDF digestion with a slower rate of NDF digestion. Averaged across forage type, immature forages contain much less lignin and have greater rate and extent of NDF digestion. The fermentation curves in Figure 4 reflect the digestion data in Table 2.

Table 2. Effect of maturity and plant type on rate, digestibility (dNDF) and lignin content

Forage	Maturity	Rate (%/h)	Digestible NDF (% of NDF)	Lignin (% of NDF)
Legume	Average	11.6	51.2	9.6
Grass	Average	9.6	68.7	6.2
L + G	Immature	15.2	72.4	4.6
L + G	Mature	6.0	47.4	11.2

For average grasses and legumes the rumen fermentation curves cross at approximately 24 to 30 hours. Beyond this time frame, the inherently greater extent of NDF digestion in grasses should be a nutritional advantage. An important consideration is the average time that a forage particle spends in the rumen in comparison to the point when grass NDF digestion exceeds that of legumes. If rumen residence time is too short, then the greater extent of NDF digestion for grasses will be of relatively little use to the cow.

Figure 4. Rumen retention time and typical NDF digestion profiles for grasses and legumes. The outlined area illustrates measured retention time of fiber particles in highly productive dairy cows.



Grass Fiber Digestion and Rumen Retention Time

Recent research conducted at Miner Institute indicates that the mean rumen retention time for marked haycrop silage and corn silage of medium length (1.18-4.75 mm) is approximately 35 to 45 hours for cows consuming about 27 kg/d of dry matter and producing about 45 kg/d of milk. Mean retention time for small forage particles (<1.18 mm) is about 30 to 35 hours. In separate studies with similarly productive and rumen-fistulated cows, we have observed a consistent relationship between undigested NDF (uNDF; measured at 240 hours of in vitro fermentation) in the TMR versus uNDF in the rumen of approximately 0.625. On a 24-hour basis, this equates to a passage rate of about 2.6%/h or a mean retention time of about 38 hours. So, we see consistency among several studies that all indicate that high-producing cows can do quite well on grass forages based on their inherent rumen dynamics.

The inference of this research is that highly productive dairy cows can effectively use grass forage as a source of fermentable NDF. The retention time in the rumen is sufficiently lengthy that the greater extent of NDF digestion of grasses can be effectively exploited. Of course, the data in Table 2 make it clear that maturity at harvest has a far larger impact on NDF digestion that type of forage (grass or legume) and the grass must be harvested early to support high feed intake and milk production. An important forage management goal is to shorten the fermentation time required for the two NDF digestion profiles to cross. This can be accomplished by harvesting grass forage at earlier maturities with less lignified NDF or other approaches that enhance NDF digestion rate. A recently published data set from Dairy One Forage Lab (Chase, 2012) shows that the normal range in 30-hour NDF digestibility for grass silage in the US is about 55 to 70% (normal range defined as the average ± one standard deviation). We need to target the upper end of this digestibility range to maximize response to grass forages when fed to highly productive dairy cows.

Perspectives

The goal of current research is to optimize the cow response to forage NDF whether the situation is a high-forage diet or more strategic, limited use of forages. Understanding the role of NDF digestibility and indigestibility is critical for predicting cow response. The digestibility (indigestibility) of NDF influences: rumen fill, time budgeting and feeding management, chewing responses to peNDF and ruminal pH, and efficiency of milk production. To optimize grasses for high producing cows, we must take advantage of their lower lignin content and greater extent of NDF digestion. Fortunately, measured forage passage kinetics in high-producing dairy cattle indicates that grass NDF may be effectively used at typical mean retention times.

We are entering a new era in our ability to measure forage NDF digestion characteristics and to accurately model cow response to forage quality. The uNDF fraction is the "ballast" that serves as an intake constraint. The relative proportions of the fast and slow NDF determine the flux of NDF through the rumen. In particular, we believe the uNDF plus the slow-NDF govern ruminal space available and consequently dry matter intake. The proportion of fast and slow NDF within a forage or diet determines the relationship between digestion rate, rate of particle breakdown, and passage from the rumen. We should be able to optimize efficiency of feed use by identifying the optimal ratio of fast-NDF:slow-NDF:uNDF. Over a wide range of dietary forage bases, the ratio of rumen to intake uNDF is about 1.60. In other words, uNDF in the rumen is about 1.6x uNDF in the diet. Or, it passes out of the rumen at about 2.6%/hour. If the cow eats more uNDF, then there is more uNDF in the rumen, up to a maximum amount. It also appears that uNDF intake balances uNDF output in the feces on a daily basis. So, we are close to developing a system for accurately determining intake and turnover based on assessment of fast-NDF, slow-NDF, and uNDF.

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Corn Silage: Fungal Disease, the Silent Killer?

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Take Home Message

- In 2012, 9% (583 million bu) of corn yield loss in the top 4 corn producing states (IA, MN, NE, IL) was attributed to plant disease, of that loss, fungal disease accounted for 60% of the total loss (approximately 360 million bu).
- Fungal diseases loss can be lessened by proper corn hybrid selection, good management, and by chemical means such as fungicide.
- Fungal diseases not only cause yield loss but may also decrease feed quality for animals since it can increase the amount of fiber present in a feed, increase the amount of lignin as part of that fiber, and decrease the fat content of the feed.
- Foliar fungicide applied to corn silage seems to increase the DM yield, decrease the amount of fiber present, increase sugar content, increase the amount of rumen degradable silage, and increase predicted milk per ton and milk per acre production.
- Corn silage treated with foliar fungicide and fed to lactating Holstein cows seems to increase feed conversion.

Introduction

Corn silage is a common feedstuff used in many different ruminant feeding systems. The National Agricultural Statistics Service (NASS) estimated that in 2014, 6,371,000 acres of corn were harvested for silage, which is more than acres harvested in 2011, which was estimated at 5,567,0000 thousand acres. In 2014, total corn silage production was 128 million tons, and average as fed production was estimated at 20.1 tons/acre. Corn silage has been increasing in popularity in recent decades due to its ability to keep the nutritive value of a feedstuff over a long period of time such as a winter or dry season when less or no feed can be grown (Wilkinson et al., 2003), and due to its high yielding nature (Allen et al., 2003). However, ensiling is a very complex process which may result in poor fermentation if proper management is lacking. The process of proper management starts at the field level, and thus appropriate consideration must be given to the standing crop as well as the chopped or ensiled forage.

One major concern for corn silage yield and quality at the field level is pest control. The infection of a plant by disease in its simplest form is often described by the disease triangle which includes host, pathogen, and environment, each at one of the three points. This relationship is unique in its simplicity because of the plants immobility, and lack of a complex immune system. There are many different pathogens that can infect corn, but for the purpose of this paper we will focus on fungal infections. These pathogens can cause a decrease in corn silage yields, and have potential negative effects on the quality of the corn silage. In 2012 the 22 top producing corn states and Ontario total corn loss to disease was estimated at 10.9%, in Illinois, Iowa, Minnesota, and Nebraska, corn loss was estimated at 9% or about 583 million bushels total. After further analysis it was found that losses in these states were primarily due to Fusarium ear rot (67.7 million bushels lost), Pythium damping off (63.3 million bushels lost), Aspergillus ear rot (56.3 million bushels lost), Fusarium stalk rot (50.9 million bushels lost), gray leaf spot (50.3 million bushels lost), southern rust (42.6 million bushels lost), and Goss's wilt (34.7 million bushels lost; Wise and Mueller, 2014). Of the total corn production in these 22 states and Ontario, 24.4% of harvested grain samples had mycotoxin contamination. Concern for fungal infection has increased since the introduction of the Highly Erodible Land Act which was included in the 1985 farm bill, giving farmers incentives to implement practices which limited erosion such as no till or conservation tillage (Glaser, 2012). These practices leave crop residues on the ground which can be inoculum that can impact the crop in the next season.

Common Fungal Diseases of Corn

Fusarium ear rot can be caused be F. monoliforme, F. proliferatum, and F. subglutinans. Fusarium ear rot is most common in hot dry conditions. The major concern surrounding this disease is the production of fumonosin mycotoxins which include commonly known mycotoxins such as deoxynivalenol, and HT-2, T-2, and zearalenone (Miller et al., 1983). Symptoms include white to purple colored fungal growth on the kernels or silks. These organisms can overwinter in crop residue, and can be spread as airborne conidia. The F. Monoliforme species may also cause further infection in the plant leading to stalk rots and further yield losses. Hybrids have varying degrees of resistance to Fusarium ear rot and much work has been done to examine if breeding can be used to create resistance in corn against the Fusarium spp. But, because there are such a wide variety of species and

toxins produced, it has been difficult to accomplish. The proper storage of the kernels (i.e. moisture and temperature) can help the decrease the mycotoxin contamination (White, 1999).

Pythium damping off happens when seedlings rot and die pre or post germination. These fungi are present in the soil. Pythium damping off is of greatest concern when weather conditions are cool and wet, when weather is cool plant growth is slower and allows more time for the fungi to infect and kill the seedling. Seeds are at higher risk when there is damage to the pericarp where the fungi can gain access to the seed. Some hybrids carry less susceptibility to damping off and seedling blights, however there is much genetic advancement needed in this area to help better control the disease (White, 1999; Sweets and Wright, 2008).

Aspergillus ear rot was another main disease responsible for crop loss in the top corn producing states. Like Fusarium spp., it also poses a concern in regards to mycotoxin production, more specifically the production of aflatoxins. The main species responsible for this ear rot are A. flavus, and A. parasiticus. The pathogen presents itself in field as a yellow-green mold present on or between kernels, and is most commonly found on the tip of the ear. These species can survive overwinter in soil residue, and thrive in warm and dry conditions. It can also be spread between plants by wind and insects. The major method for control of this species is hybrid selection, however, this may not be effective in severe drought conditions. Irrigation during drought may help to decrease the spread of this fungi. Other methods of control include insect control, and tillage to reduce inoculum. A study by Windham et al. (1999) showed that when the southwestern corn borer also was present in corn, A. flavus infestation was higher, along with aflatoxin production, and resistance breeding to the fungi was no longer effective. (White, 1999; Sweets and Wright, 2008).

Fusarium stalk rot is caused by the same agents as Fusarium ear rots, F. monoliforme, F. proliferatum, and F. subglutinans. This stalk rot is hard to distinguish and lacks the presence of a noticeable fungus, however, the pith may have some white or pink discoloration. This disease happens most commonly in warm dry climates, and usually occurs after pollination. This pathogen can also overwinter in crop residues, and can infect the seed at planting. Hybrids specific to Fusarium stalk rot are not extensively used (White, 1999; Sweets and Wright, 2008).

Gray leaf spot is most common in temperate climates in warm and humid conditions. This disease is caused by C. zeae-maydis. It was originally only a major problem in the eastern states; however, now can cause serious damage to crops in the Corn Belt as well. This disease can develop rapidly, and can cause leaf blight, and premature leaf death. This pathogen can overwinter in crop residue, and is more common when corn is planted following corn while implementing conservation tilling. This disease causes rectangular lesions that begin with small necrotic spots on leaf tissue. There have been hybrids developed that carry resistance to this disease; however, they may not be available for all corn maturities. Conventional tillage may also aid in some disease control, however, may not be as effective in areas where the pathogen is well developed. Foliar fungicide may also offer economic benefit in high yielding susceptible hybrids when risk for loss is high (White, 1999; Sweets and Wright, 2008).

Southern rust is caused by the fungus Puccina polyspora. This disease is primarily present in tropical or subtropical climates but can be found in temperate regions. This disease manifests itself as small circular yellow-green spots which then turn into reddish oval pustules. The pustules eventually rupture releasing powdery spores. This pathogen is mostly spread by wind or infected plant tissue. Resistant hybrids exist, and are the primary method for control of this pathogen; however, chemical methods such as foliar fungicides are also a very effective method of control.

Negative Effects of Mycotoxins

These infections can lead to yield losses, and loss of plant quality and digestibility. As briefly mentioned before one major concern when crops are exposed to fungal infections is mycotoxin contamination. Mycotoxins are produced by the secondary metabolism of the genera Aspergillus, Penicillium, Fusarium, and Alternaria, and are low molecular weight substances (Keller et al., 2013). Visual observation is often done to assess the degree of fungal infection and to evaluate whether a pesticide is needed to control the infection; however, visual observation may not be adequate to estimate infection and contamination level. A study done by Eckard et al. (2011) reported that when corn was disease-scored in the field, few disease symptoms were seen. In a study of 1,100 ears of corn that were disease scored, only 61 ears were infected, and of those only 43 ears were visibly infected on the surface. When looking at stalks only 1.7% showed signs of disease. These samples were then plated on agar medium formulated for mold growth, and this time 67% of all samples were found to be infected, and 25-75% of these infections

were attributed to Fusarium species or spores from this genera. This means that even though there may not be visible symptoms of corn infection, the fungal spores can still be present and given the right environmental conditions may grow, and toxins could then be produced and be present in these feeds.

Some common mycotoxins in corn silage are aflatoxin, deoxynivlenol, zearalenone (ZEA), T-2 toxins, fumonisin, and ochratoxin (OTA) (Allen et al., 2003). Mycotoxin contamination is favored in situations of poor storage which include excessive moisture, dryness, condensation, heating, leaking, and insect infestation (Dos Santos et al., 2003). Alonso et al. (2013) reported that fungal spoilage and mycotoxin contamination can lead to loss of nutrients, dry matter, palatability, and dry matter intake which can negatively affect animal performance. Scudamore and Livesy (1998) concluded that concentrations of fungi greater than 1 x 104 CFU/g-1 can cause respiratory problems, abnormal rumen fermentation, decreased rumen fermentation, decreased reproductive performance, kidney damage, and skin and eye irritation; although exact fungi species were not indicated in this statement. Mycotoxins are a major concern in today's dairy industry due to their possible impact on animal performance, and employee exposure to mycotoxins while working on the farm and thus mycotoxins pose a threat to the profitability and safety of dairy farms (Richard et al., 2007).

F. moniliforme may be responsible for the production of fumonisin B1 (Mesterházy et al., 2012). However, in post fermented silage, F. verticilliodes is the most common Fusarium spp. pathogen found (Keller et al., 2013). Fumonisin contamination in feed can lead to pulmonary edema in pigs, and esophageal cancer in humans; however, ruminants are more resistant to fumonisin contamination (Keller, 2003). The effects of ZEA and OTA include alteration of immune-mediated activities in bovines (Keller et al., 2003). Aflatoxins are produced by A. Flavus, as mentioned above, has been found to have potential carcinogenic effects, and thus pose a threat to human and animal health if consumed. Aflatoxin is known to be carcinogenic and can be transferred to milk; therefore, the aflatoxin concentration in milk is strictly regulated by the FDA. Acute aflatoxicosis is also a possible concern with aflatoxin contamination (Keller et al., 2003). Overall fungal contamination of feeds can lead to mycotoxin production, decreased palatability, decreased feed intake, may impair the rumen microbiota, and can cause negative health events in dairy cattle. This may also exacerbate the stress at which the animal under due to high milk demand which may decrease the overall efficiency of the animal (Alonso, 2013).

Decrease in Plant Quality

Besides the more common concerns of mycotoxin contamination, fungal infestation may also decrease plant quality for animal feed. Many factors affect corn silage nutrient content and digestibility. Fiber, or the cell wall portion of the plant cells (made up of hemicellulose, cellulose and lignin) comprises a major portion of corn silage (70%) and thus is a major contributor to corn silage quality. The amount of fiber present in the feed differs depending on the tissue of the plant it comes from. Because corn silage is processed from the whole plant, the amount of fiber can vary greatly and, if proper sampling techniques are not used, poor nutrient composition estimates may result. One possible concern with feeding high amounts of silage to high producing dairy cows is high NDF (hemicellulose, cellulose, and lignin) content which may lead to decreased dry matter intake. Corn silage has been included in levels of 63% of DM in some dairy cattle diets (Weiss and Wyatt, 2000). Van Soest (1965) proposed that when forage NDF ranged from 55-60% it had little effect on DMI. However, Kendall et al. (2009) found that a four percentage unit decrease in NDF, going from 32% dietary NDF to 28% dietary NDF, increased feed intake (22 kg/d for high NDF compared with 23 kg/d for low NDF diets), total milk production increased approximately 3 kg/d, milk fat increased 0.1 kg/d, and milk protein increased by 0.15 kg/d for cows fed the lower NDF diet. The NDFD is a measure of how digestible the NDF present in the forage will be in the rumen. This value is often found using in vitro laboratory techniques. NDFD can be highly variable, and is not as important for determining energy content; however it does play an important role in determining dry matter intake which can limit total energy intake, and thus milk production (Allen, 1993). One study found that when feeding a brown midrib variety of sorghum silage with higher NDFD (44.8% for normal vs 46.7% for BMR), the cows ate 5 kg/ day more DMI, and produced 6 kg/d more milk (Grant et al., 1995).

One factor affecting NDFD is lignin content, which is a phenolic compound considered to be indigestible by the animal microbial systems (Jung and Deetz, 1993). It has also been found that when researching varieties of corn bred for low lignin content, there is an increase of 5 kg/ day of milk and 9 kg/ day of DMI when compared to corn with a higher lignin content (Jung et al., 2011). Feeding lower levels of lignin also may increase production of VFA by rumen microbiota thus providing more energy for the cow (Oba and Allen, 1999). Allen et al. (2003) also found that lignification of NDF was closely correlated to in vitro

NDFD (IVNDFD); IVNDFD decreasing as lignification increased. There are different types of lignin present in the cell wall, and some suggest that all lignins do not have the same impact on digestibility; however, lignin is often used as a direct indicator of NDF quality and digestibility (Jung and Allen, 1995).

One study reported that corn kernels from corn infected with Fusarium moniliforme tended to have more overall fiber content when compared with non-infected corn (Williams et al., 1992). Many factors can affect the content of lignin in a plant, such as environmental condition, forage hybrid (as discussed briefly above), and plant maturity. Lignin content can be influenced by plant stress as a response to drought, cold, or other disease such as fungal infestation. Lee et al. (2007) found that in white clover, drought stress does not decrease plant biomass, but can lead to an increase in overall lignification, by causing an increase in the enzymes responsible for lignification (primarily phenylalanine ammonia lyase). It has also been shown that cold and heat stress can cause an increase in phenolic compounds (Rivero et al., 2001). When looking at corn seedlings, it was found that infestation of the root by an endophyte caused increased plant rigidity, and increased the structural components of the plant; this may be due to the plant attempting to protect itself from further fungal infection (Yates et al., 1997).

A competition for nutrients between the plant and the fungus can lead to a decrease in non-fiber carbohydrates (NFC) as well as the fat content of plants, which may decrease feed value for use as animal feed. Sugars provide a rapidly degradable energy source for the rumen microbes; however, these can also be readily used by the fungal colonies on infected corn plants. This may decrease the amount present in the corn silage, and decrease its energy content if these nutrients are selectively used by the fungus. These colonies may also use fat from the plant as an energy source as evidenced by a study done by Williams et al. (1992) which found that corn infected with a fungus had less crude fat content when compared to non-infected kernels; however, the infection did not have an effect on gross energy content of the corn. This could be due to higher protein content found in infected plants, which attribute value to the gross energy value. Weiss and Wyatt (2000) found that an increase of 3% of TDN% in a high-oil corn silage led to higher 3.5% FCM (23.9 vs. 22.6 kg/d) when fed to dairy cattle. Fat also accounts for 2.25 times more energy when compared with NDF or starch, and can influence digestibility and energy content (Allen et al., 2003). Fungal pathogens may also use N for its own growth, and decrease the availability of nitrogen use for the plant Therefore, a decrease in fat, protein or sugar content due to fungal infection can have negative effects on plant nutritive value.

Management of Fungal Disease

As briefly discussed above there are many different methods of controlling fungal diseases which include hybrid selection, management practices such as crop rotation and tillage, and chemical methods of control such as fungicides. There are benefits and disadvantages to each of the above methods and all methods can be used in combinations with other methods to help mitigate disease. Hybrids are the most common method for disease control, however technology is still being developed for some disease, and hybrid does not completely eliminate the risk for disease. In some cases disease can occur with a resistant hybrid, and the effect of the resistance may be decreased or in some cases almost eliminated when insect infestation occurs (Windham el al., 1999). Crop rotation may be a helpful tool however, may not be possible in some systems, this is also true for tillage due to the aforementioned farm bill. Finally, chemical means such as seed fungicide or foliar fungicide may also be a helpful tool to be used alone or in combination with the above methods.

Fungicides may be a potential management tool to control the growth of an already present fungal infection or to help prevent a possible infection. Different types of foliar fungicide may be used depending on the goal of the producer. Two of the most common classes of foliar fungicides are triazoles and strobilurin or a combination of both (Wise and Mueller, 2011). Stobilurin fungicides may also have positive plant health effects outside of preventing, and treating fungal disease (Kohle et al., 2002). A meta-analysis by Paul et al. (2011) reported an average increase of 255 kg/ha (4.5 bu/acre) of grain yield with pyraclostrobin fungicide application. However, this article also mentions that the grain yield response is higher when disease severity was higher, and return on investment was higher when crop prices were high. One important point to make about this particular study is that, although positive results were seen, the authors concluded that the increase in corn yield did not always make up for the cost of the fungicide application. Another compilation of fungicide yield effects was completed by University of Illinois Extension and results are shown in Figure 1. This figure shows the mean yield response when a foliar fungicide was applied to corn, with disease severity less than or greater than 10%. Yield response was greater when disease severity was greater than 10%; however, the frequency of receiving an overall yield response ≥ 3 was 55%. One common recommendation for application of foliar fungicide is score disease

severity at various points in the growing season and assess the need for foliar fungicide based on disease scoring. Foliar fungicide offers a management strategy that allows producers to be flexible depending on environmental conditions, and other variables that may influence the return on investment.

Effects of Fungicide on Corn Silage Quality

Several studies have been done by the University of Wisconsin extension and University of Minnesota extension examining the potential benefits of foliar fungicide on corn silage quality and yield. One study done found foliar fungicide application on corn silage significantly increased corn silage output by 0.7 tons DM per acre compared to untreated corn silage. Fungicide use also led to a numerical increase in nutrients such as CP, and starch, while also significantly decreasing the amount of NDF and increasing its NDFD. There was also an estimated increase of 75lbs of milk/ton of silage, and increase of 2,500lbs of milk per acre of silage, this was calculated using the MILK 2006 system. The MILK 2006 system was developed by the University of Wisconsin in order to aid in determining the relative quality of a forage or feed based on energy value which is predicted from ADF, and potential intake using NDF and NDFD. These plants also showed less premature plant death as well as decreased signs of disease. A 2011 study from the University of Wisconsin extension analyzed the use of foliar fungicide at the R1 and V5 stages of crop growth and concluded that Headline AMP® when applied at the R1 stage had the highest yield for 1 of 3 counties tested, and had higher moisture in 2 of the 3 counties tested. The corn treated with Headline AMP® also had lower disease severity when compared to untreated corn silage in 1 of the 3 counties (Esker et al., 2012). Another study done in 2013 showed no significant difference in nutritive value, dry matter yield, milk per ton, or foliar disease scores at harvest for corn treated with various types of fungicide when compared with untreated corn silage. However, another study was done by the same parties in 2008 which evaluated fungicide use on two different hybrids. The fungicides evaluated were Headline®, Quilt®, and Stratego®. It was concluded that using Headline® on the DeKalb DJC57-79 lead to the highest DM yield/ acre at 10.9; however, the Pioneer P34A98 hybrid showed more NDFD overall. No significant difference for milk per ton was found; however, milk per acre was highest for the Dekalb hybrid when Headline® was applied, and for the Pioneer hybrid with no fungicide.

Recently, one study done at the University of Illinois looked at corn treated with various applications of foliar fungicide and its effect on corn silage quality and in situ digestibility. This study found that corn treated with 1 (1X), 2 (2X), or 3 (3X) applications of foliar fungicide had higher sugar content when compared with the untreated control (1.21 vs 0.72 % DM). There was also a linear treatment effect for content of sugar and fat with the concentration of fat and sugar increasing as the numbers of applications increased. There was also a decrease in the amount of fiber (ADF and NDF) in the treated silages when compared to the untreated control (27.72 vs 29.24% for ADF and 45.52 vs 47.32% for NDF). There was also a treatment linear effect for ADF with the amount of ADF decreasing as the number of treatments increased. This study also showed that corn silage treated with foliar fungicide had a higher portion of rumen degradable feed when compared with untreated corn silage (0.43 vs. 0.36%). Finally this study concluded that when corn silage treated with fungicide was fed to lactating dairy cows, the cows receiving treated silage tended to have higher fat-corrected milk (FCM) and energy-corrected milk (ECM) feed conversion when compared to control (1.65 vs 1.47 for FCM/DMI, 1.60 vs 1.43 for ECM/DMI). An economic analysis was then completed to evaluate potential benefits associated with the increase in efficiency. Interestingly, the total income from milk yield over feed cost was 7.35, 7.54, 8.31, and 7.83 dollars for CON, 1X, 2X, and 3X respectively (Table 1)

Conclusion

Fungal disease can cause significant losses in corn yield and can decrease feed quality. These diseases are very widespread in the areas of the plant they infect, the damage they cause, and the environmental conditions which they favor. As prices of feed increase producers must find ways to increase the nutritive value of forages. Foliar fungicides have been shown to decrease disease severity in corn plants and increase yields. Lactating Holstein cows fed corn silage treated with foliar fungicide had higher feed conversion, and corn silage treated with foliar fungicide had less fiber (ADF and NDF) and more sugar content than corn not treated with foliar fungicide. Increasing nutritive value of corn silage may have economic benefits to dairy farmers.

Tables and Figures on next page

Figure 1. Disease severity vs yield response

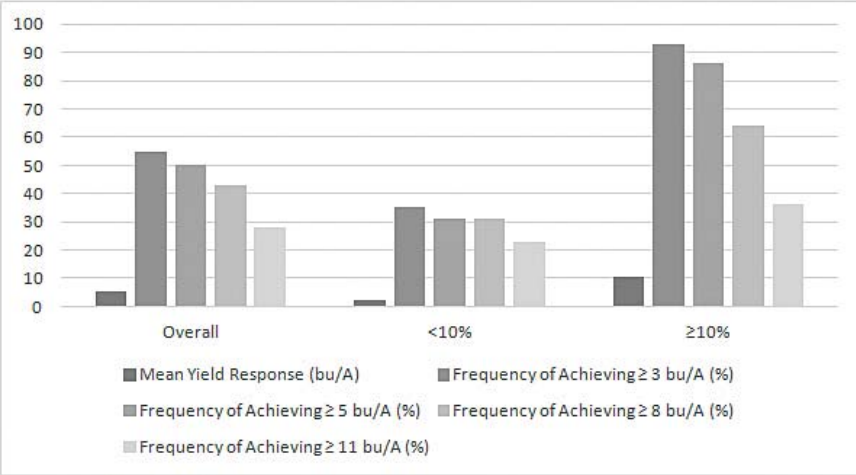

Table 1. Income over feed cost associated with feeding cows corn silage treated with no application of foliar fungicide (CON), one application of foliar fungicide (1X), two applications of foliar fungicide (2X), or three applications of foliar fungicide (3X).

