Impact of Yeast Probiotics on Rumen Environment, and NDF and ADF Digestion

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Introduction

The yeast Saccharomyces cerevisiae has been used in ruminants for over 20 years. Globally, live yeast *Saccharomyces cerevisiae* promotes a better diet utilization by the animal: Julien et al. (2015) observed an increase of total organic matter (OM) digestibility in a range of 0.8 to 3.7 points for early-lactating dairy cows supplemented with probiotic yeast (Saccharomyces cerevisiae Sc47, Actisaf®, 10^{10} CFU/g, Phileo Lesaffre Animal Care, France). Regarding fiber degradation, Marden et al. (2008) stated that ACTISAF® supplementation in dairy cows suffering digestive trouble such as SARA definitely increase total fiber digestibility from 29.6% to 41.6%. Probiotic yeast effect at ruminal ecosystem level are mainly driving this impact at animal level.

Ruminal ecosystem

Ecosystem is a functional ecological unit endowed with a certain stability, constituted by a set of living organisms (the biocenosis) exploiting a given environment (the biotope or the milieu). This notion also integrates the interactions between the different species constituting the biocenosis and the interactions between these species and the environment. So there is an ecosystem whenever there is interaction between organisms and a milieu (Fonty and Chaucheyras-Durand, 2007). Therefore, the rumen is an amazing ecosystem: fermentation occurring in the rumen provide the ability to ruminants to produce human edible food. Given the high importance of the rumen fermentation, large part of nutrition research has been designed to optimize the system specifically to improve fibrolysis and microbial synthesis: a great deal of effort has been devoted to investigating methods for manipulating or "engineering" this complex ecosystem (Ungerfeld and Newbold, 2018).

Regarding rumen environment, pH has been identified as a key parameter of characterization of this milieu considering that bacteria composing the biocenosis present their own range of pH sensitivity. More specifically, ruminant depend on cellulolytic ruminal bacteria, but these bacteria cannot resist the low pH, pH sensitivity of cellulolitic bacteria dealing with alteration of enzymatic activity and/or growth (Russell and Wilson, 1996). Specifically, acidity in the reticulo-rumen during Subacute Ruminal Acidosis (**SARA**) has been clearly stated in last decades and threshold values of pH have been proposed considering that the functionality of many rumen bacteria is reduced when the pH drop below these levels (Plaizier et al., 2018). Indeed, background acid–base reactions are essential for the maintenance of all living organisms but also oxidation-reduction or redox potential ($\mathbf{E}_h$) are

also. Unlike in soil, redox potential has received little attention in rumen as pH was regarded as a master variable (Husson, 2013; Huang et al., 2018). In his review, Huang et al. (2018) put forward that in vivo measurement of ruminal E_h are scarce mainly due to the difficulties of accurate measurement. By the way, as Husson (2013) for soil, Huang et al. (2017) give evidence that E_h and pH are respectively and jointly major drivers of microorganism systems.

Redox potential for rumen environment assessment

Julien et al. (unpublished data) reported that positive E_h values recorded in a buffered sterile rumen fluid, *i.e.* deprived of any living organism, revealed oxidative conditions (+ 270 mV). On the contrary, in vivo E_h values ranged generally between −220 and −110 mV which confirmed that ruminal reducing conditions directly originated from microbial activity. Furthermore, considering that the evolution of pH with time around meal reveals ruminal metabolism, the simultaneous E_h evolution seemed to reflect the varying energetic transfers involved (Julien et al., 2010).

A meta-analysis conducted by Huang et al. (2017) gave evidence that dietary characteristics affected ruminal pH but also ruminal E_h such as NDF, starch or soluble sugars respective contents.

Even if ruminal Eh is not easy to assess in field conditions, it proved to be an endogenous parameter as meaningful as ruminal pH or fermentative profiles, allowing a different focus on rumen metabolism. As a consequence, it has been considered as a precious and interesting tool for investigations in probiotic yeast effect on ruminal ecosystem.

What about yeast impact on ruminal ecosystem

Probiotic yeast (Actisaf®) viability in rumen

It is already known that probiotic yeast is unable to colonize the digestive tract of ruminants, even if large proportion is alive. Monteils et al. (2006) demonstrated that Actisaf® numbers in the rumen, ileum and feces actually decreased to a negligible level 48h after administration of a single dose to dairy cows. However, viable cells recovered from ileal content indicated that Actisaf® could reach cow intestines alive. Actisaf® is not totally destroyed by conditions in the rumen, abomasum and duodenum and can survive in these parts of the digestive tract. Moreover, Julien et al. (2016) demonstrated that probiotic yeast ruminal concentration varied little, as demonstrated by CFU counts taken over 3 days after 18 days of daily supplementation, independently of the diet fed the cows. So it is conclusive that daily supplementation with probiotic yeast Actisaf® is essential to maintain a stable threshold concentration in the rumen ecosystem.

Probiotic yeast (Actisaf®) mode of action in ruminal ecosystem

Different hypotheses were put forward to explain the mechanisms involved in live yeast effects. Therefore, research works brought forward arguments to gain a better insight of the mode of action

of live yeast: globally, by inducing higher reducing conditions in rumen (lower oxydo-reducing potential - E_h - values) and pH stabilization, live yeast prevented accumulation of lactate and allowed better fiber digestion for ruminants receiving a diet rich in high fermentative carbohydrates (Marden et al. 2008). This thermodynamic approach of live yeast effect in rumen was unique and still contributes to have a better insight in global rumen metabolism (Marden et al., 2009; Julien et al., 2010). Indeed, the quantitative analysis of the effect of probiotic yeast (Actisaf®) on ruminal redox potential in dairy cow shows that the E_h is a physicochemical parameter of interest for understanding not only the functioning of the rumen but also the ruminal action of the probiotic yeast (Huang et al., 2016). Regulation of ruminal by living yeasts is particularly effective when the risk of ruminal dysfunction is sufficiently high.

According to the results obtained by Marden et al. (2008) who compared live yeast and sodium bicarbonate ($NaHCO_3$), the E_h confirmed to be an important parameter complementing pH measurements. It allows a better understanding of the fermentative activity of the rumen and helps to clarify the mode of action of the probiotic yeast. Supplementation with $NaHCO_3$ and live yeast had the same ability to stabilize ruminal pH after feeding but $NaHCO_3$ had smaller effects than live yeast on ruminal E_h (Figure 1), fermentation, and total tract digestibility (Figure 2).

Figure 1: Effect of Actisaf® and sodium bicarbonate on rumen physicochemical parameters in 3 dairy cows suffering from SARA and allocated in a 3x3 Latin square design (Marden et al., 2008)

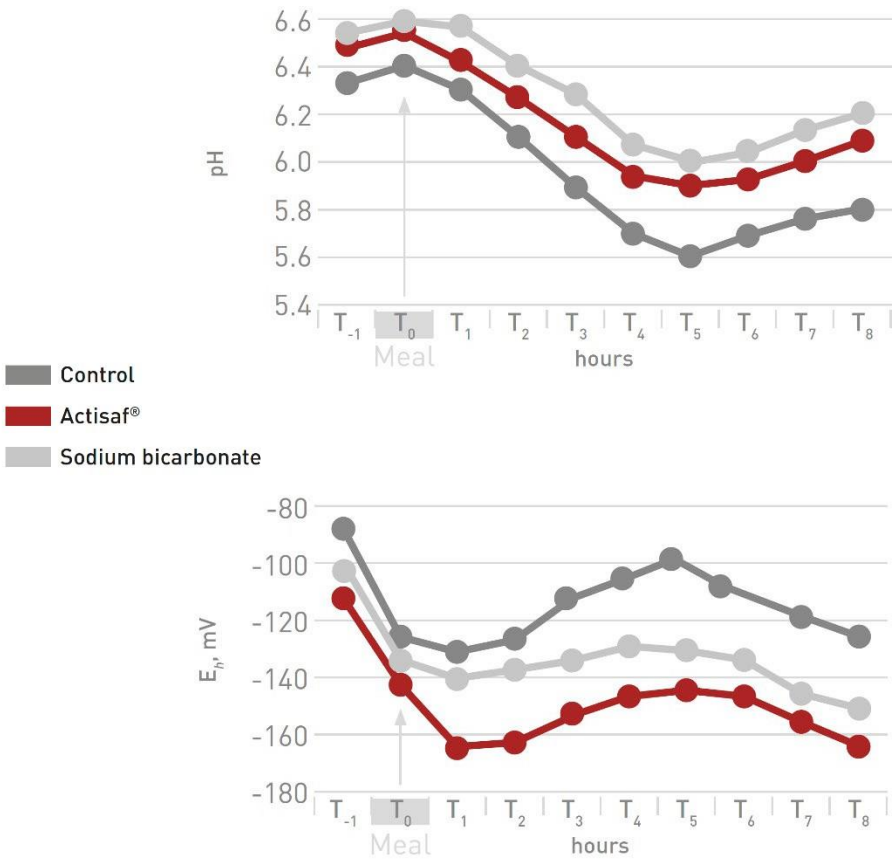
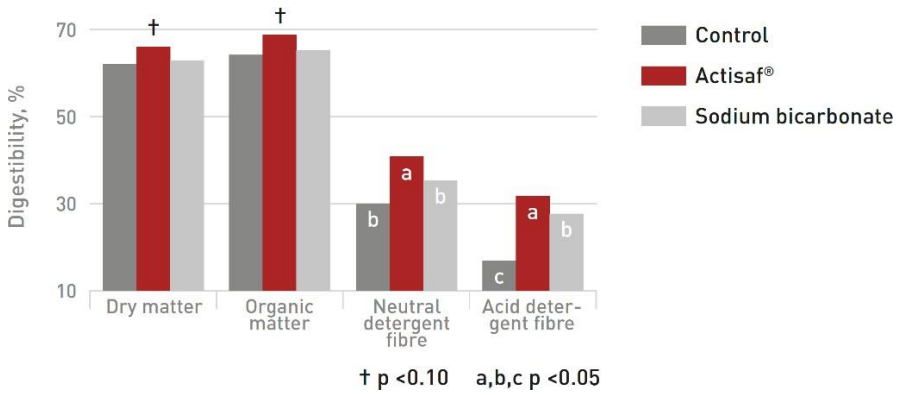


Figure 2: Effect of Actisaf® and sodium bicarbonate on total tract digestibility in 3 dairy cows suffering from SARA and allocated in a 3x3 Latin square design (Marden et al., 2008)



That clearly underlined that the mode of action of $NaHCO_3$ was only to buffer excess acid in the rumen whereas live yeast prevents the accumulation of lactate and allowed better fiber digestion by strengthening reducing conditions of ruminal environment.

In this same school of thought, Pinloche et al. (2013) verified that the effects of live yeast on physico-chemical (pH, Eh) and fermentative (volatile fatty acid and lactate) parameters were accompanied with a shift in the main fibrolytic group *Fibrobacter* and *Ruminococcus* and lactate utilizing bacteria *Megasphaera* and *Selenomonas* species. Also, Julien et al. (2012) confirming the role of probiotic yeast (Actisaf®) as potent microbiota modulator in ruminants considering that probiotic yeast supplementation might permit to decrease inter-individual variability of ruminal bacterial community suggesting a possible stabilizing effect of probiotic yeast (Actisaf®) on microbiota, at least among the 177 genus highlighted in this study.

Conclusion

Probiotic yeast Actisaf® can balance the rumen ecosytem, preventing rumen disorders and improving digestibility and performance in highly productive ruminant. Actisaf® can optimize the rumen environment by reducing the Eh, stimulating the growth and activity of obligate anaerobic bacteria such as lactate-fermenting bacteria and fibrolytic bacteria, leading to feed digestibility and animal performance improvement.

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Impact of Chronic Inflammation on the Health of Dairy Cattle, Swine, and Beef Cattle

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Introduction

There are a variety of situations in an animal's life where nutrient utilization is reprioritized from productive towards agriculturally unproductive purposes. Heat stress represents one of these annual situations where productivity is markedly reduced in all animal agriculture. Decreased feed intake experienced during heat stress is unable to fully explain decreases in productivity. Additionally, animals exposed to a thermal load exhibit altered post-absorptive metabolism, characterized by increased basal and stimulated circulating insulin. While the metabolism of heat stress has been thoroughly studied for the last 40 years, the initial insult in the cascade of events ultimately reducing productivity in heat-stressed animals has not been identified. Although certainly multifactorial, many of the negative consequences of heat stress on animal health and productivity may be mediated by reduced intestinal integrity. To that end, we have generated preliminary data strongly implicating a metabolic disruptor, endotoxin, as the etiological culprit of environmental hyperthermia. Endotoxin initiates an immune response characterized by inflammation, and both acute and chronic inflammation require a substantial amount of nutrients (amino acids and glucose).

Heat Stress

Many reports indicate the global surface temperature is expected to increase (IPCC, 2007). High ambient temperature, especially when coupled with elevated humidity, imposes severe thermal stress and reduces performance in all agriculturally important species (Baumgard et al., 2011, Baumgard and Rhoads, 2013; Belhadj Slimen et al., 2015). Heat stress interferes with animal comfort and suppresses production efficiency (Fuquay, 1981; Strong et al., 2015). Furthermore, it is well-known that genetically selecting animals based on productivity increases their metabolic heat production which makes them less heat resistant. In other words, increased production decreases heat tolerance (Brown-Brandl et al., 2004; Spiers et al., 2004). Despite advances in management practices and nutritional mitigation strategies, heat stress continues to be a financial burden. In the U.S. livestock industry, annual losses associated with environmental

hyperthermia are estimated to be nearly $900 million for dairy, $369 million for beef, and $1 billion for swine (St. Pierre, 2003; Pollmann, 2010). These economic constraints are mainly explained by the negative consequences of heat stress on productive parameters including milk yield and composition, growth, reproduction, and carcass traits (Baumgard and Rhoads, 2013).

During periods of heat stress, animals initiate major thermo-regulatory adaptations in order to maintain euthermia. It has traditionally been assumed that inadequate feed intake caused by the thermal load was responsible for decreased milk production (Fuquay, 1981; West, 2003; Strong et al., 2015). Presumably, reduced feed intake is a survival strategy as digesting and processing nutrients generates heat, especially in ruminants (i.e., thermic effect of feed; Collin et al., 2001; West, 2003). However, reduced feed intake only explains approximately 35-50% of the decreased milk yield during environmental-induced hyperthermia (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). Therefore, heat stress affects many production parameters either indirectly (i.e., via decreased feed intake; Collier et al., 2006; Adin et al., 2009; Hansen 2009; Baumgard et al., 2011, Baumgard and Rhoads, 2013; Mahjoubi et al., 2014) or directly (i.e., decreased milk yield, increased mortality). Direct mechanisms contributing to heat stress milk yield losses involve an altered endocrine profile, including reciprocal changes in circulating anabolic and catabolic hormones (Bernabucci et al., 2010; Baumgard and Rhoads, 2012). Such changes are characterized by increased circulating insulin, lack of adipose tissue lipid mobilization, and reduced adipocyte responsiveness to lipolytic stimuli. Cellular bioenergetics in the liver and skeletal muscle also exhibit clear differences in carbohydrate production and use due to heat stress. Thus, the heat stress response markedly alters post-absorptive carbohydrate, lipid, and protein metabolism through coordinated changes in fuel supply and utilization across tissues in a manner distinct from commonly recognizable changes that occur in animals on a reduced plane of nutrition (Baumgard and Rhoads, 2013). Altogether, the result of heat stress is underachievement of an animal's full genetic potential.

Heat stress and leaky gut

Mechanisms responsible for altered nutrient partitioning during heat stress are not clear; however, they might be mediated by the effects of heat stress on gastrointestinal health and function as we and others have demonstrated heat stress compromised intestinal barrier function (Lambert et al., 2002; Dokladny et al., 2006; Pearce et al., 2013; Sanz Fernandez et al., 2014). During heat stress, blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat, leading to intestinal hypoxia (Hall et al., 1999). Enterocytes are particularly sensitive to hypoxia and nutrient restriction (Rollwagen et al., 2006), resulting in ATP depletion and increased oxidative and nitrosative stress (Hall et al., 2001). This contributes to tight junction dysfunction and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013). As a result, heat stress increases the passage of luminal content into portal and systemic blood (Hall et al., 2001; Pearce et al., 2013). Endotoxin, otherwise referred to as lipopolysaccharide (LPS), is a glycolipid embedded in the outer membrane of Gram-negative bacteria, which are abundant and prolific in luminal content, and is a well-characterized potent immune stimulator in multiple species (Berczi et al., 1966; Giri et al., 1990; Tough et al., 1997). Activation of the immune system occurs when LPS binding protein (LBP) initially binds LPS and together with CD14 and

TLR4 delivers LPS for removal and detoxification, thus LBP is frequently used as a biomarker for LPS infiltration (Ceciliani et al., 2012). For a detailed description of how livestock and other species detoxify LPS see our recent review (Mani et al., 2012). Endotoxin infiltration into the bloodstream during heats tress is a common observation among heat stroke patients (Leon, 2007) and is thought to play a central role in heat stroke pathophysiology as survival increases when intestinal bacterial load is reduced or when plasma LPS is neutralized (Bynum et al., 1979; Gathiram et al., 1987). It is remarkable how animals suffering from heat stroke or severe endotoxemia share many physiological and metabolic similarities to heats tress, such as increase circulating insulin (Lim et al., 2007). Infusing LPS into the mammary gland increased (~2-fold) circulating insulin in lactating cows (Waldron et al., 2006). In addition, we intravenously infused LPS into growing steers and pigs and demonstrated > 10-fold increase in circulating insulin (Kvidera et al., 2016, 2017b,c). Interestingly, increased insulin occurs prior to increased inflammation and the temporal pattern agrees with our previous in vivo data and a recent in vitro report (Bhat et al., 2014) suggesting LPS stimulates insulin secretion, either directly or via GLP-1 (Kahles et al., 2014). The possibility that LPS increases insulin secretion likely explains the hyperinsulinemia we have repeatedly reported in a variety of heat-stressed agriculture models (Baumgard and Rhoads, 2013). Again, the increase in insulin in both models is energetically difficult to explain as feed intake was severely depressed in both experiments (Figure 1).

Leaky gut's contribution to decreased animal productivity

Distinguishing between the direct and indirect effects of leaky gut on metabolism and productivity is difficult as situations responsible for decreased intestinal integrity are highly variable in nature (i.e., heat stress, transition period). Therefore, to isolate leaky gut and evaluate its effects on metabolism, production, and inflammation, we intentionally induced intestinal permeability in otherwise healthy mid-lactation dairy cows using a gamma secretase inhibitor (GSI), a compound that specifically inhibits crypt stem cell differentiation into enterocytes via disrupting Notch signaling (van Es et al., 2005). We anticipated feed intake of GSI-administered cows would decrease, so we pair-fed controls in order to eliminate the confounding effect of dissimilar feed intake. Treatment with GSI decreased feed intake and altered jejunum morphology consistent with characteristics of leaky gut (shortened crypt depth, decreased villus height, decreased villus height to crypt depth ratio). Circulating insulin and LBP were increased in GSI cows relative to controls. Interestingly in our GSI model, acute phase proteins serum amyloid A and haptoglobin increased for both GSI and pair-fed treatments over time, indicating inflammation was occurring in pair-fed controls as well (Kvidera et al., 2017a). This is not surprising, as pair-fed controls were receiving ~20% of their ad libitum intake and decreased feed intake has been shown to increase intestinal permeability in feed restricted rodents and humans (Rodriguez et al., 1996; Welsh et al., 1998).

This is of particular relevance as suboptimal feed intake, either voluntarily (i.e., weaning, heat stress, transition period, phycological stress) or involuntarily (i.e., off-feed events, drought, shipping, overcrowding) is a common observation in animal production settings. In fact, we've repeatedly reported reduced intestinal barrier integrity in thermal neutral pigs that were pair-fed to their heat-stressed counterparts (Pearce et al., 2013; Sanz Fernandez et al., 2014). Therefore, to further elucidate the effects

of feed restriction alone, we subjected mid-lactation cows to different levels of feed restriction. Results from this study confirmed the detrimental effects of feed restriction by demonstrating a linear increase in circulating acute phase proteins and endotoxin with increasing severity of feed restriction. Furthermore, cows fed 40% of ad libitum intake had shortened ileum villous height and crypt depth, indicating reduced intestinal health (Kvidera et al., 2017d).

Ketosis and its association with leaky gut

The periparturient period is associated with substantial metabolic changes involving normal homeorhetic adaptations to support milk production (Baumgard et al., 2006; Baumgard et al., 2017). Unfortunately, a disproportionate amount of herd culling occurs before cows reach 60 days in milk (Godden, 2003). Ketosis is defined as an excess of circulating ketone bodies and is characterized by decreases in feed intake, milk production, and increased risk of developing other transition period diseases (Chapinal et al., 2012). Epidemiological data indicate about 20% of transitioning dairy cows clinically experience ketosis (BHBA > 3.0 mM; Gillund et al., 2001) while the incidence of subclinical ketosis (>1.2 mM BHBA) is thought to be much higher (> 40%; McArt et al., 2012). Ketosis is a costly disorder (estimated at ~$300 per case; McArt et al., 2015) and thus it represents a major hurdle to farm profitability. Traditionally, ketosis is thought to result from excessive adipose tissue mobilization (Baird, 1982; Grummer, 1993; Drackley, 1999) which in turn contributes to fatty liver (hepatic steatosis) and excessive ketone body synthesis (Grummer, 1993). However, our recent study demonstrated increased inflammatory markers in cows diagnosed with ketosis only and no other health disorders. When compared with healthy controls, ketotic cows had increased circulating LPS prior to calving and post-partum acute phase proteins such as LPS-binding protein, serum amyloid A, and haptoglobin were also increased (Figure 2; Abuajamieh et al., 2016). Although endotoxin can originate from a variety of locations and obvious sources in transitioning dairy cows include the uterus (metritis), and mammary gland (mastitis) (Mani et al., 2012), we believe intestinal permeability may also be responsible for inflammation observed in the transition dairy cow.