	$/lb DM	Feed Cost[1]	Milk Income[2]	IOFC[3]
CON	$0.121	$6.30	$13.65	$7.35
1X	$0.121	$6.11	$13.66	$7.55
2X	$0.122	$5.23	$13.54	$8.31
3x	$0.122	$5.79	$13.62	$7.83

[1] Daily dry matter intake X $/lb DM.
[2] Daily milk production X $0.18/pound of milk.
[3] Income over feed cost (Milk Income – Feed Cost).

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Serotonin and Calcium Homeostasis During the Transition Period

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Summary

The transition from pregnancy to lactation puts significant demands on maternal energy and calcium reserves. While most lactating mammals are able to effectively manage these metabolic adaptations, the lactating dairy cow is acutely susceptible to transition-related disorders due to their milk production demands. Given that calcium is the major mineral component of milk, periparturient hypocalcemia is one of the most common disorders that affects dairy cows, particularly at the time of parturition. Hypocalcemia is characterized by a range of clinical symptoms that have been correlated with production losses, as well as detrimental impacts on animal health and welfare. In addition, cows that develop hypocalcemia are more susceptible to a host of other diseases and metabolic challenges, emphasizing the need for effective prevention strategies. Different feeding tactics, including manipulating the dietary cation-anion difference and administering low calcium diets, are commonly used preventative strategies. Yet the incidence of hypocalcemia in the subclinical form is still as high as 25 to 30% in the United States dairy cow population, with 5-10% incidence of clinical hypocalcemia. Additionally, while there are various effective oral and intravenous treatments in place, they are administered only after the cow has become noticeably ill, at which point there is already significant metabolic damage. Serotonin has been implicated as a potential therapeutic target in the prevention of hypocalcemia. Our research in rodents has shown that serotonin is necessary for the production of mammary parathyroid hormone related protein (PTHrP), which is critical for the mobilization of bone tissue and subsequent restoration of maternal calcium stores during lactation. We have shown that circulating serotonin concentrations are positively correlated with ionized calcium in serum on the first day of lactation in dairy cattle. Administration of serotonin's immediate precursor through feeding, injection, and infusion to various species, including mice, rats, and dairy cows, has been shown to increase circulating serotonin concentrations, while having positive effects on other components of maternal metabolism. Finally, preliminary data from a recently completed study suggests that manipulation of the serotonergic axis pre-calving may positively affect post-calving calcium dynamics. Combined, these data suggest a potential mechanism by which serotonin acts on the mammary gland through autocrine / paracrine dynamics to maintain circulating maternal calcium stores. Elucidation of this mechanism and further research into serotonin's potential as a therapeutic target could contribute significantly to the arsenal of prevention strategies against hypocalcemia in early lactation dairy cows.

Hypocalemia and current prevention/treatment strategies

The transition period in dairy cows is defined as three weeks pre-calving and three weeks post-calving, during which time maternal metabolism changes rapidly. On the day of parturition, a dairy cow produces 10 liters or more of colostrum containing at least 23 grams of calcium (Ca) (Goff, 2008) and by later lactation as much as 50 grams per day of calcium are lost into milk (DeGaris and Lean, 2009). Typically, circulating Ca concentrations are tightly regulated between 2.0-2.5 mM in dairy cows. At the onset of lactation, however, increased demand by the mammary gland for milk synthesis often leads to depletion of circulating maternal Ca stores, provoking periparturient hypocalcemia (milk fever). Clinical hypocalcemia (defined as 1.4 mM or less of circulating calcium) has an incidence of between 5-10% in the United States dairy cow population, while the subclinical form (1.4-2.0 mM) has a much higher incidence of 25-50% (Goff 2008; Reinhardt et al., 2011). Additionally, several recent studies suggest that the threshold for subclinical hypocalcemia is underestimated based on poor reproductive outcomes, increased displaced abomasums, and higher incidence of ketosis (Chapinal et al., 2012; Martinez et al., 2012). Older cows are at a much higher risk for developing hypocalcemia due to their decreased ability to mobilize calcium from bone; in fact the risk for milk fever increases by 9% with each lactation (Lean et al., 2006). In terms of breed, Jersey cattle are the most susceptible, likely due to the increased calcium content in their milk and higher milk production per unit of body weight

(Oetzel, 1988). Hypocalcemia is a particularly difficult disease to manage because of its manifestation: the early symptoms in stage I of the disease are often short-lived and hard to detect. By the time the cow has moved on to stage II, characterized by decreased body temperature, lack of coordination when walking, and muscle tremors, significant intervention in the form of intravenous calcium administration is often required, resulting in an estimated 14% production loss (Adams et al., 1996; Guard, 1996). The economic impact of hypocalcemia is enormous: considering the 9.2 million cows in the U.S. dairy industry with a cost of $125 and $300 per case of subclinical and clinical hypocalcemia, respectively, given treatments and lost milk yield, there is an estimated cost of $900,000,000 annually. Translating these numbers to the 1.27 million cows in Wisconsin, the annual average cost of hypocalcemia to a WI farmer is approximately $12,000 (Oetzel, 2013). While these estimates are purely economic, there are also animal welfare concerns, given that the cow may be unable to stand or walk until identified by the farmer. Potentially more troubling than the physical and economic ramifications of hypocalcemia is the fact that the subclinical form is nearly impossible to identify in a production setting, as cows do not display obvious clinical symptoms (Oetzel and Miller, 2012).

Hypocalcemia can be considered a "gateway disease", because its incidence is positively correlated with a variety of other health concerns (Goff, 2008; Reinhardt et al., 2011). Calcium is required for both smooth muscle contraction and proper immune function, among other essential functions. In the dairy cow, the contraction of smooth muscle is responsible for rumen and gut motility, and both uterine and teat sphincter contraction. Dysregulation of these systems leads to a host of common transition disorders, including ketosis and fatty liver, displaced abomasum, dystocia, metritis, and mastitis, in addition to increased susceptibility to infectious disease (Kimura et al., 2006; Goff, 2008; Martín-Tereso and Verstegen, 2011; Chapinal et al., 2011). Furthermore, subclinical hypocalcemia specifically has been linked to greater risk of fever and metritis, as well as decreased pregnancy rates and longer intervals to pregnancy (Martinez et al., 2012). The consequences of hypocalcemia, therefore, must be considered beyond the immediate treatment of the disease and into the cow's entire lactation and subsequent lactations.

While there are prevention strategies currently utilized in the United States, they are often difficult to implement effectively. The primary target for prevention is through manipulation of the diet at the end of the dry period. The two major strategies are administration of low calcium diets (LCD) and adjustment of the dietary cation-anion difference (DCAD). Feeding of a LCD works by stimulating a transient hypocalcemia, inducing calcium resorption from the bone and increased absorption from the small intestine, in order to increase available calcium reserves (Bethard and Smith, 1998). For the prevention of milk fever, a diet of 8-10 grams of calcium per day has been shown to have the greatest effect, but LCD with this little calcium are difficult to achieve mainly because the primary forage of alfalfa is quite high in calcium (Horst et al., 1997). Conversely, the strategy of DCAD manipulation is to increase availability of absorbable dietary anions and decrease the number of absorbable dietary cations through use of dietary anionic salts (Goff, 2008). While there is no doubt that this strategy aids in the prevention of milk fever (Charbonneau et al. 2006), there are two major concerns: the first is that the salts decrease palatability, reducing feed intake and predisposing the cow to other energy-related transition disorders. The second issue is that anionic salts are quite expensive, adding additional cost onto an already costly period in the cow's life (Bethard and Smith, 1998). Additionally, the low DCAD diet is typically implemented during the 3 weeks immediately pre-partum, creating the need to have two separate groups of cows in the dry pen. Further work has been done with respect to vitamin D3 or oral calcium/metabolite administration, but these results have been shown to be largely impractical and largely dependent on timing of administration (Martín-Tereso and Verstegen, 2011). Improvement of these prevention strategies depends on a solid understanding of the physiological mechanisms that govern calcium homeostasis in the dairy cow. Our lab has shown that manipulation of a key regulator of calcium dynamics, serotonin, may have significant impact as a novel therapeutic target in the prevention of hypocalcemia.

Early lactation calcium homeostasis

Proper maintenance of circulating calcium concentrations is essential to a successful lactation. The main source of calcium in lactating dairy cows is through bone resorption because dietary calcium is insufficient to support mineral demand by the mammary gland. During the first 30 days in milk, a dairy cow will mobilize between 9 and 13% of her bone mass (Goff, 2014) in an attempt to maintain calcium homeostasis. Therefore, despite decreased active transport in the kidney and increased passive transport from the intestine, resorption of bone tissue is the main mechanism for maintaining calcium homeostasis in the lactating dairy cow. The process of bone resorption during lactation is regulated largely by parathyroid hormone related protein (PTHrP), which is detectable

in the circulation only during lactation and certain metastatic epithelial cancers that are osteolytic in nature, and can also be detected in milk (Fiaschi-Taesch and Stewart, 2003; Stewart, 2005; Kovacs, 2011; Wysolmerski, 2012). In rodent models, mammary-specific deletion of PTHrP results in decreased concentrations of bone turnover markers (VanHouten et al., 2003) and the PTHrP responsible for bone turnover during lactation is derived from the mammary gland (VanHouten, 2005; Wysolmerski, 2010). PTHrP signals through the same G-protein coupled receptor as parathyroid hormone (PTH) (Wysolmerski, 2012). PTHrP communication with the skeletal system is essential for bone resorption during lactation.

The skeletal system maintains its structural and functional roles via communication between two cell types, osteoblasts (OB), which are responsible for bone formation, and osteoclasts (OC), which are responsible for bone resorption and therefore, the release of calcium. PTHrP signals through G-protein coupled receptor PTH1R on OB to decrease OB cell proliferation and up-regulate genes responsible for OC differentiation. This signal is critical for the stimulation of bone resorption of calcium. In this way, mammary-derived PTHrP is responsible for the induction of signaling cascades at the site of the bone that drive mineral dissolution and calcium release, restoring maternal calcium concentrations. Understanding of this mechanism is critical to the prevention and treatment of calcium-related disorders, particularly PTHrP. Yet despite this fact, very little research has been performed investigating the role of PTHrP in maintaining calcium homeostasis in lactating dairy cows (Onda et al., 2006; Filipović et al., 2008). Our lab has pioneered the correlation between PTHrP and serotonin in lactating dairy cows.

Serotonin is a homeostatic regulator of lactation

Serotonin is an evolutionarily conserved monoamine that has a multitude of functions throughout the body, including acting as the homeostatic regulator of lactation (Matsuda et al., 2004; Stull et al., 2007; Hernandez et al., 2012). Serotonin exerts its actions physiologically by signaling through approximately 15 different receptors throughout the body (Hannon et al., 2008). It is synthesized in a two-step pathway from L-tryptophan, which is converted to 5-hydroxy-L-tryptophan (5-HTP) by the rate-limiting enzyme tryptophan hydroxylase 1 (TPH1) in non-neuronal tissues, and TPH2 in neuronal tissues. Aromatic amino acid decarboxylase then converts 5-HTP to 5-hydroxytryptamine (5-HT), also known as serotonin (Wang et al., 2002). There have been five 5-HT receptor subtypes identified in bovine mammary epithelial cells including 5-HTR1B, 5-HTR2A, 5-HTR2B, 5-HTR4, and 5-HTR7, and 5-HTR4 was also detected in the myoepithelium (Hernandez et al., 2009; Collier et al., 2012). Mutations of the 5-HTR1B subtype have shown to increase milk yield in cattle (Zhang et al., 2008) and very recently it was shown that dams lacking the 5-HT7 receptor are insufficient in the ability to sustain their pups, have malformed mammary glands, and an inability to transition from lactation to involution (Pai et al., 2015). Serotonin has also been shown to regulate milk protein gene expression, as well as the disassembly of tight junctions that occurs during mammary gland involution (Stull et al., 2007; Hernandez et al., 2008; Pai and Horseman, 2008). Our lab has shown a direct association with serotonin and calcium homeostasis in that mice deficient for the rate-limiting enzyme TPH1. These dams had decreased gene and protein expression of key calcium transporters including calcium sensing receptor (CaSR), plasma membrane Ca2+ ATPase (PMCA2), and calcium release-activated calcium channel protein 1 (ORAI1). These effects could be reversed by the administration of 5-HTP, which bypasses the rate-limiting step for serotonin synthesis (Laporta et al., 2014a).

In addition to autocrine/paracrine action at the mammary gland, serotonin contributes to calcium homeostasis by regulating bone mass (Bliziotes et al., 2001; Yadav et al., 2008; Modder et al., 2010; Chabbi-Achengli et al., 2012). Our research focus has been on the role of serotonin on production of PTHrP by the mammary gland, and subsequent effects on bone tissue. We have demonstrated that mice deficient in the rate-limiting enzyme TPH1 have decreased circulating and mammary PTHrP concentrations along with reduced femoral OC during lactation (Hernandez et al., 2012; Laporta et al., 2014a). This indicates that dams deficient in non-neuronal serotonin during lactation have an inability to efficiently mobilize calcium from bone tissue. Therefore, these dams would have an impaired ability to maintain circulating calcium concentrations. Injecting the immediate precursor to serotonin, 5-HTP, restored mammary PTHrP synthesis and femur OC populations. Additionally, we have shown that serotonin's induction of PTHrP involves signaling through the 5-HT2B receptor (Horseman and Hernandez, 2014; Laporta et al., 2014a) and that serotonin acts via the 5-HT2B receptor subtype to modulate mammary Ca transport (Laporta et al., 2014a). In support of this mechanism, feeding 5-HTP to rats during the periparturient period increased post-partum maternal serotonin and calcium concentrations and maternal bone turnover (Laporta et al., 2013a) with marked increases in expression of genes related to calcium resorption from bone in rat femurs (Laporta et al., 2013a). Our work in rodent models demonstrated that serotonin is a key regulator of calcium homeostasis at the site of the mammary gland and the bone. Further work in dairy cows has only

supported the role of serotonin in positively regulating calcium homeostasis, suggesting its promise as a therapeutic target in the prevention of hypocalcemia.

Serotonin and calcium homeostasis in the dairy cow

We conducted several studies in rodent models to better understand how serotonin interacts with calcium homeostasis during lactation. This provided us with necessary information to design experiments in dairy cows. In order to discern if our research was applicable in the bovine, we initially conducted a small study in 42 multiparous Holstein dairy cows and observed that serotonin and PTHrP concentrations on d 1 of lactation were positively correlated with total calcium concentrations (Laporta et al., 2013b). In a second study conducted at two commercial dairy farms in South-central Wisconsin, we sought to determine if serotonin concentrations were dynamic over the course of a lactation, with a heavy focus on the transition period. Additionally, we sought to establish normal circulating concentrations of serotonin in dairy cows, as this was previously unknown. We observed that serotonin concentrations are dynamic over the course of a lactation, and decrease around the time of calving (d 0-2 lactation), rebounding by approximately ten days into lactation. Once again, there was a positive correlation between serotonin and calcium on the days immediately following calving (Moore et al., 2015). The overall average serotonin concentration in dairy cows is approximately 1700 ng/ml. However, serotonin concentrations fluctuate dependent on stage of lactation. These results combined with our rodent data support our hypothesis that serotonin and PTHrP are important in the regulation of calcium homeostasis in dairy cows.

After establishing that serotonin was indeed relevant to calcium status in a dairy cow, we wanted to determine if administration of the serotonin precursor 5-HTP would impact calcium status in a lactating dairy cow, and what the optimum dose would be. Elucidation of these questions would support the possibility that manipulation of the serotonin-PTHrP axis could prove to be useful for the modulation of hypocalcemia. To this end, we performed a preliminary experiment in which we infused 5-HTP intravenously (IV) for one hour in late-lactation, non-pregnant dairy cows (333 DIM) at varying doses (0, 0.5, 1.0, or 1.5 mg/kg) to determine an optimum dose of 5-HTP necessary to produce significant changes in Ca. All three doses of 5-HTP significantly increased circulating serotonin concentrations (Laporta et al., 2015 under review) to a similar extent in the two hours after dosing, with concentrations returning to baseline values observed in the saline controls by two hours after infusion. In addition to serotonin concentrations, we measured circulating total calcium concentrations following the same time course post-infusion. While initially counter-intuitive, our data demonstrated that total calcium concentrations decreased in immediate response to 5-HTP treatments (Laporta et al., under review). In order to determine where the circulating calcium was being used in the body after 5-HTP infusion, we measured urine calcium concentrations prior to the start of infusion and two hours after the end of the infusion. Our results showed that there was a decrease in urine calcium output with higher doses of 5-HTP treatment. This suggests that calcium is not being lost into the urine. Therefore, we measured total calcium concentrations in the milk during the infusion periods and observed that the highest dose of 5-HTP increased total milk calcium concentrations. Given this data, we believe that serotonin causes transient hypocalcemia by increasing calcium transport into the mammary gland in order to stimulate PTHrP-induced bone resorption necessary to raise systemic calcium.

Upon determination of an optimal dose of 5-HTP to manipulate the serotonin-PTHrP axis, we wanted to determine if administration of 5-HTP pre-calving would improve post-calving calcium concentrations. Given that we chose 1.0 mg/kg as the optimal dose of 5-HTP, we treated multiparous Holstein cows with daily IV infusions of 1.0 mg/kg of 5-HTP beginning 7 d before the estimated calving date until calving. Our preliminary data demonstrates that IV infusions of 5-HTP pre-calving tended to increase ($P = 0.07$) post-calving total calcium concentrations compared to saline treated controls. Additionally, mRNA expression of the CaSR ($P = 0.03$) and PMCA2 ($P = 0.018$), two key regulators of mammary gland calcium transport, were significantly increased on both d 1 and d 7 of lactation of cows treated with 1.0 mg/kg 5-HTP pre-calving. This preliminary data in dairy cows appears to mirror results observed in our rodent experiments. Therefore, we believe that 5-HTP treatment pre-calving could be used as a preventative measure for both subclinical and clinical hypocalcemia post-calving. Further work in this area will involve investigating 5-HTP absorption dynamics in the rumen, potentially aiming towards rumen-protecting the serotonin precursor and administering it in the feed.

In conclusion, we propose the following model for serotonin's action to modulate calcium homeostasis during lactation. Collectively, our data in rodents and now in dairy cows supports the hypothesis that serotonin increases calcium transport into the mammary epithelial cells, transiently decreasing maternal circulating calcium concentrations. Decreased calcium levels in serum are detected by the mammary epithelial CaSR, which then signals for increased production of PTHrP from mammary epithelial cells. Increased PTHrP production by the mammary epithelium dur-

ing lactation allows for increased calcium mobilization from bone tissue, allowing for the restoration of maternal calcium homeostasis and thereby alleviating hypocalcemia. Delineation of this pathway and the associated mechanisms in the dairy cow has the potential to result in a novel therapeutic intervention for the prevention of hypocalcemia and its associated disorders in the U.S. dairy cow population.

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Euthanasia of Cattle: Are You Doing it Correctly?

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Methods recognized as appropriate for euthanasia of cattle are: barbiturates and barbituric acid derivatives, gunshot and captive bolt. Penetrating captive bolt is required in adult cattle. In contrast, penetrating and non-penetrating captive bolt are suitable for euthanasia of calves. Whether used in mature animals or in calves captive bolt requires an "Adjunctive" method to assure death. All are described in greater detail below.

Injectable Anesthetics

Barbiturates and barbituric acid derivatives—Barbiturates are preferred by some because of their rapid action and ability to induce a smooth transition from consciousness to unconsciousness and death. Drawbacks to the use of these agents for euthanasia include: cost, the need for restraint to deliver the drug, necessity to maintain a careful accounting of amounts used, regulatory requirements specifying that these agents be administered only by a veterinarian and residues that limit carcass disposal options.

Research and clinical observation indicates that barbiturates readily cross the placenta resulting in fetal depression; however death of the dam precedes death of the fetus by as much as 20-25 minutes. Fetal welfare is preserved by the fact that while in utero, the fetus is maintained in sleep-like state of unconscious. On the other hand, if removed from the uterus prior to 20-25 minutes beyond death of the dam, the fetus may regain consciousness. In cases involving euthanasia, any fetus removed from uterus prior to the amount of time required to cause death should be carefully observed for evidence of life and immediately euthanized if there is any doubt.

Firearms

"Free Bullet" from Gunshot A 2008 study by Fulwider found that gunshot is the most common method used for on-farm euthanasia of cattle. Death by means of a "free bullet" is caused by massive destruction of brain tissue. Despite its popularity and effectiveness for the purpose of euthanasia, those who are less familiar with firearms often find gunshot violent and objectionable. However, as stated in a previous edition of the Guidelines:

"Properly applied, "euthanasia by either gunshot or penetrating captive bolt, causes less fear and anxiety and induces a more rapid, painless, and humane death than can be achieved by most other methods."

Recommendations on Firearms for Euthanasia

Handguns Handguns or pistols are short-barreled firearms that may be fired with one hand. For the purposes of euthanasia, handguns are limited to close-range shooting (within 1 to 2 feet or 30 to 60 cm) of the intended target. Calibers ranging from .32 to .45 are recommended for euthanasia of cattle. Solid-point lead bullets are recommended over hollow points because they are more likely traverse the skull. Hollow point bullets are designed to expand and fragment on impact with their targets which reduces the depth of penetration. The .22 caliber handgun is not recommended for routine euthanasia of adult cattle regardless of the type of bullet used, because of the inability to consistently achieve desirable muzzle energies with standard commercial loads.

Rifles A rifle is a long barreled firearm that is usually fired from the shoulder. Unlike the barrel of a shotgun which has a smooth bore for shot shells, the bore of a rifle barrel contains a series of helical grooves (called rifling) that cause the bullet to spin as it travels through the barrel. Rifling imparts stability to the bullet and improves accuracy. For this reason, rifles are the preferred firearm for euthanasia when it is necessary to shoot from a distance. Rifles are capable of delivering bullets at much higher muzzle velocities and energies and are therefore not the ideal choice for euthanasia of animals in indoor or short range conditions. General recommendations on rifle selection for use in euthanasia of cattle include; .22 magnum, .223, .243, .270 and .308 and others.

Shotguns Shotguns loaded with buckshot or slugs (solid lead projectiles specifically designed for shot-

guns) are appropriate from a distance of 1 to 2 yards (.9 to 1.8 meters). Although all shotguns are lethal at close range, the preferred gauges for euthanasia of mature cattle are 20, 16, or 12. Birdshot begins to disperse as it leaves the end of the gun barrel; however, if the operator stays within short range of the intended anatomic site, the birdshot will strike the skull as a compact bolus or mass of BBs with ballistic characteristics on impact and entry that are similar to a solid lead bullet. At close range penetration of the skull is assured with massive destruction of brain tissue from the dispersion of birdshot into the brain that results in immediate loss of consciousness and rapid death.

One advantage of euthanasia using a shotgun is that within close range and when properly directed, birdshot has sufficient energy to penetrate the skull, but is unlikely to exit the skull. In the case of a free bullet or shotgun slug there is always the possibility of the bullet or slug exiting the skull creating an injury risk for the operator or by-standers. For safety reasons it is important that the muzzle of a shotgun (or any other firearm) never be held directly against the animal's head. Discharge of the firearm results in the development of enormous pressure within the barrel that can result in explosion of the barrel and potential for injury of the operator and by-standers if the muzzle end is obstructed or blocked.

Captive Bolt

Captive bolt is a popular method of euthanasia for cattle in field situations. Unlike euthanasia with firearms, once the animal is rendered unconscious, an adjunctive method to insure death must be applied. Styles of penetrating captive bolt include an in-line (cylindrical) and pistol grip (resembling a handgun) versions. Pneumatic captive bolt guns (air powered) are limited to use in slaughter plant environments. Models using gunpowder charges are more often used in farm environments. Depending upon model, the bolt may automatically retract or require manual placement back into the barrel through the muzzle.

Accurate placement of the captive bolt over the ideal anatomical site, energy (i.e. bolt velocity) and depth of penetration of the bolt determine effectiveness of the device to cause a loss of consciousness and death. Bolt velocity is dependent on maintenance, in particular cleaning and proper storage of the cartridge charges. Captive bolt guns should be cleaned regularly using the same or similar solvents used in the cleaning of firearms. Powder charges for captive bolt should be stored in air tight containers to prevent damage from the absorption of moisture in hot and humid conditions. Non-penetrating captive bolt is not recommended for euthanasia of adult cattle. On the other hand, non-penetrating captive bolt is appropriate for euthanasia of calves when followed by the use of an adjunctive (secondary step) method to assure death.

In general, captive bolt guns, whether penetrating or non-penetrating, induce immediate loss of consciousness, but death is not always assured with the use of this device alone. A recent study by Gilliam et al. found that death was likely to occur approximately 90% of the time without the use of a secondary adjunctive step. An adjunctive method such as exsanguination, pithing or the intravenous injection of a saturated solution of potassium chloride (KCl) is recommended to ensure death when penetrating captive bolt is used. A newer version of penetrating captive bolt has emerged in recent years. This device is equipped with an extended bolt with sufficient length and cartridge power to increase damage to the brain including the brainstem.

Unlike techniques described for gunshot, the animal must be restrained for accurate placement of the captive bolt. And, unlike use of a firearm, proper use of the captive bolt requires that the muzzle of the device be held firmly against the animal's head. Once the animal is restrained, discharge of the captive bolt should occur with little or no delay so that animal distress is minimized. Adjunctive methods should be implemented as soon as the animal is rendered unconscious to avoid a possible return to sensibility. Thus, when conducting euthanasia by captive bolt, pre-planning and preparation is necessary to achieve the desired results.

Visual Indicators of Unconsciousness

Visual indicators that an animal has been rendered unconscious from captive bolt or gunshot include the following: immediate collapse; brief tetanic spasms followed by uncoordinated hind limb movements; immediate and sustained cessation of rhythmic breathing; lack of coordinated attempts to rise; absence of vocalization; glazed or glassy appearance to the eyes; centralized eye position with a dilated pupil; and absence of eye reflexes. Nervous system control of the blink or corneal reflex is located in the brain stem; therefore, the presence of a corneal reflex is highly suggestive that an animal is still conscious.

Anatomical Landmarks for Euthanasia of Cattle

The objective in euthanasia is to cause sufficient damage to the brain to result in immediate loss of consciousness and death. Accomplishment of this objective requires the accurate delivery of a bullet or captive bolt at an anatomical site that is most likely

to cause damage to the brainstem. Several methods may be used to determine the proper anatomical site (See Diagram) for conducting euthanasia with either a firearm or captive bolt. The method published in the 2013 Euthanasia Guidelines recommends that the point of entry for a projectile be at (or slightly above) the intersection of two imaginary lines, each drawn from the outside corner (lateral canthus) of the eye to the center of the base of the opposite horn. Alternatives to this recommendation include the following: 1) approximately 3 inches (7.6 cm) anterior to the poll in a mature Holstein cow or approximately 2 ½ inches (6.35 cm) for a feedlot steer of 800 to 1200 lb. (365 kg to 545 kg), 2) in the center of the forehead on a line drawn laterally from ear to ear, and 3) half-way between 2 lines drawn laterally; one across the poll and the other from lateral canthus to lateral canthus of the eyes.

Unacceptable Methods

The methods of euthanasia deemed unacceptable include: 1) manually applied blunt force trauma (as with a large hammer), 2) injection of chemical agents or other substances not specifically designed or labeled for euthanasia (i.e. disinfectants, cleaning solutions, etc.), 3) air injection into the vein, 4) electrocution as with a 120 volt electrical cord, 5) drowning, 6) exsanguination of conscious animals, and 7) deep tranquilization as with xylazine or other alpha-2 agonist followed by potassium chloride or magnesium sulfate. While some have been forced out of desperation to resort to one or more of these methods, readers are strongly advised against their use. Several of these methods are known to result in a less than humane death and for others the level of pain or distress associated with these methods is unknown. For example, use of xylazine to create a deep

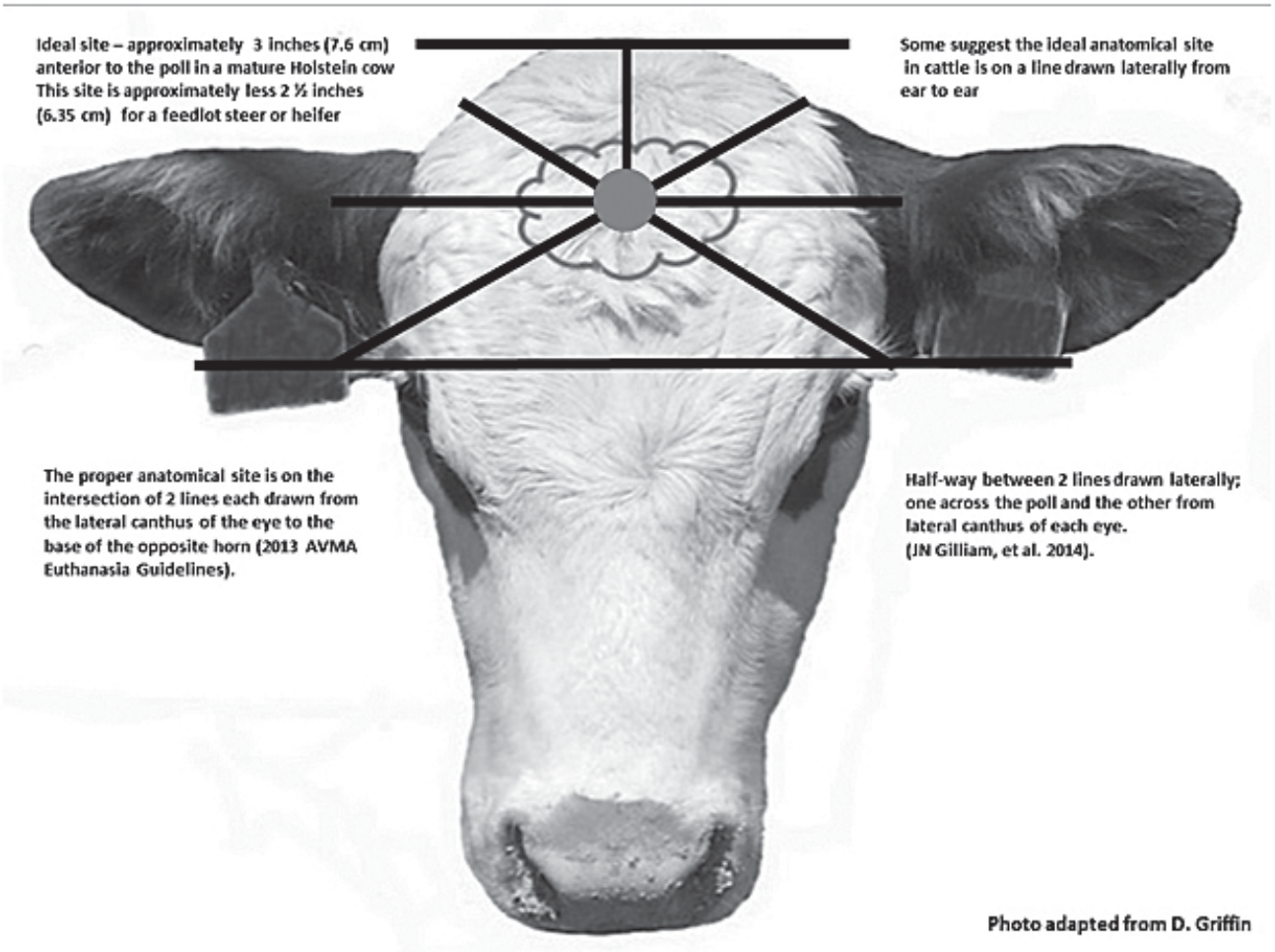

Figure 1. The above photo and captions identify several ways to determine the proper anatomical site for conducting euthanasia procedures in cattle.

state of tranquilization followed by the rapid administration of KCl is used by some veterinarians. The position of the AVMA is as that stated in Goodman and Gilman's Pharmacological Basis of Therapeutics, 11th Edition: "Although large doses of alpha-2 agonists can produce a state resembling general anesthesia, they are recognized as being unreliable for that purpose." Therefore, until such time as we have better information on this method in terms of its ability to cause a humane death, it is best to utilize alternate techniques.

Confirmation of Death

Regardless of method used for conducting euthanasia procedures it is important to confirm death. It is sometimes more easily said than done. However, the most reliable criteria include lack of pulse, breathing, corneal reflex and response to firm toe pinch, inability to hear respiratory sounds and heart beat by use of a stethoscope, graying of the mucous membranes, and rigor mortis. None of these signs alone, with exception of rigor mortis, confirms death.

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Ash-free fiber (aNDFom): Why the change and what are the consequences?

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Introduction

- For ruminants, fiber in plants should contain the cellulose, hemicellulose and lignin in cell walls
  - Pectin in cell walls is easily fermented
  - Digestible cellulose and hemicellulose are organic structural carbohydrates that contribute energy to ruminants via VFAs
  - Neutral detergent fiber is the best measure of total insoluble fiber for ruminants

Introduction

- Fiber is a nutritional entity
  - The indigestible or slowly digesting fraction of a feed that occupies space in the gut
  - Differs with the type of animal
- Fiber is not a chemical entity
  - We attempt to use chemical extraction to measure the nutritional entity (fiber)
  - Analytical methods for fiber are empirical
    - The method defines the fiber fraction measured
    - There are compromises to make methods effective and efficient

Introduction

- Depending on the method used, NDF contains some contaminants
  - aNDF (AOAC Official Method) used amylase and sulfite and was isolated without ashing
    - Contaminated with less CP and some ash
    - Rapid method because there was no ashing step
  - aNDFom (AOAC Official Method) used amylase and sulfite, and is ash-free
    - Contaminated with some CP
    - Only the organic matter in fiber contributes energy to the animal

Introduction

- For ruminants, fiber in plants should contain the cellulose, hemicellulose and lignin in cell walls
  - Pectin in cell walls is easily fermented
  - Digestible cellulose and hemicellulose are organic structural carbohydrates that contribute energy to ruminants via VFAs
  - Neutral detergent fiber is the best measure of total insoluble fiber for ruminants

Introduction

- Depending on the method used, NDF contains some contaminants
  - aNDFom (AOAC Official Method) used amylase and sulfite, and is ash-free
    - aNDF and aNDFom will result in less aNDICP, so separate NIR calibrations are needed
    - Ashing crucibles typically results in loss of weight from empty crucibles, so a blank correction after ashing is needed to accurately measure aNDFom

Introduction

- Methods and their acronyms are important
  - NDF
  - NDR
  - aNDF
  - aNDFom
  - They are all a measure of fiber, but not the same measure of fiber!
- Everyone has to be more accurate in fiber measurement, reporting and usage
  - My patience is wearing thin!

Why worry about NDF contamination?
Crude Protein Contamination

Feed	CP	NDR	NDICP (w/o sulfite)	aNDF	NDICP (w/ sulfite)
Grasses	8.69	66.82	2.32	65.02	1.73
Corn silage	7.65	36.08	0.72	34.74	0.50
Legumes	17.32	40.32	2.71	38.91	1.65

CP contamination of forages is small especially for the aNDF method, which uses sulfite

Introduction

- Methods and their acronyms are important
  - NDF
  - NDR
  - aNDF
  - aNDFom
  - They are all a measure of fiber, but not the same measure of fiber!
- Everyone has to be more accurate in fiber measurement, reporting and usage
  - My patience is wearing thin!

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Why worry about NDF contamination?
CP versus Ash Contamination

Feed	NDICP (w/ sulfite)	aNDF (w/ sulfite)	aNDFom (w/ sulfite)	aNDF ash (w/ sulfite)
Grasses	1.73	55.60	53.64	1.96
Corn silage	0.50	36.31	35.36	0.95
Legumes	1.65	40.45	38.40	2.05

For the aNDF method, the correction for ash contamination is typically greater than that for CP contamination

If we need to "correct" aNDF for NDICP, then we also need "correct" for aNDF ash to more accurately measure fiber for models, summative equations, and the calculation of NFC

Why worry about NDF contamination?
Crude Protein Contamination

Feed	CP	NDR	NDICP (w/o sulfite)	aNDF	NDICP (w/ sulfite)
Fish meal	53.94	30.44	10.43	6.27	1.29
Brewer's grains	30.44	52.32	12.16	40.87	4.65
Distiller's grains	25.57	38.58	11.01	27.89	3.68
Soybean meal	46.15	18.48	3.63	12.44	0.48
Sunflower meal	31.86	38.52	2.38	35.20	1.14
Canola meal	40.83	23.73	4.33	20.88	2.09
Citrus pulp	6.53	21.27	2.06	20.20	1.59

There can be a huge difference in NDF and NDICP between the NDR and aNDF methods
CANNOT use NDICP measured by NDR to adjust aNDF for protein contamination

Why worry about NDF contamination?
CP versus Ash Contamination

Feed	NDICP (w/ sulfite)	aNDF (w/ sulfite)	aNDFom (w/ sulfite)	aNDF ash (w/ sulfite)
Grasses	1.73	55.60	53.64	1.96
Corn silage	0.50	36.31	35.36	0.95
Legumes	1.65	40.45	38.40	2.05

For routine use, the correction for NDFash is more important that correction for NDICP because fiber-containing feeds are typically fed to provide energy, which can only be obtained from organic matter (OM)

Correct measurement and partitioning of OM is crucial, and soil contamination of aNDF can be large

Why be concerned about ash contamination of aNDF?

- Conceptually, OM should be the basis for energy evaluation of feeds
  - Only OM can be used to generate energy
  - Europeans use digestible OM (dOM or "d value") to measure the energy potential of feeds
  - US used TDN, which was also ash-free
    - TDN = dCP + dCF + dNFE + 2.25*dEE
  - Digestible DM (dDM) is correlated with dOM but is affected by digestible ash

Why be concerned about ash contamination of aNDF?

- Results in over estimation of fiber in the diet
  - Crucial when low fiber dairy rations are fed
  - Example, dairy farmer fed 30% of ration DM as alfalfa silage containing 36% aNDF, but experienced milk fat depression.
    - The aNDFom of the alfalfa was only 28%, so the peNDF of the ration was below minimum requirements

What is Ash Contamination of aNDF

- Ash intrinsic to fiber
  - Ash that is a part of fiber (silica) or ash that is not extracted by ND
  - Typically 15 to 25% of total ash in forages
    - Corn silage = 3-5% ash
    - Grasses = 6-8% ash
    - Legumes = 8-10% ash
- Ash from soil contamination 0-10% of DM
  - Raking, splashing during rains, flooding
  - Gravel pads for silage or hays
  - Soil is insoluble in ND

Why be concerned about ash contamination of aNDF?

- Results in over estimation of fiber in the diet
  - Can cause a significant under estimation of NFC in feeds
    - NFC = 100 – ash – CP – Fat – aNDF
    - Example (alfalfa with soil contamination):
      - NFC = 100 – 17 – 20 – 3 – 36 = 24
      - NFC = 100 – 17 – 20 – 3 – 28 = 32
    - The error in NFC creates an under estimation of TDN or NE when summative equations are used because true digestibility of NFC is 0.98, which is more that the digestibility of aNDF

Why be concerned about ash contamination of aNDF?

- Need to detect soil contamination and accurately measure aNDFom
- Need to be more accurate in the calculation of NFC
- Need to accurately calculate usable energy in feeds (TDN and NE)

Why be concerned about ash contamination of aNDF?

- TDN_{1X} = tdCP + tdFA*2.25 + tdNDF + tdNFC – 7 (2001 Nutrient Requirements of Dairy Cattle)
  - tdCP(for) = CP X exp(-1.2 X ADICP/CP)
  - tdCP(conc) = CP X (1 – 0.4 X ADICP/CP)
  - tdFA = (EE – 1) X 1.00
  - tdNDF = (NDF – NDICP – Lignin) X .75 X [1 – (Lignin/(NDF – NDICP))$^{0.667}$]
  - OR tdNDF = IVNDFomD*aNDFom
    - If aNDFom is measured then IVNDFomD must also be measured
    - Lignin equation is inappropriate for aNDFom

Why be concerned about ash contamination of aNDF?

- Over estimation of fiber causes inaccurate estimates of TDN and NEL

Ash	NDR	aNDF	aNDFom	aNDFash	NDICP	aNDICP	NFC	IVNDFD	IVNDFomD	TDN
10.0	40.0				3.0		31.0	0.377		58.9
10.0		38.6				2.0	31.4	0.391		59.3
10.0			36.6	2.0		2.0	33.4	0.412		61.2
15.0	45.0				3.0		21.0		0.335	49.1
15.0		43.6				2.0	21.4		0.346	49.5
15.0			36.6	7.0		2.0	28.4		0.412	56.3

For normal samples, using aNDFom increases NFC and TDN slightly
For soil contaminated samples, not using aNDFom reduces NFC and TDN dramatically

Why be concerned about ash contamination of aNDF?

- Results in over estimation of fiber in the diet
  - Paradoxically, aNDF generates better estimates of NE when regression equations are used
    - Grasses: NEL = 2.860 - .0262*NDF
    - Legumes: NEL = 2.323 - .0216*NDF
    - These equations SHOULD NOT be used with aNDFom
      - They imply that the lower NE is due to fiber, when it is actually lower due to ash
      - Summative equations not only are more accurate when aNDFom is used but also clearly indicates that the problem is ash and its impact on NFC

Why be concerned about ash contamination of aNDF?

- Results in over estimation of fiber in the diet
  - Paradoxically aNDF generates better estimates of NE when regression equations are used
    - Grasses: NEL = 2.860 - .0262*NDF
    - Legumes: NEL = 2.323 - .0216*NDF
    - Example (soil contamination):
      - NEL = 2.323 - .0216*36 = 1.545 Mcal NE/kg DM
      - NEL = 2.323 - .0216*28 = 1.761 Mcal NE/kg DM
      - A soil-contaminated sample should have less NE because the added ash generates no NE, but using aNDFom results in a higher estimate of NE
      - In this situation aNDF gives a better estimate of NE than does aNDFom, but for the wrong reasons.

What are the consequences of using aNDFom?

- NFC, OM partitioning and energy estimation are more accurate if aNDFom is used
- Regression equations developed using NDF to predict TDN or NE cannot be used with aNDFom

Why be concerned about ash contamination of aNDF?

- Results in over estimation of fiber in the diet
  - Paradoxically, aNDF generates better estimates of NE when regression equations are used
    - Grasses: NEL = 2.860 - .0262*NDF
    - Legumes: NEL = 2.323 - .0216*NDF
    - These equations were generated from research samples that had little or no soil contamination
    - Both the intercept and regression coefficients were generated assuming typical intrinsic ash in NDF

What are the consequences of using aNDFom?

- For most forages that are not heated, “correction” of aNDFom for NDICP (using sulfite) is not needed to adequately measure NFC and energy value
  - The assumption that all N is protein by multiplying N X 6.25 is probably a larger error in summative equations than is aNDICP contamination

What are the consequences of using aNDFom?

- If aNDFom is used, the minimum peNDF and optimum aNDF recommendations should be reduced to 95%
  - Most (all?) recommendations for NDF were based on NDF, NDF or aNDF measurements that were not corrected for ash

Conclusions

- aNDFom is the most accurate measure of fiber that can generate energy for the animal
- When using aNDFom summative equations must be used to estimate TDN or NE
- When using aNDFom in summative equations IVNDFomD must be used to estimate dNDFom

Conclusions

- aNDFom should not be substituted into regression equations developed for predicting TDN or NE using NDF
- Recommendations for optimal NDFom and minimum peNDFom should be about .95 of the current recommendations for optimal NDF and minimum peNDF

Ash-free fiber (aNDFom): Why the change and what are the consequences?

Questions ?

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Precision Dairy Monitoring Technology Investment Considerations

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Extension Dairy Specialist

Precision Dairy Monitoring Technology Investment Considerations

Jeffrey Bewley, PhD, Extension Dairy Specialist

Amanda Stone, Randi Black, Barbara Wadsworth, Di Liang, Karmella Dolecheck, Matthew Borchers, Lauren Mayo, Nicky Tsai, Maegan Weatherly, Melissa Cornett, Samantha Smith,

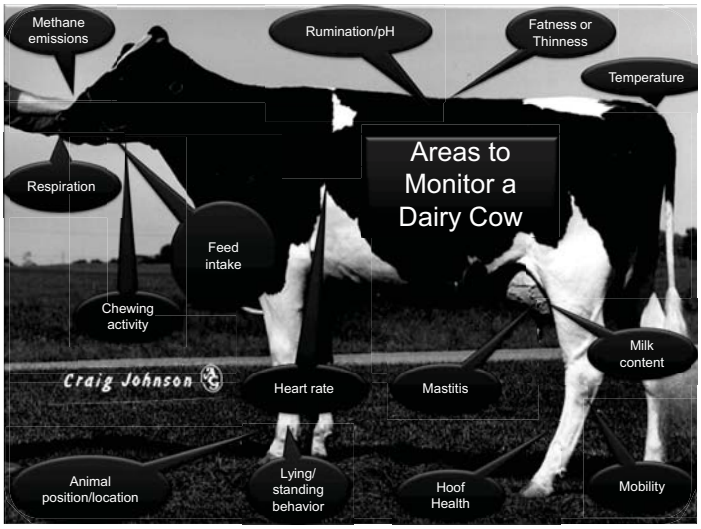
Precision Dairy Monitoring

- Technologies to monitor
  - Physiology
  - Behavior
  - Milk content

- Focus on preventive health and performance at the cow level
- Make more timely and informed decisions

Happy Cows via Technology?

University of Kentucky Research



Ideal Technology

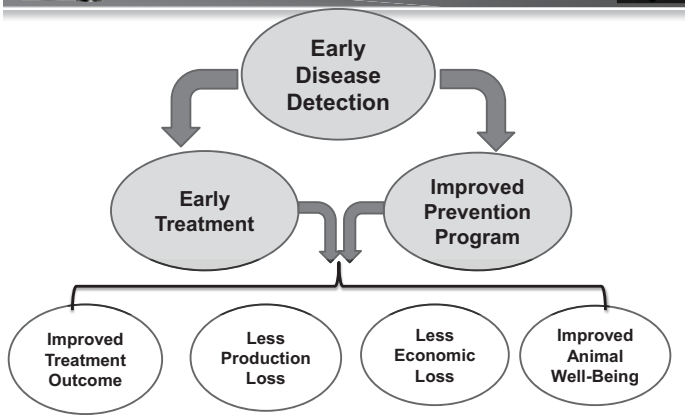
- Explains an underlying biological process
- Can be translated to a meaningful action
- Cost-effective
- Flexible, robust, reliable
- Simple and solution focused
- Information readily available to farmer

So Many Options!!!!

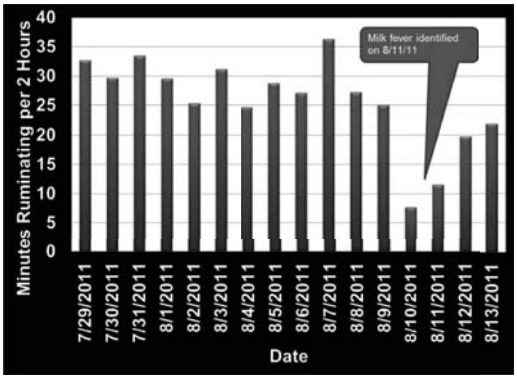
Precision Dairy Benefits

- Improved animal health and well-being
- Early detection
- Increased efficiency
- Improved product quality
- Minimized adverse environmental impacts
- More objective measures

Disease Detection Benefits

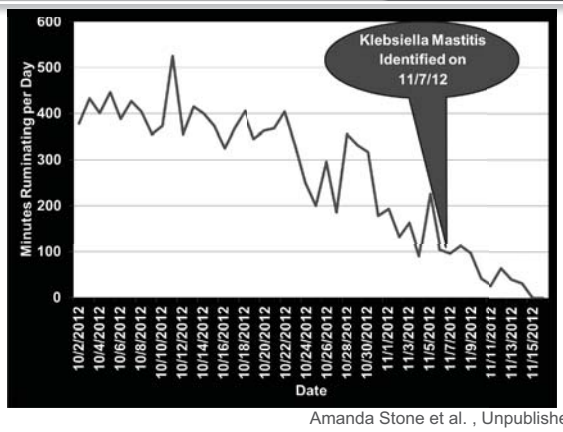


SCR HR Tag for Milk Fever Detection



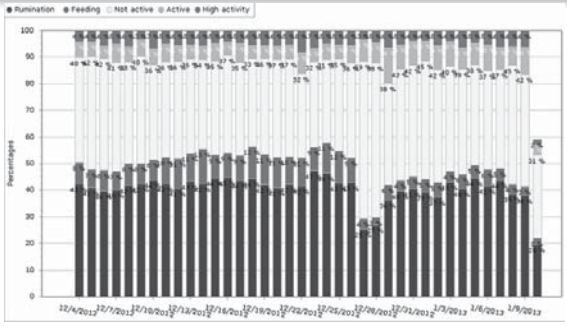
Amanda Stone et al. , Unpublished Data

SCR Rumination Time



Amanda Stone et al. , Unpublished Data

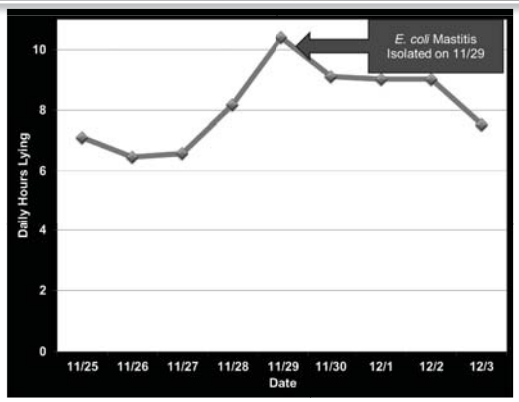
Agis Health Alert



Staph aureus **isolated on 12/29/12. Health alert on 12/27/12.**

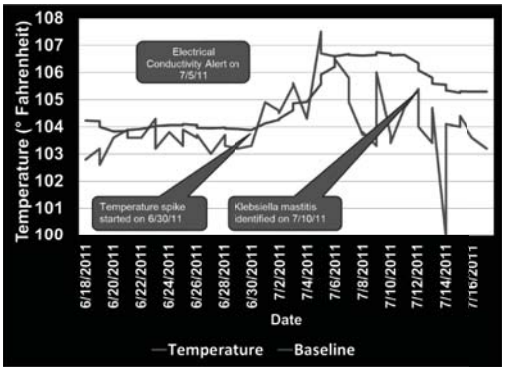
Stone et al. , Unpublished Data

IceQube Lying Time



Stone et al. , Unpublished Data

DVM Systems Temperature and Milkline Individual Quarter Conductivity



Stone et al. , Unpublished Data

Estrus Detection

Estrus Detection

- Efforts in the US have increased dramatically
- Satisfaction closely tied to reproductive performance before investment
- Only catches cows in heat
- Balanced approach with selective hormone intervention
- Tag management, data management, and algorithms matter

SCR HR Tag/AI24

GEA Rescounter II

DairyMaster MooMonitor/ SelectDetect

AFI Pedometer +

BouMatic HeatSeeker II

Track a Cow

Comparison of timed artificial insemination and automatic activity monitoring as reproductive management strategies in three commercial dairy herds

K.A. Dolecheck, W.J. Wilvia, G. Heersche Jr., C.L. Wood, K.J. McQuerry, and J.M. Bewley

Study Cows

- **Three commercial Holstein herds in Kentucky**
- **No clinical metabolic diseases**
- **Veterinary check**
  - **Normal ovarian activity**
- **Body condition score ≥ 2.50**

Dolecheck et al., 2014

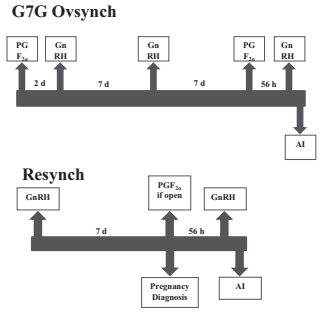
Cow Treatment Allocation

- 90 d study period
  - No visual estrus detection
- Balanced for
  - Parity (primiparous or multiparous)
  - Predicted milk yield (above or below herd average)

Dolecheck et al., 2014

Timed Artificial Insemination (TAI)

- Combination of G7G, Ovsynch, and Resynch
- Up to three services (maximum possible in 90 d)



Dolecheck et al., 2014

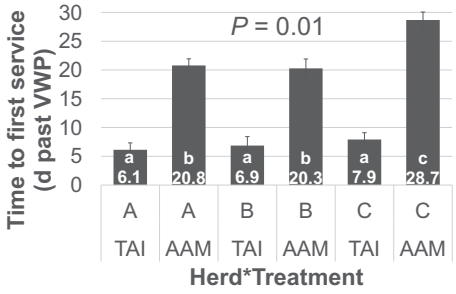
Automated Activity Monitoring (AAM)

- AfiTag Pedometer™ Plus (Afimilk®, Kibbutz Afikim, Israel)
  - Number of steps, rest time, rest bouts
  - "Cows to be bred" report
- Veterinary examination determined hormone intervention ($PGF_{2\alpha}$ or GnRH) if no alert was created for a cow for > 32 days

Dolecheck et al., 2014

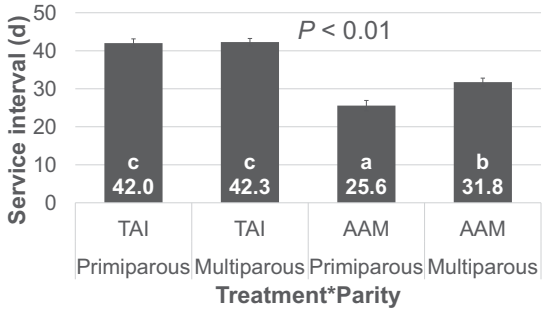
Time to First Service

- Time to first service was significantly lower for TAI bred cows (15 d); the difference was greatest in Herd C



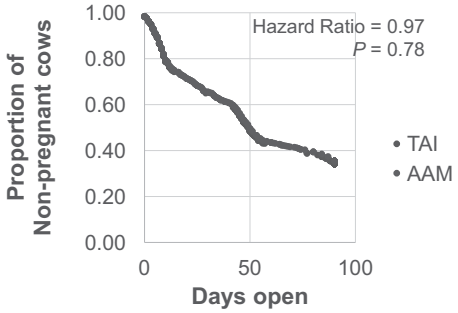
Service Interval

- Service interval was shorter in AAM cows than TAI cows and shortest in primiparous AAM cows



Rate of Pregnancy

- No significant difference



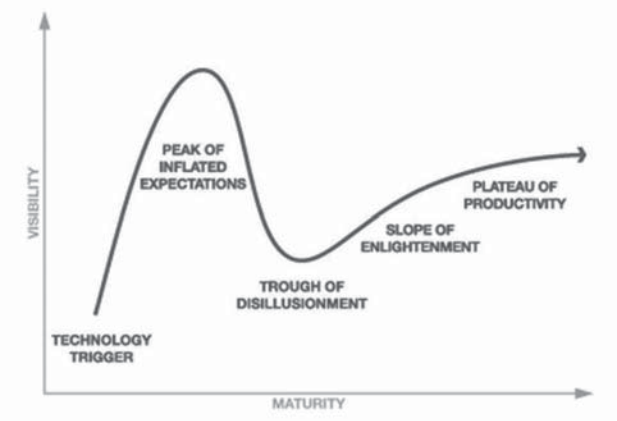
Dolecheck et al., 2014

Other Analysis

Parameter	n	TAI	AAM	*P*-value
First service conception rate (%)	539	41.5 ± 3.2	41.7 ± 3.5	0.97
Repeat service conception rate (%)	293	41.5 ± 4.4	49.9 ± 5.3	0.12
Services per pregnancy	356	1.58 ± 0.06	1.55 ± 0.06	0.70
Pregnancy loss (%)	397	10.5 ± 2.3	7.1 ± 2.0	0.20
Days open (d past VWP)	356	31.3 ± 1.9	35.3 ± 2.0	0.13
Proportion pregnant at 90 d (%)	543	67.5 ± 3.1	68.3 ± 3.2	0.84

Dolecheck et al., 2014

Gartner Life Cycle



What Are the Limitations of Precision Dairy Farming?