Metabolism of inflammation

LPS-induced inflammation has an energetic cost which redirects nutrients away from anabolic process that support milk and muscle synthesis (see review by Johnson, 1997, 1998) and thus compromises productivity and efficiency. Interestingly, immune cells become more insulin sensitive and consume copious amounts of glucose upon activation in order to support rapid proliferation and biosynthetic processes (Calder et al., 2007; Palsson-McDermott and O'Neill, 2013). In contrast, inflammation induces an insulin resistant state in skeletal muscle and adipose tissue (Liang et al., 2013; Poggi et al., 2007). Recent data has also demonstrated a decrease in ketone oxidation during LPS infiltration (Suagee et al., 2011; Frisard et al., 2015) which we believe may partly explain increased ketone body concentrations during the transition period.

Endotoxin has previously been recognized to be involved with metabolic dysfunction. In humans, both obesity and high fat diets are linked to endotoxemia (Cani et al., 2007, Gregor and Hotamisligil, 2011). Furthermore, LPS is involved with the development of fatty liver (Ilan, 2012), and cytokines are linked to lipid accumulation and cholesterol retention (Ma et al., 2008; Clément et al., 2008). Experimentally-induced endotoxemia in dairy cattle has been linked to several metabolic and endocrine disturbances including decreased circulating glucose, termination of pregnancy, leukopenia, disruption of ruminal metabolism, and altered calcium homeostasis (Griel et al., 1975; Giri et al., 1990; Waldron et al., 2003; Jing et al., 2014). The aforementioned pathological conditions are likely mediated by LPS-induced inflammation and the subsequent changes in nutrient partitioning caused by immune system activation.

What are the energetic costs of immune activation?

The energetic costs of immunoactivation are substantial. Upon activation, immune cells become obligate glucose utilizers and switch their metabolism from oxidative phosphorylation to aerobic glycolysis in a phenomenon known as the Warburg Effect (Vander Hiden et al., 2009). Although quantifying the energetic demand of the immune system is difficult due to its ubiquitous nature, we have recently employed a series of LPS-euglycemic clamps to calculate the energetic cost of an activated immune system. Using this model, we estimated glucose consumption by an activated immune system in lactating Holstein cows, growing steers, and growing pigs. Interestingly, on a metabolic body weight basis the amount of glucose utilized by LPS-activated immune system in lactating cows, growing steers, and growing pigs was 0.64, 1.0, and 1.1 g glucose/kg $BW^{0.75}$/h, respectively (Kvidera et al., 2016, 2017b,c). Additional data generated in two separated studies in lactating cows revealed a similar number (1.0 and 0.93 g/kg BW^{075}; Horst et al., 2018a,b), suggesting the response is conserved across species and life stages. A limitation to our model is the inability to account for liver's contribution to the circulating glucose pool (i.e., glycogenolysis and gluconeogenesis). However, both glycogenolytic and gluconeogenic rates have been shown to be increased during infection (Spitzer et al., 1985; Waldron et al., 2003). Furthermore, we have observed both increased circulating glucagon and cortisol (indirect markers of hepatic glucose output) following LPS administration (Horst et al., 2018c,d) suggesting we are underestimating the energetic cost of immunoactivation.

Conclusion

Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake: this coupling of enhanced nutrient requirements with hypophagia obviously decrease the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus). We and others have now demonstrated that both heat-stressed and ketotic animals have increased circulating endotoxin and markers of inflammation. We believe that circulating LPS in both maladies originates from the intestine and thus both likely have an activated immune system. This inflammation can redirect resources normally used for growth, milk production, muscle synthesis, and reproduction toward agriculturally unproductive purposes. More research is still needed to understand the mechanisms and consequences

of intestinal permeability and associated inflammation in order to provide foundational information for developing strategies aimed at maintaining productivity under these circumstances.

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Figures

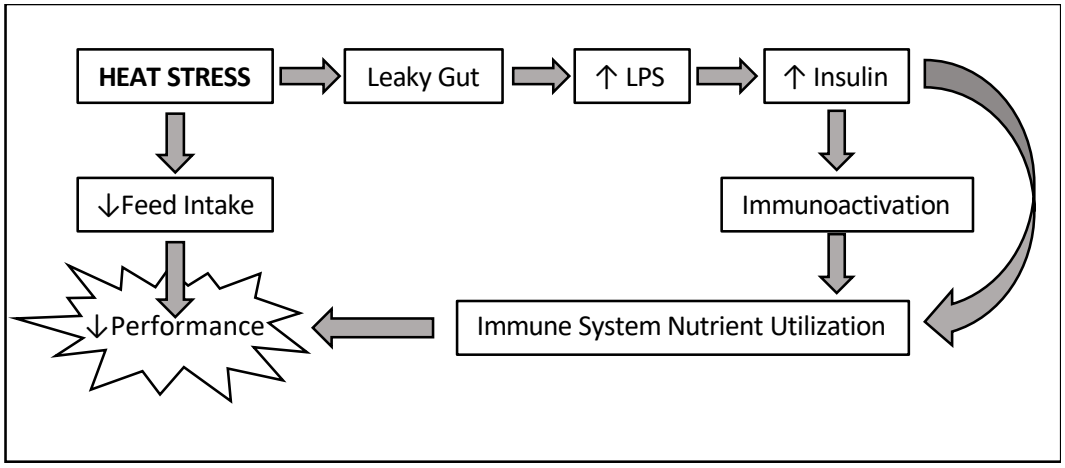

Figure 1. Consequences of leaky gut on metabolism and inflammation during heat stress.

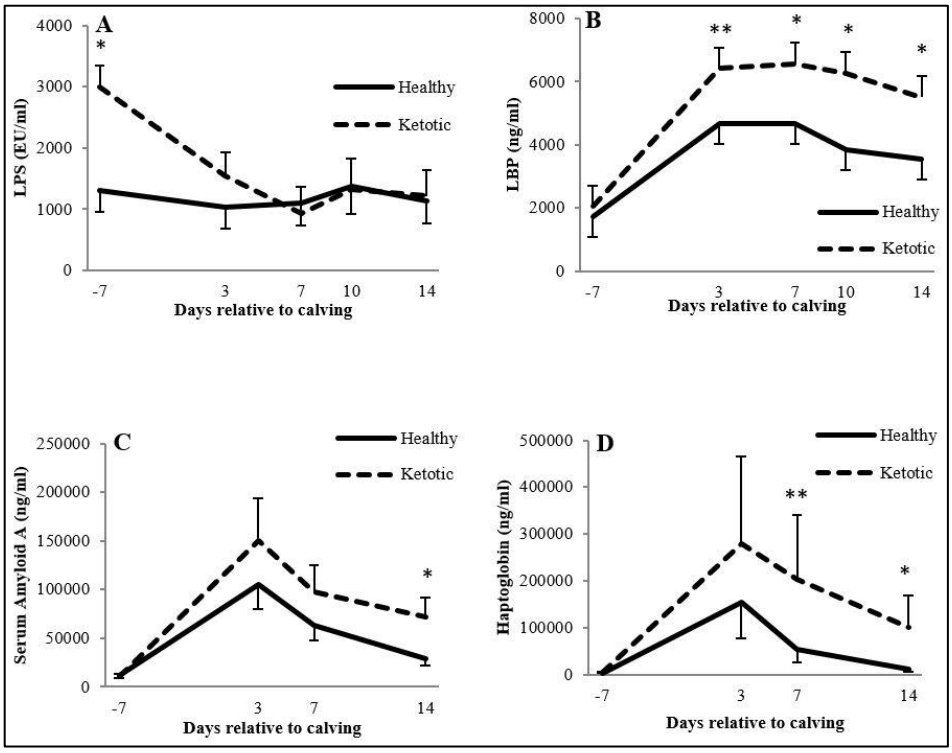

Figure 2. Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows.

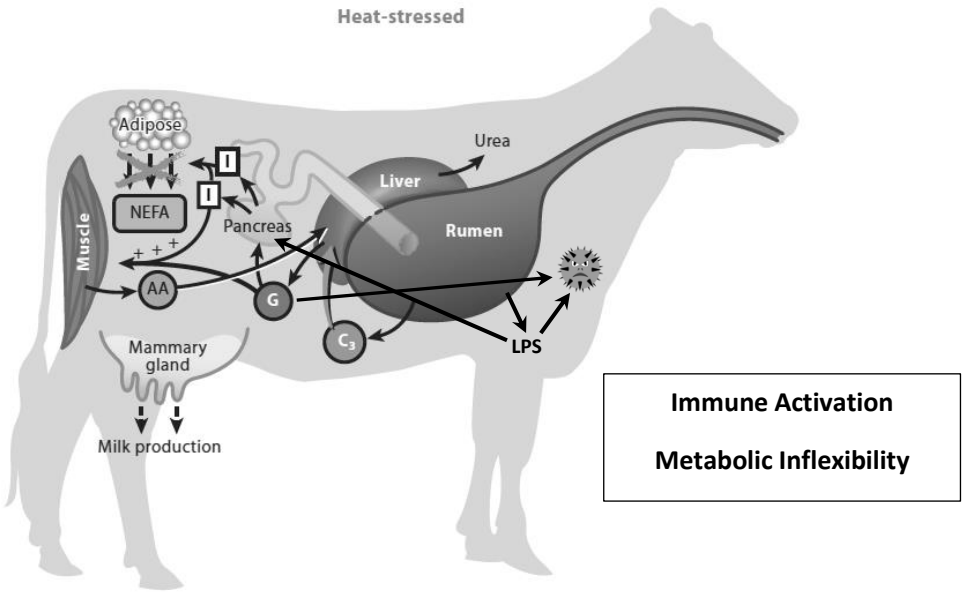

Figure 3. Nutrient partitioning in a heat-stressed lactating cow. Intestinal dysfunction resulting from heat stress results in lipopolysaccharide (LPS) translocation into portal and systemic circulation. Immunoactivation and the subsequent inflammatory increase glucose uptake by immune cells. The pancreas secretes more insulin preventing the cow from sparing glucose for milk synthesis. The heat-stressed cow enters a metabolically inflexible state characterized by minimal use of body fat reserves. Abbreviations: AA, amino acids; C_3, propionate; E, energy, G. glucose; I, insulin, LPS; lipopolysaccharide; NEFA, nonesterified fatty acid. (Adapted from Baumgard and Rhoads, 2013).

Show Me the Money! What is the Return on Investment (ROI) When Using Yeast Probiotics in Dairy Cattle Diets

Stephen M. Emanuele, Ph.D., PAS

Phileo Lesaffre Animal Care

Head, North American Dairy Program

Saccharomyces cerevisiae- the oldest industrial microbe

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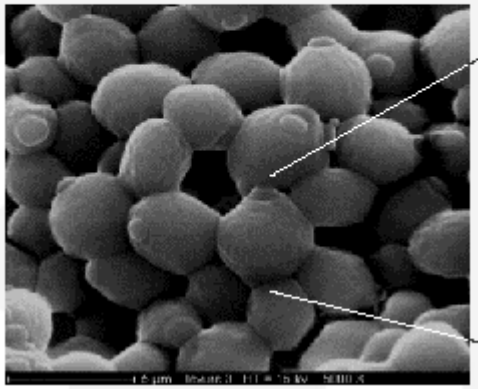
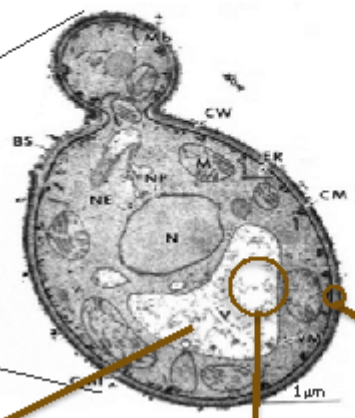
Yeast Prebiotics and Probiotics: What are they?





Probiotic is a live organism such as live yeast.

Prebiotic is a killed organism such as yeast culture or yeast cell wall.

- Whole Yeast
  - Live yeast
  - Dead yeast
    - Yeast culture
- Cellular Content
  - Yeast extract
- Yeast Cell Fractions
  - General YCW
  - Purified YCW

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Yeast Products Functionality

Live yeast	Yeast Culture	Yeast extract
Metabolically active. Optimizes the environment to enhance beneficial bacteria growth.	Nutrient source for rumen bacteria and for the animal.	Highly concentrated nutrient source. Water soluble.

General YCW	Purified YCW
Undefined structure and composition that may vary. Able to bind certain mycotoxins. Weak binding of certain bacteria	Defined structure and composition. Reliable mycotoxin binding Strong bacteria binding Immune system modulation.

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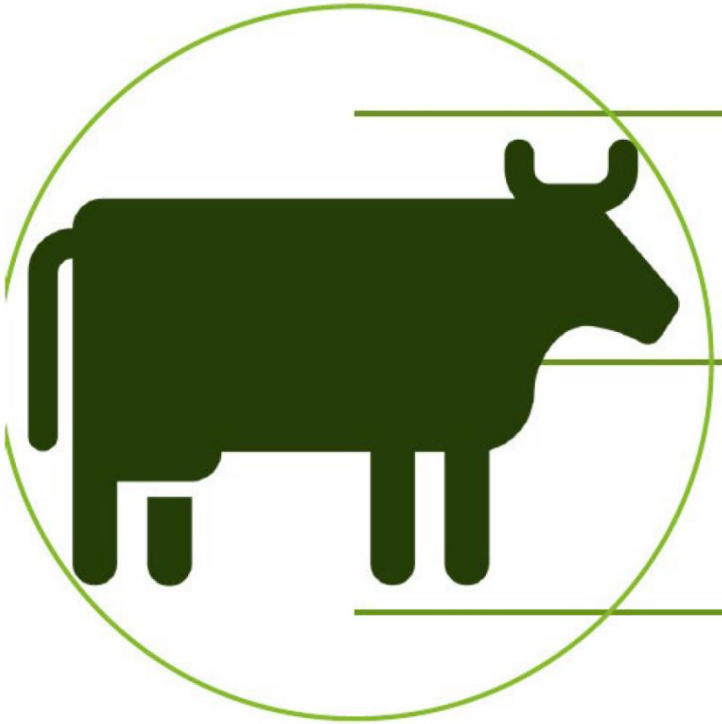
Yeast solutions: probiotic yeast



Rumen enhancer

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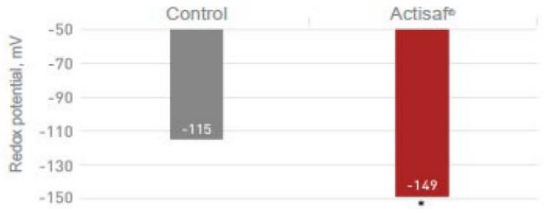
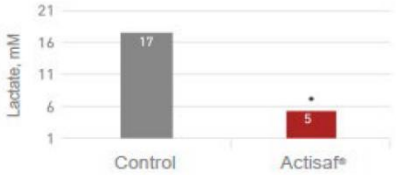
Mode of Action for Yeast Probiotics

Actisaf Action on Rumen Ecosytem Balance

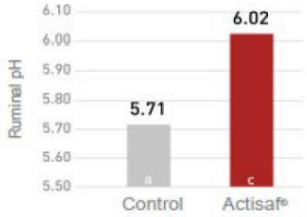
↘ Rumen Redox potential



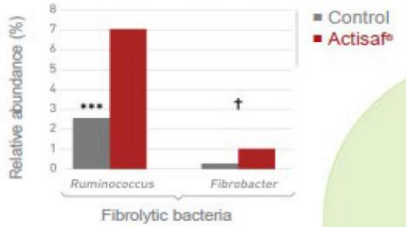
↗ Lactate-utilizing bacteria



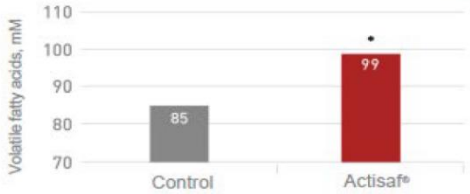
↗ Ruminal pH



↗ Fiber-digesting bacteria



↗ Volatil Fatty Acids

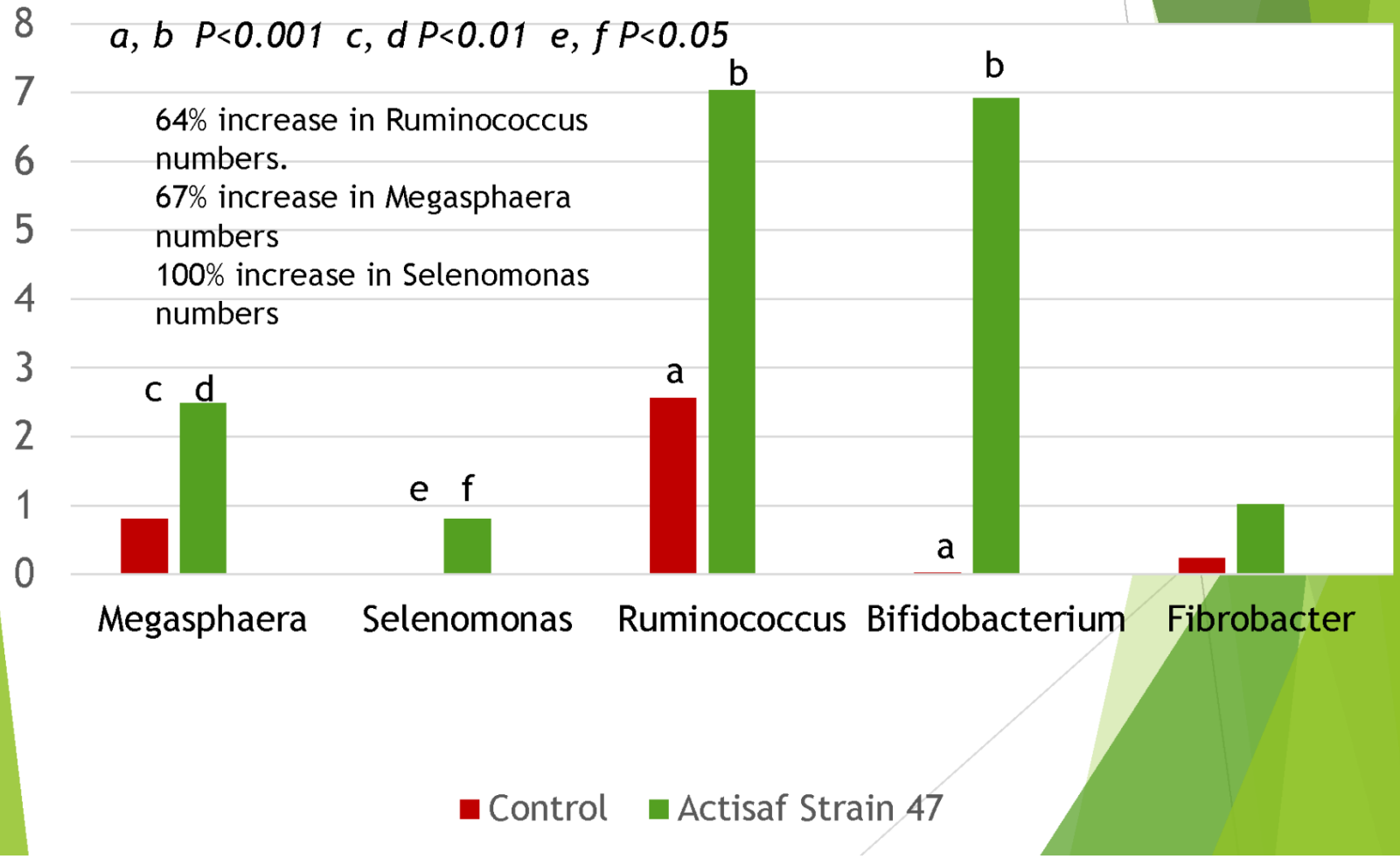


➡ **Reduced risk of acidosis**

➡ **Increased digestibility**

Impact of Actisaf Strain 47 on Microbial Species in the Rumen, % of Total Bacterial DNA

Pinloche et. al., 2013 Plos one 8(7) e6784



Mode of Action of Yeast Probiotics

- Actisaf increase feed conversion and VFA production in rumen

→ This leads to **higher glucose synthesis from the liver**

An increase of blood glucose during heat stress benefits milk production

Ivati *et al.*, 2015. Response of lactating cows to live yeast supplementation in summer. J dairy Sci.:98: 1-12

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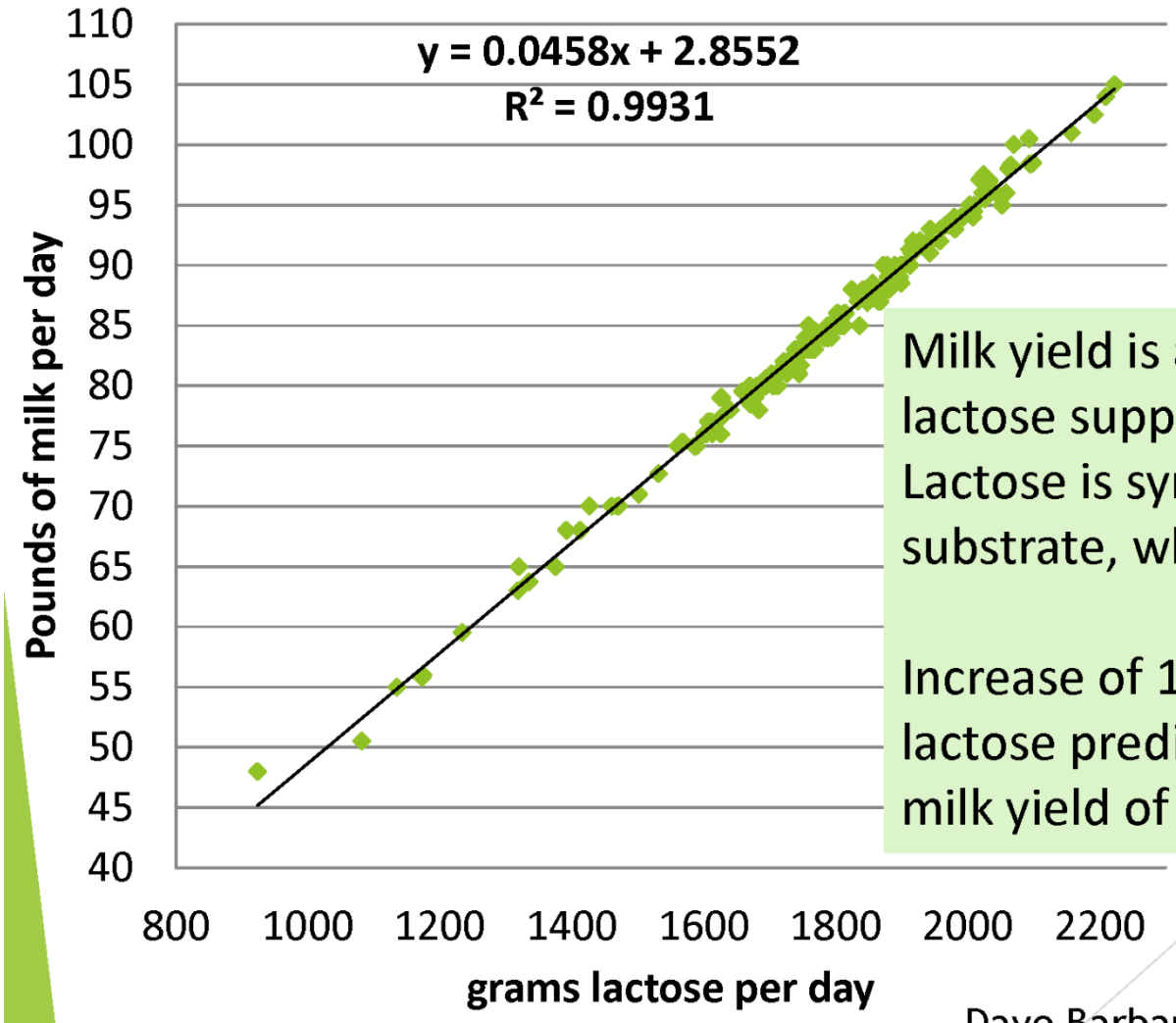
Relationship Between Lactose Yield, g/d and Milk Yield, lbs./day



Milk yield is a function of lactose supply.
Lactose is synthesized from one substrate, which is glucose.