Technology Pitfalls

- "Plug and play," "Plug and pray," or "Plug and pay"
- Technologies go to market too quickly
- Not fully-developed
- Software not user-friendly
- Developed independently without consideration of integration with other technologies and farmer work patterns

- **Maybe not be #1 priority for commercial dairy producers (yet)**
- **Many technologies are in infancy stage**
- **Not all technologies are good investments**
- **Economics and people factors**

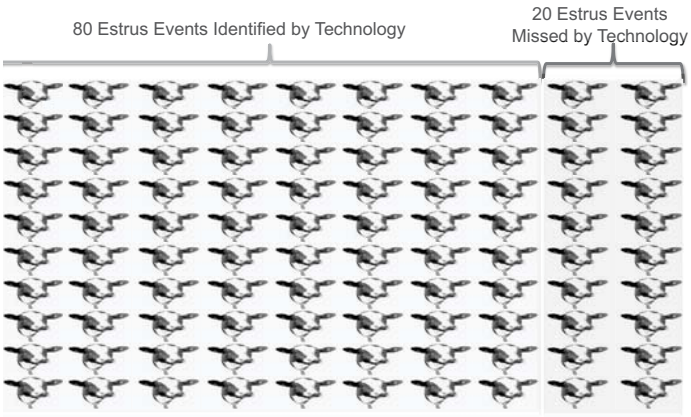
Technology Pitfalls

- Too many single measurement systems
- Lack of large-scale commercial field trials and demonstrations
- Technology marketed without adequate interpretation of biological significance of data
- Information provided with no clear action plan

PDF Reality Check

- **Maybe not be #1 priority for commercial dairy producers (yet)**
- **Many technologies are in infancy stage**
- **Not all technologies are good investments**
- **Economics must be examined**
- **People factors must be considered**

How Many Cows With Condition Do We Find?

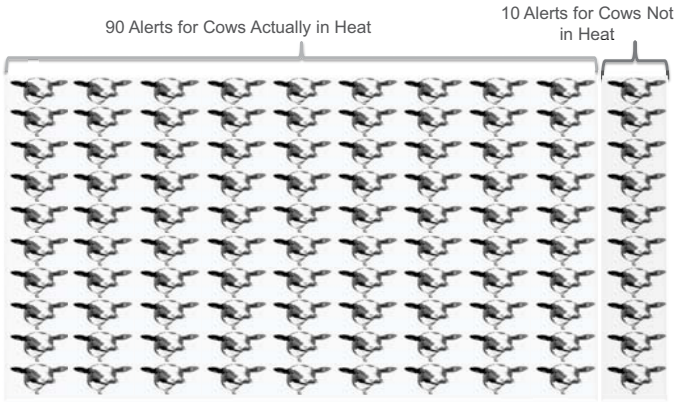


Example: 100 estrus events

Lessons Learned

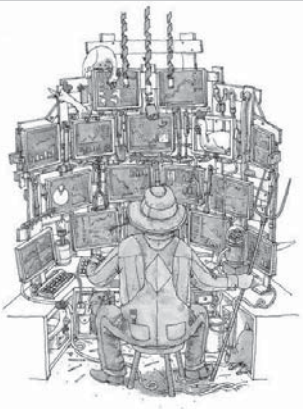
- Be careful with early stage technologies
- Need a few months to learn how to use data

How Many Alerts Coincide with an Actual Event?



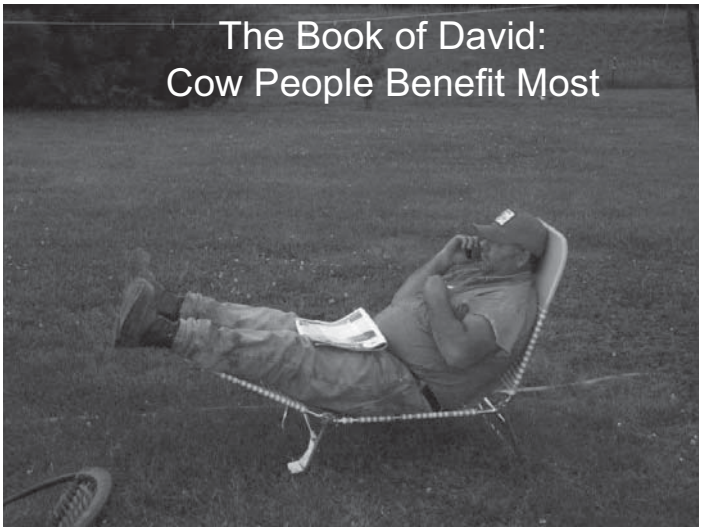
Example: 100 estrus events

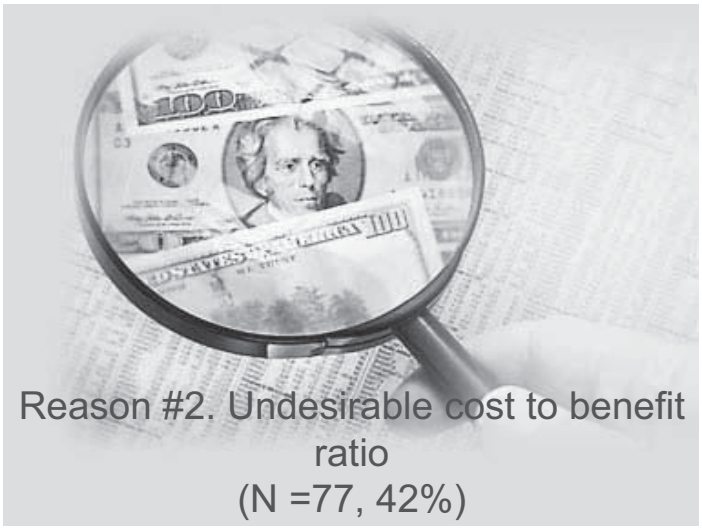
UK Herdsman Office



What's the Sweet Spot?

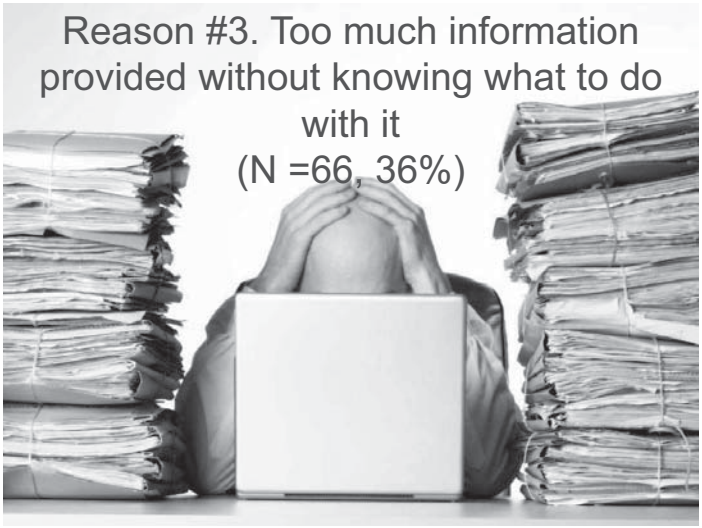
- Cost of missed event
  - – High for estrus
  - – Lower for diseases?
- Cost of false positive
  - – Low for estrus
  - – High for mastitis
- Farm dependent

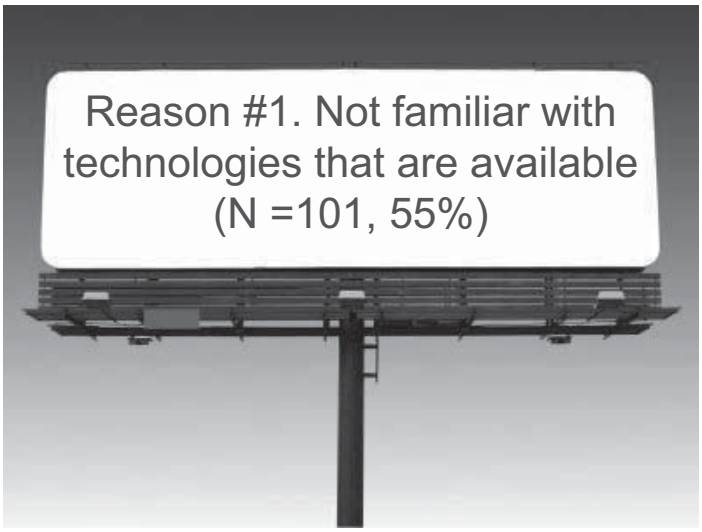




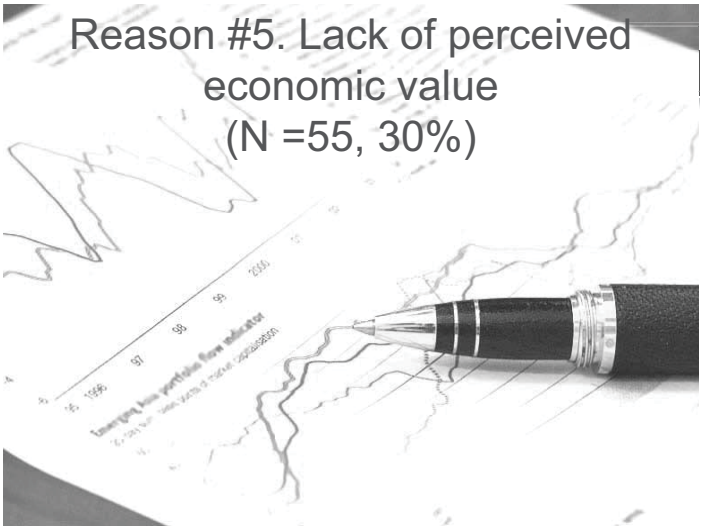
Why Have Adoption Rates Been Slow?

Rebecca Russell, 2013



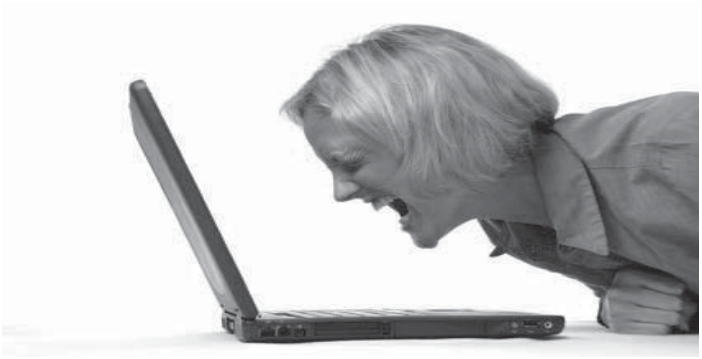


Reason #5. Lack of perceived economic value
(N =55, 30%)



Reason #8. Better alternatives/easier to accomplish manually
(N =43, 23%)

Reason #6. Too Difficult or Complex to Use
(N =53, 29%)



Reason #9. Failure in fitting with farmer patterns of work
(N =40, 22%)

Reason #7. Poor technical support/training
(N =52, 28%)

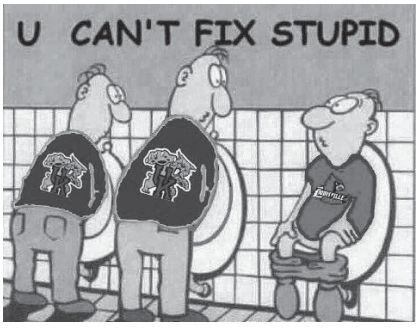
Reason #10. Fear of technology/computer illiteracy
(N =39, 21%)

Reason #11. Not reliable or flexible enough (N =33, 18%)

What do producers consider before purchasing one of these technologies?

Matthew Borchers, 2014

Reason #99. Wrong College Degree (N =289, 100%)



Consideration #1. Benefit: cost ratio (4.57 ± 0.66)

Matthew Borchers, 2014

Precision Dairy Technologies: A Producer Assessment

Matthew R. Borchers and Jeffrey M. Bewley
University of Kentucky
Department of Animal and Food Sciences

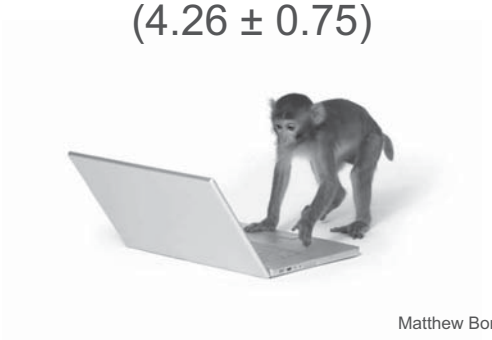
seeblue.

UKAg DAIRY UNIVERSITY OF KENTUCKY

Consideration #2 Total investment cost (4.28 ± 0.83)

Matthew Borchers, 2014

Consideration #3. Simplicity and ease of use
(4.26 ± 0.75)



Matthew Borchers, 2014

Important Parameter #2
Standing heat
(4.75 ± 0.55)

Matthew Borchers, 2014

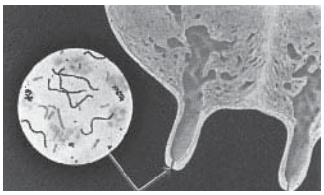
What parameters do producers find most useful in technologies?

Matthew Borchers, 2014

Important Parameter #3 Daily milk yield
(4.72 ± 0.62)

Matthew Borchers, 2014

Important Parameter #1. Mastitis
(4.77 ± 0.47)



Matthew Borchers, 2014

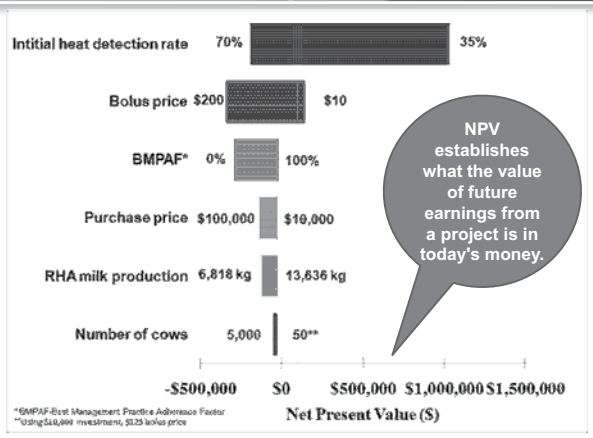
Economic Considerations

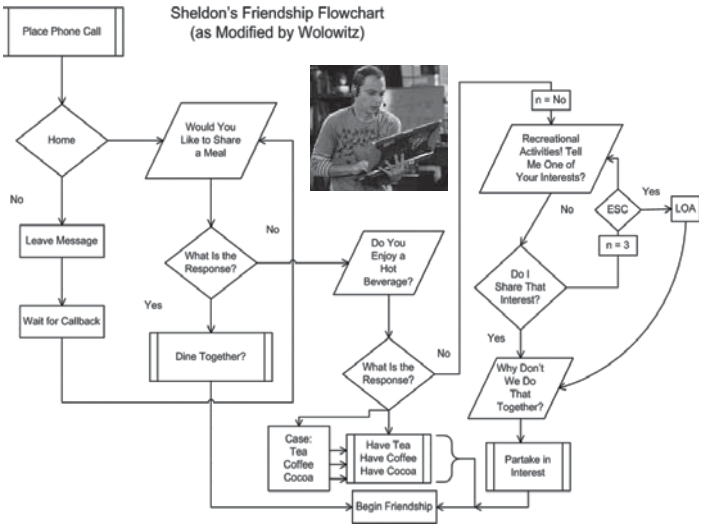
- Need to do investment analysis
- Not one size fits all
- Economic benefits observed quickest for heat detection/reproduction
- If you don't do anything with the information, it was useless
- Systems that measure multiple parameters make most sense
- Systems with low fixed costs work best for small farms

Purdue/Kentucky Investment Model

- Investment decisions for PDF technologies
- Flexible, partial-budget, farm-specific
- Simulates dairy for 10 years
- Includes hundreds of random values
- Measures benefits from improvements in productivity, animal health, and reproduction
- Models both biology and economics

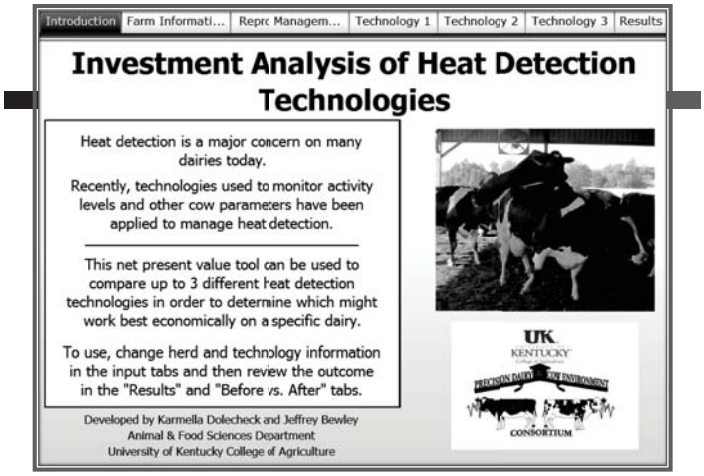
Tornado Diagram for Deterministic Factors Affecting NPV



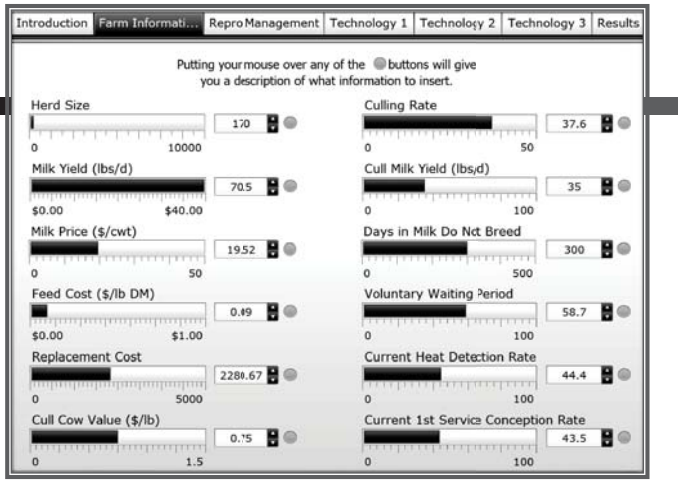


Investment Analysis of Automated Estrus Detection Technologies

K.A. Dolecheck, G. Heersche Jr., and J.M. Bewley
University of Kentucky



Dashboard available at: www2.ca.uky.edu/afsdairy/HeatDetectionTechnologies



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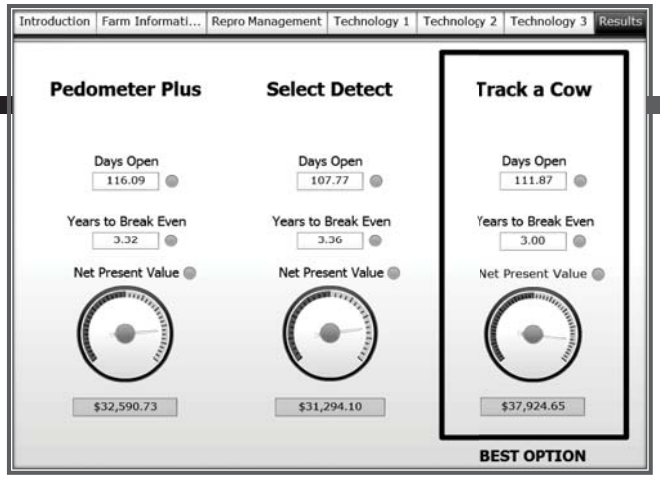


Dashboard available at: www2.ca.uky.edu/afsdairy/HeatDetectionTechnologies

Path to Success

- Continue this rapid innovation
- Maintain realistic expectations
- Respond to farmer questions and feedback
- Never lose sight of the cow
- Educate, communicate, and collaborate

Customer Service is Key

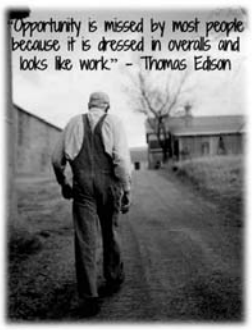
- More important than the gadget
- Computer literacy
- Not engineers
- Time limits
- Failure of hardware and software

Future Vision

- New era in dairy management
- Exciting technologies
- New ways of monitoring and improving animal health, well-being, and reproduction
- Analytics as competitive advantage
- Economics and human factors are key

Cautious Optimism

- Critics say it is too technical or challenging
- We are just beginning
- Precision Dairy won't change cows or people
- Will change how they work together
- Improve farmer and cow well-being



Mark your calendars!

CONFERENCE & EXPO ON
PRECISION DAIRY FARMING 2015
Mayo Civic Center, ROCHESTER, MN
24-25 June

Questions?

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Rumen Lipid Submodels in Nutrition Programs: Are they ready to help you?

Tom Jenkins
Clemson University
Clemson, South Carolina

Rumen Lipid Submodels in Nutrition Programs: Are they ready to help you?

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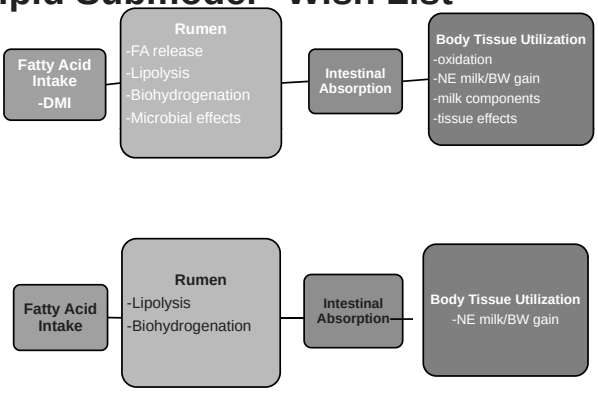
Clemson, South Carolina

Fatty Acid Sources

Ingredient	DMI, lb/d	RUFAL, g/d
Corn Silage, Med Chppd	21.95	152
AlfHay2 20Cp40Ndf17LNDF	5.78	26
CrnGrn56DryFine	9.34	139
Citrus Pulp Grnd	1.03	6
Cottonsd WLint	2.30	142
Megalac	0.29	48
Soybean ML 47.5 Solv	6.95	60
Other (mineral, vitamin, trace supplements)	1.32	0
Total	48.96	573

Lipid Submodel "Wish List"



Forage FA Variation

FA, % DM	Netherlands[1] Grass Silage	Netherlands[1] Corn Silage	USA[2] Corn Silage
Mean	1.9	2.0	2.5
Minimum	0.8	1.2	1.6
Maximum	3.3	3.5	3.6

[1]Khan et al., 2012 Anim Feed Sci Tech. 174: 36-45
[2]Klein, Ploetz, Jenkins, *& Lock.2013 ADSA Abstract #73*

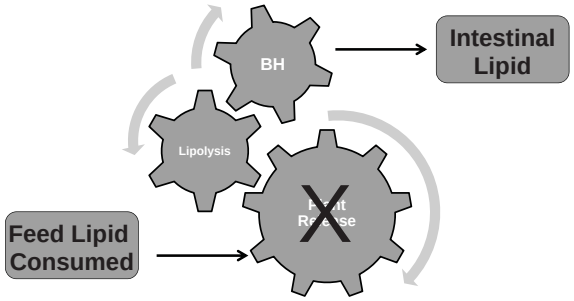
Fatty Acid Intake

Lipid Library

- Foundation of a good model
- Advancements
  - Fatty acids becoming primary/ ether extract secondary
  - Several commercial ag labs offering fatty acid analysis
- Challenges (aside from DM intake)
  - Variation within a feed – models assume same FA even as other nutrients vary considerably.
  - Mindset that fat supplements are the only significant source of lipids.