Increase of 170 grams of lactose predicts an increase in milk yield of 7.8 pounds

Dave Barbano data set

Return on Investment (ROI)

- Inputs: Actisaf SC 47, 5 grams = 5 cents
- 4.8 lbs. more DMI = 48 cents
- Total inputs = 53 cents

- Output = 7.8 lbs. milk.
- Milk Price = $16.00/CWT.
- Output, $ = 1.25
- ROI = (1.25/0.53) = 2.4:1
- Net revenue/Cow = $0.72/day

Economic Value of Improved Fiber Digestion

More Dry Matter Intake
More Milk Components

Impact of the Yeast Probiotic, Actisaf Strain 47 on Fiber Digestion

Early Lactation Cows producing 45 kg (100 lbs.) of Milk

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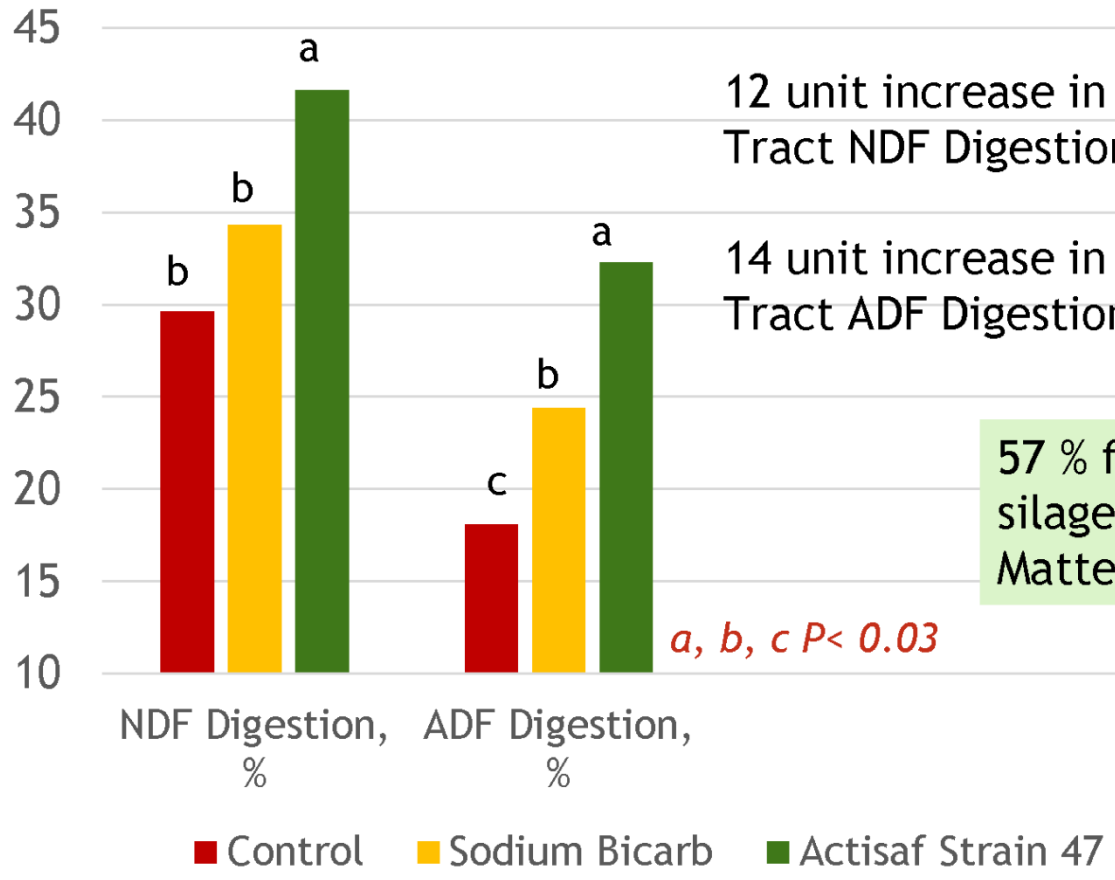
Impact of Actisaf strain 47 on Total Tract NDF and ADF digestion.



12 unit increase in Total Tract NDF Digestion

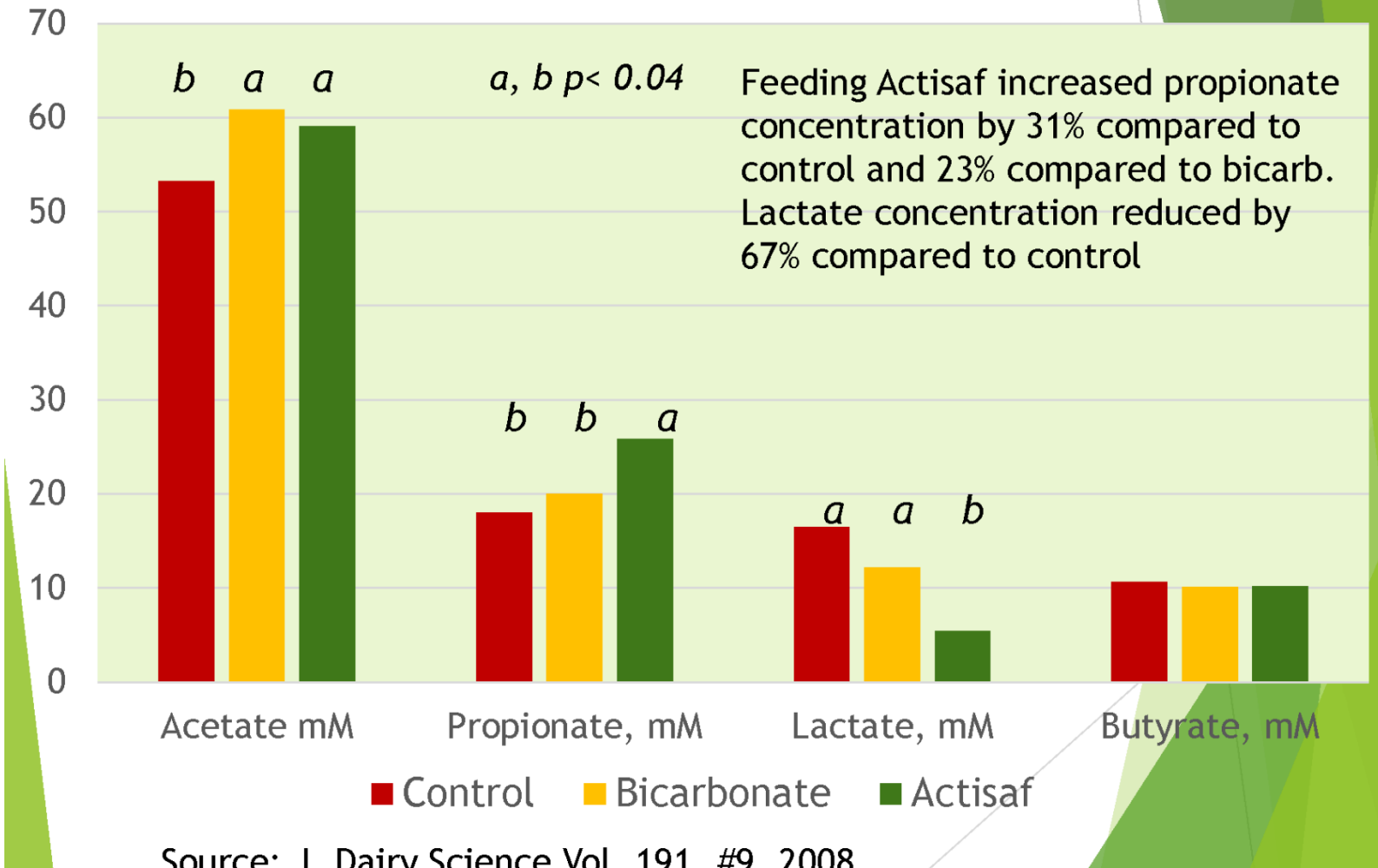
14 unit increase in Total Tract ADF Digestion

a, b, c P< 0.03

57 % forage diet, 51% corn silage, 6% alfalfa hay. Dry Matter intake = 62 pounds.

Source: J. Dairy Science Vol. 191, #9, 2008.

Impact of Actisaf strain 47 on Rumen VFA Concentration



Source: J. Dairy Science Vol. 191, #9, 2008.

Each one unit increase in NDF digestibility increases dry matter intake by 0.17 kg

- 12 unit increase in NDF Digestion
- 2 kg or 4.4 pound increase in DMI.
- Inputs - 5 grams Actisaf SC 47 = 5 cents
- Inputs - 4.4 lbs. DMI = 44 cents
- Total Inputs = 49 cents

- Outputs - 3 kg (6.6 lbs.) 4% FCM
- 6.6 lbs. x $16.00/CWT. = $1.06
- ROI = (1.06/49) = 2.2:1
- Net revenue/Cow = (1.06-0.49)= $0.57

Impact of Yeast Probiotic (Actisaf strain 47) on Performance of Lactating Cows

Journal of Dairy Science (2009). 92:343-351

21 Holstein cows per Treatment
114 DIM ± 54
Trial conducted mid-July through mid-Oct.in Israel.
Cows cooled 5 times per day, 30 minutes of cooling each session.
Individual cow feed intake with milk yield and composition.(Afimilk)
90 day trial

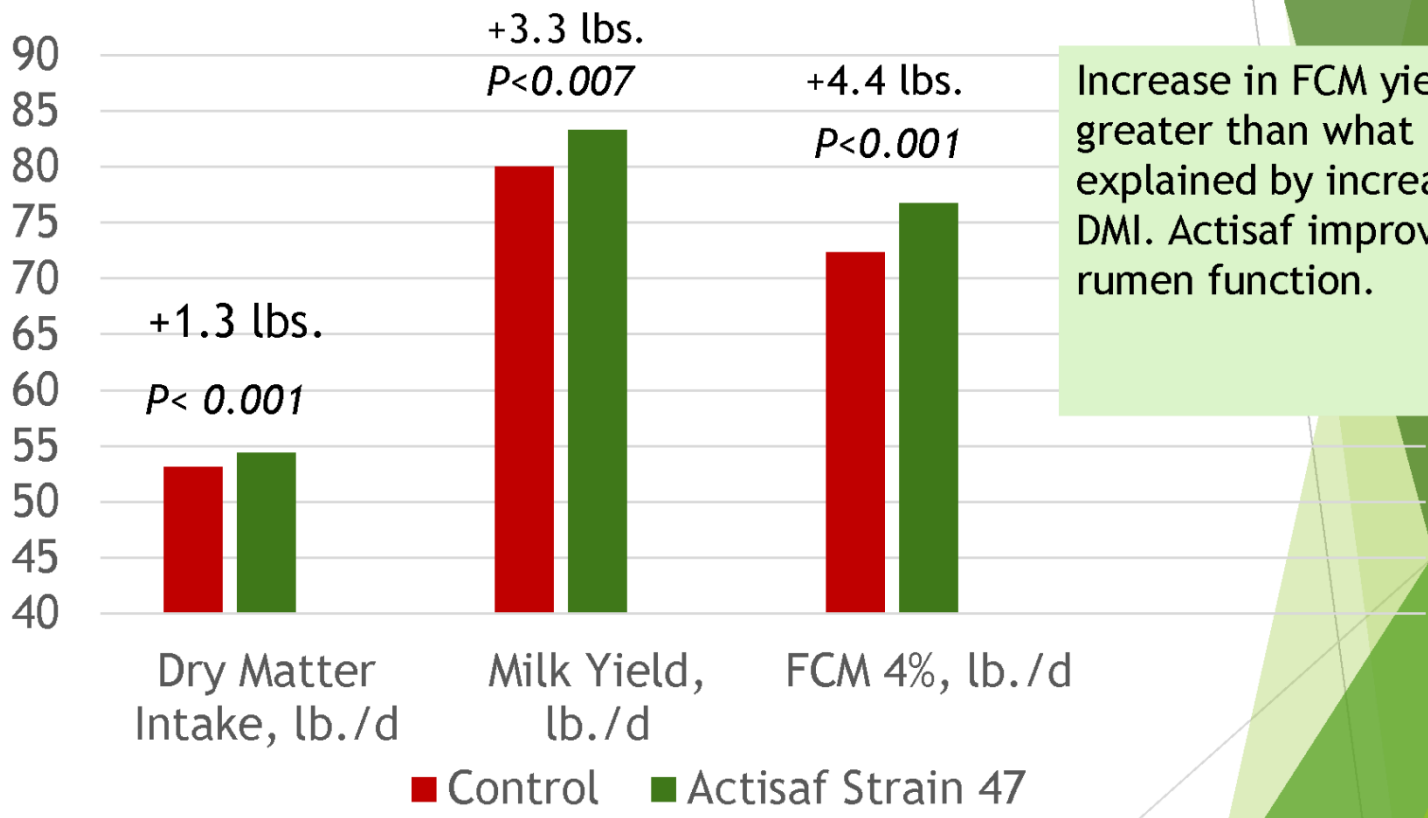
Cows housed in shaded lots with access to unshaded area.

Experimental Treatments and Diets

- Cows fed a TMR once daily.
- Yeast Probiotic mixed into 100 grams of ground corn and mixed into TMR.
- 2 treatments, control and Actisaf (6 grams/cow/day)

Chemical Composition of TMR	
NEL, mcal/lb. DM	0.79
Crude Protein, % DM	16.50
Forage NDF, % DM	17.2
NDF, % DM	31.7
ADF, % DM	16.1
RDP, % DM	11.4
RUP, % DM	5.1

Impact of Yeast Probiotic on Dry Matter Intake and Milk Yield



Increase in FCM yield greater than what can be explained by increased DMI. Actisaf improved rumen function.

Impact of Yeast Probiotic (Actisaf strain 47) on Milk Composition

Variable	Control	Actisaf (SC 47)	P value
Milk Fat, %	3.47	3.63	0.15
Milk Protein, %	3.20	3.24	NS
Milk Lactose, %	4.86	4.91	0.02
Milk Fat Yield, lb./cow	2.80	3.00	0.03
Milk Protein Yield, lb./cow	2.58	2.69	0.12 (trend up)
Lactose Yield, grams/cow	1811	1889	0.15
FCM 4% / kg. DMI	1.36	1.41	0.03

Greater lactose percent with greater lactose yield on yeast probiotic treatment indicates that more glucose was reaching the mammary gland.

Yeast Probiotics (Actisaf): Economic Analysis
Show Me the Money !!!

INPUTS	Cost/Cow
Actisaf 5 grams/cow	5 cents
Additional Dry Matter Intake (+1.3 lbs. at 10 cents/lb.)	13 cents
Total Inputs	18 cents
Outputs	
0.20 lb. of additional milk fat $2.53/lb.	51 cents
0.11 lb. of additional milk protein, $1.36/lb.	15 cents
ROI (66/18)	3.7:1
Net revenue/cow, $ (0.66-0.18)	0.48

Easy way to cover the cost of the Actisaf.

Option 1: replace Diamond V yeast culture with 5 grams of Actisaf.

Option 2: replace 0.33 pounds of bicarb with 5 grams of Actisaf.

Impact of Yeast Probiotic (Actisaf strain 47) on Performance of Lactating Cows

United Kingdom Trial

80 Holstein Cows per Treatment
Control or Actisaf SC 47

Actisaf SC 47 - 4.9 grams/cow

Dry Matter Intake on Control Diet = 48.5 lbs.

47% forage diet.

26% Corn silage, 15% Grass silage, 5.5% chopped straw

23% Starch + Sugars

Impact of Actisaf SC 47 on Milk Yield and Milk Components.

	Control	Actisaf	SEM	P Value
Milk Yield, lbs.	84.8	89.0	1.02	<0.05
Fat, %	3.67	3.87	0.17	NS
Fat Yield, lbs.	3.11	3.44		
Protein, %	3.11	3.15	0.27	NS
Protein Yield, lbs.	2.64	2.80		
Total Fat + Protein, lbs.	5.75	6.24		

Yeast Probiotics (Actisaf): Economic Analysis
Show Me the Money !!!

INPUTS	Cost/Cow
Actisaf 5 grams/cow	5 cents
Additional Dry Matter Intake (+2.5 lbs. at 10 cents/lb.)	25 cents
Total Inputs	30 cents
Outputs	
0.33 lb. of additional milk fat $2.53/lb.	83.5 cents
0.16 lb. of additional milk protein, $1.36/lb.	21.8 cents
ROI (1.05/0.30)	3.5:1
Net revenue/cow, $ (1.05 -0.30)	0.75

Easy way to cover the cost of the Actisaf.

Option 1: replace Diamond V yeast culture with 5 grams of Actisaf.

Option 2: replace 0.33 pounds of bicarb with 5 grams of Actisaf.

Somatic Cell Counts and Milk Economics

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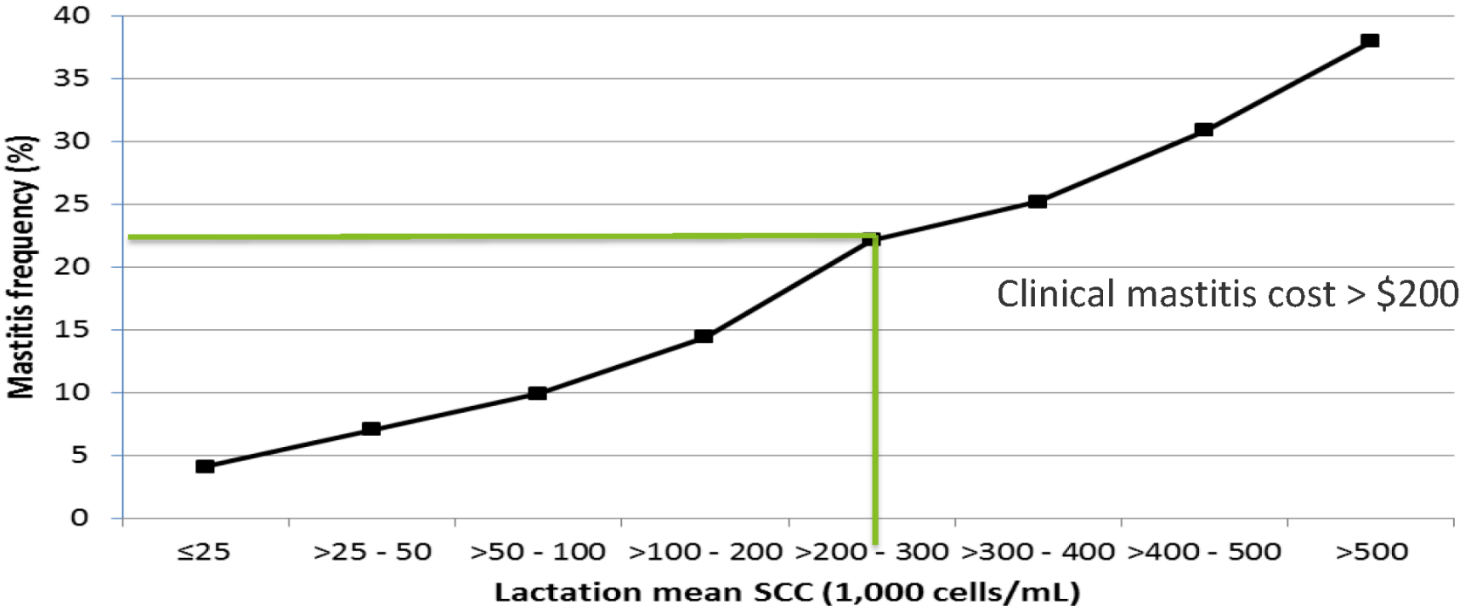
SCC economic impact

- SCC ≥ 200 000 = subclinical mastitis
  - High risk of clinical mastitis



Association between lactation mean somatic cell count (5 to 305 DIM) and mastitis frequency.

*A. Koeck et. Al. University of Guelph.