Ingredient	1.5 % CS	3.5 % CS
Corn Silage, Med Chppd	152	349
AlfHay2 20Cp40Ndf17LNDF	26	26
CrnGrn56DryFine	139	139
Citrus Pulp Grnd	6	6
Cottonsd WLint	142	142
Megalac	48	48
Soybean ML 47.5 Solv	60	60
Other (mineral, vitamin, trace supplements)	0	0
Total	573	770
RUFAL, % DM	2.57	3.47

Three Interlocking Events in Rumen Lipid Metabolism

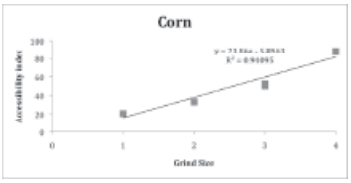


Samples

- 4 grind sizes from whole to finely ground
  - Soybean seeds
  - Corn
  - Cottonseed
  - Alfalfa pellets

1 lb fat	Microbial Exposure	Shift Microbial Population/Lipolysis
Corn Oil (1 lb)	Immediate	High
Corn silage (30 lb)	Slower release from plant structure	Medium
Cottonseed (5-6 lb)	Slow release from outer seed coat	Low

Corn



Lipid Accessibility Index (AI)

Goal: Develop a AI that can be used in the industry to predict exposure of plant lipid to the microbial population.

Approach: Given that lipids in plant matter must be converted to methyl esters prior to analysis by gas chromatography, and that contact of reagents with plant lipids is needed for methylation, we explored how completeness of lipid methylation might be used as a measure of AI.

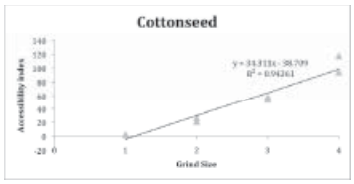
Hypothesis: Methylation for 10 min will not give the same result for fatty acid content relative to methylation for 2 h because plant lipids are not accessible to the reagents.

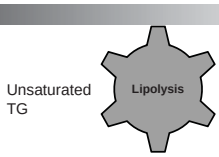
Based on this,

AI = FA 10 min methylation at particle size x / FA 2 h methylation in finely ground sample X 100

Soybeans

Cottonseed





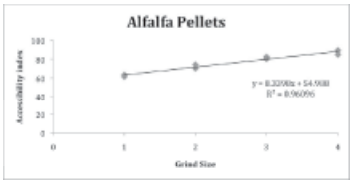
Bound

- Mainly bound to glycerol
- No biohydrogenation
- Little effect on rumen fermentation

Free (FFA)

- Released by plant or rumen lipases
- Biohydrogenation
- Disrupt rumen fermentation

Alfalfa Pellets



Release from Ensiling!

	FFA, % of total lipid		
	Fresh	Ensiled	Reference
Ryegrass	2	27-73	Elsgersma et al. 2003
Timothy	15	56	Vanhatalo et. al. 2007
Red Clover	8	45	Vanhatalo et. al. 2007

Plant lipases release FFA after cutting (Thomas, 1986) or during ensiling (Chow et al., 2004).

Questions

- Can you use TMR without grinding?
- Doesn't account for breakdown by chewing/rumination.
- Does methylation AI equate to microbial AI?
  - Tested in vitro

FFA in WCS

	WCS Source		
	Normal	No Heating	Overheated
Moisture, %	9.4	10.6	11.9
Oil, %	18.4	17.1	15.9
FFA, % of oil	6.8[b]	24.1[a]	22.3[a]
DMI, kg/d			
Milk, kg/d			
Fat, %			

[ab] $P < 0.05$
Cooke et al. 2007. J. Dairy Sci. 90:2329.

FFA in WCS

	WCS Source		
	Normal	No Heating	Overheated
Moisture, %	9.4	10.6	11.9
Oil, %	18.4	17.1	15.9
FFA, % of oil	6.8[b]	24.1[a]	22.3[a]
DMI, lb/d	47.5	48.4	51.7
Milk, lb/d	77.0	74.8	77.2
Fat, %	4.22[a]	3.64[b]	3.58[b]

[ab] $P < 0.05$
Cooke et al. 2007. J. Dairy Sci. 90:2329.

Klip and Kb in CPM

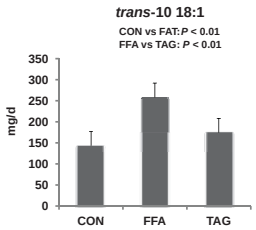
- **An XL Spreadsheet Linear Program "Solver"**
- **Solver Was Used to Iteratively Alter Klip and Kb to Obtain a Least Squares "Best Fit " of Model Predictions to Observed Duodenal Flows of LCFA.**
- **K_{lip} and K_{BH} were computer generated**

"Maybe we don't have all the answers but it's a foundation to build on."

Dr. Bill Chalupa, University of Pennsylvania

USA Corn Silage-75 corn silage samples from 2011 harvest

	TFA, %DM	FFA, %TFA
Mean	2.5	20
Min	1.6	13
Max	3.6	31

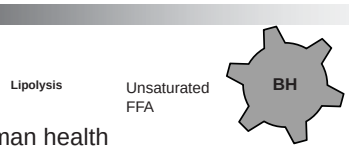


Klein, Ploetz, Jenkins, & Lock.2013 ADSA Abstract #73

Data and Methodology

- **Data Used Were From 27 Dietary Ingredients in 36 Diets in 8 Published Experiments That Reported Dietary Intakes and Flows of Specific Fatty Acids (g/cow/day) to the Duodenum of Dairy Cows.**

23



- Affects human health
  - Meat and milk higher in saturated fat
  - Bioactive intermediates
    - Anti-carinogenic
    - Anti-atherogenic
    - Enhance immune system
- Affects animal performance
  - Supply of essential fatty acids – reproduction, immune
  - Fatty acid antimicrobial effects - production
  - CLA
    - Milk fat depresson
    - Growth regulators

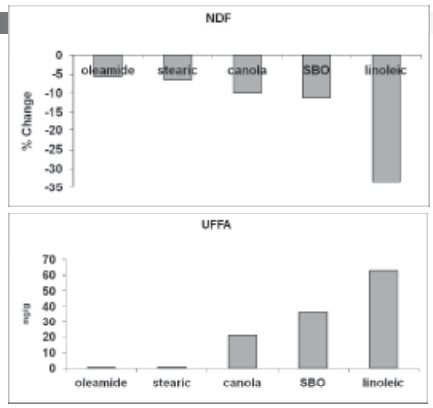
Concerns

- Sometimes there were very few published studies on a specific feed ingredient leading to considerable uncertainty about their lipolysis rate.
  - Klip
    - WCS = 500%/h n=2 diets in 1 exp.
    - sunflower oil 52%/h n=2 diets in 1 exp.
- As of now, there is not an easy, inexpensive way to have a feed ingredient analyzed for its lipolysis rates.

Microbial Effects

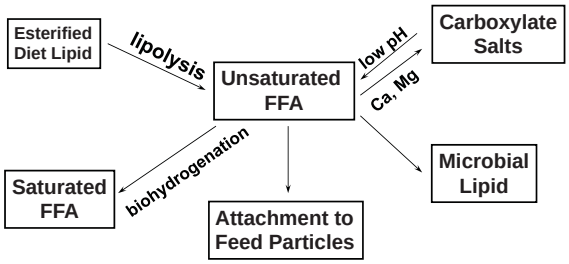
Rumen
-FA release
-Lipolysis
-Biohydrogenation
-Microbial effects

- Shift microbial population
- Affect fermentation and fiber digestion
- Affect biohydrogenation
  - Rumen outflow of unsaturated fatty acids
  - CLA and milk fat depression
- Difficult to model only knowing dietary fat levels
- The best predictor is how the fat contributes to the rumen UFFA concentration.
  - Models already half way there.

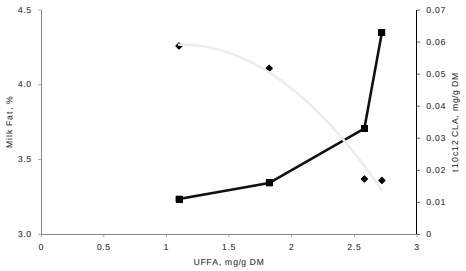


Jenkins. 2002. Gastrointestinal Microbiology in Animals.

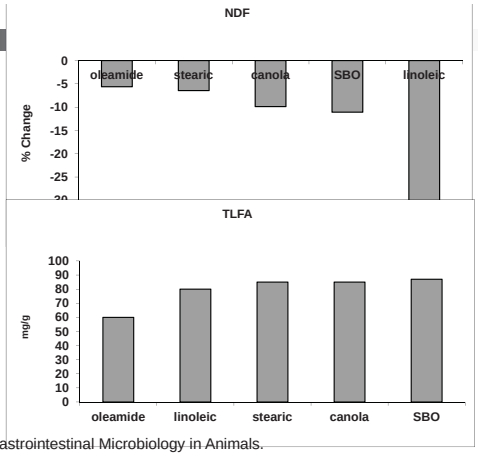
Factors Modifying the Rumen Pool Size of UFFA



Increasing RUFFA on CLA and Milk Fat %



29



Jenkins. 2002. Gastrointestinal Microbiology in Animals.

Intestinal Absorption

Importance

- Affects lipid energy available for a production response.
- Affects the delivery of specific fatty acids to tissues.
  - Milk fatty acid composition
  - Reproductive performance
  - Immune function and disease resistance
- Affects the expected performance and profitability of a commercial fat product.

Variations in Calculations of Intestinal FA Digestibility

- Meta-analysis
  - Some species deleted?
  - Some fat sources deleted?
    - Hydrogenated TG
  - Some study designs deleted?
    - Duodenal, ileal canulations (eliminate hindgut BH)?
- Calculations
  - Apparent based on flows
  - True digestion (eliminate endogenous secretions)

Lipid Model "Wish List"

- Feed FA release – not being worked on
- Feed FFA – analytical and can't use info
- Rumen antimicrobial effects – not yet but most of the way there
- Test any fat for K_{lip} and K_{bh} – not yet but can be done in vitro
- Intestinal digestion – meta-analysis but in vitro model possible
- Tissue effects – not yet (milk fat, repro, etc.)

Table 2. Digestibility of FA between the duodenum and ileum or feces

FA, %	Lock et al. (2006)	Glasser et al. (2008b)	Doreau and Chilliard (1997)
Palmitic (C16:0)	75		79
Stearic (C18:0)	72	74	77
Oleic (C18:1)	80	79	85
Linoleic (C18:2)	78	72	83
Linolenic (C18:3)	77	70	76
Total	74		

From Loften et al., 2014. JDS 97:4661-4674.

Thank You!!!

Lipid Submodel "Givens"

- Library fatty acids
- Rumen lipolysis/BH
- Intestinal digestibility
- Lipid energy contribution to milk

Practical Recommendations for Trace Minerals for Lactating Dairy Cows

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Summary

Providing adequate trace minerals to dairy cows is essential for high production and good health. Providing excess trace minerals inflates feed costs and could be detrimental to production and cow health. Basal ingredients such as corn silage and hay provide absorbable trace minerals to cows. Concentrations of trace minerals in basal ingredients should not be set to 0. The 2001 NRC recommendations for most trace minerals (Mn is an exception) appear adequate and should be the starting point for ration formulation. Because of uncertainty regarding absorption and requirements, a modest safety factor of 1.2 to 1.5 X NRC requirements is appropriate for most trace minerals under normal conditions. The NRC does not consider antagonism and for Cu, antagonism can be quite common (high intake of S from diet or feed, grazing, and dietary Mo). In those cases, absorption coefficients should be reduced (perhaps more than 50%) so that cows are fed diets with adequate absorbable Cu. However, feeding excess Cu over the long term (months or years) can result in high concentrations of Cu in the liver which may be detrimental to cows. The 2001 NRC recommendation for Mn is too low and may need to be increased by a factor of 1.8. The NRC recommendation for Co may be too low, but in many cases the basal diet may be adequate. The NRC did not establish a requirement for Cr, but the majority of production studies with transition cows have shown increased milk yield.

Currently Used Requirement System (e.g., NRC, 2001)

Several nutrition models are used in the U.S. (e.g., NRC, CNCPS, AminoCow) to formulate diets for dairy cows and they often differ substantially in their recommendations regarding energy and protein. However, mineral requirements from probably every nutrition model currently used in the US are derived directly or almost directly from the NRC (2001). The requirements for most trace minerals (Se, I, and Co are exceptions) are calculated using the factorial approach. Mineral needed for maintenance plus mineral deposited in the growing fetus (gestation requirement) and body (growth requirement) and mineral secreted in milk (lactation requirement) were summed to generate the requirement for absorbed mineral in either gram or milligrams/day. Because requirements were calculated on an absorbed mineral basis, absorption coefficients (AC) for all the minerals had to be generated and multiplied by mineral concentrations to calculate the concentration of absorbed mineral in the diet.

The factorial system has been used for decades to determine requirements for energy and protein and more recently for minerals. However, conceptually, separating requirements into maintenance, gestation, growth, and lactation components is flawed and because of their biological functions the factorial approach may be extremely flawed for trace minerals. A major problem is defining maintenance. For example, if extra copper is needed by the immune system to prevent mastitis is that a maintenance function or a lactation function? If extra selenium is needed to prevent retained placenta, is that a maintenance function or a reproduction function? The problem with partitioning mineral requirements into various function is not simply an academic exercise, it can result in erroneous estimate of mineral requirements. At least conceptually, the current system could underestimate the requirements for many trace minerals. In addition, certain disease states such as a severe infection, increase loss of certain minerals via feces and urine. This may mean that an immune or health requirement needs to be considered and if necessary included in the factorial system.

Mineral Supply

A major change that occurred in NRC (2001) was that requirements were calculated for absorbed mineral rather than total mineral. This was a major advance because we know mineral from some sources are more absorbable than minerals from other sources. However the use of absorbable mineral has limitations:

- Measuring absorption of some minerals is extremely difficult
- Actual absorption data and absorption coefficients are limited. Many values are estimates
- Absorption is affected by physiological state of the animal and by numerous dietary factors (many of which have not been quantified).

- For many of the trace mineral, the AC is extremely small and because it is in the denominator (i.e., Dietary mineral required = absorbed requirement/AC) a small numerical change in the AC can have a huge effect on dietary requirement.

Concentrations of Minerals in Basal Ingredients

For most minerals of nutritional interest good analytical methods that can be conducted on a commercial scale at reasonable costs are available. Assuming the feed sample is representative, a standard feed analysis (using wet chemistry methods for minerals) should provide accurate concentration data for most trace minerals. Although chromium, cobalt, and selenium are of nutritional importance, most labs do not routinely measure these because the concentrations commonly found in feeds are lower than what commercial labs can reliably measure or because of contamination caused by routine sample processing such as using a steel feed grinder (a major concern for Cr).

Concentrations of trace minerals in feeds are low. For example 1 ton of average corn silage (35% dry matter) only contains about 2.5 grams of Cu (to put this in perspective a penny weighs about 2.5 g). Sampling error is a problem for most nutrients and when concentrations are low, sampling error is usually larger. From a survey we conducted on forages, sampling variation for trace minerals was greater than true variation. This means that mineral concentration data from a single sample should be viewed suspiciously. The mineral concentration of soils is a major factor affecting the concentrations of most minerals in forages. Therefore means of samples taken from a farm over time (up to a few years) or from a group of farms within a small geographic area (e.g., a few counties) should be a truer estimate of the actual mineral concentration of a forage than a single sample.

Besides sampling issues, the concentrations of many minerals in feeds are not normally distributed (a normal distribution is the classic bell shaped curve). In a normal distribution about half the samples have less than the mean or average concentration, about half the samples have more than the average, and about 95% of the samples are within + 2 standard deviation (SD) unit of average. This means that if you know the average concentration and the SD you have a good description of the population. This information helps with risk assessment. However when distributions are skewed, the average and the SD may not be good descriptors of the population, and for many minerals, concentrations within feeds are not normally distributed (Figure 1). Often the distributions have long tails often because some samples are contaminated with soil. The more skewed that data, the less valuable the average and SD become in describing the feed. The median is the concentration where half of the samples have a lower mineral concentration and half of the samples have more mineral. For concentrations of trace minerals, the median is usually less than the average because their distributions are skewed. What this means is that for most situations, using the average, overestimates the trace mineral concentration in the majority of samples. The bottom line is that averages for trace mineral concentrations in forages (and perhaps other feeds) found in tables should be used with caution but because of substantial sampling variation, data from a single sample should also not be used. The best advice is to generate mean values for trace minerals for forages grown within a limited geographical area.

Do the trace minerals in basal feeds have nutritional value ?

Essentially every feedstuff used in dairy diets contains some minerals. The question is, are those minerals biologically available to cows? Based on personal observations it is not uncommon for nutritionists to set trace mineral concentrations in basal ingredients or at least forages, at 0. This approach would be valid if the trace minerals in feedstuffs were not biologically available to cows. Although substantial uncertainty exists regarding the absorption coefficients for most minerals in most feeds (this includes mineral supplements), a portion of the trace minerals found in all feedstuffs is clearly available to cows. On average, unsupplemented diets for lactating cows in Ohio based mostly on corn silage, alfalfa, corn grain and soybean meal contain 7 to 9 ppm Cu and 30 to 40 ppm Zn. For an average Holstein cow (75 lbs of milk/day and 53 lbs of dry matter intake) basal ingredients supply about 80% and 75% of NRC requirements for Cu and Zn. Ignoring minerals supplied by basal ingredients can result in substantial over formulation for trace minerals.

The NRC (2001) estimates that Cu, Mn, and Zn from basal ingredients are 4, 0.75 and 15% absorbable. The AC assigned to basal ingredients are usually lower than AC for the sulfate form of trace minerals even though most of the trace minerals contained within plant cells would be in an organic form. The lower AC for trace minerals in basal ingredients may reflect an adjustment for soil contamination. Some of the trace minerals in basal feeds, especially forages, are in the soil that is attached to the feed and those minerals are often in the oxide form (i.e., low availability). This suggests that feeds with substantially higher ash and trace mineral concentration than typical (i.e., the tails

discussed above) likely have AC that are lower than the NRC values for trace minerals. Concentrations of trace minerals substantially greater than median value should be discounted but an exact discount cannot be calculated at this time, but those feeds would still contain some available mineral.

Recommendations

The primary trace minerals of interest in dairy nutrition are chromium (Cr), cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), selenium (Se) and zinc (Zn). The NRC (2001) did not establish a requirement for Cr, but for the other trace minerals, the NRC should be the starting point. Iron will not be discussed because basal diets almost always contain adequate Fe. Iodine also will not be discussed because of limited new information.

Chromium

Feeding diets with more than 0.5 ppm of supplemental Cr or from sources other than Cr propionate is not legal in the U.S. Chromium is a required nutrient, however, the NRC (2001) did not provide a quantitative recommendation. Cr is needed to transport glucose into cells that are sensitive to insulin. Because of analytical difficulties (e.g., grinding feeds prior to chemical analysis can contaminate them with Cr) we do not have good data on Cr concentrations in feedstuffs. Some studies with cattle have shown that supplemental Cr (usually fed at 0.4 to 0.5 ppm of diet DM) reduced the insulin response to a glucose tolerance test. Elevated insulin reduces glucose production by the liver and enhances glucose uptake by skeletal muscle and adipose tissue. These actions reduce the amount of glucose available to the mammary gland for lactose synthesis and this may be one mode of action for the increased milk yield when Cr is supplemented. Most of the production studies evaluating Cr supplementation started supplementation a few weeks before calving and most ended by about 42 DIM. Supplementation rates varied but most were 0.3 to 0.5 mg Cr/kg of diet DM. The median milk response from 30 treatments from 14 experiments (treatments that fed supplemental Cr well in excess of the permitted 0.5 ppm were excluded) was +4.1 lbs/day (the SD among responses was 3.5 lbs/day). About 75% of the treatment comparison yielded an increase in milk of more than 2 lbs/day. Although a comprehensive meta-analysis is needed, based on this preliminary analysis of studies, increased milk yield of at least 2 lbs/day is highly probable when approximately 0.5 ppm Cr is supplemented to early lactation cows. Whether this response would be observed throughout lactation is not known. The potential return on investment from milk can be calculated by using the value of milk and cost of increased feed intake plus the cost of the supplement and assuming a median response of about 4 lbs of milk, an expected increase in DMI of about 2.8 lbs. At this time, a milk response should only be assumed to occur up to about 42 DIM.

Cobalt

The current NRC requirement for Co is expressed on a dietary concentration basis (i.e., 0.11 ppm in diet DM). This was done because Co is mostly (perhaps only) required by ruminal bacteria and the amount they need is a function of how much energy (i.e., feed) is available to them. Although data is limited, studies have reported Co concentrations of 0.3 to 2 ppm in the basal diets which is often adequate to meet the Co requirement. Based on older research (<1970), diets with 0.11 ppm Co maintained adequate concentrations of vitamin B-12 in the liver of cows, but B-12 production in an in vitro ruminal system increased as Co increased up to 1 ppm in the incubation media (Tiffany et al., 2006). The greatest response was when Co was increased from 0 to 0.1 ppm (B-12 concentration increased about 60%). When Co was increased ten-fold (0.1 to 1.0 ppm), B-12 increased only an additional 40%. Data using growing beef animals (Stangl et al., 2000) found that liver B-12 was maximal when diets contain 0.22 ppm Co. With dairy cows, liver B-12 concentrations continued to increase as supplemental Co (from Co glucoheptonate) increased up to 3.6 ppm ((Akins et al., 2013). In that study elevated liver B-12 did not translate into any health or production benefits. Indicating that maximal liver B-12 may not be necessary. Milk production responses to increased Co supplementation has been variable. One study (Kincaid et al., 2003) reported a linear increase in milk yield in multiparious cows, but no effect in first lactation animals when supplemental Co increased from 0 to about 1 ppm. Older cows tend to have lower concentrations of B-12 in their livers which could explain the parity effect.

Copper

The NRC (2001) requirement for Cu and over a wide range of milk yields (40 to 150 lbs), range from about 7 to 15 mg of absorbed Cu /day under normal conditions. Because Cu is secreted in milk, as milk yield increases, the NRC requirement for Cu increases. However, because basal ingredients contain Cu and because DMI usually increases as milk yield, the dietary concentration of Cu needed to meet the requirement may actually decrease as milk yield increases (Table 1). Contrary to popular practice, diets for pens of high producing cows often do not need to contain higher concentrations of many trace minerals than diets for lower producing cows. Whereas fresh cow pens, because of low DMI often need to be fed diets with increased concentrations of trace minerals.

Table 1. Effect of intake and milk production on requirements (NRC, 2001) of certain trace minerals.

	Dry cow 28 lbs DMI		High producing cow 120 lbs milk; 67 lbs DMI		Average cow 75 lbs milk; 53 lbs DMI	
	Absorbed requirement, mg/d	Dietary requirement, mg/kg of diet DM	Absorbed requirement, mg/d	Dietary requirement, mg/kg of diet DM	Absorbed requirement, mg/d	Dietary requirement, mg/kg of diet DM
Cu	6.8	12	12.8	10	9.7	10
Fe	18.0	14	54.4	18	34.0	14
Mn	1.7	18	2.9	13	2.3	13
Zn	42.6	26	247.0	49	165.3	43

1 Basal diets were assumed to contain 8, 225, 30, and 35 ppm Cu, Fe, Mn, and Zn. Basal absorption coefficients were 0.04, 0.10, 0.0075, and 0.15 for Cu, Fe, Mn, and Zn. If supplemental minerals were needed, absorption coefficients for sulfate forms were used.

All trace minerals have antagonists that reduce absorption but often these do not occur in real situations. All trace minerals are toxic but for most of the minerals the intakes needed to produce toxicity are usually quite high. Copper, however, is unique among nutritionally important trace minerals in that it is toxic at relatively low intakes (~3 to 4 times requirement) which should dictate caution regarding over supplementation. On the other hand, Cu has numerous real world antagonists which mandate the need to over supplement in several situations. The NRC requirement assumes no antagonism; however several situations commonly exist which result in reduced Cu absorption including:

- Excess intake of sulfur (provided by the diet and water)
- Excess intake of molybdenum (effect is much worse if excess S is also present)
- Excess intake of reduced iron (may reduce absorption and increase Cu requirement)
- Pasture consumption (probably related with intake of clay in soil)
- Feeding clay-based 'binders'

Most of these antagonisms have not been quantitatively modeled, and specific recommendations cannot be provided. When dietary S equivalent (this includes S provided by the diet and the drinking water) is >0.25 to 0.3%, additional absorbable Cu should be fed. In most situations dietary S will be <0.25% of the DM. Diets with high inclusion rates of distillers grains and diets that contain forages that have been fertilized heavily with ammonium sulfate can have substantially higher concentrations of S. Water S concentration is dependent on source. Water should be sampled and assayed on a regular basis (at least annually) to determine whether water is adding to the S load in the diet. A spreadsheet to calculate dietary S equivalent concentration and it can be found at: dairy.osu.edu/resource/OSU%20Dairy%20Pubs.html#computer

As an approximation, for an average Holstein cow, for every 100 mg/L (ppm) of S in water add 0.05 percentage units to the S concentration in the diet to estimate dietary equivalent S. For example, if your diet has 0.26% S and your water has 400 mg/L of S, dietary equivalent S = 0.26 + 4*0.05 = 0.46%. Some labs report concentrations of sulfate, not S. If your lab reports sulfate, multiply that value by 0.333 to obtain concentration of S.

Although the presence of antagonist justifies feeding additional Cu or using Cu sources that are more resistant to antagonism, no data are available indicating that the current NRC requirement is not adequate under normal conditions. Because of uncertainties associated with AC and the actual requirement, a **modest** safety factor should be used when formulating diets. Under normal situations, feeding 1.2 to 1.5 X NRC can be justified for risk management and it also should prevent excessive accumulation of Cu in tissues over the life of the cow. For an average lactating cow, NRC requirement for absorbed Cu is about 10 mg/day. Applying the 1.2 to 1.5 X safety factor, the diet should be formulated to provide between 12 and 15 mg of absorbed Cu/day. For an average

Holstein cow fed a diet without any antagonists and using Cu sulfate as the source of supplemental Cu, the diet should be formulated to contain 12 to 15 ppm of **total** Cu (i.e., basal + supplemental). If using a Cu source that has higher availability than Cu sulfate, the safety factor would be the same but because of a greater AC, the concentration of total Cu in the diet would be less because less supplemental Cu would be needed.

If antagonists are present, the NRC model will overestimate absorbed Cu supply and adjustments should be made to the AC. For an average Holstein cow fed a diet with substantial antagonists, total dietary Cu may need to be 20 to 30 ppm to provide 12 to 15 mg/d of absorbed Cu (when Cu sulfate is fed). Some specialty Cu supplements have been shown to be much less affected by antagonism (Spears, 2003) and if those products are used total Cu concentration should reflect the higher bioavailability of those products.

Adequate absorbable Cu must be fed to maintain good health in dairy cows, however excess Cu is detrimental to cows. Acute Cu toxicity can occur but of a greater concern are the effects of long term overfeeding of Cu. When cows are overfed Cu, liver Cu concentrations increase. If Cu is overfed for a short period of time (i.e., weeks to a few months) the change in liver Cu may be insignificant but when Cu is overfed for the lifetime of the animal, liver Cu concentrations can become dangerously elevated. Although Jerseys are at a higher risk of Cu toxicity because they accumulate greater amounts of Cu in the liver than Holsteins when fed the same diet (Du et al., 1996), toxicity can occur in Holsteins.