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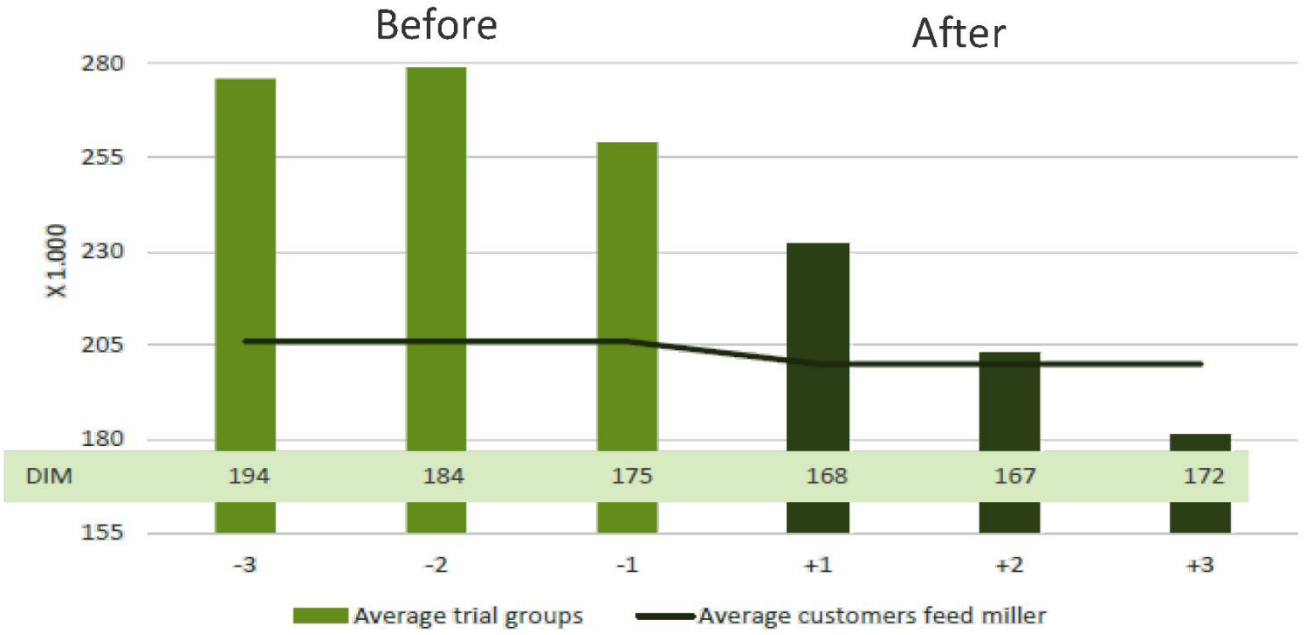
Safmannan – Health through Nutrition



	Before Safmannan supplementation	After Safmannan supplementation	Difference in %
Average trial groups	271	205	- 23.7 %

Field trial, The Netherlands, 8 dairy farms. 2015.
Actisaf dose - 5g/c/d
Safmannan dose – 10g/c/d

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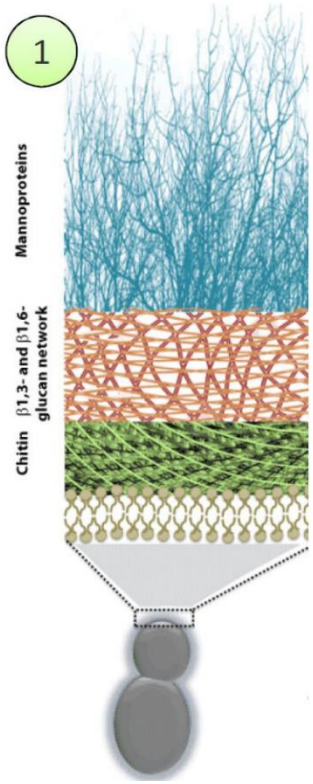
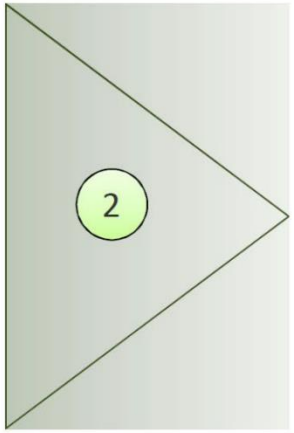
Safmannan – Health through Nutrition

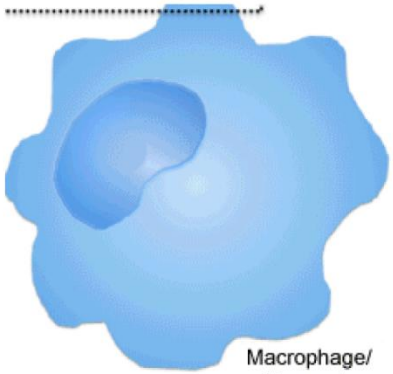


YCW - Safmannan

Recognition



Immune cell activation



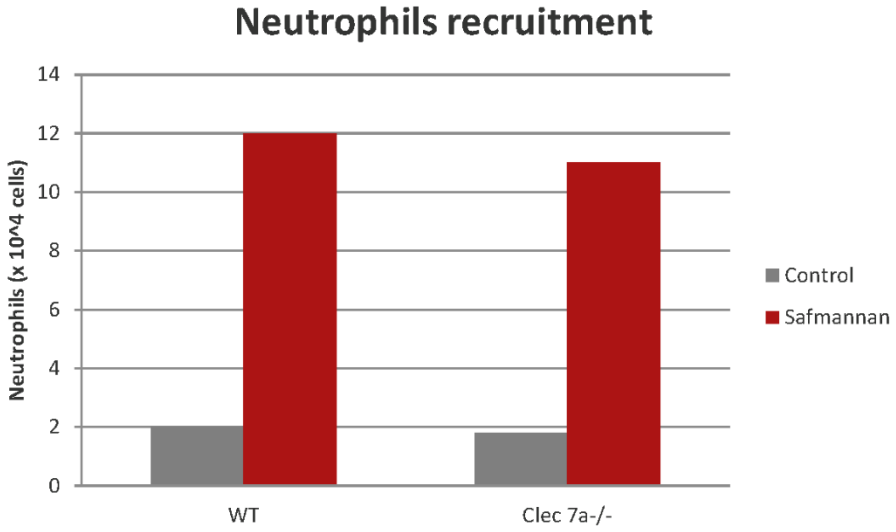
Immune mediators production

4

Cytokines
Free radicals
Lipids
Etc.

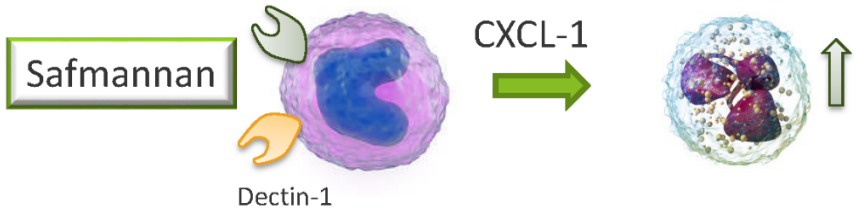
1. YCW is made of various molecules
2. These structural motifs are recognized by dedicated immune receptors
3. Ligand/receptor event triggers immune pathways
4. Immune functions are induced

Activation of the Neutrophils



In-vitro experiment using two different cell cultures.

Safmannan demonstrates high capacity to activate the neutrophils.



- **Safmannan** triggers early neutrophils recruitment

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Economic Impact of Reducing SCC using Safmannan

Variable	Before Safmannan (10 grams/cow/d)	After Safmannan (10 grams/cow)
SCC	271,000	180,000
Incidence of Mastitis, %	23.0	15.0
Cases per 100 cows	23	15
Cost, $ per Mastitis case	315.00	315.00
Cost of Mastitis /100 cows, $	7,245	4,725
Cost, $ to Feed Safmannan 21-d pre-fresh through 150 DIM per 100 cows		855.00
ROI (2520/855)		2.95:1

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The Big Takeaways

ROI on the use of Yeast Probiotics Depends on How You Measure it.

When You account for the increase in DMI, ROI = 2.2 to 3.7:1

At Current Prices, Net Revenue is 48 to 75 cents per cow/day.

Can Lower Diet Cost When Used to replace yeast culture or part of the buffer in the diet.

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Thank you!

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Opportunities for Improved Cow Comfort through Freestall Barn Renovations

Jeffrey Bewley
Alltech Dairy Housing and Analytics Specialist

The environment in which lactating dairy cows spend the majority of their time has considerable influence on productivity, health, milk quality, reproduction, animal well-being, and farm profitability. When discussing this environment, we often talk about maximizing cow comfort. Cow comfort generally refers to minimizing animal stress in efforts to maximize milk production and animal well-being. Many dairy producers provide shelter for dairy animals within a freestall barn. A properly managed and designed freestall barn can support high levels of milk production and animal well-being. On the other hand, mismanaged or poorly designed freestalls can contribute to mastitis, lameness, hock abrasions, and injuries. Through years of experience observing and studying cow behavior in freestall barns, farmers, researchers, and engineers have refined recommendations for freestall design and management. Additionally, as cow size has increased so has the amount of resting space required within a freestall, effectively changing the recommendations for freestall dimensions. Today's freestall barns provide a more desirable environment for dairy cows than those that were constructed 20, 30, or 40 years ago. Dairy producers still housing their cows in these older facilities could observe dramatic improvements in cow comfort by making some minor, relatively simple changes to existing freestalls. The economic assessment renovations in facilities is difficult because of wide variation in herd responses to modifications. However, the potential economic impacts of increased production, reduced lameness, improved milk quality, reduce culling rates, and increased longevity are immense. Further, just as important, as public concern for animal well-being increases, freestall barn renovations may help minimize the impact of future concerns, policies, or third-party audit programs.

Freestalls and Lying Behavior

The purpose of a freestall is to provide a cow with a comfortable, clean, dry resting area. A good freestall allows the cow to enter and exit the stall with a natural reclining, resting, and rising motion without striking the stall structure. These freestalls present minimal opportunity for injury, pain or frustration. Some freestall design considerations involve trade-offs between optimal stall usage and cow cleanliness. Providing the largest cows in the herd with maximum resting space may mean that even slightly smaller cows may have more opportunity to soil the rear of the stall with manure and urine. For example, research has shown that cows spend more time lying in wider freestalls; however, these same stalls were not as clean as the narrower stalls. Thus, the maintenance requirements for stalls that provide better conditions for cows to lie may increase.

Lying behavior plays a critical role in the production, profitability, and well-being of dairy cattle. The amount of time a cow spends lying is influenced by many factors including facilities, management, and the physiological status (i.e. days in milk, milk yield, pregnancy status) of the animal. Grant (2007) proposed that the requirement for lying may be as high as 14 hours per day, based upon lying behavior observed in high producing cows. Production benefits of increased lying time have been reported to be as much as 2.0-3.5 lbs of milk per day for each extra hour of lying time (Grant, 2007). Increasing lying time may increase rumination, improve immune status, increase blood flow to the mammary system, reduce stress on the hoof, and

reduce the incidence of lameness in a herd. Research has demonstrated that depriving cows of adequate lying time may result in physiological and behavioral stress, increased lameness, altered feeding behavior, and reduced milk yield. Cows strive to attain a fixed amount of lying time even at the expense of feeding time. Lying time has higher priority than eating time and social contact in both early and late lactation cows (Munksgaard et al., 2005). In managing dairy cows, we need to do everything we can to ensure that cows have the opportunity to fulfill their lying time requirements. Additionally, a good freestall helps keep the cow clean and minimizes the chances of injury to the cow while moving in and out of the stall.

Behavior Observations

Often, dairy producers wonder why their cows do not spend as much time lying in their freestalls as they should. This situation is particularly problematic when cows choose to lie in manure-covered alleys rather than in the freestalls (Figure 1). The most effective way to determine if your freestall barn is meeting your cows' needs is to simply watch the cows. In comfortable, well-designed freestalls, cows will spend most of their time lying or standing straight (parallel to the length of the stall) in the stall (Figure 2). Approximately 2 hours after milking, about 90% of the stalls should be occupied (McFarland, 2007). Continuous monitoring of stall use and cow behavior while getting into and out of stalls is essential for assessing cow comfort. By understanding what behaviors to look for, you can learn what minor adjustments need to be made to the standard recommendations to best fit the needs of your cows in your facilities.

Watch the cows as they lie in the stalls. Think about how a cow gets up when she is on pasture (Figure 3). Their behavior in freestalls should be similar to this.Typically, a cow will first shift to move her front knees beneath her body. Then, she lunges her head forward transferring weight to the front of her body which also allows her rear end to be easily lifted. Next, she shifts weight to one knee and straightens the other front leg with the foot in front of the shoulder. Finally, she shifts her weight to the straightened leg pushing up and straightening the other front leg to finalize her standing position (McFarland, 2003).

Do the cows enter the stalls with ease and with minimal hesitation? Do they come into contact with any part of the stall while lying down? Watch cows as they rise from a resting position. Do they come into contact with any part of the stall while getting up? Is there adequate lunge space for their heads as they rise? Do you see any potential for injury as the cows get into and out of stalls? Do cows spend considerable time standing in the stall, showing hesitation, before lying down in the stall? Do they push their nose or mouth against pipes or stall structures? Do cows stand in the freestall, swinging their heads to the left and right? This behavior has been termed "the hesitation waltz" (Anderson, 2008a). Once cows are lying, do they appear calm or restless? Restlessness, or frequent changing of positions while lying, may be another sign of potential cow comfort shortcomings (Anderson, 2008a). Finally, spend some time looking at the cows focusing on the hocks, knees, and rumps. Do you see any evidence of injury, abrasions, abscesses, bumps, or bruises that may have resulted from getting into and out of the freestalls? If you stand in front of the stall and drop to your knees, is it a painful process? If so, how do you think this "knee test" reflects the cow's experience in using the stall? All of these observations may indicate potential improvements can be made through freestall modifications.

Figure 1. When a high proportion of cows choose to lie in the freestall alleys rather than in the stalls, this may be an indication that the freestalls do not provide a comfortable resting area.

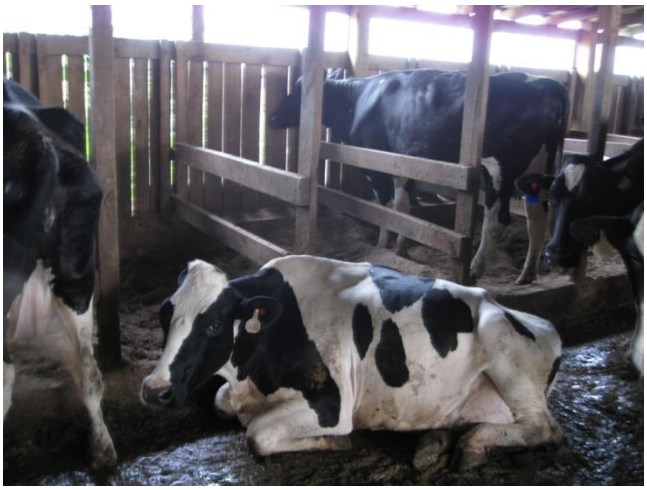
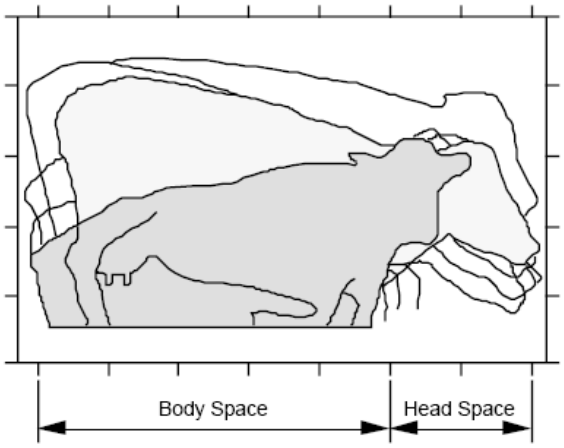


Figure 2. In well-designed and well-maintained freestalls, most cows will be observed resting comfortably or standing straight in the stall. In both positions, cows should be located parallel to the length of the stall.

Figure 3. A diagram depicting the normal rising motion of a dairy cow (Irish and Merrill, 1986).



Selecting the Right Stall Dimensions

Once you have observed the cows, use a tape measure to assess the dimensions of your existing stalls. Be sure to collect measurements for all types of stalls in your barns. For example, the dimensions may be different for stalls on the outside walls or if a different type of loop is used in one row versus the others. Once you have collected this information, compare your dimensions to the recommendations listed below (Table 1). Select freestall dimensions for the largest cows in your herd. Varying cow sizes within a herd should lead to varying stall sizes. A one-size-fits-all approach to freestall design is not conducive to optimal cow comfort. When possible, first lactation cows should be provided a separate pen with smaller freestalls to accommodate their smaller frame size.

Table 1. Recommended freestall dimensions by cow size (Graves et al., 2005, Cow Freestall (Cubicle) Types and Details).

Animal Weight (lbs)	Total Stall Length Closed Front (in)	Total Stall Length Open Front (in)	Length to Brisket Tube or Board (in)	Length to Neck Rail (in)	Stall Width Center to Center (in)	Height to Top of Partition (in)	Height to Neck Rail (in)	Brisket Board or Tube Height (in)
900-1100	90-96	78-82	64-66	62-64	41-43	42-44	42-44	4-6
1100-1300	96-102	80-86	66-68	64-66	43-45	44-46	44-46	4-6
1300-1500	102-108	90-96	68-70	66-68	45-48	46-48	46-48	4-6
1500-1700	108-114	96-102	70-72	68-70	48-52	48-52	48-52	4-6

Renovating a freestall barn. Before renovating a freestall barn, it is important to first determine whether observed cow comfort or cleanliness problems are the result of ineffective maintenance rather than actual design problems. Spending time grooming and cleaning freestalls can have a dramatic impact on cow cleanliness (Figures 4-5). Often, the first step to renovating a freestall barn is to fix what is broken in the existing freestall barn. All too often cow comfort is compromised by broken or detached freestall dividers and stall structures (Figure 6). Not only can these stalls lead to sub-optimal stall use but they also can result in serious injuries. Reattaching or repairing stall dividers and structures is a simple step toward improving cow comfort. Sometimes, the best idea is to replace the existing stall dividers with new or slightly used stall dividers that may be more conducive to increased stall use. It is important to recognize that optimal stall use and lying behavior are the result of a combination of factors. Changing one factor may not necessarily remedy the situation immediately. Some trial and error may be needed during the renovation process. One should not expect to see immediate results and improvements. It may take time for cows to adjust to the redesigned freestalls and old problems (lameness, hock injuries, etc.) will not disappear overnight. When renovation is not a viable option, it may be best to tear down the existing barn and start over with a new one. When renovation is a viable option, here are some cow comfort bottlenecks that you may find in your facility. In addition, a description of the desired situation and potential solutions for fixing the problems are outlined.

Figure 4. Frequent, scheduled stall grooming can have a dramatic impact on stall usage and cow cleanliness.

Figure 5. Cow cleanliness problems can often be attributed to infrequent or inadequate removal of manure and urine from freestall alleys.

Figure 6. Detached or broken stall dividers or structures can lead to poor stall usage, dirty cows, or cow injury and entrapment.

Poor or Inadequate Resting Surface

Identifying the Problem. When cows are not provided with a comfortable place to rest, they will not utilize or occupy the stalls well. Hock injuries are commonly observed in situations where cows are forced to lie on a hard surface or when insufficient bedding is provided (Figure 7). Of course, the worst scenario is when cows are lying on concrete without any bedding. Bedding helps to minimize friction between the hock and the stall surface. In deep-bedded stalls, cows may dig out the bedding and effectively reduce their resting area if bedding is not replaced (Figure 8). This situation may also increase the effective height of the brisket board and stall dividers. In turn, cows may have difficulty getting in and out of the stall. Moreover, the potential for abrasions between the now-protruding rear curb and the cows' hocks can lead to severe hock abrasions and ulcers. When mattress or mats are used, inadequate bedding may also lead to hock injuries and poor stall use. This problem is worsened when the mattress cushions have lost their flexibility and are utilized past their useful life.

Understanding the Desired Situation. Providing a comfortable, soft surface cushion may be the most important factor affecting stall usage and lying time. An ideal stall bed conforms to the cow's shape, provides cushion while the cow is getting up and lying down, maintains effective traction to minimize slipping, and remains dry to minimize bacterial growth and promote optimal udder health. Many different combinations of stall bases and bedding types can be effective; however, sand bedding generally best meets the cows' needs. Stall usage and lying time tends to be higher for sand bedded freestalls than for mattress freestalls (Cook, 2006). Keeping sand filled to the top of the curb increases stall use. In one study, daily lying time was 1.15 hours longer when sand stalls were filled to the top of the curb compared to stalls with sand levels 2.44" below the curb (Drissler et al., 2005). Although mattresses, waterbeds, and mats may reduce the amount of bedding needed, bedding still must be used to minimize friction while the cow rises from the stall and to absorb moisture (Figure 9). In a British Columbia study, cows spent 1.5 hours more lying down in mattress freestalls bedded with 16.5 pounds of sawdust than those with no sawdust (Tucker and Weary, 2004). Thus, lying time can be improved considerably by providing cows with more bedding (Figure 10).

Selecting and Implementing a Solution. The solution to this problem may often be as simple as using more bedding. This is particularly true for sand. Sand provides such a good resting material for cows that it will often mask other freestall design limitations. Hard or worn-out surfaces may need to be replaced with deep-bedded sand or new mattresses (Figure 11). When adding a mattress on top of concrete, caution must be used to be sure that the height for the cows stepping into the stalls does not exceed 8 to 10." In a deep-bedded scenario without a mattress or mat, a minimum of 6" of bedding material is required. When mattresses or mats are used, at least 3" of bedding must be added to the top of the stall base. Freestalls should be groomed, removing manure and wet areas 2 to 3 times per day. Deep-bedded stalls should be leveled at least twice per week. Bedding should be added at least once per week and possibly once per day depending on the type of bedding used, environmental conditions, and observations of cow cleanliness. Bedding savers may be used to minimize bedding waste.

Figure 7. When cows do not have an adequate resting surface or when bedding levels are insufficient, the resulting friction that occurs as the hocks rub against rough surfaces may result in hock abrasions and injuries.

Figure 8. Over time, cows will pull sand out of stalls. This sand must be replaced frequently to maintain a comfortable resting area.

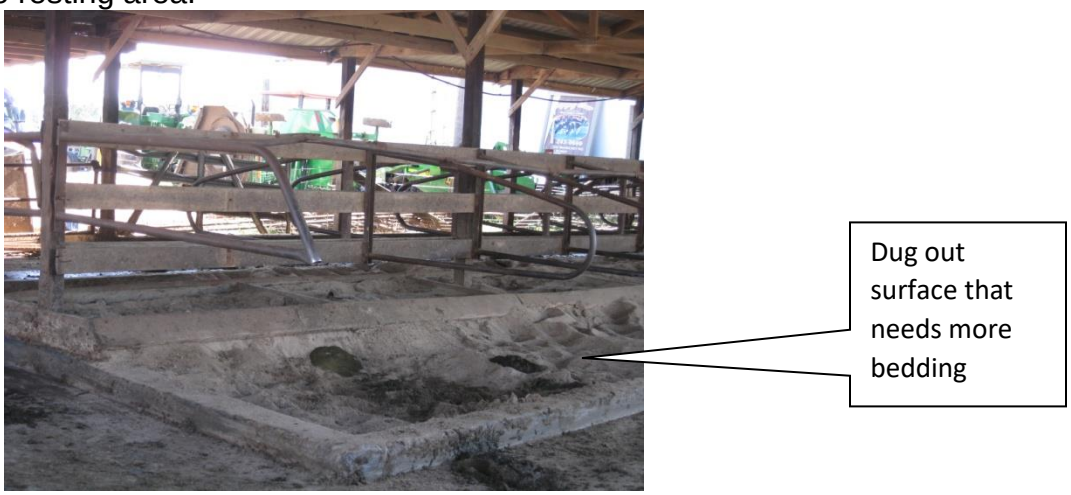


Figure 9. Although mattresses provide cushion for cows, adding bedding on top of the stalls is still essential.

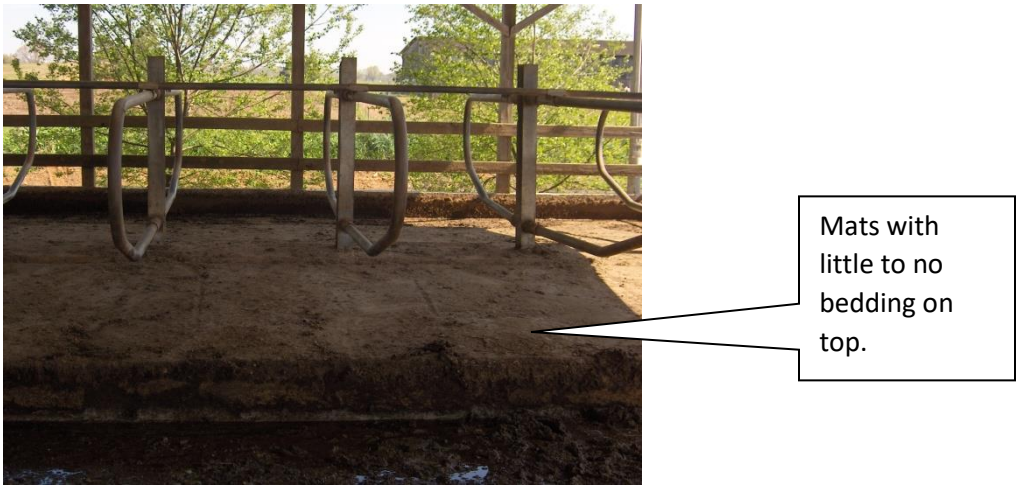
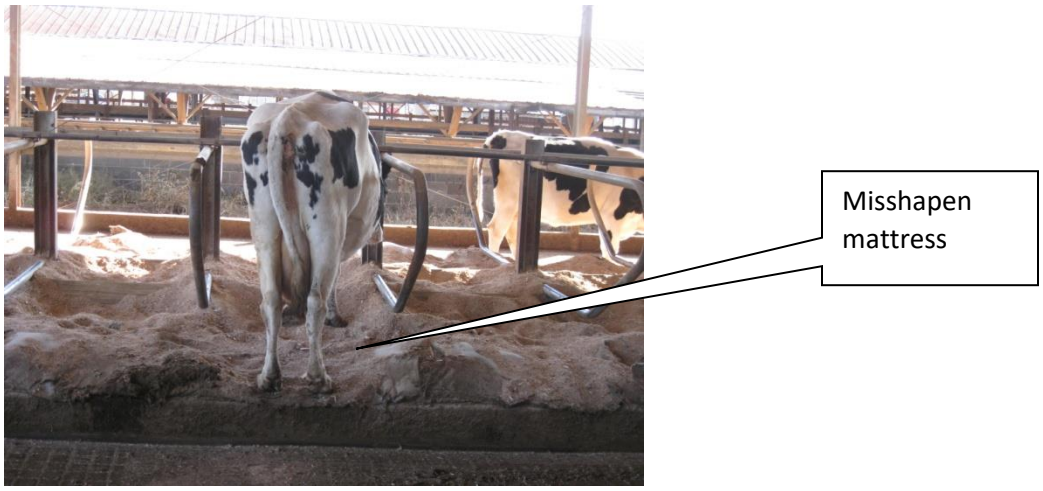


Figure 10. Deep bedding minimizes potential for hock injuries, improves stall usage, and increases lying times.

Figure 11. Over time, mattresses may become misshaped or damaged and may need to be replaced.



Inadequate Forward Lunge Space

Identifying the Problem. Forward lunge space is often blocked by walls or boards directly in front of the cows' heads (Figure 12). Generally, cows prefer to lunge forward when rising from a resting position. Thus, when obstructions are placed in front of the cows, there is no room for their heads to go in this natural rising motion. When cows do not have the ability to lunge forward, they may have difficulty rising from stalls. They may even become trapped against the wall while rising from the stall. Standing or lying diagonally in the stalls may also be a sign of cows searching for a way to preserve forward lunge space. Dog-sitting, where cows sit like dogs with weight placed on the rear end of their body and their front legs extended may indicate a lack of lunge space (Figure 13). Stalls that lack adequate lunge space are also characterized by overall poor stall usage and may contribute to perching (standing with front legs in the stall and rear legs in the alley).

Understanding the Desired Situation. Stalls must be long enough to allow cows to lunge forward when rising from the stall. Cows prefer to lunge forward rather than lunge to the side. To provide the cow with adequate forward lunge space, give 30 to 44" of space ahead of

where their front knee is positioned while resting. Thus, closed front stalls (such as stalls that face an outside wall) should be at least 1 foot longer than open front stalls to preserve this lunge space .

Selecting and Implementing a Solution. The key to solving this problem is to remove the lunging obstacles (Figure 14). For head-to-head stalls or inside stalls, remove walls and boards that may impede lunging leaving at least 6” above the stall surface and 32” of vertical clearance. Depending on how the stall dividers are attached to the support structure, removing these obstacles may require moving posts or modifying where the stall dividers are attached. If the stalls are located on an outside wall, building a sloping adjustable sidewall curtain support along the outside wall will give the cows ample opportunity to lunge forward while still protecting cows from adverse weather (McFarland, 2007). Another possible solution would be to use a stall divider that allows for side-lunging into the adjacent stall. In this case, the lower rail should be no higher than 11 inches above the stall surface and the upper rail should be no lower than 40 inches. Avoid piling bedding in front of the stall as this can unintentionally block lunge space. Some producers express concern that with open-front, head-to-head stalls, cows may attempt to go through the section between the stalls into the facing stall. This situation can lead to injury or cows restrained between the stalls. To remedy this problem, a deterrent bar, rope or strap may be placed 40 to 42" above the stall surface in 16 foot stalls (2 rows of head-to-head 8 foot stalls) or 34 to 36" above the stall surface in 18 foot stalls (2 rows of head-to-head 9 foot stalls). This deterrent may be wood, metal, galvanized pipe, nylon strapping, or rope (Anderson, 2008b).

Figure 12. Forward lunge space is often blocked by walls or boards placed in front of the cows’ resting space.

Figure 13. Dog-sitting may indicate a lack of lunge space or other freestall design problems.


Figure 14. Lunge space can be preserved by keeping the area in front of the cows' heads free of obstructions.


Improperly Placed Neck Rail

Identifying the Problem. One way we can evaluate neck rail placement is by observing cows for perching behavior. "Perching" refers to the behavior when cows stand with their front feet in the stall and their rear feet in the alley behind the stall (Figure 15). Generally, this behavior indicates improper neck rail placement. When the neck rail is too low, cows will sometimes stand with their head above the neck rail. If the bottom side of the neck rail has a polished appearance, the cows are likely hitting their neck against the neck rail when rising from the stall. If neck rails are too low, cows may also be hesitant to enter the stalls and have difficulty standing up. When the neck rail is too close to the rear of the stall, cows may lie diagonally rather than parallel to the length of the stall. If cows do not have enough space to lie down because the neck rail is too far back, hock injuries may be observed. If the neck rail is placed too far forward, lunge space will be limited and cows may become trapped while rising from the stall. Additionally, excessive manure and soil may be deposited in the rear of the stall. Early freestall designs recommended a much shorter neck rail height than we recommend today; however, experience and research have shown that these older recommendations were incorrect.

Understanding the Desired Situation. The neck rail helps position the cow when she enters the stall or when she is standing in the stall before or after standing up. Additionally, the neck rail helps encourage cows to preserve lunge space. When the neck rail is in the proper position, cows will stand with all four feet placed squarely within the stall, level backs, and the top of their necks gently touching the neck rail (Figure 16). The neck rail is typically a few inches lower and forward from the cow's withers. Wisconsin researchers (Fulwider and Palmer, 2005) demonstrated that the percentage of stalls with cows lying in mattress based freestalls was significantly higher with a 50 inch neck rail (51.4%) when compared to stalls with a 45 inch neck rail (40.0%). In one study, cows spent less time perching when the neck rail was further from the rear curb but cows were more likely to defecate in these stalls and had dirtier udders (Fregonesi et al., 2009). Cows showed significantly more evidence of lameness when the neck rail was positioned closer to the rear curb in a British Columbia study (Bernardi et al., 2009). For large-frame dairy cattle, the distance between the top of the stall bed (including bedding) and the bottom of the neck rail (also referred to as neck rail height) should be 48 to 52 inches. The horizontal distance from the alley side of the rear curb to the neck rail (also referred to as neck rail length) should be 68 to 70 inches.

Selecting and Implementing a Solution. In many situations, the neck rail can be moved without any major modifications. Increasing or decreasing the neck rail length generally involves unbolting the neck rail and moving it forward or backward along the stall divider to the desired length. Increasing the neck rail height may be a bit more challenging. Dairy producers should use their engineering ingenuity to determine the best modification for their facility. Wood blocks, box steel, welded pipe fixtures (Figure 17) and clamps are examples of strategies used to raise neck rail height (McFarland, 2007). In some situations, it may be possible to move the entire stall divider up though caution must be used to make sure that the distance between the divider and the stall base does not leave opportunity for cows to become lodged beneath the stall divider. The lower rail should be no higher than 11 inches above the stall surface and the upper rail should be no lower than 40 inches.

Figure 15. Examples of "perching" with cows standing half in and half out of stalls. Perching may indicate that the neck rail should be moved.

Figure 16. Examples of freestalls with proper neck rail placement. Notice that most cows are standing with their four feet squarely placed in the freestall.

Figure 17. An example of a welded pipe fixture used to raise the height of a neck rail.

Undesirable Curb Height

Identifying the Problem. If the curb height is too high, cows may be reluctant to use the stalls or hesitant and uneasy when exiting the stalls. This problem may be more evident in lame cows than in non-lame cows. With high curb heights, some cows may drag their teats and udders on the curb or bed when entering the stall. If the curb height is too low, manure from the alley may be pushed into the stalls while scraping or tracked into the stall by cows upon entry. Additionally, cows may back into stalls and lie facing outward.

Understanding the Desired Situation. The primary purpose of the curb is to keep manure from the alley from entering the back of the stall. When a cow places her rear leg on the concrete alley behind the stall, a tremendous amount of weight must be supported by that leg. Thus, the curb height plays a critical role in minimizing this stressful process. The ideal curb height is 8" though curb heights up to 12" may be tolerated.

Selecting and Implementing a Solution. When the curb height is too high, efforts to reduce the curb height through concrete removal may prove labor intensive and expensive. In some cases, it may be more feasible to raise the alley height. When the curb height is too low, additional concrete may be added to the curb. Alternatively, adding a mattress or bedding saver may effectively increase the curb height.

Narrow Stalls

Identifying the Problem. When cows invade the space of other cows in adjacent stalls or prevent the use of the adjacent stall because they are taking up part of the stall, stalls are likely too narrow. Stalls that are too narrow are often characterized by excessive body contact with the stall divider while lying down and rising from the stall (Figure 18). Cows may also not use stalls well and they may lie diagonally.

Understanding the Desired Situation. The stall divider helps position the cow in the stall and encourages cows to lie parallel to each other and to the length of the stall. British Columbia researchers found that cows in 52 inch wide freestalls spent 42 more minutes per day lying down than cows in 44 inch wide freestalls (Tucker et al., 2004). In this same study, cows spent more time perching in the narrowest freestalls. The wider stalls tended to be dirtier than the narrower stalls.

Selecting and Implementing a Solution. Increase the width of the stalls to accommodate your largest cows. Unfortunately, depending on how the stall dividers are attached to support posts, this modification may entail considerable effort and structural modifications to achieve. Additionally, this may reduce the total number of stalls in a barn.

Figure 18. When stalls are too narrow, cows may lie diagonally or come into direct contact with the stall dividers or structures while resting.

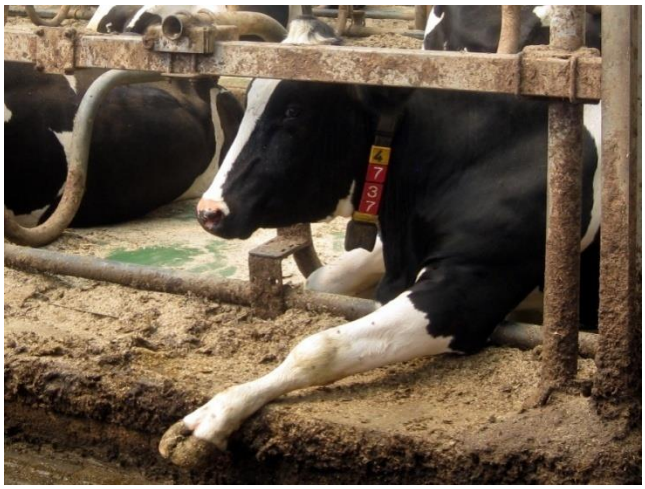
Brisket Locator/Board Position and Size

Identifying the Problem. When the brisket board is placed too close to the curb, diagonal resting may occur. If cows do not have enough space to lie down because the brisket board is too far back, hock injuries may be observed. Moreover, this situation can lead to perching just as an improperly placed neck rail does. If the brisket locator is placed too far forward, cows may become trapped while rising from the stall. Additionally, excessive manure and soil may be deposited in the rear of the stall. If the brisket board extends more than 6" above the stall surface, it may actually block forward lunging as the cow rises from the stall and prevent them from extending their front legs forward during the rising motion. Abrasions on the inside of the cows' front legs may be observed if the brisket board is too high or has rough edges.

Understanding the Desired Situation. The brisket locator keeps the cow from moving forward while resting and helps position the cow in the stall and preserve forward lunge space. It also provides a bracing point for cows as they get up. When positioned properly, the brisket locator provides all cows with ample space to lie down comfortably within the stall. The brisket board is positioned directly underneath the neck rail or slightly further toward the rear of the stall. In mattress or mat freestalls, the brisket locator should be 68 to 72" from the rear edge of the mattress or mat. In deep-bedded freestalls, the brisket locator should be 68 to 72" from the cow side of the rear curb. The best brisket locator is one that provides the cow with an opportunity to extend her front leg over the locator while resting (Figure 19).

Selecting and Implementing a Solution. If the existing brisket locator impedes forward lunge-space or does not provide a smooth surface for the cow to extend her leg over, the existing brisket locator may need to be removed and replaced with a smooth brisket locator no more than 4 to 6" above the stall surface. Flexible plastic barriers with rounded edges (i.e. PVC pipes) generally perform best. It may be possible to shorten the existing wood brisket board to the desired height, but care must be taken to avoid rough edges. The brisket locator should be attached to the stall surface and not to the stall divider.

Figure 19. Cows often extend their front leg over the brisket locator while resting. To allow for this behavior, a brisket locator with rounded edges is preferred over sharp or straight edges.



Short Stalls

Identifying the Problem. The most obvious sign of short stalls is when the cow's rear end hangs over the edge of the curb (Figure 20). This situation may also cause poor stall usage. Short stalls may also be characterized by diagonal standing, lying, and rising and may contribute to perching.

Understanding the Desired Situation. Each freestall should provide enough space for the cow to rest with additional space allotted for lunging and bobbing while the cow is getting up. For large frame cows, this equates to a total length of 8 to 9 feet with at least 7 to 8 feet of actual resting space. Stalls may be too short because the actual length of the stall is inadequate or because the neck rail/brisket locator combination has limited the space for the cows to rest.

Selecting and Implementing a Solution. If the stall length problem is related to inadequate forward lunge space, the solutions listed above will apply here also. Stalls facing an outside wall should typically be 10 feet long. Moving the brisket board and/or neck rail forward may increase the amount of resting space available to cows. Producers may consider adding additional concrete to the rear of the stall to increase the length of the stall. One precaution for this strategy is to be sure not to create cow traffic problems through narrow alleys. Alleys should be 8 to 10 feet wide.

Figure 20. When cows do not have enough space to lie down, they may be found lying diagonally in stalls or “half-in, half-out” with the front part of their body on the stall surface and rear part of the body in the freestall alley.

Excessive Space behind Stall Dividers

Identifying the Problem. The primary sign of having too much space behind the stall dividers is the observation of cows walking behind the stall divider on the stall surface (Figure 21). Additionally, cows may often be seen lying backwards in the stalls (Figure 22). Both of these behaviors may lead to dirtier stalls. Too much space behind stalls may also increase the likelihood of cows becoming trapped under the stall divider.

Understanding the Desired Situation. To keep cows in the stall but prevent them from walking behind the stalls and minimize backwards lying, less than 14" should remain between the end of the stall divider and the rear curb.

Selecting and Implementing a Solution. Any solution to this problem would involve moving the stall divider toward the rear of the stall. Accomplishing this task may be challenging, because it is impossible to stretch the stall divider. Solutions could involve replacing the stall dividers, moving the existing dividers toward the curb where possible, or adding a welded extension to existing dividers to increase their length.

Figure 21. If too much space is left between the stall divider and the rear curb, cows may be able to walk on the stall surface.

Figure 22. Cows may also be more apt to lie backwards in the stall when space is open at the end of the stall.

Poor Ventilation

Identifying the Problem. During warmer temperatures, poor ventilation may result in cows expressing obvious signs of heat stress (i.e. panting, breathing heavily, Figure 23). Cook et al. (2007) illustrated that mean lying time decreased from 10.9 to 7.9 hours per day as temperature increased. Thus, stall usage may be altered if barns are inadequately ventilated. When temperatures are cooler, poor ventilation can result in increased respiratory problems and transmission of other diseases (Figure 24). Lack of proper ventilation can lead to high moisture levels, manure gases, pathogens, and dust concentrations which create an adverse environment for dairy cows (Palmer, 2005).

Understanding the Desired Situation. For optimal production and well-being, dairy cows should be provided with a constant supply of fresh, clean air. Frequently exchanging air removes or reduces the concentrations of dust, gases, odors, airborne disease organisms, and moisture. Maximizing natural ventilation is the first step toward improving ventilation. Natural ventilation relies on barn openings and orientation to remove heat and humidity from the animal's environment. Exhausted air generally leaves the barn through sidewalls or ridge openings. Although old barn designs suggested closed-in barns, current recommendations are to open the barns up to allow for better air exchange. Sidewalls allow for air, heat and humidity to be easily and continuously removed from the barn (Figure 25). This is particularly critical during the summer. If producers are concerned about the potential negative effects of open sidewalls during the winter, sidewall curtains, which can be raised in the summer and lowered during the winter, may be added to eliminate this concern. A ridge opening should also be provided at the top of the building to facilitate air removal through the top of the barn. Warm, moist air rises and exits through the ridge opening even on calm days. The steeper the roof slope the better the movement of the warm moist air out of the ridge vent. The roof slope should be at least 3/12, 3 inches of rise for every 12 inches of run. A slope of 4/12 is preferred. The ridge opening should be at least 2 inches for each 10 feet of building width. With overshot roofs, this opening should be at least 3 inches per 10 feet of building. Producers are often

resistant to this change because of fears of precipitation entering the barn through the ridge opening. Although this is generally not a major problem, a ridge cap may be added to eliminate this concern (Bickert et al., 2000).

Selecting and Implementing a Solution. For many older barns with ventilation issues, the main opportunity for improvement is removing tin or wood sidewalls that block natural winds from entering the barn. Before removing these obstructions, consider how this change might affect the structural integrity of the building. Strive for at least 8 feet of sidewall opening. A 3 to 4 foot overhang should be provided to prevent precipitation from entering the barn. Curtains may be needed to block adverse weather during the winter (Bickert et al., 2000). Similarly, opening the endwalls may also prove beneficial. In some cases, there may benefit in raising the height of the roof to increase the amount of air flowing through the sidewalls. Adding or increasing the size of the ridge opening can dramatically improve natural ventilation. Natural ventilation can also be supplemented with mechanical ventilation with the addition of fans. Adding fans to an existing freestall barn is one of the highest return investments a dairy producer can make.

Figure 23. Cows housed in barns with poor ventilation are more likely to be affected by heat stress.

Figure 24. Barns that are completely enclosed do not allow for adequate air exchange resulting in a damp, dark environment and can lead to heat stress, respiratory problems, and increased transmission of disease.


Figure 25. The ideal freestall barn maximizes natural ventilation with high, open sidewalls, a ridge vent opening and supplements natural ventilation with fans used to increase air flow and exchange.


Conclusions

Cow comfort can be improved dramatically through modification of existing freestalls. Often, these changes can be made with minimal expense. Before undertaking such an effort, one should be evaluate long-term plans. It is important to determine whether the existing facility truly has enough positive attributes to renovate or if building a new facility would prove more beneficial and cost effective. Observing cow behavior can provide clues for evaluating what changes could be made. Modifying one shortcoming may not always improve the situation if other bottlenecks still exist. But, cow comfort improvements achieved through freestall modification can provide immense benefits to animal well-being, milk yield, cow longevity all while minimizing farmer frustration and stress.

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Understand fiber: TMR NDF breakdown and reduced lignin feed potential. Make profitable decisions within these areas[1]

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Fiber in dairy diets

Carbohydrate impact upon animal and ruminant nutrition is not a new focal point for nutritionists. Hall and Mertens (2017) recently reviewed 100 years of carbohydrate research relative to ruminant nutrition. Fiber, defined as Neutral Detergent Fiber (NDF; Goering and Van Soest, 1970**)** in dairy nutrition, contributes two major facets of dairy diets. It is important for both physical and energetic aspects, but energetically fiber provides the least energy per pound of all nutrients in the total mixed ration (TMR). The balance of the diet is then more readily digestible carbohydrates (primarily sugar and starch), protein and fatty acid. It's important to simultaneously consider both fiber's physically effective and energetic attributes, and at times these are inter-related.