In non-lactating cows that were in good (or excess) Cu status based on liver Cu concentrations and fed diets with approximately 20 ppm total Cu, liver Cu accumulated at an average rate of 0.8 mg/kg DM per day (Balemi et al., 2010). This accumulation of liver Cu is likely similar to a lactating cow fed a diet with 20 ppm Cu. Over a 305 day lactation, a cow fed a diet with ~20 ppm Cu (without antagonists) could accumulate ~250 mg/kg DM in the liver. Over 2 or 3 lactations, liver Cu concentrations would become extremely high. Classic toxicity is thought to occur when liver Cu concentrations are >2000 mg/kg DM. Beef cattle are tolerant to extremely high liver Cu concentrations (Felix et al., 2012), and many of the studies used to establish the upper limit for liver Cu used beef cattle. However, beef cattle usually have short lifespans and may not be good models for dairy cows. Chronic copper poisoning is subclinical and can cause liver degeneration, which is evident based on liver enzyme (AST and GGT) activities in plasma (Bidewell et al., 2012). Accumulating evidence suggests problems may start occurring at much lower concentrations (500 or 600 mg/kg DM). Elevated activity of AST, and GGT can indicate liver dysfunction, and activity of those enzymes were significantly greater in heifers and bulls that had average liver Cu concentrations of 640 mg/kg DM compared with animals with average liver Cu of 175 mg/kg DM (Gummow, 1996). What may be considered acceptable overfeeding of Cu (e.g., ~15 or 20 ppm supplemental Cu) may result in problems because of the duration of the overfeeding.

Manganese

The 2001 NRC greatly reduced the requirement for Mn compared with the earlier NRC. Based on NRC (2001) most lactating cows need between 2 and 3 mg/d of absorbable Mn which based on typical DMI translates to 14 to 16 ppm of total Mn in the diet. About 70% of the calves borne from beef heifers fed a diet with about 16 ppm Mn the last 6 month of gestation expressed clinical defects directly related to Mn deficiency (Hansen et al., 2006). Using Mn balance studies in lactating cows (Weiss and Socha, 2005), we estimated that lactating cows needed to consume 580 mg of Mn to be in Mn balance (approximately 28 ppm for total dietary Mn). Lactating cows may need additional Mn is because they have high requirements for Ca and P, and those minerals can reduce absorption of Mn. As discussed above uncertainty exists and reasonable safety factors (i.e., 1.2 to 1.5 X) should be applied. For Mn, the starting point is 28 ppm and after the safety factor is applied, diets for lactating cows should have 33 to 42 ppm **total** Mn.

Selenium

Per US FDA regulations, the amount of supplemental Se in dairy cow diets cannot exceed 0.3 ppm. Fortunately, in the vast majority of situations, diets with 0.3 to 0.4 ppm total Se (basal at 0.1 + 0.3 supplemental) is adequate. Excess S (from water and diet) reduces the absorption of Se substantially (Ivancic and Weiss, 2001), however the only legal option to overcome that problem is to use a high quality Se-yeast product rather than selenite or selenate. Under normal conditions, inorganic Se provides adequate available Se to the cow. However, Se from Se yeast results in substantially greater concentrations of Se in milk and colostrum and in the newborn calf if the dam was fed Se yeast during the dry period (Weiss, 2005). Clinical measures such as mastitis prevalence or immune function have not shown any consistent differences when inorganic Se or Se yeast was fed. Because of increased transfer of Se to the fetus and into colostrum, feeding a portion of Se as Se-yeast to

dry cows is a good idea. Using Se-yeast in situations with excess S should also be considered.

Zinc

The Zn requirement in NRC (2001) for lactating cows ranges from about 110 to 260 mg of absorbed Zn/day (dependent on milk yield). Assuming typical AC and DMI, diets with 40 to 50 ppm **total** Zn should be adequate. No new data are available contradicting the current NRC recommendation. Real world antagonists for Zn are not a major concern; therefore the current requirement plus a modest safety factor (1.2 to 1.5 X NRC) for risk management is adequate. As with Cu, if you are using forms of Zn with greater bioavailability, dietary concentrations should be less than if diets are based on Zn sulfate. Suppliers of those minerals should have data on relative (usually relative to Zn sulfate) bioavailability of their products.

Conclusions

Adequate supply of trace minerals improves the health and productivity of dairy cows; excess or inadequate trace minerals have the opposite effect. The 2001 NRC requirements (or the FDA regulation) for Cu, Zn, and Se are adequate in most situations and only a modest safety factor should be applied for risk management. Because of regulations, no safety factor can be applied to Se. For most minerals, diets should be formulated for total absorbable minerals and the minerals provide by basal ingredients must be included. This also means that diets that include sources of supplemental mineral that have higher bioavailability should have lower total concentrations of trace minerals than diets based on trace mineral sulfates. For Cu, numerous antagonist exist and in those cases, diets need to provide substantially more Cu than recommended by NRC. Although many situations dictate higher concentrations of dietary Cu, be aware of excessive Cu supplementation. Overfeeding Cu for months or years can result in high liver Cu concentrations that may be negatively affecting cow health. The bottom line is to feed slightly more than adequate, but not excessive, amounts of trace minerals.

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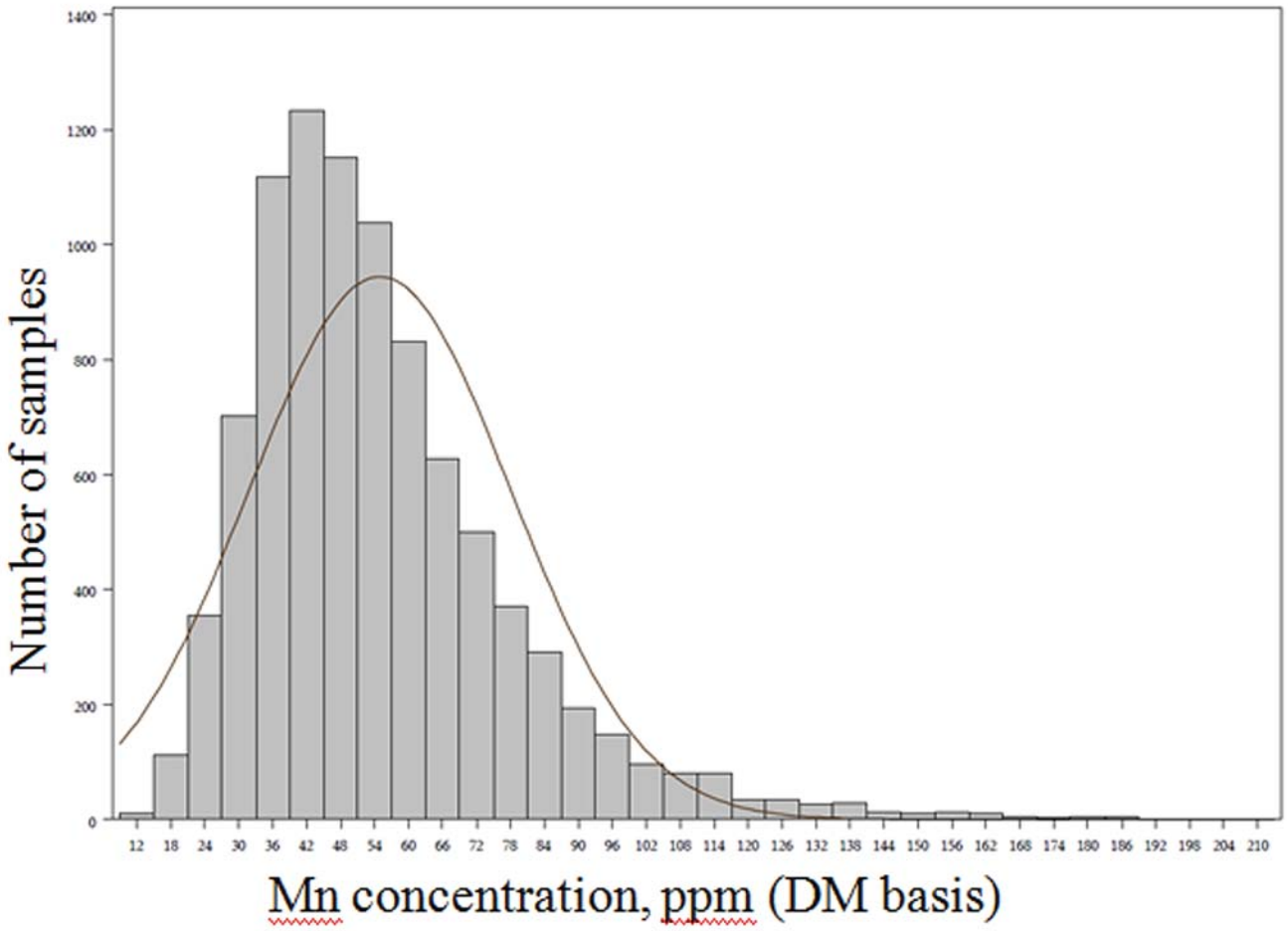
Figure 2. Distribution of Cu concentrations in mixed, mostly legume silage grown throughout the U.S. The smooth line indicates a normal distribution would while the bars indicate the actual distribution. Figure courtesy of J. Knapp (Knapp et al., 2015). Note the long tail.



What Useful Information Can We Get From a TMR Fat Analysis?

Tom Jenkins
Clemson University
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What Useful Information Can We Get From a TMR Fat Analysis?

Tom Jenkins
Animal & Veterinary Sciences
Clemson University

Compounds extracted in EE?

- Lipids
  - Nonglycerol-based
    - waxes, alkanes
  - Glycerol-based
    - Triglycerides
    - Phospholipid
    - Galactolipids
- Non-lipid
  - Water
  - Fat-soluble vitamins
  - Pigments

What can I do with a TMR fatty acid analysis?

- Make decisions about limiting fat supplements to maximize productive efficiency.
- Make decisions about managing dietary lipid to overcome milk fat depression problems.
- Verify lipid intakes when unsure about book values.
- Verify if a unique fatty acid is being fed.

2

EE vs Acid EE

	EE			
	Lab 1	Lab 2	Lab 3	Acid EE
Corn	3.1	2.7	4.0	5.8
Alfalfa	3.6	3.7	3.8	6.2
TMR	4.5	4.1	4.5	6.0
Ca Salt	1.2	2.4		85.1

Feed Fat Analysis

- Total Lipid (ether extract)
  - Includes fatty acids
  - Non-lipid contaminants
- Acid-Ether Extract
  - Extruded and high Ca fats
  - Includes fatty acids and non-lipids
- Fatty acids
  - Best predictor of animal performance

Ether Extract vs Fatty Acids

Forage	Ether Extract (%)	Fatty Acid (% of EE)
Alfalfa	3.50	2.28
Corn grain	4.23	4.03
Corn Silage	3.19	2.21

From CPM for Dairy

Fatty Acid Analysis of TMR

Dry Matter: 94.2%
Moisture: 45.8%

		As Sampled %	Dry Matter Basis %
	Fat (ether extract)	N/A	N/A
	Fat (acid hydrolysis)	N/A	N/A
	Total Fatty Acid	3.00	5.54

		Relative Basis %	Dry Matter Sample Basis %
C12:0	Lauric Acid	0.09	0.01
C14:0	Myristic Acid	3.80	0.04
C16:0	Palmitic Acid	28.47	1.30
C16:1	Palmitoleic Acid	3.47	0.03
C18:0	Stearic Acid	2.84	0.16
C18:1	Oleic Acid	25.06	1.89
C18:2	Linoleic Acid	41.80	2.32
C18:3	Linolenic Acid	4.09	0.22
C20:0	Arachidic Acid	0.53	0.03
C20:1	11-Eicosenoic Acid	0.16	0.01
C20:2	11-14 Eicosadienoic Acid	N/D	N/D
C22:0	Behenic Acid	0.90	0.02
C22:1	Erucic Acid	N/D	N/D
C24:0	Lignoceric Acid	0.42	0.02
C24:1	Nervonic Acid	N/D	N/D
	Total	100.0	5.54

There is a point of diminishing return for all fat supplements

LIMITING FAT SUPPLEMENTS

8

Responses to Whole Cottonseed

	lb WCS			
Item	*0*	*4.2*	*6.3*	*8.4*
Milk, lb/d	53.6	55.0	56.2	55.8
Fat, %	3.19	3.45	3.51	3.61

From DePeters et al. 1985. J. Dairy Sci 68:897.

Responses to Condensed Corn Distillers Solubles

	lbs CCDS		
Item	*0*	*1.2*	*2.25*
Milk, lb/d	75.0	78.1	78.8
Fat, %	3.54	3.33	3.43

From De Cruz et al. 2005. J. Dairy Sci88:4000.

Responses to Soybean Oil

	lbs SBO		
Item	*0*	*0.77*	*1.56*
Milk, lb/d	60.5	64.9	64.7
Fat, %	3.76	3.59	3.14

From AlZahal et al. 2008. J. Dairy Sci 91:1166.

Responses to Fish Oil

	lb FO			
Item	*0*	*0.64*	*1.03*	*1.35*
Milk, lb/d	69.7	75.2	71.1	60.3
Fat, %	2.97	2.79	2.37	2.36

From Donovan et al. 2000. J. Dairy Sci 83:2620.

Fat-Feeding Recommendations I've Heard

- Absolute maximum of 7% total Fat (DM basis)
  - 6% maximum preferred
- Limit Rumen-Active Fat to not more than 5% of DM
- Avoid excessive levels of unsaturated fats
- Feed up to 2% Bypass Fat (DM basis)

Rumen Unsaturated FA Load (RUFAL)

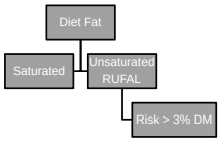
18:1 (oleic)
+ 18:2 (linoleic)
+ 18:3 (linolenic)

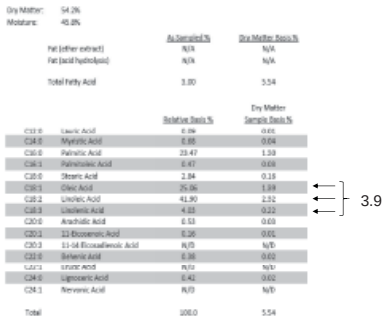
A Way to Account for The High Risk Fatty Acids

Limits to Fat Utilization by Dairy Cattle

- Metabolic Limit
  - Specific FA don't matter
  - Estimated equal to lbs milk fat produced
- Rumen Limit
  - Only UFA matter
  - Estimated equal to 4*NDF/UFA

Fat Risk Factor
Rumen Unsaturated FA Load (RUFAL)

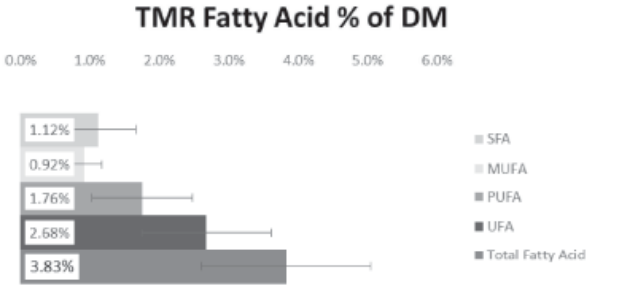




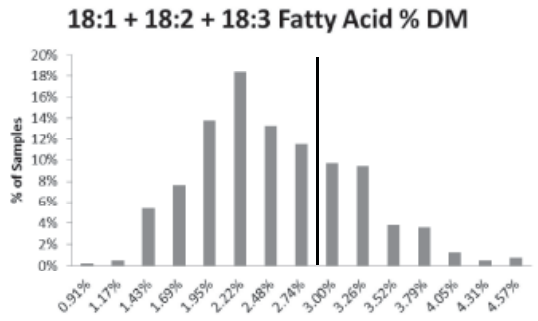
Too much unsaturated fatty acids is a classic cause

MILK FAT DEPRESSION

15



Courtesy of Kyle Taysom
Business Development Manager
Dairyland Laboratories, Inc. n=397



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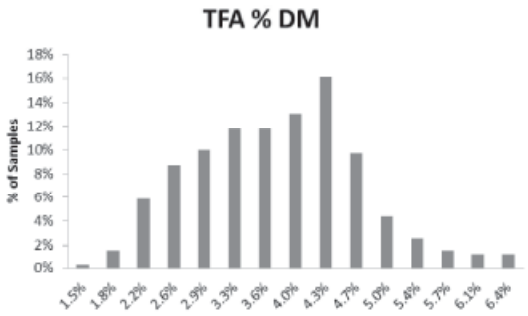

Courtesy of Kyle Taysom
Business Development Manager
Dairyland Laboratories, Inc. n=397

Reasons Why RUFAL > 3 % Might Not Correlate Well with MFD

	Why antimicrobial effects decrease
Reduced rate of lipolysis	Only FFA shift microbes
Increased rate of biohydrogenation	Only unsaturated FA have antimicrobial effects
Ca salt formation	Ca salts have little to no antimicrobial effects
Binding to feed particles	RUFAL must bind to microbial cell for antimicrobial effects
Direct uptake by microbial cell	Shields from binding to bacterial membranes

Forage FA Variation

FA, % DM	Netherlands[1]		USA[2]
	Grass Silage	Corn Silage	Corn Silage
Mean	1.9	2.0	2.5
Minimum	0.8	1.2	1.6
Maximum	3.3	3.5	3.6

[1]Khan et al., 2012 Anim Feed Sci Tech. 174: 36-45
[2]Klein, Ploetz, Jenkins, *& Lock.2013 ADSA Abstract #73*

You can't always rely on book values

VERIFY FATTY ACID INTAKES

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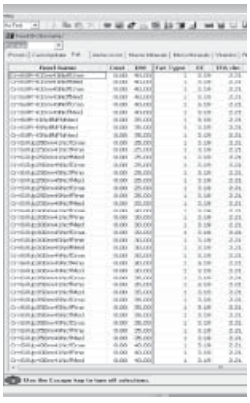
Feed Libraries – use the same fat values for all corn silages



Ingredient	1.5 % CS	3.5 % CS
Corn Silage, Med Chppd	152	349
AlfHay2 20Cp40Ndf17LNDF	26	26
CrnGrn56DryFine	139	139
Citrus Pulp Grnd	6	6
Cottonsd WLint	142	142
Megalac	48	48
Soybean ML 47.5 Solv	60	60
Other (mineral, vitamin, trace supplements)	0	0
Total	573	770

		As Sampled %	Dry Matter %
	Fat (ether extract)	N/A	N/A
	Fat (acid hydrolysis)	N/A	N/A
	Total Fatty Acid	2.2	3.99
		Relative Basis %	Dry Matter Sample Basis %
C12:0	Lauric Acid	0.10	0.004
C14:0	Myristic Acid	1.26	0.050
C16:0	Palmitic Acid	22.20	0.886
C16:1	Palmitoleic Acid	1.47	0.058
C18:0	Stearic Acid	9.94	0.397
C18:1	Oleic Acid	20.78	0.829
C18:2	Linoleic Acid	36.72	1.464
C18:3	Linolenic Acid	3.65	0.145
C20:0	Arachidic Acid	0.41	0.017
C20:1	Eicosanoic Acid	0.22	0.008
C20:2	Eicosadienoic Acid	ND	ND
C20:5w3	Eicosapentaenoic Acid	0.63	0.025
C22:0	Behenic Acid	0.26	0.010
C22:1	Erucic Acid	ND	ND
C22:6w3	Docosahexaenoic Acid	0.31	0.012
C24:0	Lignoceric Acid	0.27	0.010
C24:1	Nervonic Acid	ND	ND
Other	N/A	1.76	0.071
Total		100.0	3.987

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Predicted vs. Actual Dietary Fat Contents

Farm	Wet Chem (% DM)	Model (% DM)	Actual Difference (% DM)	% Difference
1	6.7	5.5	**1.2**	**17%**
2	7.7	6.1	**1.6**	**21%**
3	6.9	5.3	**1.6**	**23%**
4	7.2	6.0	**1.2**	**17%**
5	6.0	5.0	**1.0**	**17%**
6	5.4	5.7	-0.3	-6%
7	5.3	5.3	-	-
8	5.3	5.8	-0.5	-9%

Slide courtesy of Dr. Adam Lock

Does a product contain what it is supposed to contain?

VERIFY IF A UNIQUE FATTY ACID IS BEING FED

27

Sources of Variation in Nutrient Composition and Their Effects on Cows

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Introduction

Regardless of the sophistication of the nutritional model or software used to formulate a diet, good feed composition data is essential, and the foundation of feed composition data is a feed sample. Nutrient composition of feeds is not constant; feeds must be sampled repeatedly. The nutrient composition of diets can change because of changes in the nutrient composition of the ingredients or because of formulation changes by the nutritionist. At times ingredient composition will change unknowingly (for example, the silage being fed today came from a weedy part of the field), but at other times compositional changes may be expected (for example, a new load of hay was delivered). Ideally, a diet is reformulated to reflect a real change in the nutrient composition of the ingredients; however, if a diet is reformulated based on bad feed composition data, the diet will not have the expected nutrient profile.

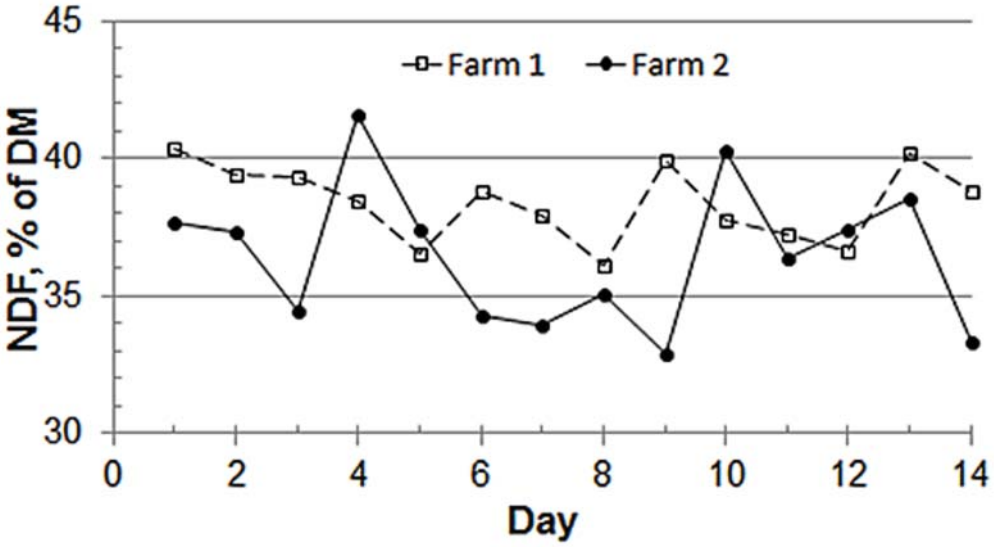
Is Sampling Error an Issue?

An ideal sample perfectly reflects the population from which it was taken. If you ground and analyzed an entire 1000 lb. bale of hay and it was 19% CP you would know the exact protein concentration of the hay (assuming the analysis was perfect), but you would have nothing left to feed. On the other hand, if you took a perfect 0.25 lb sample of hay from a 1000 lb bale and assayed it you would know the hay contained 19% CP and still would have about 1000 lbs of hay left to feed. However, if the sample was not perfect you could obtain a CP concentration of 17 or perhaps 23%. If either of those values were used to formulate the diet, the resulting diet would not contain the desired concentration of CP.

The heterogeneity of the nutrient composition of the physical components of a feed is probably the major factor related to the ability to obtain a representative sample. If a feedstuff is comprised of nutritionally uniform particles, obtaining a biased sample would in fact be extremely difficult. For example, suppose that you are sampling a container of salt that is a blend of large salt crystals and fines (salt dust). If your sample contained only large crystals or only salt dust, upon assay both samples would have about 39% sodium and 61% chloride because the individual particles of salt are nutritionally homogeneous. However, many common feeds are comprised of physical components that are extremely heterogeneous with respect to nutritional composition. Corn silage has particles of corn cob, corn grain, corn leaves and corn stalks. The different plant components are in particles of different size and shape and have different nutrient composition. Pieces of stalk and cob are high in NDF and low in starch whereas pieces of kernel are high in starch and low in NDF. The in vitro NDF digestibility (IVNDFD) differs greatly between stalk, cob and leaves (Thomas et al., 2001). If your sample had too many pieces of stalk relative to the silage (for example, small pieces of kernel and leaf fell out of your hand before you put the sample in the bag enriching the stalk portion of the sample), the IVNDFD of the sample were likely be lower than the IVNDFD of the silage. Likewise, if your sample was enriched in kernel pieces relative to the silage, your sample would have a misleadingly high concentration of starch.

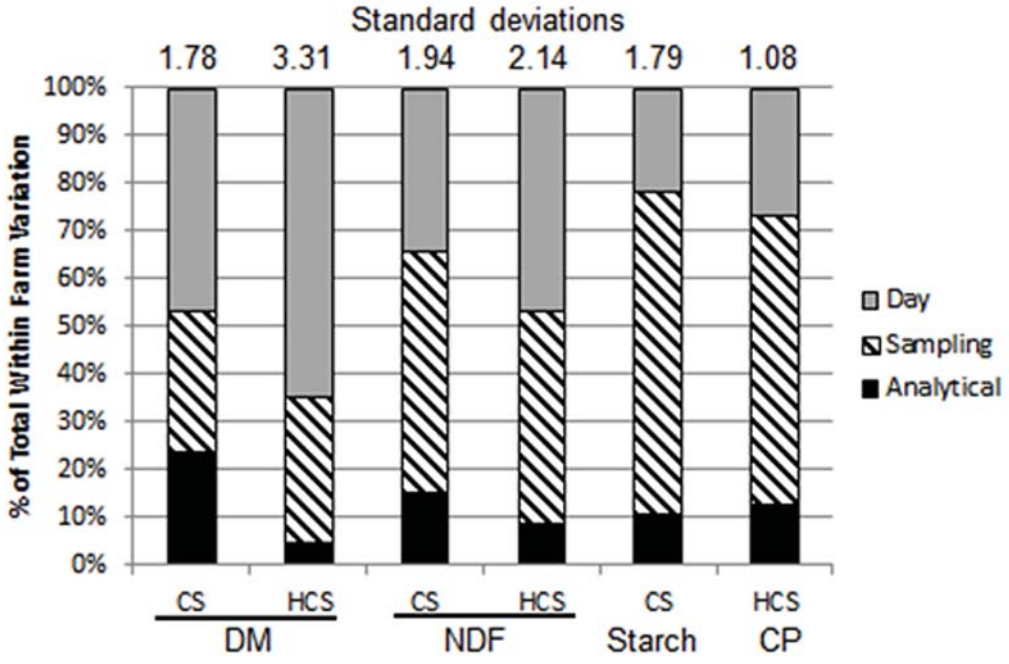
The concentrations of NDF in corn silage on two commercial dairy farms over a 14 day period are shown in Figure 1. Each data point represents a value from a single analysis of a single daily sample. From Figure 1, one could reach the conclusion that the corn silage on Farm 1 is relatively consistent with respect to NDF because its range was only 4 percentage units or about + 2 percentage units from the mean. Corn silage from Farm 2 appears much more variable (range of 10 percentage units). An alternative and just as plausible explanation to the data in Figure 1 is that the day to day variation is not caused by the silage actually changing but rather by unrepresentative samples. Perhaps the person taking the samples from Farm 1 was just a better sampler than the person taking samples from Farm 2. The usual way we sample forages does not allow separating sampling variation from real day to day variation. If you were formulating diets for Farm 2 (Figure 1) and you sampled on day 4 you would formulate a diet assuming the corn silage had 42% NDF. If you sampled again on day 14, you would reformulate the diet assuming the silage had 33% NDF. The silage may have actually changed; however, just as plausibly, the silage never changed and actually contains about 38% NDF.

Figure 1. Concentrations of NDF in corn silage from two different dairy farms over a 14 day period. Each data point represents the value from a single assay of a single sample. The coefficient of variation (CV) for Farm 1 is 3.7% and 7.1% for Farm 2.



To determine whether sampling error was a major issue in the field, we undertook a project in which corn silages and haycrop silages were sampled over 14 consecutive days on 11 farms located near Wooster OH and Ferrisburgh VT. Every day, 2 independent samples of each silage were taken on each farm. Those samples were sent to the OARDC Dairy Nutrition Lab and analyzed in duplicate using standard wet chemistry methods for DM, NDF, starch (corn silage only) and CP (haycrop only). This design (multiple farms, duplicate samples and duplicate assays) allowed us to partition the overall variation into that caused by farm, sampling, and analytical. Any variation remaining was assumed to be true day to day variation.

Figure 2. Partitioning within farm variation for corn silage (CS) and hay crop silage (HCS) with 14 daily samples and each assay duplicated by a single lab.



As expected, farm to farm variation for all measured nutrients in both corn silage and haycrop silage was the greatest contributor to overall variation. Farm contributed between about 70 and 90% of the total variation. Although farm is by far the greatest contributor to variation, it really is not that important. Large farm to farm variation means that you should not take data from corn silage or haycrop silage collected on one farm and use it to formulate diets on another farm. Most nutritionists are well aware of that. Because farm to farm variation was not of major importance, we expressed analytical, sampling and day to day variation as a percent of total within farm variation (Figure 2). Except for corn silage DM, analytical variation usually comprised 10% or less of the total within farm variation. Because the same procedure is used to measure DM in all feeds, the high analytical variation for corn silage DM was likely caused by subsampling error. The average DM concentration of the ear (cob, husk, and grain) portion of corn silage is about twice as high as the DM concentration of the stover portion of silage (Hunt et al., 1989). Overall, this data suggest that analytical (or lab) variation is not a major contributor to within farm variation. However, only one lab (a research scale lab) was evaluated. Lab variation may be more or less with other labs. Sampling variation ranged from about 30 to 70% of the total within farm variation, and it was the major source of within farm variation for NDF and starch in corn silage and CP in haycrop silage. True day to day variation ranged from about 20 to 65% of total within farm variation. It was the majority source of within farm variation only for haycrop DM concentration, but the proportion of within farm variation from day to day variation was also high for corn silage DM. True day to day variation in haycrop silage and corn silage DM is expected. The DM concentration of haycrop silage at the time of harvest can change over very short periods of time because of drying conditions. Multiple fields (with different drying rates) could be represented and moisture content can change because of precipitation during storage for both haycrop and corn silage depending on storage method. The proportion of within farm variation caused by day to day changes was also high for haycrop NDF concentration. This could be caused by multiple fields or cuttings being represented over the sampling period. Within field variation of NDF concentrations could also be high because of changing proportions of grass and legume within the field that the silage was grown.

The large contribution sampling makes to within farm variation has important ramifications for ration formulation. First, high sampling variation means that a single sample of a silage is probably not a good representation of the actual silage; multiple samples are needed to obtain an accurate nutrient description of the silage. Second, high sample variation means that very often what appears to be a change in silage composition (e.g., comparing data from a sample of corn silage taken in May to one in April) actually did not occur. A nutritionist may reformulate a diet because of an apparent change in forage composition when the silage actually did not change. This reformulation based on bad data could result in a poorly balanced diet and a loss in milk yield or perhaps increases health problems such as ruminal acidosis.

What Can Be Done About Sampling Error?

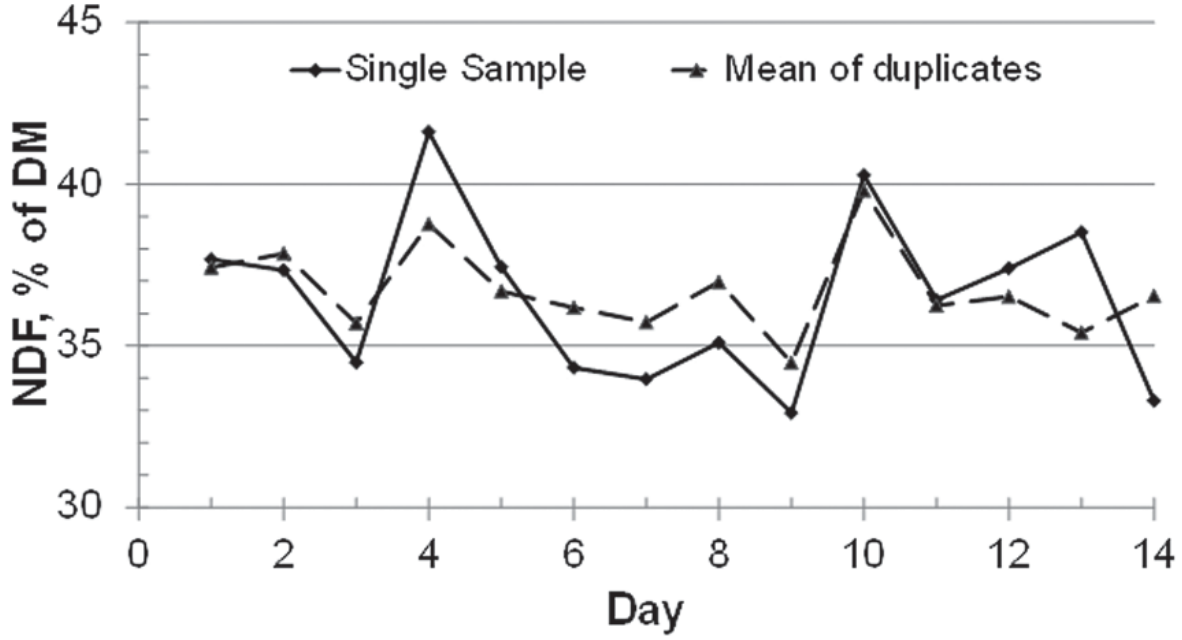
Sampling error can be eliminated by using a sampling protocol that always results in perfectly representative samples. Although this probably is an unobtainable goal, sampling techniques often can be improved which should reduce sampling error. Mix what you going to sample as much as possible before sampling. If you take a grab sample from the face of a bag of corn silage, the sample represents that specific site in the silo. However if you take several loader buckets of the silage, put it in a mixer wagon and sample that, your sample represents a substantially larger amount of silage. We sample physical components of a feed (e.g., a piece of corn cob) we do not sample specific nutrients. Therefore sampling procedures that allow for segregation of different particles will increase sampling variation if the different particles have different nutrient composition. Corn silage is arguably the most difficult feed to sample properly. It is comprised of particles that differ greatly in shape, size, density and nutrient composition. Sampling techniques that can result in an enrichment of specific types of particles include: pulling a handful of silage from a face of a bag or bunker silo. Not only should the face of a bunker silo never be sampled because of the real risk of getting killed by a silage avalanche it also can result in a biased sample. Longer pieces (usually leaves and stalks) can be stuck in the silage mass and the handful of silage you pull away will be enriched with smaller particles (likely higher starch particles). Removing a sample with your palm facing down allow smaller particles to drop away which could reduce the starch concentration of the sample and enrich its NDF concentration. Because of size and density, with movement, larger particles tend to rise to the top of a pile and small particles migrate to the bottom. Not sampling all the vertical strata of a pile could result in a biased sample.

Evaluating Sampling Techniques

A good sampling technique should reduce sampling error (i.e., the nutrient composition of repeated samples is similar) and should be accurate (sample results are similar to the true composition of the feed). Accuracy is difficult to determine because you

never know the true composition of the feed you are sampling. Sampling error, however, can be evaluated by repeated sampling. Consider developing a written standard operating procedure (SOP) for sampling. Then over a relatively short period (1 or 2 weeks) take 4 samples of the forage following your SOP, send the samples to a good lab (use a single lab) and have them analyzed for DM and NDF. On larger farms that are removing substantial amounts of silage, the repeated sampling could occur during the same day. Calculate the standard deviation (SD) and mean and then calculate the coefficient of variation (CV) by dividing the standard deviation by the mean and multiplying by 100. This process should be done on more than one of your client's farms. Based on data we collected from multiple farms, a CV of 4% or less indicates consistent sampling. If the CV you obtained is greater than 4%, make modifications to your SOP (write down the modifications) and repeat. Once you have developed good sampling techniques, occasionally test yourself by repeating this process.

Figure 3. Effect of duplicate daily sampling on reducing variation in corn silage NDF. The solid line is data from a single assay of a single daily sample. The dashed line is the mean of the sample used in the solid line plus its duplicate sample. The coefficient of variation for the Single sample line is 7.1% and 3.8% for the duplicate sample line.



The Value of Multiple Samples

Once you have developed good sampling techniques, taking multiple independent samples of the same forage still has value. For this discussion, multiple samples mean samples of the same silage taken over a short period of time (days or a few weeks). Independent means that the repeated samples are not subsamples. Using the average of repeated samples for diet formulation, rather than a single sample reduces the likelihood that a really bad diet will be formulated because of bad feed composition data. Figure 3 shows the NDF concentration of corn silage from a single farm over a 14 day period. The dashed line represents data from a single sample per day from a single assay. The range, mean, SD, and CV for that line are: 9 percentage units, 36.5%, 2.61, and 7.1%. The solid line in Figure 3 represents the mean of duplicate samples taken each day (single assay per sample). The range, mean, SD, and CV for that line are: 5 percentage units, 36.7%, 1.38, and 3.8%. Duplicate sampling had almost no effect on the overall mean but reduced measures of variation by about 50%. A single sample could have been as much as 5.2 percentage units from the overall mean; whereas the mean of duplicate samples was at most 3 percentage units from the mean. Using means of repeated samples greatly reduces the risk of a bad sample.

Does Variation Matter to a Cow?

Although sampling error is a major cause of short term variation in composition of feed ingredients and TMR, feeds do have real variation. If you have read articles or attended conferences about dairy cattle nutrition, you have likely heard or read something to the effect, "cows do better when fed a diet that is consistent day to day". Although this seems to make

sense, essentially no research has evaluated the effect of diet inconsistency on dairy cows. In the past few years we have conducted 4 studies at Ohio State to address the question, does short term variation or transient changes in diet composition affect dairy cows. We have evaluated effects of varying silage dry matter concentration (McBeth et al., 2013) and dietary concentrations of long chain fatty acids (Weiss et al., 2013), crude protein (Brown and Weiss, 2014), and forage NDF (Yoder et al., 2013). Extreme variation in concentrations of dietary fatty acids (from corn oil and distillers grains) reduced dry matter intake and milk yield but considering the degree of variation (diets changed from 4.8 to 7.0% long chain fatty acids), the effects were small. In another experiment cows were fed a diet with 16.4% crude protein (CP) or 13.4% CP every day or a diet that contained 10.3% CP for 2 days followed by a diet with 16.4% CP for 2 days over a 28 day period. The average CP concentration of the oscillating treatment was 13.4%. Milk urea nitrogen accurately reflected the oscillation in dietary protein however it had a 1 day lag. Milk yield also followed a cyclic pattern in cows fed the oscillating treatment, but average milk yield for the entire period was not significantly different between treatments (78, 76, and 74 lbs/day for cows fed the 16.4%, 13.4% or oscillating treatments). Although not statistically different, if the experiment went longer, milk yield by cows on the oscillating treatment would likely be lower. Even though milk yield was likely reduced because of variation in dietary protein concentration, the imposed variation was extreme (10.3% to 16.4% CP).

Effects of transient variation in silage dry matter

Transient changes in silage DM concentrations can occur because of weather events (e.g., unprotected silage in a bunker gets rained upon). We conducted an experiment to determine whether short terms changes in silage DM affected cows and whether as-fed rations should be adjusted to account for the short term change in silage DM (McBeth et al., 2013). One treatment was a consistent diet over the 21 day experiment that contained 55% forage (2/3 alfalfa silage and 1/3 corn silage) on a DM basis and 45% concentrate. The second treatment was the same as the first treatment except during two 3-day bouts when wetted silage was fed. Wetted silage was made by adding enough water to the mix of alfalfa and corn silage to reduce its DM percentage by 10 units. During those two 3-day bouts the wetted silage replaced the normal silage on an equal as-fed basis. Because the silage was wetter, the forage to concentrate ratio during the bouts for this treatment was reduced to 49:51 on a DM basis. During the bouts the NDF concentration was lower for this treatment and the starch concentration was higher. The third treatment was the same as the second treatment except that during the bouts the amount of as-fed forage offered was increased to maintain the same forage to concentrate ratio, and concentrations of NDF and starch (on a DM basis) as the control diet. Over the 21 day experiment, DM intake of the two wet silage treatments did not differ from the control but milk yield was higher than control for the unbalanced, wetted silage treatment (87.6 vs. 86.5 lbs./day). The increased milk yield is likely in response to the increased concentrate in the diet during the bouts. Milk yield was the same for cows fed the control or fed the diet with wetted silage that was reformulated to account for the added water. **In this experiment, cows were offered excess feed so that when the wetter diets were fed, the cows did not run out of feed.** This approach was likely the reason we did not observe any negative effects. When fed the wetted silage, as-fed intake of the cows increased immediately; this could not have happened if excess feed was not offered to the cows. As-fed intake continued to increase during the second day of the bouts and it was not until the second day of feeding wetted silage that DM intake returned to normal for those cows.

An interesting finding of this experiment, which has practical application, is the intake pattern of cows when they switched from the wetted silage back to their normal diet. The day following each bout, DM intake was higher than control. Cows appeared to consume about the same amount of as-fed feed on the day when they returned to the normal DM silage but because the diet was drier, DM intake increased compared to control. This implies that extra feed should be offered to cows when they are switched from wet silage back to the normal silage. From our study, rebalancing diets for a short term (a few days) change in silage DM is not necessary. However, increasing the amount of feed offered is probably important to maintain production, and excess feed should be offered for a day or two after the silage DM returns to normal.

Extreme Day to Day Variation in Forage Quality

Because of variation within fields, the composition of a mixed legume-grass silage can be extremely variable. This experiment (Yoder et al., 2013) was conducted to evaluate the effects of extreme daily variation in forage quality. The experiment had 3 treatments but because of space limitations, only 2 treatments will be discussed. One treatment was the control and forage quality was as consistent as possible day to day (SD for dietary concentration of forage NDF = 0.5). The second treatment (Variable) had a constant forage to concentrate ratio (same as the

control), but the ratio of alfalfa to grass varied daily in a pre-selected random pattern resulting in large variation in the concentration of forage NDF in the diet (fNDF SD = 2.0). On average, over the 21 day period, treatments were equal in percent forage, alfalfa to grass ratio, forage NDF (25%), CP, and starch.

Over the 21 day experiment, cows on the Variable treatment consumed similar amounts of DM and produced similar amounts of milk compared to the Control. Daily within cow variation in milk yield and DM intake were significantly greater for cows on the Variable treatment compared with Control. Based on other measurements we made, there are two likely reasons cows were not negatively affected by extreme daily variation in forage quality in this study. First excess feed was provided to cows every day. On days when cows were fed a high forage NDF diet, dry matter intake was reduced but then on days when lower forage NDF diets were fed, the excess feed delivery allowed cows to consume additional feed. Effects of diet variation were also probably mitigated by transient mobilization of body energy. On days when cows were fed high concentrations of grass (i.e., lower quality forage), DM intake was reduced but cows mobilized energy to maintain milk yield. On days when cows were fed a better diet (more alfalfa and less grass), cows ate more and produced more milk. This suggests that over a longer time period (this experiment only lasted 3 weeks) a highly variable diet could reduce body condition which can have long term negative impacts on reproduction and production. Long term losses in body condition is a negative, however, the very modest effects on body condition must be put in context of the extreme variation imposed in this experiment.

Conclusions

Good samples are the cornerstone of good diet formulation; however sampling error for some feeds is large. If sampling technique is poor and the uncertainty surrounding feed composition data is expressed as plus or minus several percentage units, using nutritional models that formulate diets to the tenth decimal place will not result in well formulated, consistent diets. Good SOP for sampling should be developed and followed. Multiple samples of feeds should be taken to monitor sampling variation and averages of composition data should be used rather than data from a single sample to reduce the impact of improper sampling. Although sampling is a major source of variation in diet composition, real variation does exist but substantial day to day variation in nutrient composition did not have large negative effects on cows. This may mean that a 24 hour day is not the correct periodicity for assessing variation. Some of our data suggest that a period of 2 or 3 days may be more appropriate. In other words, if nutrient composition differed between two successive 3-day periods, cows might be more likely to respond to that variation. We have some evidence that diet variation may have cumulative negative effects and that over a longer term (months), negative effects of variation may increase. A key management factor that appeared to reduce the effects of variation was ensuring cows had access to adequate feed on all days. If the diet changes and cows need to consume more feed (e.g., the diet becomes wetter) or the diet changes and the cow can consume more feed (e.g., diet changes from a higher concentration of NDF to a lower concentration), feed must be available to allow the cow to compensate. If this compensation cannot occur, the effects of variation would likely be exacerbated. Although providing excess feed may mitigate some negative effects of variation, it will also increase feed costs.

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Using the KetoMonitor to Access Cow and Milk Data to Manage Herd-Level Ketosis Prevalence

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The transition to lactation period is known to be the most challenging period in the dairy cow life cycle, specifically in terms of metabolic disorders. Hyperketonemia, or ketosis, is defined as elevated ketone bodies in the blood and is a critical challenge to transition dairy cows that has negative impacts to milk production, animal health, and profitability. Cows with ketosis produce less milk, are more likely to develop a displaced abomasum (DA), and are more likely to be culled from the herd. As with many disorders, ketosis has been historically separated into either clinical ketosis (hyperketonemia with clinical signs) or subclinical ketosis (hyperketonemia without clinical signs; SCK). Incidence of SCK ranges from 40 to 60% of cows while clinical ketosis occurs in 2 to 15% of cows. It has been demonstrated that subclinical ketosis is just as costly and detrimental to animal health as clinical ketosis, largely because it can go undetected without active testing and management protocols. Each case of hyperketonemia costs approximately $361 and $247 for cows and first-calf heifers, respectively.

Ketosis Onset and Treatment

Better understanding the tissue-level metabolism that leads to ketosis (clinical and sub-clinical throughout) onset has allowed for better understanding of disease etiology. Ketosis is an early, fresh cow disorder (onset most commonly detected within 4 to 9 days in milk) and is tightly related to energy balance at, and shortly after, calving. Decreased feed intake prior to and around the time of calving, coupled with increases in energy requirements to meet the needs of lactation, result in cows entering a state of negative energy balance (NEB) after calving. During periods of NEB, stored body fat is mobilized and transported to the liver to aid in meeting energy and glucose demands. Triglycerides (TG) mobilized from the adipose tissue are transported through the blood stream as nonesterified fatty acids (NEFA) and glycerol, and absorbed by the liver, where fatty acids are broken down for four possible fates: complete oxidation through the tricarboxylic acid (TCA) cycle, incomplete oxidation through ketogenesis, TG synthesis and packaging as very-low density lipoprotein for export from the liver, or TG synthesis for storage as liver lipids. When available acetyl-CoA exceeds the capacity of the TCA cycle, there are increases in production of ketones and deposition of TG, leading to the onset of ketosis and fatty liver syndrome.

While circulating ketones can be used to a certain extent as a fuel source by heart, brain, liver, and mammary tissue, excessive blood ketones can have negative effects. Widely accepted cutoffs for SCK are blood beta-Hydroxybutyrate (BHBA) ≥ 1.2 mmol/L and for clinical ketosis blood BHBA ≥ 3.0 mmol/L. These cutoffs have been established based on increased negative effects and increased relative risk for other diseases and complications (ex. DA, culling, decreased reproductive efficiency, lost milk production) as blood BHBA concentration increase beyond 1.2 mmol/L. Negative impacts and relative risk for other disorders are further increased, based on day of onset and blood concentration of BHBA. Cows with ketosis onset within the first week of lactation are at further increased risk for developing a DA and being culled. Additionally, increases in blood BHBA concentrations above 1.2 mmol/L increase risk for DA and culling as well as result in exponential milk losses. This highlights the importance of early detection and treatment protocols.

Historically, ketosis has been most commonly treated with intravenous dextrose. However, this treatment may not be ideal. The dose of glucose typically administered (500 mL of 50% dextrose) increases blood glucose concentrations eight times the normal concentration immediately after administration; blood glucose then returns to pretreatment concentrations within 2 hours. This elevation in blood glu-

cose initiates a regulatory cascade that begins with a 12-fold increase in insulin concentration and ends with downregulation of liver glucose production, decreased mobilization of fat stores, and decreased oxidation of mobilized NEFA within the liver. Glucose not transported into the cell during this insulin peak is excreted through the kidneys adding a risk of electrolyte imbalance. High glucose concentrations have also been linked to abomasal dysfunction, decreased mobility, and DA. The benefit of dextrose treatment lasts less than 24 hours and therefore must be repeated for sustained benefit. Decreased liver production of glucose, coupled with quick disappearance of intravenous glucose sources, results in a secondary blood glucose "crash". Thus, it is recommended that IV dextrose treatments be reserved for clinical ketosis cases, be limited to 250 mL or 50% dextrose, and always be followed by oral treatment with propylene glycol. Cows with clinical ketosis need the glucose boost provided by the IV dextrose and a 250 mL dose of 50% dextrose does not downregulate liver metabolism as severely.

In contrast to treating ketosis with intravenous glucose, propylene glycol appears to have many advantages. Propylene glycol is delivered as an oral drench and serves as a glucose precursor to the animal. In the rumen, propylene glycol is either converted to propionate or absorbed directly. Propylene glycol generated propionate and directly absorbed propylene glycol can enter the TCA cycle and gluconeogenesis to produce glucose. By providing a precursor that is still dependent on liver gluconeogenesis and TCA cycle oxidation, we are providing a fuel source without leading to a secondary "crash". Collectively, metabolism of propylene glycol provides a glucose precursor that most closely mimics glucose metabolism in a healthy cow and requires liver metabolism to be maintained, providing an optimal treatment. Glycerol and calcium propionate may also be effective oral treatments for ketosis, but have not been evaluated as fully as propylene glycol.

Application of current research regarding the negative impacts of ketosis and optimal treatment protocols to commercial dairy farm settings is absolutely dependent on accurate and practical detection methods. Ketones are transferred from blood into blood, urine, and milk and concentrations that reflect hyperketonemia in all three fluids have been defined.

On-Farm Ketosis Testing

In order to tailor a detection protocol to a farm, an approximate ketosis prevalence (percent of cows that have the disease on any one day) is needed. The average herd prevalence is between 15 and 25%; however, it is important to remember that prevalence varies by farm and within farm over time. To determine prevalence, start by testing fresh cows between 4 and 20 DIM (or a 30 to 40 cow subset of this group in larger herds) on a few separate dates to establish the prevalence. Then, multiply the herd prevalence by 2.5 to get the herd incidence (the percent of cows in the herd that get ketosis each year).

Weekly testing protocols can be adapted to each farm but should strive to monitor prevalence and to catch early cases of SCK to allow for treating cows and reducing the negative impact of the disease. For any testing strategy, sick or off-feed early lactation cows should always be promptly tested and treated as necessary. Testing cows two days a week will allow checking every cow twice between 3 and 9 days in milk, and will identify 80% of cows with SCK. An alternative testing strategy is to test one day a week, checking all cows between 3 and 16 DIM and aiming to test each cow twice which will successfully identify 70% of cases. Both testing strategies are justified in herds with a ketosis prevalence greater than 7%. If the herd's ketosis prevalence is greater than 25%, blanket treatment protocols should be considered until the underlying causes can be corrected and prevalence decreased. Work with the veterinarian and herdsman to update treatment protocols to ensure that cows are being treated appropriately, depending on disease severity.

These tests have good specificity but poor to moderate sensitivity (27 to 78%) depending on the test. Urine test strips are typically the cheapest but require obtaining a urine sample, which can be challenging. Validation of a cowside blood ketone test (Precision Xtra®) has provided a cowside test with a much better sensitivity of 95% and specificity of 94%. While this test costs more than the Ketostix urine test strips or KetoCheck powder for milk and about the same as the KetoTest milk test, it provides a highly sensitive on-farm diagnostic tool.

The KetoMonitor as a Herd Ketosis Prevalence Tool

Regardless of what type of ketone test is used, testing protocols require time and money, highlighting the need for new technologies and tools to routinely identify herd ketosis prevalence. The KetoMonitor is a tool that utilizes milk data and herd records to determine prevalence of ketosis during monthly milk testing. The KetoMonitor tool resulted from research that examined milk data and herd records in over 700 Holstein dairy cows on commercial dairies and used that information to predict blood BHBA quantified using the colorimetric laboratory assay. On the day of milk test, blood and milk samples were collected from cows between 5 and 20 DIM. Regression models were built for first lactation and 2+ lactation

cows. Cows were also separated into 5 to 11 DIM and 12 to 20 DIM reflective of the early-onset etiology of ketosis. The four models produced are able to predict blood BHBA with 85 to 90% accuracy. Although the models were originally designed to determine herd-level prevalence, the strong accuracy means that the models can also identify individual cows that are predicted as positive for ketosis. A few things to remember are that the ketosis prevalence reported is a snapshot taken on test day. Typically, the incidence, or the actual number of fresh cows with ketosis, is 2 to 2.5 times the prevalence levels found on the report. Because only cows between 5 and 20 DIM are analyzed in the KetoMonitor, only about 45 to 50% of fresh cows will be within the 5 to 20 DIM window on test day for farms that milk test every 4 weeks. The size of the farm can also influence how the KetoMonitor is reported. Herds with, on average, more the 20 cows freshening each month will be summarized using fresh cows for a single test day. Herds freshening, on average, 10 to 20 cows per month will use cows fresh reported spanning two test days, and herds with less than 10 cows fresh each month will be summarized using fresh cows reported spanning three test days.