Physical attributes

With dairy diets, we typically feed adequate fiber to maintain sound rumen function and metabolism. There is often a perception of rampant clinical acidosis or sub-acute rumen acidosis (SARA). However, my belief, founded upon working with many consulting nutritionists across the US and reviewing diets, is that very few formulated diets today are responsible for clinical symptoms. Rather, management factors such as feed delivery timing or feed mixing are often the contributing factors toward rumen health and SARA.

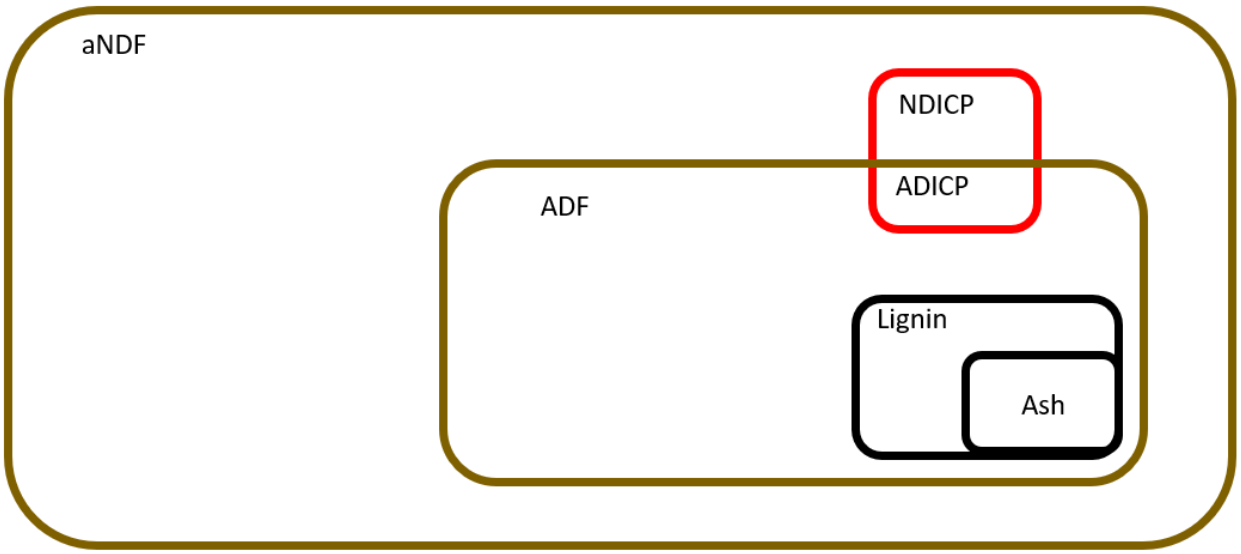
To date, there is not a readily accepted "standard" in quantifying the aNDF percentage that is physically effective (peNDF, % of aNDF or DM). Prof Mertens' work suggested the 1.18 mm size was ideal, yet other work from Penn State and others suggested the 4 mm size may be more accurate in determining effectiveness. Both 1.18 and 4 mm sieves are now incorporated within the Penn State particle size separator and the aNDF percentage greater than these sizes can be readily determined (Heinrichs, 2013). Of note, the NRC (2001) held back from making recommendations for fiber effectiveness. Rather, the National Research Council committee provided recommendations for forage NDF, % of DM, at varying fiber to starch ratios.

[1] This article has been adapted and modified from that originally written for, and published, in the Proceedings to the 2018 Four State Dairy Nutrition Conference, Dubuque, IA.

Fragility (i.e. alfalfa fiber being more fragile than grass fiber; Allen, 2000) is another concept contributing to fiber's effectiveness that warrants further exploration but is vaguely understood and characterized today.

Prior to discussing the energy side of fiber, the detergent fiber complex warrants discussion as considerable confusion exists yet today within the industry. **Figure 1** demonstrates the concept of various fiber fractions, each nested within aNDF. Forage analysis laboratories sequentially rinse (like a laundry machine) feed samples with neutral, mildly acidic and then strongly acidic solutions to wash away feed components and ultimately determine the fractions outlined in figure 1. Each is determined by relating the remaining sample weight to original mass after sequential rinses or burning in an often (ash).

Figure 1: The fiber nesting doll. The acid detergent fiber (ADF), neutral and acid detergent insoluble crude protein (NDICP, ADICP), lignin and some ash are nested within aNDF. Image Adapted from the March 10, 2018 Hoard's Dairyman article, "Dairy nutrition's tribal language: speaking fiber."



Energetic attributes

Starch and fiber contain the same calorie content per pound, around 4 calories per gram. Both starch and fiber (cellulose) are generally chains of glucose bonded together. Yet as nutritionists, we understand the energy available to the cow varies greatly between these two nutrients. The enormous difference in energy available is due both the type of glucose-glucose bond (alpha- vs beta- bond configurations) as well as lignin and cell wall cross linking that further zippers cellulose into a less digestible complex. In 2014, I surveyed several meta-analyses and summarized fiber and starch digestion data from more recent published lactating cow feeding studies. Total-tract fiber digestion in lactating cows averages about 40 to 50% (Table 1) whereas total-tract starch digestion averages over 90% (Goeser, 2014). Further, commercial dairy cow-level digestion (apparent digestion, % of nutrient) appear similar to published research (**Figure 3**). In the 2014

summary, my aim was to revisit laboratory fiber and starch digestion measures relative to real, *in vivo* data and recognized that 30h *in vitro* NDF digestion values often over-estimate cow level digestion thus questioned the utility.

Since the 2014 survey and time, the industry has better embraced the notion that single time point fiber digestion measures (i.e. NDFD30) are inadequate to describe complex rumen nutrient digestion. In conjunction with this better recognition, forage analyses laboratories have advanced multi-time point rumen fiber digestion predictions by near infrared reflectance (NIR) spectroscopy.

To merge the two points together and bring functional nutrition decision making tools to the field, two practical nutrition models have come online in the US:

1. Cornell Net Carbohydrate and Protein System v6.5 (Van Amburgh et al., 2015)
2. Total Tract NDF Digestibility (Combs, 2013)

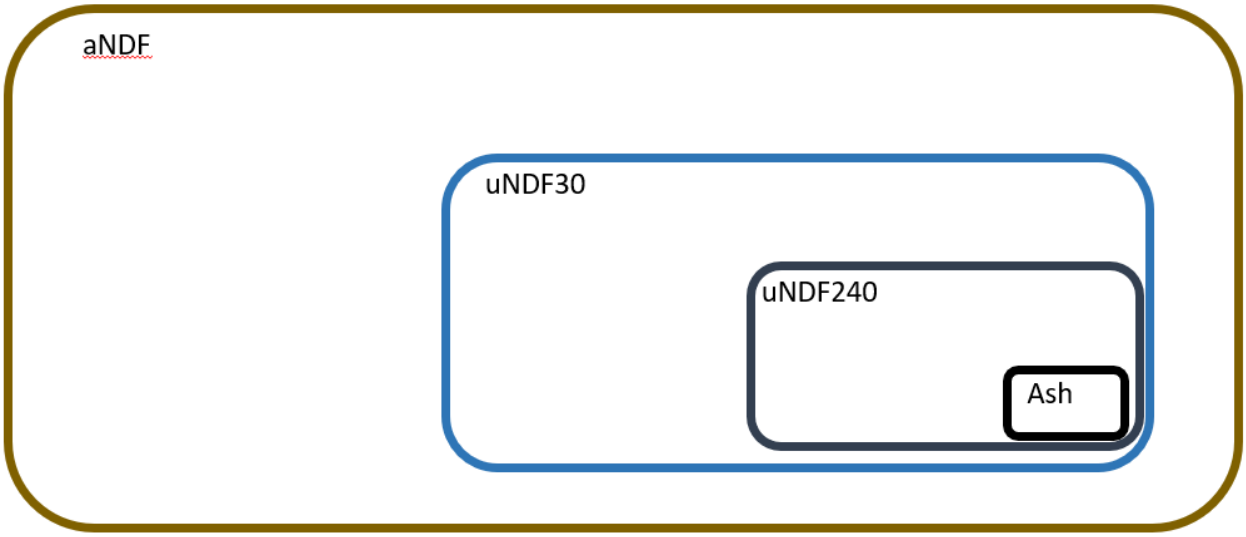
Another multi-time point analytic tool warrants recognition, Fermentrics™ (www.fermentrics.com, accessed online; Johnston, personal communication), which was developed using methodology and concepts described by Pell and Schofield (1993). Gas production is intriguing as these models allow one to consider thousands of data measures over time. However, the model fiber and starch digestion rates are determined via gas production curve peeling and not direct fiber quantification.

Each of these tools incorporate digestible nutrient pool sizes and nutrient digestion rates into compartmental models to predict fiber digestibility within the rumen or total-tract. To better understand both nutrient digestible pool size and digestion rate consider the following analogy and story.

uNDF and NDFD meaning and relationship

Similar to how the detergent fiber parameters can be depicted with a nesting doll analogy, uNDF30 and uNDF240 (% of DM or NDF) can be better understood relative to aNDF with a picture (**Figure 2**). Within the laboratory, the sample (and it's fiber) is digested for a time period and then it's washed with neutral detergent to determine the amount of fiber that's left. This ends up being a gram divided by gram type equation and NDF digested at time = x ($NDFD_x$, % of NDF) is then calculated by: (aNDF – $uNDF_x$) / aNDF x 100. Alternatively, the amount of fiber left after 30 or 240 hours may be a better lignified fiber indicator, thus comparing uNDF (% of DM) has become another measure we evaluation. In this case, the uNDF is looked at as a % of the original sample. Just like is the case with aNDF.

Figure 2: The undigested fiber nesting doll. Each uNDF30 and uNDF240 are nested within aNDF (% of DM).



Building a camp fire within the rumen: kindling and a bundle of fire wood

Continuing with the analogies, rumen fiber (or any other nutrient) digestion can be more simply understood by comparing to our experience with building a campfire. Both the wood pile size and moisture (i.e. dry vs wet wood) contribute the heat we feel through the night from the fire pit. Similarly, digestible fiber pool size (akin to the wood pile size) and fiber digestion rate (akin to wood moisture) must be accounted for to accurately predict rumen fiber digestion across different diets and intake levels. The same forage consumed in a high cow or dry cow TMR will actually be digested differently due to passage rate (i.e. rumen retention time). The only way this can be accurately predicted is by combining digestible fiber pool size and digestion rate in a model that also includes a passage rate. Reason being, fiber leaves the rumen in two ways; digestion or passage. Both the CNCPS and TTNDFD models combine passage rate (k_p, % hr^{-1}) with potentially digestible fiber pool and digestion rate (aNDFom k_d, % hr^{-1}) in the following equation:

Rumen NDF digestion (% of aNDFom) = potentially digestible fiber pool x fiber [kd / (kd + kp)], where:

- *pdNDF, % of aNDFom = NDFD240om = (aNDFom – uNDF240om)/aNDFom x 100*
- *fiber k_d, % pdNDF hr^{-1} = non-linear model determined using multi-time point NDFD (i.e. 24, 30, 48 or 30, 120, 240)*

Fiber digestion term dictionary

- aNDF = NDF determined with amylase in the neutral detergent solution
- aNDFom = aNDF corrected for ash
- uNDF = undigested aNDF following a discrete digestion time (i.e. 30 or 240 h)
- iNDF = indigestible aNDF, theoretical value determined only by nonlinear modelling
- uNDFom = undigested fiber corrected for ash
- NDFD = Digested aNDF, expressed as a percent of aNDF

- pdNDF = potentially digestible NDF
- NDF k_d = fiber digestion rate

Semantics

Often, "k_d rate" has been used to describe fiber or starch digestion rates. Like how Prof. Mertens helped the industry's understanding of uNDF (undigested NDF at time = x) vs iNDF (indigestible NDF at time = infinity), I'll attempt to help us understand rate coefficient terminology; "k_d rate" is grammatically incorrect as the "k" is defined as the *rate coefficient* and the "d" is defined as *digestion*. Hence, "k_d rate" is redundant and akin to stating, "Digestion rate rate".

Helping growers manager toward better feed and margins

While uNDF and digestion rate are related to one another, they both can theoretically be improved. Reduced lignin forages have lesser uNDF levels and correspondingly greater digestible NDF pools. This does not mean though that reduced lignin forage fiber digests faster, it just means there is more fiber to digestion similar to how a large bundle of wood offers more energy than does a small bundle.

Reducing uNDF in feeds can be achieved in two ways; 1) diluting the uNDF with more digestible nutrients such as starch, protein or sugar or 2) managing to lessen the uNDF in relation to total aNDF. The second strategy is the route that brown midrib corn mutants lessen uNDF and theoretically how reduced or low-lignin alfalfa varieties improve quality. Going forward, Prof Combs' (personal communication) has suggested that digestion rate may be heritable, which could then lead to advances in fiber digestion speed along with decreasing uNDF and increasing digestible NDF pool size.

In the field, harvesting alfalfa and grass crops earlier should result in both lesser uNDF and faster digestion rates. Cross linking within cell walls develops as plants mature and will be related to bacterial cellulose access, thus decreasing both digestion speed and extent as maturity advances. Cut first crop each year at 22 to 24" PEAQ (Hintz and Albrecht, 1993). Do not assume 28 day cutting intervals result in dairy quality forage, I suggest scouting fields starting about 17 days after the prior cutting and monitoring plant maturity every 3 to 5 days with scissor clipping.

Managing what the dairy has provided us with the campfire in mind

Balancing diets with 30 or 48 h NDFD could not be considered "old school" as the days of using a single NDFD measure to formulate are behind us. Given better information available from labs, I now recommend considering both pdNDF and aNDF k_d in formulation to accurately formulate with the same forage at different intake levels and passage rates. The aNDF k_d should not be used by itself under any circumstances as it depends upon the uNDF level. However, uNDF values have utility as "the new lignin" measures.

I suggest monitoring uNDF30 and 240 levels (% of DM) in diets on a herd by herd basis. To my knowledge, there is not an industry accepted or published benchmark for a certain uNDF level that will limit intakes, however within a herd these metrics can prove valuable to help formulate forage inclusion rates when switching forage sources. Further, uNDF level could be used within diet projections to evaluate potential income over feed costs within partial budgets. I've appreciated also learning from Dr. Sam Fessenden recently (AMTS technical services) to use uNDF (g CHO-C) as a tool to consider when forecasting an intake response due to lesser uNDF content in feeds. Sam has suggested that diet projections can be compared by using different forages at similar dry matter intakes but further by also comparing the diet scenarios and maintaining CHO-C relatively constant between diets.

On farm, consider using Prof Combs' TTNDFD as a forage analysis level tool to make decisions and allocate feeds. Many consultants have had success coaching their clients to focus on TTNDFD as a "new RFQ on steroids" in better projecting forage quality.

Speak a different language on farm

Lastly, try and change the language you speak on farm as the terms discussed in this paper are difficult to convey to those not skilled in the art. Rather than speak of uNDF or NDFD or NDF k_d, speak in terms of total fiber in the diet, pounds of fiber digested by the cow or the amount of fiber that washes out the back end in manure. For example, at 55 pounds dry matter intake and 28% aNDFom, this approximates to 15 pounds of fiber cows consume each day in the TMR. If diet digestibility is recognized to be only 40% whereas the goal is 50%, talk about the 15 pounds being digested at both 40 and 50% results in 6 versus 7.5 pounds of fiber digested. The 40 versus 50% may seem vague, but when we're talking about 1.5 pounds of digestible nutrient at hand it may spur change. This 1.5 pounds of digestible nutrient could correspond to 3 pounds of milk or more!

Economically balancing reduced lignin (and uNDF) feeds based upon published nutrition research, yield and digestible tons production potential, and disease resistance considerations

Research investigating reduced lignin corn silage, published by both plant breeders and animal scientists, dates back decades and *brown-midrib* mutations appear to largely impact the pdNDF and not the pdNDF digestion rate (Cherney et al., 1991). In many published studies, reduced lignin forages correspond to an increase in intake and performance. The production response is relatively well understood relative to other economically relevant factors related to growing and feeding *brown-midrib* or reduced lignin forages. These factors should also be evaluated when doing projections: feed conversion potential, yield and digestible nutrient yield, and disease resistance.

Feed conversion: the balance between intake and performance gain needs to be considered when evaluating reduced lignin feed potential. The aim should be to outpace increased intake with performance gains, thus increasing feed conversion efficiency. According to Prof Allen and

colleague, a 1-unit gain in *in vitro* rumen NDF digestion corresponds to a 0.26 and 0.47 unit increase in DMI and 4% fat corrected milk production per cow, respectively (Oba and Allen, 2005). With a roughly 2:1 milk to intake increase per unit ivNDFD, theoretically feed conversion should improve via reduced lignin forages assuming ivNDFD increases. However Stone et al., (2012) reported no improvement in feed conversion with *brown-midrib* corn silage relative to convention. Feed conversion is not always reported within published studies yet is an increasingly important key performance indicator to track with dairies and feedlots during challenging economic periods. Interesting research coming from Dr. Rick Grant's group at the Miner Institute may also better help us understand intake and uNDF relationships (Grant, 2018).

Forage yield: lesser yield with *brown-midrib* or other reduced-lignin technologies are often expected. For example, data summarized from several years of Prof. Joe Lauer's hybrid trials detailed less yield with *brown-midrib* mutant corn relative to other conventional varieties (Lauer et al., 2016 and prior years; accessed online, http://corn.agronomy.wisc.edu/HT/Default.aspx). And a more recent publication with transgenic alfalfa reported lesser yield when managed in a similar manner to conventional lines. The reduced lignin alfalfa though may better maintain quality though with extended cutting intervals (Getachew et al., 2018).

However raw yield is not as economically relevant as the digestible nutrient yield. I suggest determining digestible yield with plot efforts by combining variety total yield (DM basis) with variety total digestible nutrient (TDN) measures, determined at a reputable forage laboratory, which incorporate the advanced fiber digestion concepts discussed here. Total digestible nutrient yield will more properly project energy harvested per acre.

Disease resistance: lastly, learning from Prof Damon Smith, among others, lignin is a plant defense mechanism. Seed genetics with a lesser ability to lignify may also be less able to withstand added disease pressure and could warrant additional crop protection. Crop protection inputs should also be considered in partial budgets evaluating reduced lignin seed economic impact.

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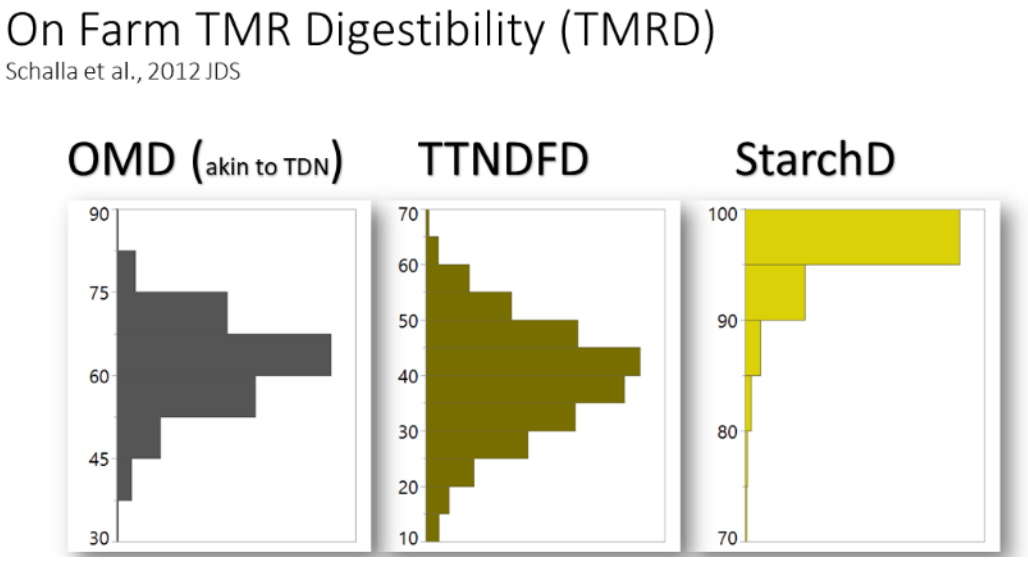
Table 1: Rumen and total-tract fiber digestibility measures for lactating dairy cattle in published research. Table adapted from Goeser (2014).

Description	Digestion Site	Author(s)	Treatment means	Digestion Coefficient, %	SD

Mixed TMRs	Rumen	Firkins et al. (2001)	121	43.5	11.3
Mixed TMRs	Rumen	Hannigan et al. (2013)	152	42.8	12.8
Corn silage based TMRs	Rumen	Ferraretto and Shaver (2012)	39	41.9	NA
TMRs containing barley based grain	Rumen	Ferraretto et al. (2013)	30	39.4	NA
TMRs containing corn based grain	Rumen	Ferraretto et al. (2013)	82	39.3	NA
n or Weighted means	**Rumen**		**424**	**42.0**	**12.0**
Alfalfa and Grass Forage based TMRs	Total Tract	Goeser (2008)	75	47.4	8.0
Corn and Sorghum Forage based TMRs	Total Tract	Goeser and Combs (unpublished)	85	42.7	10.5
Mixed TMRs	Total Tract	Firkins et al. (2001)	75	48.0	10.9
Mixed TMRs	Total Tract	Hannigan et al. (2013)	137	49.2	10.7
TMRs	Total Tract	Krizsan et al. (2010)	172	59.7	12.8
Corn silage based TMRs	Total Tract	Ferraretto and Shaver (2012)	105	44.7	NA

TMRs containing barley based grain	Total Tract	Ferraretto et al. (2013)	62	47.2	NA
TMRs containing corn based grain	Total Tract	Ferraretto et al. (2013)	335	45.6	NA
n or Weighted means	**Total Tract**		**1046**	**48.5**	**10.7**

Figure 3: Apparent total-tract fiber digestibility measures for commercial dairies in the Midwestern US (Rock River Laboratory, Inc; unpublished data since 2015). Commercial measures performed using methods described by Schalla et al. (2012). Organic matter digestibility (% OM), total tract NDF digestibility (TTNDFD; % of NDF) and total tract starch digestibility (StarchD; % of starch) histograms.