The KetoMonitor can be used as a tool to aid in ketosis management. The KetoMonitor estimates herd ketosis prevalence on the day of milk test, guides management and nutrition decisions, alerts you when blood testing protocols should be employed, and flags changes that have had an impact on transition cow health. The KetoMonitor report is quick and easy to read. As mentioned, ketosis behaves differently by age and breed, so prevalence thresholds differ for first calf heifers (<5%), cows (<15%) and overall (<10%), therefore, KetoMonitor charts and graphs the information for both age groups and the herd's overall prevalence separately. In addition, graphs illustrate current test-day information and previous 12 months to aid in identifying trends.

Cows predicted to have ketosis on the current test day are likely in need of immediate attention, and are listed on the backside of the report. If available, their pen, lactation number, DIM, days dry and age at first calving are also recorded. These information can be helpful for treating individual cows and for identifying farm-specific common triggers for ketosis onset, which may include extended dry period or older cows (ex. greater than 4 lactations). Knowing this information can help identify criteria for monitoring certain animals closer post-calving.

When prevalence is between 7 and 25%, research shows the expense of blood testing every fresh cow twice is justified. However, when herd prevalence levels fall below 7%, time and money spent on blood testing can be saved. If herd prevalence levels exceed 25%, it is most economical to consider blanket treatment. The economics and practicality of blood testing are different across farms, but the KetoMonitor can play a valuable role in any detection protocol by providing monthly prevalence indicators. Using the KetoMonitor to quantify monthly ketosis prevalence, an informed decision on whether individual cow testing is economically justified can be made. The KetoMonitor report tracks levels over a period of 12 months, further allowing producers to recognize the impact of seasonal, forage and nutrition, and management changes on ketosis prevalence.

Summary

Ketosis is a costly, but manageable disease. KetoMonitor provides an effective way to monitor herd level prevalence. It offers a new approach to herd level testing and can be used in conjunction with blood testing. KetoMonitor provides an economical option for farms that don't always need to do blood testing, or don't have the labor to do blood testing.

Practical Strategies to Adress to Improve Foot Health

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Introduction

Lameness is a painful, costly disease that affects productivity of cows through its effect on milk production, culling and reproductive performance. In addition, lameness is also a major animal welfare concern as it is highly prevalent and more importantly recognizable by consumers.

Worldwide, clinical lameness prevalence estimates range from 20 to 30%. Estimates of the prevalence of foot lesions found at hoof trimming are much higher however, ranging from 40 to 70% of cows (Cramer et al, 2008). Types of lameness due to foot lesions can be broadly categorized into infectious (digital dermatitis heel horn erosion, foot rot) and hoof horn (ulcers, white line disease, hemorrhage). Although infectious lesions are the most common type of lesions in most herds, hoof horn lesions are far more costly due to their effects on milk production and culling.

Economic losses due to hoof horn lesions are difficult to quantify yet it is becoming apparent that cows affected with hoof horn lesions are usually cows with higher production potential and production losses start prior to a lameness diagnosis. Typical production losses for cows with hoof horn lesions range from 200-500 kg plus these cows are also at increased risk of culling. Infectious lesions, on the other hand, do not appear to have an association with long term productivity and are a source of short term inconvenience.

Fortunately for the dairy industry the knowledge exists to prevent and reduce the impact of lameness. This knowledge can be summarized into the following four success factors.

1. Low infection pressure
2. Good horn quality and hoof shape
3. Low forces on the feet
   a. Good cow comfort
   b. Good cow flow
4. Early detection and prompt effective treatment of lame cows

The implementation of these success factors requires a management approach that is similar to the dedication and approach most producers have to improving udder health.

The focus of this paper is on the process of the developing a foot health program. It will outline a foot health program that can be used to reduce the level and impact of lameness. This foot health program has 6 components and focuses on controlling the major risk factors for both infectious and hoof horn lesions. The reader will find specific recommendations absent. This is due to the fact that recommendations are farm specific and on farm particulars need to be considered.

Foot Health Programs Components:

1. Record and use lesion data from lame cow trimmings.
2. Find lame cows early and treat them quickly and appropriately.
3. Provide a housing environment that ensures cows' feet are comfortable, clean and dry.
4. Disinfect and clean cows' feet regularly.
5. Ensure cows' feet have a proper weight bearing surface through proper hoof trimming by a trained individual.
6. Minimize metabolic stresses especially nutritional and transition problems

1. Record and Use Lesion Data:

The recording and use of foot lesion data from clinically lame cows is necessary to the development of a foot health program and for its continuation. This data is necessary for the design of a good foot health program as knowledge of the type and stage of lactation of the lameness event allows the prevention program to be tailored to the specific farm instead of being created for the average dairy farm. Continued recording of foot lesion data allows for the monitoring and adjusting of the foot heath program as farm dynamics evolve.

Recording of foot lesion data starts with the person doing the hoof trimming. Ideally this person records lesions in a standardized manner to allow proper communication between the hoof trimmer and the farm's advisory team. It is equally important that

the person who identifies and treats the lame cows uses the same terms as the person doing the routine preventative hoof trimming so there is continuity in the data collected.

The recording of foot lesion data does not have to be complicated. At minimum what is recorded is the cow's ID, the date, the lesion and the treatment. Additional data on location and size of the lesion is of lesser value from a monitoring perspective and should not become an impediment to the recording of the necessary basic information. Regardless of recording method it is necessary that this data gets entered into the on-farm software to allow both cow and herd level interpretations to be made.

2. Find and Treat Lame Cows Early

The second and probably the most important part of the foot health program is to create a protocol for early detection and treatment of lame cows. It is quite likely that the dairy industry can make the biggest change in lameness prevalence by addressing the lack of detection and treatment of lameness. The primary reason to focus on the detection and treatment of lameness is to improve the well-being of the cow. Compared to a cow with either metritis, mastitis or a displaced abomasum, the time between noticing her as diseased and implementing a treatment is usually delayed considerably for the lame cow. Typical comments are: "Oh we'll see how she does in a couple of days", or "The hoof trimmer is coming in a month", or "Maybe a shot of antibiotics will fix that swollen claw". Since lameness can quickly develop into a chronic disease, early intervention will result in reduced duration of pain, quicker return to productivity and reduced chance of chronicity.

3. Clean, Dry and Comfortable

This part of the foot health program focuses on the key risk factors for both infectious and hoof horn lesions.

3.1 Clean and Dry

The organisms responsible for digital dermatitis, foot rot and heel horn erosion are anaerobic bacteria that thrive in wet and moist conditions. For this reason the major focus to control infectious foot lesions should be to ensure that the cow's feet are clean and dry. No amount of foot bathing will overcome an environment where the cow's feet are constantly coated with manure. In free stalls manure and wetness are a fact of life, but measures can still be taken to reduce exposure to wetness by ensuring proper drainage and avoiding pools of water in cow traffic areas.

Although alley scrapers are used a as labour saving device, several research studies have shown an association with increased scraping frequency and higher prevalence of digital dermatitis (Cramer et al., 2009). Therefore scraping of alleys should occur at times when cows' feet do not get coated by a "tsunami" of manure several times a day and timing of the scraper should be such that the majority of cows are not standing in the alleys when it is running. For barns with slats, alleys should also be scraped and robotic alleys scrapers are an effective way to accomplish this.

Currently, no clinical trial has been done with alley scrapers to prove the association with digital dermatitis prevalence. However, observations of feet in alley scraper barns reveal a thicker coat of manure on the front wall of the claw as opposed to manually scraped barns. This thicker coat would create a more anaerobic environment.

One of the best ways to reduce exposure to manure is to increase the amount of time cows spend lying down in a well bedded stall. A well bedded stall will serve 2 functions; entice the cow to lie in it thereby reducing manure exposure and the secondly the deep bedding will have a cleansing action on the feet.

3.2 Comfortable

Hoof horn lesions such as sole ulcers, white line lesions and hemorrhage are caused in a large part by movement of 3rd phalanx (P3) in the claw capsule. The downward movement of P3 causes compression of the corium resulting in the production of inferior horn. Depending on several factors including the duration and extent of movement by P3, different lesions can develop. The exact cause of the movement of P3 is still open for debate, but enzymes and mediators that act on ligaments and the thickness of the digital cushion are all thought to play a role.

For hoof horn lesions to develop there needs to forces acting on the corium both from the exterior and interior of the claw. This occurs when a cow is standing as there is pressure exerted on the corium by P3 and a counter pressure by the surface she is standing on.

The major risk factor that should be controlled for to prevent hoof horn lesions is standing time. Any change to cows' environment that can be made to reduce standing time is going to result in less lameness as it removes weight bearing from the corium.

This focus on cow comfort needs to go beyond the stall and needs to consider the cow's time budget to discover areas of "avoidable" standing time. A typical cow stands approximately 12 hours/day split up in 2.7 hrs for milking, 4.3 hrs for feeding 2.5 hrs for time in the alley and 2.7 hrs in the stall (Gomez and Cook, 2010). Herd level factors that influence standing time on individual farms include parlour and holding pen size, stocking density, social make up of groups, heat abatement strategies and management procedures like fresh checks and synchronizing programs.

The above factors all affect standing time and are in addition to the effects that stall design and management has on standing time. There is not enough space to address each of these factors individually in this paper. For the design a foot health program the impact of each of these factors needs to considered and if short comings are identified, additional management efforts will need to be devoted to other areas to compensate for these deficiencies.

4. Disinfect and Clean Regularly

Once we have addressed the cleanliness of the cow's feet, the reality is that most herds still require the regular use of a proper footbath to clean and disinfect feet. For most herds it is likely not the type of product used that is responsible for the lack of apparent control of infectious lesions. Even though there are few clinical studies to prove the efficacy and economics of most current foot bath products, no product will be effective if it is not used regularly and effectively. What defines regular is likely herd dependent but just like teat dipping is a standard practice twice daily, foot bathing should be standard practice daily on all free stall herds.

A good footbath protocol starts with thinking of a footbath as a preventative tool, similar to teat dipping, and not as a treatment tool. There is a role for antibiotics in footbaths as a treatment solution, however in most cases these should be short term in nature and not used on an ongoing basis.

On most farms digital dermatitis control would improve if footbaths were run more frequently. Does this mean that there needs to be disinfectant in the bath every time? Potentially, but even having a cow walk through a footbath with water alone or with a small amount of soap will have a cleansing action and over time remove the caked manure on the foot. This cleansing will result in a cleaner foot so when a disinfectant is used 3-5x/week, it will be more effective. An additional benefit to running cows through a footbath more frequently is that the footbath becomes part of the cow's routine and running a footbath does not automatically mean a longer milking time.

For a footbath to be effective we need contact time with the disinfectant and in this case more is better. One way to do this is to increase frequency of use, but the other way is to increase the number of "dips". If we consider the length of a cow and how far apart her feet are and then watch cows walk through a six foot footbath, it becomes obvious that 6 foot footbaths were meant for the cow to stand in and not to walk through. Recent work out of Wisconsin has shown that over 60% of cows get less than 2 "dips" in a 6 foot footbath (Cook, 2010 pers. comm.). Unfortunately, 6 foot long footbaths are common both in the portable and permanent concrete form. The ideal footbath is at least 8-10 feet long, narrow (20 inches) and have a minimum of 2 feet high side walls to avoid cows stepping on the side and to keep solution in the bath. Minimum water depth should be at 4-6 inches. Higher curbs at the entrance and exit of the footbath will force cows to take more steps again increasing the number of "dips". To create good cow flow through the footbath the ideal location for a footbath is not in the return lane but in the area that links the parlour to the barn. If this is not possible, then having the footbath at the very end of the return alley will allow for better cow flow out of the parlour.

Spraying the cows feet either in head locks or in the parlour is an alternative to a regular foot bath program but can quickly become a labour issue. Whether spraying or foot bathing, it is important to remember to include dry cows and heifers in the control program.

5. Proper Balanced Weight Bearing

Hoof trimming plays an important preventative role in a foot health program. In most of our current housing environments an imbalance is created between horn growth and wear. Preventative hoof trimming attempts to remove the excessive growth and redistribute the forces that occur within a cows' foot to avoid excessive pressure on the sole ulcer location. Several excellent texts exist that describe a functional trimming technique based on the method developed by Dr. Toussaint Raven. The basis of this method is to transfer weight bearing from the overgrown outside claw to the inside claw and to create a flat weight bearing surface to walk on. Unfortunately, no research exists that evaluates different trimming techniques. However, for any trimming method the goal of trimming is to prevent or treat lameness and any horn that is removed from cows' foot should meet these criteria.

Hoof trimming should only be done by trained personnel, who have knowledge of the anatomy of the foot as it is possible to do a lot of damage with improper hoof trimming. The required frequency of

hoof trimming is cow dependent but in most cases cows should be examined at least twice a year. An examination does not necessarily mean that the foot is trimmed, but twice a year a judgment is made about the length and shape of her feet. Some chronically lame cows will benefit from more frequent trimmings and if a hoof trimmer makes regular visits to a herd this becomes much easier to implement.

6. Minimize Metabolic Stress

The transition period is also a time of great metabolic stress thus in a foot health program this time period cannot be ignored. Recent work has shown increased standing behaviour in transition cows not only leads to traditional transition cow problems, but also foot lesions (Proudfoot et al., 2010) This finding provides another reason to treat transition cows properly and ensure they go through a stress free calving. Additionally, recent work from Cornell has shown that there is a relationship between body condition score, the thickness of the digital cushion and lameness rates. Although still preliminary, these findings suggest that cows that lose a lot of body fat during early lactation also lose a lot of shock absorptive capacity in their feet increasing their risk of lameness (Bicalho, et al., 2009).

Traditionally nutritional factors and nutritionists have received a lot of the blame for lameness problems in herds. Surprisingly, the evidence in the literature for a causal relationship between subclinical acidosis and lameness is very weak. Based on our current understanding of the digital cushion, suspensory apparatus and the effect that mediators and enzymes have on the tissues and structures inside the claw, the diet the cow eat is likely less important than how she eats it. Factors that increase standing time or create periods of slug feeding such as available bunk space, consistency and quality of the actual feeds and ration delivery, and behavioural factors likely play a bigger role than the actual "paper" ration.

To minimize metabolic stress and to promote proper horn growth and integrity the role of trace minerals and vitamins in a foot health program cannot be ignored. Whilst supplementing trace minerals should be considered in most herds it is important to remember that to gain the maximal benefit from these products they should be fed in the dry period and during lactation.

Conclusion

By focussing on lameness success factors the dairy industry can prevent lameness from becoming a major animal welfare issue. The implementation of this knowledge requires a dedicated management approach to foot health similar to the one that exists for udder health. The keys of this program are to detect and treat lame cows early, focus on clean, dry and comfortable feet that are regularly disinfected and evaluated, and ensure cows do not experience metabolic stresses at key periods in their lactation. Following these principles will reduce lameness levels in the dairy industry but will require a concerted effort by all sectors of the industry including producers, hoof trimmers, veterinarians, nutritionists, researchers, dairy supply companies, and contractors.

Note: This paper was adapted from a paper presented at the Western Canadian Dairy Seminar in 2010.

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Reduced Lignin Alfalfa

Dr. Dave Combs
University of Wisconsin

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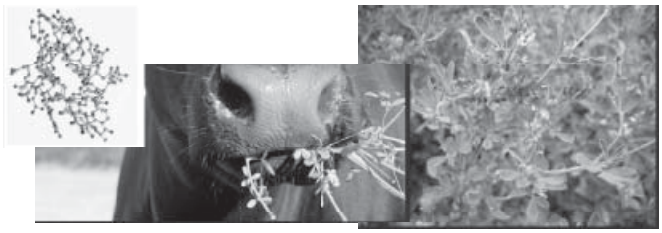
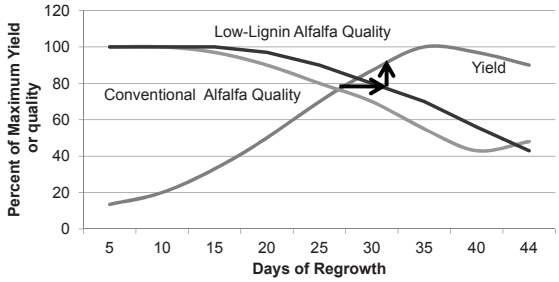


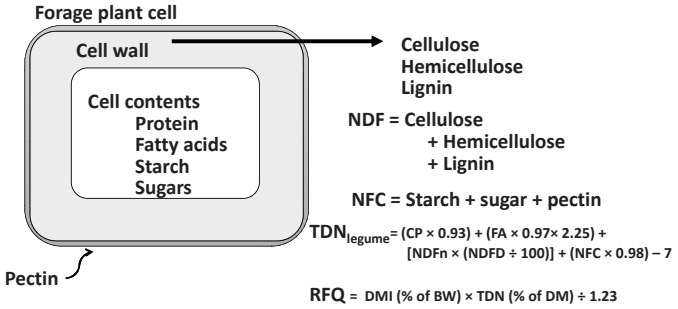
Yield and Quality Curve of Alfalfa



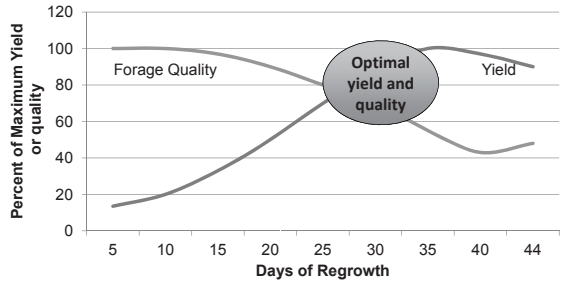
Value of Reduced Lignin Alfalfa Varieties

- Wider harvest window?
- Later harvest
  - Greater tonnage per cutting
  - Make use of full growing season
  - Reduce number of cuttings
    - a 15 to 18% lignin reduction means we could harvest 8 to 10 days later
- Improved forage quality

How Does Lignin Affect Alfalfa Quality? Carbohydrates and Lignin

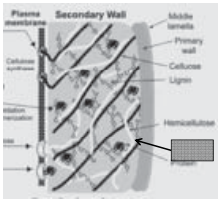


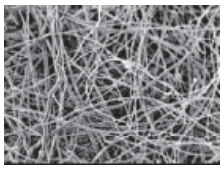
Yield and Quality Curve of Alfalfa



Cellulose

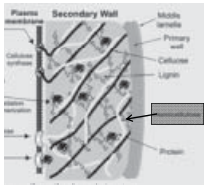
- a straight chain polymer of d-glucose: unlike starch, no coiling or branching
- Cellulose molecule has a stiff rod-like shape
- Hydrolyzed by rumen cellulase enzymes



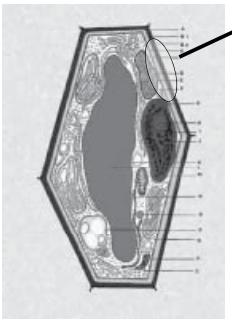


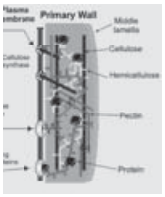
Hemicellulose

- Is a polymer of several sugars
- Has a random, amorphous structure with little strength.
- Is hydrolyzed by microbial hemicellulase enzymes.
- Lignin cross-links with hemicellulose



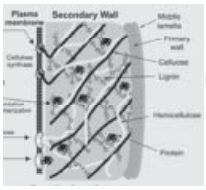
Primary and Secondary Cell Wall





Primary Cell Wall

Relatively thin-flexible membrane making up the outer layer of plant cell

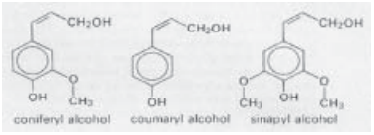


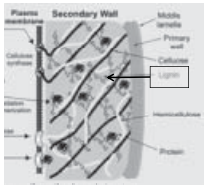
Secondary Cell Wall

Thickens as plant matures

Lignin

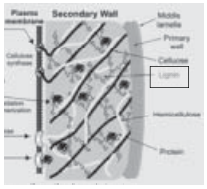
- Is a polymer of aromatic alcohols





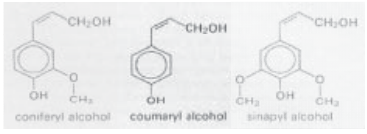
Importance of Lignin

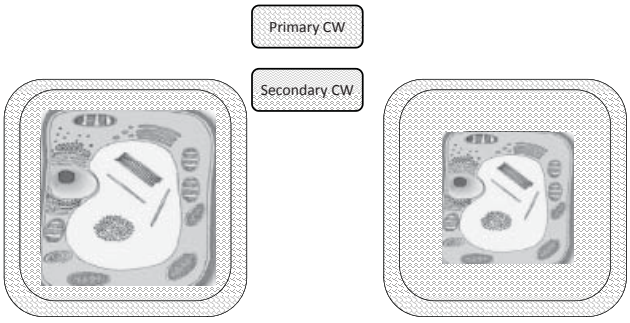
- Lignin fills spaces in the cell wall between cellulose, hemicellulose, and pectin molecules
- Lignin cross-links to hemicellulose



Plant lignins can be broadly divided into three classes

alfalfa lignin is composed principally of coniferyl alcohol units.





Vegetative Alfalfa Cell **Mature Alfalfa Cell**

Importance of Lignin to the Alfalfa Plant

- ✓Provides strength to plants
- ✓Allows the plant vascular system to transport water in the plant without leakage.
- ✓Sequesters atmospheric carbon into vegetation
- ✓Is one of the most slowly decomposing components of dead vegetation, contributing a major fraction of soil organic matter.

Alforex Introduces Hi-Gest 360

Alfalfa with Improved TTNDFD

28 Day Cut System (5 cuts)*

Alfalfa Variety	pdNDF	Dyn Kd	TTNDFD
Hi-Gest 360	73.3	7.2	55.1
Conventional Check	68.2	6.6	48.2
% Difference:	7%	10%	14%

35 day Cut System (3 cuts)*

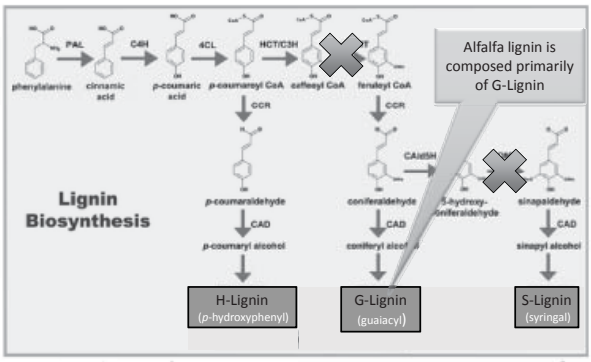
Alfalfa Variety	pdNDF	Dyn Kd	TTNDFD
Hi-Gest 360	59.1	5.9	39.3
Conventional Check	54.8	5.4	35.6
% Difference:	8%	8%	10%

Low lignin: higher fiber digestibility
TTNDFD: Tells you how fiber digestibility was improved

Composition and Digestibility of Alfalfa Changes with Maturity

	NDF	Lignin	TTNDFD	DOM
	% of DM	% of DM	% of NDF	% of DM
Immature	33	5.4	54	71
Vegetative	37	6.2	50	67
Mid-maturity	43	7.3	47	63
Mature	50	8.4	46	60

Nobel Foundation gene knockouts -low lignin alfalfa



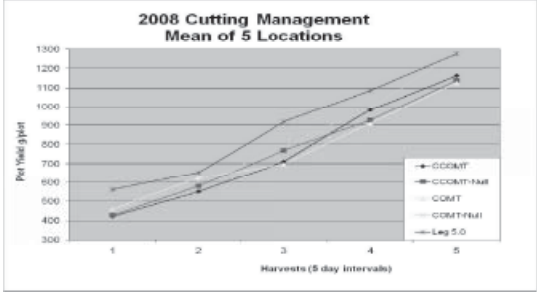
Low lignin alfalfa varieties

Company	Lignin Reduction	Unit reduction (assuming 7% lignin)
Pioneer	5%	0.35
Alforex	7 to 10%	0.49 to 0.7
Forage Genetics	10 to 15%	0.7 to 1.05

Transgenic Low Lignin Alfalfa Study

Studies were conducted at Davis, CA and Tulelake, CA, Becker, MN, Arlington, WI, and West Salem, WI.

Yield by Harvest Interval for Alfalfa with and without Genes for Down-regulation of Lignin Synthesis*

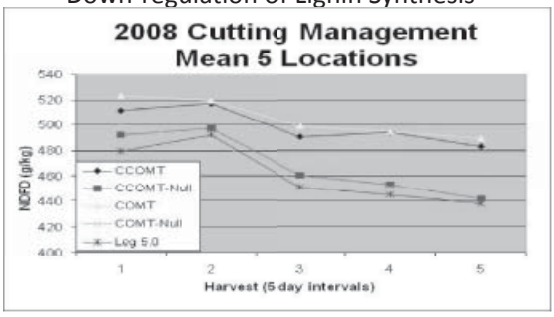


D. Undersander (UW-Madison), M. McCaslin (Forage Genetics International), C. Sheaffer (U on MN), D. Whalen (Forage Genetics International),D. Miller (Pioneer Hi-Bred International), D. Putnam (UC-Davis) and S. Orloff (UC- Cooperative Extension) **In** Proc. 2009 Western Alfalfa and Forage Conf., Published by UC Cooperative Extension (http://alfalfa.ucdavis.edu).

Effect of low lignin genes on milk production

Lactating cow responses to alfalfa hays with down- regulated lignin biosynthesis				
Alfalfa hay type[1]	CP % DM	NDF % DM	NDFD %NDF	Milk lb/day
COMT Inactive	18.1	31.1	53.5**	84.7*
COMT Active (Control)	18.4	29.3	42.5	82.1
CCOMT Inactive	18.1	42.5	48.6**	84.5
CCOMT Active (Control)	18.3	31.1	44.5	86.7
[1]TMR diets - 50 % alfalfa hay, 10 % corn silage, 40 % concentrate				
*Significant, P < 0.10; ** significant P <0.01 (different from control)				
SOURCE: Weakley et al. 2008. J. Dairy Sci. Supple. 1				

NDF digestibility for Alfalfa with and without Genes for Down-regulation of Lignin Synthesis*



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Value of Reduced Lignin Alfalfa Varieties

- Improved forage quality
- Wider harvest window?
- Later harvest
  - Greater tonnage per cutting
  - Make use of full growing season
  - Reduce number of cuttings
    - a 15 to 18% lignin reduction means we could harvest 8 to 10 days later

Effect of low lignin genes on in vivo digestibility

Digestibility of low lignin alfalfa types and controls fed to lambs, diet was 100% alfalfa hay fed ad libitum.				
100% alfalfa hay diet	aNDF % DM	ADL % DM	NDFD % NDF	DMD % DM
COMT Inactive	38.2	5.3	57.5*	67.5*
COMT Active (Control)	39.0	5.8	49.1	64.5
CCOMT Inactive	39.4	5.2	50.1	65.3
CCOMT Active (Control)	39.4	5.9	46.4	63.7
*Significant, P < 0.05				
SOURCE: Mertens et al. 2008. J. Dairy Sci. Supple. 1