MANAGING THE WEANING TRANSITION FOR SUCCESSFUL CALF PROGRAMS

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INTRODUCTION

With increased emphasis on early life feeding programs for calves, more information regarding successful weaning management is needed. Dairy farmers and calf raisers are feeding double, and sometimes nearly triple the liquid feeding rates that had been recommended for decades prior. While growth rates are often excellent when high milk feeding rates are used pre-weaning, much of the growth advantage gained is lost due to poor growth rates in the weeks following weaning. Several recently published studies have aimed to elucidate causes for reduced growth rates after weaning, or the “weaning slump”, and some have shown poor starter digestibility as a result of less accumulated starter intake before weaning is a major driver. However, poor weaning management in general can result in reduced growth rates post-weaning, as more studies have shown that length of weaning, milk-reduction procedures, starter composition and physical form, forage provision, and a myriad of other factors contribute to how well calves adjust before, during, and immediately after weaning.

MILK FEEDING RATE AND DIET DIGESTIBILITY AROUND WEANING

Much of the push for feeding high amounts of milk or milk replacer (MR) has been spurred by several recent studies suggesting greater pre-weaning average daily gain increases first lactation milk yields. The simplest way to increase weight gain is to increase energy and protein intakes by feeding more milk or milk replacer. However, recent studies from Penn State (Gelsinger et al., 2016) and the University of Minnesota (Chester-Jones et al., 2017) have shown that starter intake is equally important to pre-weaning average daily gain and the subsequent effects on milk production in the first lactation. One benefit of obtaining growth from starter versus the liquid diet is dry feeds positively impact digestive system development by providing substrate to the developing rumen microbiota (Baldwin et al., 2004) and substrate to the small intestine to jump-start digestive enzyme secretion (Swanson and Harmon, 2002). Additionally, one Cornell study (Soberon et al., 2012) linking pre-weaning weight gain to milk production in heifers also reported that pre-pubertal average daily gain had a greater impact on first lactation milk production than pre-weaning average daily gain alone. This suggests that a more balanced diet between liquid and solid feed that optimizes growth before and after weaning is needed for successful feeding programs.

The liquid feeding program can have a profound impact on solid feed intake and rumen development in pre-weaned calves. Kristensen et al. (2007) reported reticulorumen wet weights of 5 wk-old calves declined with increasing milk allowance, despite similar rumen epithelial morphology between calves fed 0.4, 0.6, 0.8, and 1.0 kg MR DM per d. These results corresponded to reductions in starter intake with increasing MR allowance, which may indicate physical rather than chemical rumen development is affected by MR feeding rate. Kosiorowska et al. (2011) observed similar responses to Kristensen et al. (2007) in rumen weights with

increasing whole milk allowance. However, Khan et al. (2008) observed increased starter intake, reticulorumen weights, papillae length, and papillae density when calves were gradually weaned from whole milk fed at 20% of BW compared to a conventional milk feeding program (10% of BW until 49 d of age). Inconsistency in the data available may be partially explained by age at weaning, weaning protocols, differences in protein and energy intake, proportion of protein to energy in the liquid diet, and starter nutrient composition and physical form.

To help identify programs that support early starter intake but also take advantage of growth from greater MR feeding rates, Hill et al. (2010) fed calves one of four MR programs: 1) 0.4 kg of DM of a 21% crude protein (CP), 21% fat MR powder fed daily for 42 d; 2) 0.7 kg of DM of a 27% CP, 17% fat MR powder fed daily for 42 d; 3) 0.7 kg of DM of a 27% CP, 17% fat MR powder daily fed for 28 d; or 4) up to 1.1 kg of DM of a 29% CP, 21% fat MR daily fed for 49 d. Digestibility estimates were made at 8 wk of age. While calves fed up to 1.1 kg/d of MR DM had the greatest growth rates to 8 wk, weight gain and feed efficiency were poorer for those calves previously fed the highest MR rate. Much of this reduction may be explained by the depression in apparent organic matter (OM) digestibility in the immediate wk after weaning. Subsequent research trials have also illustrated MR feeding rates greater than 0.7 kg/d of DM depress apparent OM, NDF, CP, and fat digestibility immediately after (Chapman et al., 2016) and up to 4 wk post-weaning (Hill et al., 2016a; Dennis et al., 2018). These studies suggest calves fed large amounts of MR pre-weaning may have difficulty digesting nutrients from solid feed during the weaning transition.

There are several implications to these findings to consider. For example, starters containing greater amounts of fibrous by-products may be difficult to digest if calves are fed large amounts of liquid pre-weaning. Also, it may be necessary to use complex MR reduction strategies to ensure starter intake and diet digestibility is adequate prior to weaning. Because fiber digestion is primarily influenced by cellulolytic fermentation in the rumen, the low digestibility of NDF observed in these studies may indicate the rumen is less prepared for solid feed digestion in calves fed greater amounts of MR (Chapman et al., 2016). To better understand the changes in diet digestibility with age, Hill et al. (2016b) fed calves a moderate (0.7 kg/d of MR DM) or high (up to 1.3 kg/d of MR DM) milk replacer feeding program and monitored changes in nutrient digestion at three time points pre- and post-weaning. Calves fed more MR exhibited lesser apparent NDF digestion at 3, 6, and 8 wk of age. In contrast, calves fed the moderate MR program consumed more starter throughout the trial, which likely hastened rumen development as NDF digestion increased from approximately 15% at 3 wk of age to approximately 35% by 8 wk of age. Digestion of NDF in calves fed the higher level of MR did not change markedly through the 8 wk study and there were few differences with advancing age. Together, these trials identify one of likely many factors contributing to reduced growth rates around the weaning transition due to high MR feeding rates.

WEANING PROTOCOLS

A possible remedy to slowed growth post-weaning when feeding high amounts of MR may be gradually reducing MR allowance over several days to stimulate starter intake before weaning.

Several researchers have tested gradual weaning protocols in calves fed large amounts of MR (Sweeney et al. 2010; Hill et al., 2012; Dennis et al., 2018) and reported some degree of success when weaning occurred over at least a 10 d period. Additionally, Hill et al. (2016a) reported increased post-weaning digestion and growth after gradually weaning high MR-fed calves over a 3 wk period compared to a 1 wk period (stepped-down from 1.1 kg/d of MR). Later weaning ages when feeding a high rate of MR have been suggested to assist with the success of feeding programs when measuring BW gain up to 13 wk of age (Eckert et al., 2015; Meale et al., 2015). Eckert et al. (2015) reported 9 kg heavier calves at 10 wk of age with 8 vs. 6 wk weaning ages and Meale et al. (2015) reported 5 kg heavier calves at 13 wk of age with 12 vs. 8 wk weaning ages. Steele et al. (2017) reported gradually weaning calves over 12 d when fed up to 1.4 kg/d of MR DM resulted in lighter weaning weights, but similar BW at 8 wk of age to calves abruptly weaned at 7 wk of age. However, all three of these studies did not report structural growth measurements and post-weaning measurements were assessed over a short period of time. More recently, Dennis et al. (2018) reported gradual weaning over 2 wk when calves were fed up to 1.1 kg/d of MR DM did not improve growth rates to 16 wk of age to any large degree compared to calves fed 0.7 kg/d or calves fed up to 1.1 kg/d and weaned abruptly at 6 or 8 wk of age, but did improve diet digestibility post-weaning compared to calves weaned abruptly and earlier than 8 wk of age. While gradual weaning over at least 10 d appears to be the best course of action when feeding higher rates of MR, there does not appear to be an advantage in growth rate or diet utilization up to 8 wk post-weaning compared to feeding less MR pre-weaning.

IMPACT OF CALF STARTER COMPOSITION

Calf starter is an important piece of the pre-weaning feeding program and is typically offered within the first few days of life in order to encourage intake and rumen fermentation early in the pre-weaning period. Composition and physical form can vary widely depending on regional feedstuffs, processing, and other factors, but most formulations include starches, sugars, and protein from cereal grains, oilseeds, and by-product feeds. Fermentable starches and fiber are utilized by newly established populations of microbiota to produce VFA, of which propionate and butyrate are stimulatory for chemical development of the rumen epithelium (Baldwin et al., 2004). Khan et al. (2008) reported corn- and wheat-based calf starters increased rumen papillae length, density, and rumen wall thickness compared to oat- and barley-based calf starters, illustrating that starch source can affect rumen development. Castells et al. (2013) observed increased rumen weight as a percent of the total gastrointestinal tract and increased papillae length for calves fed a pelleted starter without forage provision (alfalfa or oat hay). However, when feeding calf starter diets varying in starch concentration (35 vs. 11% of DM), Kosiorowska et al. (2011) did not detect differences in rumen papillae morphology or rumen weight. The effects of starch and fiber in calf diets on rumen development has not been clearly defined by the current literature, however growth responses to starch have been recently reviewed.

Hu et al. (2018) performed a meta-regression using several published trials on the effects of starch level in starter feeds for calves up to 16 wk of age. In calves under 8 wk of age, weight gain and frame growth increased with increasing starch content. However, feed efficiency (BW

gain/DM intake) was not improved in calves less than 8 wk of age in response to starch. In contrast, feed efficiency in addition to weight gain and frame growth increased with increasing starch in calf starter in calves between 8 and 16 wk of age. These results were unsurprising as metabolizable energy (ME) estimates in starters increased with increasing starch concentration. However, what is important to note is calves often have difficulty consuming enough ME through weaning to support growth rates similar to those achieved with the milk feeding program (Steele et al., 2017). Therefore, providing dry feeds with greater ME content and digestibility will help support growth rates when milk is removed from the diet.

FORAGE PROVISION BEFORE WEANING

There has been discussion in the last few years regarding offering forage or roughage to pre-weaned calves to improve growth rates and reduce rumen acidosis. Khan et al. (2016) suggested forage is necessary for better rumen health in calves transitioning to solid feed, however the literature only supports forage inclusion when calf starter particle size is small. Specifically, when pelleted feeds with moderate to high concentrations of starch (30 to 40% of dry matter) are fed to pre-ruminating calves, one can presume that starch fermentation is rapid due to both smaller particle size and heat-treatment of starch that occurs during the pelleting process. Unfortunately, data is limited investigating the effects of starch processing on rumen fermentation in pre-weaning calves. Lesmeister and Heinrichs (2004) reported feeding texturized calf starters with 30% starch, steam-flaked corn inclusion reduced starter intake compared to whole or dry-rolled corn. The authors did not measure diurnal variations in rumen pH, but given that rumen VFA concentrations were greater and rumen pH was lower for calves fed steam-flaked corn up to weaning, accumulation of fermentation acids may have resulted in reduced starter intakes and calves experiencing acidosis. However, it is important to stress that starch content was equal among formulas used by Lesmeister and Heinrichs (2004) as ingredients only differed in corn processing.

Terré et al. (2015) reported when calves were fed a pelleted starter with straw or texturized starter without straw, rumen pH was similar despite increased total dry feed intake for calves fed pelleted starter with straw. When pelleted feed was offered without straw, both rumen pH and starter intakes were lesser compared to calves fed pelleted starter with straw (Terré et al., 2015). Starter starch content was not reported, but given the inclusion of corn, wheat, barley, and oats was over 70% of the formula for both starters, starch levels would likely have been in excess of 40% on a DM basis. Greenwood et al. (1997) fed diets that were identical in ingredient and nutrient composition and only differed in processing and particle size. Bromegrass hay was included in all diets at a rate of 15% on an as-fed basis and hay was either finely ground or coarsely chopped (average particle length was not reported). Starch content was not reported by Greenwood et al. (1997), but given the inclusion of corn (41%) and oats (15%) in the starter, starch content would likely be greater than 35% of the diet on a DM basis. Small particle size coupled with high starch concentrations likely contributed to reduced pH and parakeratosis observed in calves fed a finely ground compared to a coarse diet where abrasiveness differed. Similar abrasiveness values could be achieved with coarse grain inclusion as particle size would require reduction in order to pass from the rumen to the lower gut. Prior to Greenwood et al.

(1997), Warner et al. (1973) reported starter particle size, and not fiber content, increased solid feed intake, the age at which calves began ruminating, and time spent ruminating when fed a mash compared to a pelleted starter with no forage provision or bedding. Rumination is an important behavior for reducing particle size and buffering the rumen, therefore earlier exhibition of this behavior would be considered positive for rumen development and health in calves. Unfortunately, there is limited peer-reviewed data illustrating the effects of starter physical form on rumination behavior in calves before weaning. Responses reported by Greenwood et al. (1997) and Terré et al. (2015) could support forage feeding as a way to buffer the rumen when highly processed starches are fed to calves before weaning, but forage provision should be evaluated relative to other factors in the diet, including starter particle size, starch source, and starch processing. This topic is reviewed in greater detail in Suarez-Mena et al. (2016).

POST-WEANING PERFORMANCE UP TO 4 MONTHS OF AGE

Reduced digestibility coefficients for calves with reduced starter intakes, as was evident in work from Hill et al. (2009), potentially reflects a reduction in rumen development, which would have significant effects on post-weaning performance. However, information is limited for rumen development post-weaning, despite an acknowledged difference in rumen volume from weaning to maturity. The reticulorumen increases in volume from 30% to nearly 70% of the total foregut volume from birth to weaning (Warner et al., 1956), yet weaned calves typically experience reduced growth rates and intake when fed forages and high-fiber feed sources (Jahn et al., 1970, Hill et al., 2008) generally utilized in mature ruminant diets. It also stands to reason that following weaning, there is some capacity for continued rumen development in response to increased energy intake from highly digestible carbohydrates.

Diet form and carbohydrate inclusion could also affect rumen development as energy availability may be altered by particle size and would differ between starch and forage fiber sources. Davidson et al. (2012a) evaluated physical form of grower diets for 13 to 24 wk old Holstein steers and reported similar growth and physical rumen development; however, there was a tendency to reduce rumen papillae length in cranial ventral tissue samples for calves fed texturized compared to pelleted diets. Davidson et al. (2012b) also tested different hay types fed to 13 to 22 wk old Holstein steers and observed steers fed higher CP, lower NDF alfalfa hay exhibited greater papillae surface area in ventral tissue samples compared to steers fed lower CP, higher NDF grass hay. However, baseline slaughter data were not reported and rumen development may have been affected by previous plane of nutrition. From both of these trials, it appears that diet digestibility post-weaning and forage quality may play a role in morphological development of rumen tissue.

Research from our group supports feeding diets with limited amounts of fiber and forage in order to increase ME intake from highly digestible carbohydrates (Hu et al., 2018). This is particularly important when considering the pre-weaning feeding program. Dennis et al. (2017) showed when comparing calf starter to MR feeding rate on overall growth to 16 wk of age, BW gain improved 17% (13 kg) when feeding a high starch calf starter for 16 wk whereas a high MR

feeding rate (up to 1.1 kg/d of DM) only improved BW gain 9% (7 kg). Much of this discrepancy is likely explained by previously discussed reductions in diet digestibility up to 4 wk post-weaning when calves are fed more MR. However, performance through the weaning transition is also linked to many other nutrition- and management-related factors.

SUMMARY

While growth in body weight and frame to weaning can be indicative of a successful pre-weaning feeding program, factoring performance during the weaning transition and growth from birth to at least 16 wk of age may give a better indication of the overall success of a calf program. Nutrition, including liquid feeding rates and starter composition and quality, as well as weaning protocols have a high impact on the success of the weaning transition due to the effects on rumen development and diet digestibility.

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Long-term Consequences of Clinical Diseases Postpartum on Lactation and Reproductive Performances in Dairy Cows

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Introduction

Clinical diseases caused by microbial infection and tissue injury are prevalent in postpartum dairy cows (Santos et al., 2010; Ribeiro et al., 2013; 2016a). Approximately one-third of dairy cows have at least one clinical disease in the first 3-weeks of lactation, and they represent 60 to 80% all clinical cases occurring in lactating cows. The most common clinical diseases observed in dairy herds are metritis, mastitis, digestive problems, lameness, and respiratory problems. The incidence of these diseases in the first 2-months of lactation of 8,268 cows in eight large dairy herds in USA was 21.3, 13.8, 6.4, 5.5, and 2.4%, respectively (Ribeiro and Carvalho, 2017). Combined, these diseases affected 40% of all cows.

The increased susceptibility to diseases in the early postpartum is mostly explained by reduced immunocompetence of dairy cows during this period. The nutritional status and associated metabolic scenario observed postpartum impair function of immune cells and increase the susceptibility to opportunistic microbial infections (Sordillo, 2016). In addition, the enlarged uterus postpartum contains placenta remnants and lochia that favor proliferation of microbes and development of uterine infections (Sheldon et al., 2009).

Cows diagnosed with clinical diseases postpartum are normally treated with drugs or support therapy and, in most cases, health and metabolism seem to be normal by the end of the first month postpartum. However, cows with clinical disease postpartum have reduced lactation and reproductive performances compared with cows that are healthier postpartum or more resilient to the transition challenges. This review summarizes our current understanding of the long-term effects of clinical diseases postpartum on performance of dairy cows and the associated biological mechanisms mediating such effects.

Clinical Disease Postpartum Impairs Fertility

Cows with clinical diseases have delayed resumption of estrous cyclicity postpartum (Santos et al., 2010; Ribeiro et al., 2013), which prolongs the interval between calving and first artificial insemination (AI) postpartum. In general, delayed first breeding causes reproductive inefficiency and economic losses (Ribeiro et al., 2012). Timed AI programs can be used to assure proper time of first AI postpartum. However, the odds of being diagnosed pregnant 45 days after a timed AI is 30% smaller for cows with postpartum disease compared with cows that did not have postpartum disease (Ribeiro and Carvalho, 2017). Further, the odds of pregnancy losses after day 45 of gestation are 2-times greater, and the odds of calving from first breeding postpartum are 42% smaller for cows with postpartum diseases compared with cows that did not have disease (Ribeiro and Carvalho, 2017). Therefore, the impact of diseases is significant even when cows are subjected to timed AI programs. No differences in ovulation after synchronization of the estrous cycle or expression of estrus at timed AI were observed between cows that had or did not have

postpartum diseases (Ribeiro and Carvalho, 2017). Therefore, the observed difference in pregnancy per breeding would be a result of reduced fertilization of oocytes and/or greater embryonic losses occurring before pregnancy diagnosis.

To evaluate the impact of diseases on fertilization of oocytes, early embryo development and survival to morula stage, health information of 597 lactating cows was collected from parturition until first AI postpartum, and uterine flushing for recovery of ova-embryos was performed 5 or 6 days after AI. A total of 419 ova-embryos were recovered and evaluated for stage of development and quality. Cows with diseases before AI had reduced proportion of cleaved, live, and high-quality embryos relative to ova-embryos recovered (Ribeiro et al., 2016a). Within cows with a recovered cleaved embryo, the odds of recovering a live embryo were reduced by 53.6% in cows with disease. The reduction in cleaved embryos is likely caused by reduced fertilization of oocytes. Thus, the results indicate that postpartum disease reduces fertilization of oocytes and survival of zygotes in the first week of development.

To evaluate the impact of diseases on preimplantation conceptus elongation, health information of 148 lactating cows was collected from parturition until first AI postpartum, and uterine flushing for recovery of conceptuses was performed 15 or 16 days after AI. Cows with diseases had shorter conceptuses and reduced concentration of interferon (IFN)-τ in the uterine flush (Ribeiro et al., 2016a). These results were supported by a second experiment that evaluated the transcript expression of IFN stimulated genes (ISGs) in peripheral blood leukocytes (PBL) on day 19 after AI (Ribeiro et al., 2016a). Interferon-τ produced by the elongating conceptus in utero reaches maternal circulation and induces changes in gene expression in peripheral tissues including PBL (Oliveira et al., 2008). Within cows that did not have disease before breeding, the expression of two ISGs (ISG15 and RTP4) was increased in cows later diagnosed as pregnant compared with those diagnosed not pregnant. However, this difference in gene expression of ISGs according pregnancy status was not significant in cows with diseases before AI, suggesting that production of IFN-τ by the elongating conceptuses in utero of cows that had postpartum diseases was reduced (Ribeiro et al., 2016a).

Uterine Disease and Non-Uterine Disease Cause Similar Impact on Fertility

In order to characterize the impact of diseases on reproductive biology of cattle, it is also important to understand how the impact is mediated, so that strategies to mitigate this negative association between diseases and reproduction might be developed. The site of infection or tissue injury is an important factor because the impact on reproduction and the mediator mechanism might change accordingly. Uterine diseases cause endometrial lesions that have detrimental effects on tissue integrity and physiology, hence suboptimal embryonic development and survival. Diseases that occur outside the uterus (i.e. mastitis, lameness, acidosis) might have effects on reproductive biology that are mediated by a physiological response to infection or injury to tissues.

Ribeiro et al. (2016a) compared the effects of the uterine diseases (metritis) and non-uterine diseases (mastitis, lameness, digestive and respiratory problems) on reproduction of lactating dairy cows. Uterine and non-uterine diseases had similar impact on reproduction of dairy cows.

Both type of disorder decreased pregnancy per breeding on day 45 after breeding, increased pregnancy loss after day 45 of gestation, and decreased calving per breeding. Moreover, the two types of diseases have additive negative effects on reproductive outcomes. Cows that had both uterine and non-uterine diseases were 41% less likely to be pregnant on day 45 after breeding (adjusted odds ratio [AOR] = 0.59; CI = [0.47-0.75]), 3-times more likely to lose pregnancy after day 45 of gestation (AOR = 3.06; CI = [1.67-5.60]), and 60% less likely to calved from first breeding postpartum (AOR = 0.40; CI = [0.28-0.58]) compared with cows that did not have disease before breeding. The effects of diseases on the development to morula and conceptus elongation were also similar between uterine and non-uterine diseases (Ribeiro et al., 2016a).

Clinical Diseases Impairs Both Oocyte Quality and Uterine Environment

The interval from the activation of primordial follicles to the formation of preovulatory follicle is estimated to last 180 days (Fair, 2003), in which the majority of time would be spent in the pre-antral stages (138 days), and less time in the antral stages (42 days; Lussier et al., 1987). During folliculogenesis, disease could potentially disturb the follicular environment and oocyte developmental competence without apparent effects on growth and ovulation (Bromfield et al., 2015). Thus, a potential impact of postpartum disease on preantral or antral follicles is a plausible mechanism mediating the long-lasting effects of disease on reproduction.

If reduced oocyte developmental competence was the sole explanation for the long-lasting effects of postpartum diseases on reproduction, then fertility of cows in an embryo transfer (ET) program would not be affected by the occurrence of postpartum diseases. On the other hand, if diseases had an impact on fertility of cows receiving a viable embryo on day 7 of the cycle, then uterine environment should mediate at least part of the effects of disease on fertility of cattle. To test these hypotheses, information on the incidence of postpartum diseases, pregnancy and calving per breeding, and late pregnancy losses were collected in a large dairy farm using both AI and ET as part of the reproductive management for lactating cows (Ribeiro et al., 2016a). Disease affected all reproductive outcomes, and the interaction with breeding technique was not significant. Similar results were obtained when only uterine disease or only non-uterine diseases were considered, thereby suggesting that both types of disease have long-lasting effects on the uterine environment that impairs the ability to support pregnancy to term (Ribeiro et al., 2016a).

Furthermore, ET increased the proportion of cows calving from first breeding compared with AI. The difference, however, was significant only in cows that had disease before breeding. The improvement in calving per breeding observed in cows that had disease when receiving ET suggests that oocyte quality and/or oviduct environment is also affected by disease. Supporting evidence for this interpretation is the slightly smaller change in adjusted odds ratios attributable to disease in cows receiving ET compared with those receiving AI (Ribeiro et al., 2016a). Thus, reduced oocyte competence is a likely component in the carryover effects of disease in fertility of cows receiving AI, and impaired uterine environment is a reason for carryover effects of diseases in fertility of cows receiving AI and cows receiving ET.

Conceptus cells sense changes in uterine environment and respond accordingly. Therefore, studying the transcriptome of conceptus cells could contribute to the discovery of a mechanism

mediating long-lasting effect of inflammatory diseases on uterine biology. Ribeiro et al. (2016a) compared the transcriptome of conceptuses on day 16 of development from cows that had or did not have non-uterine diseases before AI. Five conceptuses recovered from cows that had non-uterine diseases before breeding were matched with five conceptuses of cows that did not have disease before breeding and used for transcriptome analyses. Only a small number (n = 35) of transcripts were differently expressed between the two groups. Nonetheless, functional analysis of these transcripts revealed that changes in the transcriptome of conceptus cells recovered from cows with diseases before breeding resemble an inflammatory response. Three proinflammatory molecules, lipopolysaccharide, IFN-γ and tumor necrosis factor were predicted to be potential upstream regulators of the changes in transcriptome observed in conceptuses recovered from disease cows. Moreover, the potential downstream consequences of these changes would include cell activation, particularly immune cells, and possibly problems with tissue rejection by immune system. These effects could result in rejection of the conceptus tissue by the maternal immune system and pregnancy loss.

Impact of Disease Postpartum on Fertility Goes Beyond First Breeding

All data discussed up to this point refer to fertility outcomes of the first breeding postpartum, when cows are between 50 to 90 days in milk. Although it is clear that fertility in the first breeding is affected by diseases in the early postpartum, this data does not examine how long the effects of disease postpartum last. Would be possible to delay the first breeding to avoid the negative effects of disease postpartum or a bad transition period? Are later breeding also affected?

Carvalho et al. (2018) investigated the impact of clinical diseases that occurred in the first 21 days postpartum (ClinD21) on reproductive performance up to 305 days in milk. In addition, to detailed health information, records of all breeding performed from the end of voluntary waiting period up to 305 days in milk were examined. Although the interval from calving to first breeding was not different between cows that had or did not have ClinD21, pregnancy rate up to 305 DIM was reduced in cows that had ClinD21 (adjusted hazard ratio [AHR] = 0.81), which resulted in extended interval from calving to pregnancy (NoClinD21 = 133.5 vs. ClinD21 = 147.1 d) and reduced proportion of cows diagnosed pregnant within 305 DIM (NoClinD21 = 88.4 vs. ClinD21 = 81.4%). When individual breeding were analyzed, cows that had ClinD21 presented reduced pregnancy per AI for breeding performed before 150 DIM, reduced calving per AI for breeding performed before 200 DIM, and greater pregnancy losses for all breeding up to 305 DIM. Therefore, diseases in the early postpartum have consequences for reproduction of lactating cows up to 10 months after clinical resolution of the disease, and delaying first breeding postpartum is not expect to minimize the impact of disease on reproduction.

More than Fertility - Lactation Performance Is Also Impaired

In addition to reproduction, Carvalho et al. (2018) also investigated the impact of ClinD21 on milk production up to 305 days in milk. Cows that had ClinD21 produced, on average, 410 kg less milk, 17 kg less fat, and 12 kg less protein compared with cows that did not have ClinD21. The reduction in lactation performance was also associated with frequency of ClinD21. For instance, 305-d yield of milk was 357 and 703 kg lesser in cows with a single and multiple ClinD21, respectively, when compared with cows that did not have ClinD21. In addition, cows that had ClinD21 had a lower

and delayed peak in production when compared with cows that did not have ClinD21. Similar to reproduction, uterine and non-uterine diseases such as mastitis had similar impact on milk production up to 305 days in milk, and both type of disease had additive negative effects on production. The observed differences in production could not be explained by differences in production observed during the clinical presentation, suggesting that disease in the early postpartum have long-term consequences on production traits.

In a second study performed by Carvalho et al. (2018), data regarding health postpartum and 305-d yields of milk, fat and protein were collected from 2,415 primiparous cows that had genomic information from a low density panel of single nucleotide polymorphisms. Genomic estimated breeding values (EBV) values for milk, fat, and protein were used to predict 305-d yields of milk, fat, and protein. Genomic EBV values and predicted 305-d yields of milk, fat, and protein were similar between cows that had ClinD21 and those that did not have ClinD21. However, the observed 305-d yields of milk, fat, and protein were reduced by 345, 10 and 10 kg, respectively, in cows that had ClinD21 compared with cows that did not have ClinD21. Moreover, the absolute differences between predicted and observed 305-d yields were larger in cows that had ClinD21 compared with cows that did not have ClinD21. These results suggest that 1) observed differences in production between cows that had or did not have ClinD21 are not related to distinct genetic potential to produce milk, and 2) diseases in the early postpartum compromises the accuracy of genomic predictions of production traits.

Implications for Nutritional Management

Prevention of postpartum inflammatory diseases is unquestionably the best approach to reduce the impact of diseases on fertility of cattle, and strategies to minimize the incidence of postpartum diseases are mostly associated with nutritional management pre- and postpartum (LeBlanc et al., 2006; Santos and Ribeiro, 2014).Nonetheless, understanding the mechanism mediating the impact of disease on reproductive biology of cattle could lead to new strategies for mitigation of the negative consequences of diseases. Assuming that inflammation caused by clinical diseases is the major mediator of subfertility in cows with postpartum diseases, control of inflammation during the clinical presentation of the disease could potentially mitigate the effects of inflammation on reproduction. McDougall et al. (2016) performed a randomized clinical trial testing the hypothesis that addition of a nonsteroidal anti-inflammatory drug (meloxicam) to antimicrobial treatment of clinical mastitis would improve subsequent fertility of dairy cows. Cows treated with meloxicam had greater conception risk in their first insemination postpartum and greater proportion of cows pregnant by day 120 after calving compared with the control group. The results indicate that controlling inflammation during clinical presentation of an inflammatory disease might improve subsequent reproductive performance in dairy cows. A nutraceutical alternative for control of inflammation is reducing ratio of omega-6 to omega-3 fatty acids in the diet of postpartum cows (Greco et al., 2015), which could also minimize the effects of inflammatory diseases on reproduction. Moreover, growing understanding of the differences in reproductive biology of cows that had or did not had diseases postpartum might lead to the development of diets that supply specific shortages of nutrients or stimulating factors that promote oocyte developmental competence and uterine receptivity to pregnancy, consequently improving fertility of dairy cows.

Conclusions

Clinical diseases occurring before breeding are very prevalent in dairy cows and have long-lasting effects on subsequent fertility, milk production, and survival in the herd. Diseases caused by infection in the reproductive tract and diseases caused by infection outside the reproductive tract seem to have similar consequences for reproduction and lactation performance of dairy cows and, when combined, have additive negative effects. The consequences of diseases on fertility does not seem to be mediated by a single mechanism, rather a combination of multiple mechanisms that have additive negative effects, which include reduced BCS at the time of breeding, reduced developmental competence of oocytes, and altered uterine environment. Even though cows with clinical health problems early postpartum are treated and clinical resolution is generally obtained within few days of treatment, pregnancy per AI up to 150 days in milk, fetal survival of pregnancies established up to 305 days in milk, milk production and survival up to 305 days in milk are all impaired in this subgroup of cows. In addition to prevention of diseases, early diagnosis, fast intervention with adequate treatment, and control of inflammation during clinical presentation of the disease mitigate the impact of health problems on reproductive biology of cattle. It is increasingly evident that animal health, not only at the time of breeding or pregnancy development but also in the period preceding breeding, is imperative for optimal reproduction of lactating cows and should always be considered in herd evaluations and management decisions.

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Managing Mycotoxin Problems on Dairy Farms

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Introduction: Molds grow in the field, in storage or during feeding. Mycotoxins are poisons that are produced by some molds. In the U.S.A., mycotoxins are rigorously regulated, such that human exposure is limited. However, mycotoxins routinely occur in feeds. Localized weather conditions make certain mycotoxins worse in some places and in some years.

Dairy producers constantly deal with some amounts of mycotoxins. Even well-managed and highly productive herds can have mycotoxin problems. While mycotoxins can cause severe acute problems, in most cases, mycotoxins cause chronic issues that result in a less milk, digestive problems, more disease and higher culling rates. Several excellent reviews including mycotoxin concerns for ruminant animals are available (Coppock, R.W. and Jacobsen, B.J., 2009; Fink-Gremmels, J., 2008; Gallo, A., et al., 2015; Jouany, J.P., et al., 2009; Mostrom, M.S. and Jacobsen, B.J., 2011; Riet-Correa, F., et al., 2013; Rodrigues, I., 2014; Zaki, M.M., et al., 2012; Whitlow, L.W. and W.M. Hagler, Jr. 2010).

Mycotoxins: While there are over 1000 known mycotoxins and related metabolites (Brase et al., 2009), there are about 50 to 100 which are known to have caused or believed to have the potential to cause disease in humans or vertebrate animals (Frisvad et al., 2006). Most are produced by *Aspergillus*, *Fusarium, Penicillium, Claviceps* and *Stachybotrys* molds.

Table 1. A list of some important mycotoxins grouped by major producer

Aspergillus	*Fusarium*	*Penicillium*
Aflatoxins (AF)	Butenolide	PR toxin
Cyclopiazonic acid (CPA)	Fumonisins	Mycophenolic acid
Cytochalasin A	Deoxynivalenol (DON)	Roquefortine C
Gliotoxin	(3 or 15)-acetyl-deoxynivalenol	Citrinin
Ochratoxin A (OTA)	T-2 toxin	Penicillic acid
Sterigmatocystin	HT-2 toxin	Penitrem A
Verrucologen	Diacetoxyscirpenol (DAS)	Secalonic acid D and F
Fumitremorgins	Zearalenone	Ochratoxin A (OTA)

Claviceps and *Neotyphodium* produce ergot alkaloids
Stachybotrys produce satratoxins and atranones

Mycotoxin Effects: The general effects of mycotoxins include:

- Lower or inconsistent feed intake, sometimes off feed
- Poor feed efficiency, unthriftiness
- Digestive disorders, rumen upsets, intestinal lesions, diarrhea
- Production losses
- Nervous disorders, tremors, flightiness, unsteadiness
- Reproductive disorders, lower conception, embryonic losses
- Poor fresh cow transition
- Increased disease (immune suppression), increased culling and death rate
- Symptoms associated with opportunistic diseases

Many other symptoms have been reported, some of which may be dependent on exposure to specific mycotoxins, on an interaction of multiple mycotoxin(s) and on opportunistic diseases. Digestive disorders and an increase in disease incidence are the major consequences.

Prevention: Molds can grow and produce mycotoxins in the field, in storage or during the feeding process. Management practices and processes to reduce mold growth and mycotoxin formation have been reviewed (Atanda et al., 2012; Hagstrom et al., 2012; Luo et al., 2018).

Preharvest: Prevention starts in the field with practices to reduce plant stress. The two primary factors increasing mold in the field are water stress (either drought or excess rain) and insect damage. Management factors to reduce plant stress and thus the potential for mold are:

- Selection of plant varieties with fungal resistance,
- Timely planting when weather conditions create less stress
- Proper tillage
- Crop rotation (continuous corn or corn following small grains promote fungal disease)
- Good soil fertility
- Irrigation
- Insect control
- Fungicide use
- Timely harvest (when crop is mature)
- Minimization of trash and broken kernels in grain

Postharvest: At harvest, mold spores and mycotoxins are usually present. Proper storage management can reduce additional growth of molds and formation of mycotoxins.

Dry feeds should be stored dry; below 15% moisture. Molds can grow at lower moistures when storage temperature is high. Spots of high moisture and subsequent mold growth can result due to moisture migration. This can occur when there is a sizable difference in day and night temperatures. Aeration can help reduce moisture migration and reduce temperatures. Control of insects, rodents and other pests is important. Storage facilities should be well cleaned after use. Mold inhibitors such as organic acids and other chemicals can reduce mold growth.

Silage and other fermented feeds may have the most problems with molds and mycotoxins. Cheli, F. et al., 2013; Ogunade, I.M. et al., 2018; Tangni, E.K. et al., 2013; Wambacq, E., et al., 2016). The ensiling process is never perfect, and silages often contain areas of deterioration, along with unwanted bacteria, yeasts and molds. Silage, and other wet feeds, must be managed to minimize exposure to air. The often-cited recommendations for making good silage can help prevent mold growth and mycotoxin formation (Kung and Nylon, 2001; Woolford, M.K., 1984). These recommendations generally include:

- Choose adapted varieties with genetic resistance to fungal disease
- Plant at dates to reduce plant stress
- Harvest at proper state of maturity and moisture
- Fill the silo fast, with time for packing
- Proper chop length and processing (good packing and air elimination)
- Pack to achieve a high density (eliminates air)
- Cover well (oxygen barrier plastics and enough weight to prevent air exposure)
- Effective additives such as fermentation aids and/or mold inhibitors
- Manage the feeding face (remove silage as used and at a rate to prevent spoilage)
- Discard the spoilage

Silages most likely to mold are:

- High starch: HM corn, corn silage, small grain silages
- Stressed in the field – affected with fungal disease at harvest
- Dry, mature, late harvest
- Poorly packed and covered -aerated
- Poorly fermented
- Slow feed-out - aerated
- Moved and repacked - aerated
- Fed during spring warm-up and summer – warmer weather
- Prolonged poor storage
- Intermediate feeding piles

Diagnosis: Evaluation of the herd can provide clues to a mycotoxin involvement. A process of elimination can be helpful. Diagnosis is hindered due to multiple mycotoxins, mycotoxin interactions, lack of specific symptoms, lack of animal biomarkers and feed sampling difficulties. The following points can help make a presumptive diagnosis.

- Effects are general, chronic and variable, depending on specific mycotoxin(s) involved
- General symptoms are present (as listed above under effects)
- Symptoms can result from any opportunistic disease, occurring because of mycotoxin induced immune suppression
- Disease incidence increases, despite having good health mgt. and veterinary care.

- Veterinary therapy results in little or no improvement in problems.
- Crops showed signs of fungal field diseases (stalk rot, ear rot, scab).
- Crops were weather stressed or harvested late (after maturity or over-wintered).
- Feed(s) show evidence of deterioration/molding (musty odor, off color, lumps, heating).
- Tested feeds contain excessive molds and marker (typical) mycotoxins, but results can be error prone due to sampling issues.
- Cows respond to removal/dilution of contaminated feed.
- Cows respond to use of products that reduce mycotoxin effects.

Treatments: When a mycotoxin problem arises, these actions can reduce toxic effects. Use of multiple supportive therapies improves the likelihood for maintaining healthy, productive cows.

- Minimize animal stress
- Remove or dilute contaminated feeds
- Ensure feed palatability to encourage intake
- Use mold inhibitors: such as organic acids
- Add extra nutrients: protein, energy (fat)
- Maximize nutritional antioxidants: Vit E, Vit A, carotene, Cu, Zn, Mn, Se, and others
- Enhance rumen fermentation: effective fiber, buffers, direct-fed microbials
- Support gut health: yeast products, probiotics
- **Support immunity: immune modulator products***
- **Deactivate the mycotoxins: adsorbents, binders or enzymes*#**

***Important mitigation products are immune modulators and mycotoxin adsorbents.**
#The FDA has not approved any products for claims for deactivating mycotoxins.

Immune modulators are very important parts of a mitigation strategy. A primary effect of mycotoxins is to cause immune suppression and increased disease. Improving immunity can help the mycotoxin-exposed cow resist disease that might otherwise produce extensive harm.

Mycotoxin binders* have been shown in research to significantly reduce animal exposure to mycotoxins and thus reduce toxicity. FDA has not approved mycotoxin binders.

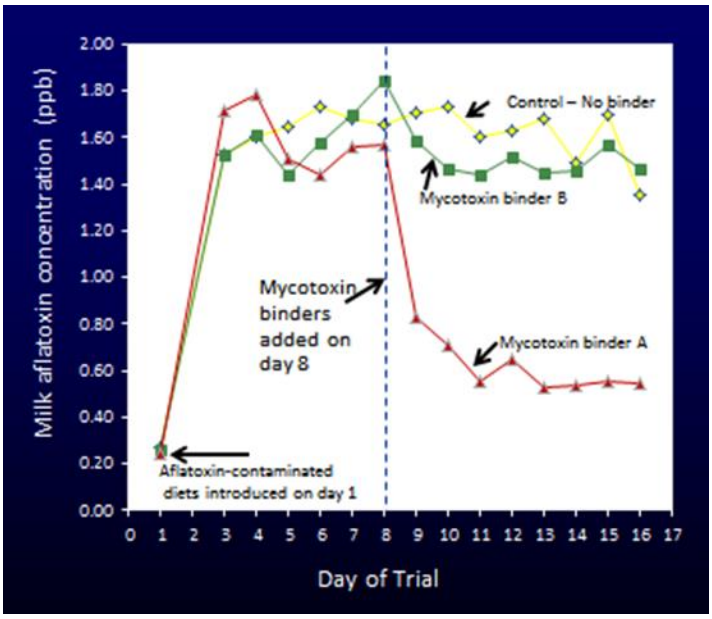
A mycotoxin binder is added to feeds in small amounts. The binder adsorbs mycotoxins, helps prevent mycotoxin absorption across the gut (into the blood supply) and results in mycotoxin elimination into the manure. Even though a mycotoxin is present in the feed, the mycotoxin binder reduces animal exposure and toxicity.

Mycotoxin binders include several types of products including silicates (bentonites, zeolites, smectites, and other clays), yeast products, bacterial products, activated carbons, chitinous products and ion-exchange resins (Boudergue, C. et al., 2009; Kolosova, A. and Stroka, J., 2011). Silicate products are the most thoroughly studied. Selection of effective products must be

based on *in vivo* research. This is because chemical composition or *in vitro* evaluations have shown poor correlations with *in vivo* binding results.

The following (figure 1) shows an example of research where dairy cattle were fed aflatoxin-contaminated diets over a 16-day period. When aflatoxin was fed, there was an aflatoxin residue in milk. In this example, two different mycotoxin binders were added to respective diets on day 8 and cows continued to consume aflatoxin. Cows receiving no binder or mycotoxin binder-B continue to have a high aflatoxin residue in their milk. On the other hand, those cows receiving mycotoxin binder-A had a dramatic decline in milk aflatoxin residue. While binding is not absolute, milk aflatoxin levels can be greatly reduced.

Figure 1. Aflatoxin residues in milk in relation to time when aflatoxin is introduced into the diet and when mycotoxin binders are added to the diet. Binder-A effectively reduced aflatoxin concentration in milk, while binder-A showed no effect with results not significantly different from the treatment with no added binder (adapted from: Kissell et al., 2012).



Summary:

- Mycotoxin occurrence is routine - especially in forages
- A diverse array of multiple mycotoxins occurs
- Chronic toxicity (resulting from long-term, low level intake) is most critical
- Symptoms are diverse, resulting from a cascade of events
- Symptoms include: digestive upsets, low feed efficiency, production loss, poor reproduction, increased disease and higher culling rates
- Disease therapy and vaccines are less effective
- Diagnosis is difficult – a mycotoxin cause may not be apparent
- Mycotoxin exposure may be an undiagnosed but key reason for problems

- Immune stimulants, adsorbents and mold inhibitors are important treatments
- Other management practices can be preventive and supportive

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Top *Eleven* Considerations for Dry Cow Cooling

(This is an updated version of an article that originally appeared in the Proceedings of the Western Dairy Management Conference, 2016)

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While producers are quite familiar with the positive effects of cooling cows during lactation, fewer understand the impact of cooling dry cows. Yet there is increasing evidence that failure to cool cows when they are dry leads to negative effects on productivity and health in the next lactation. Perhaps more critical is the emerging data that indicates a significant impact of in utero heat stress on the developing heifer, which results in long term effects on that calf's productivity and health. This paper considers those topics, along with the economic and implementation considerations.

1) How does dry period cooling affect milk yield?

Cows that experience heat stress during the dry period make 8 to 10 pounds less milk each day in the next lactation compared with herdmates that are cooled. There is no impact on milk composition, though component yields are increased with cooling. The effect is present on the first day of lactation and persists for at least 40 weeks, though all evidence suggests it persists through the entire lactation.

Mammary epithelial cell growth is depressed in heat stressed dry cows relative to cooled animals, and that is consistent with greater capacity to produce milk in the next lactation. These production responses have been confirmed in a number of studies in different locations around the world, supporting the concept that effective cooling is critical to achieve top performance in the next lactation.

2) How long do I need to cool cows in the dry period?

Some reports indicate that cooling in the transition or close-u period alone yield better performance in the next lactation. In a recent study we tested the impact of cooling cows for the first half of the dry period, the second half, or the entire dry period compared with heat stress throughout the dry period. As expected, cooling for the entire dry period improved yields relative to heat stress, but even heat stress of the first or second half of the dry period had negative effects on subsequent yield. Thus, our recommendation is that cows be cooled for the entire dry period to realize the benefits on performance.

3) What are the metabolic effects?

Similar to lactating cows, heat stressed dry cows consume less feed compared with cooled cows. Under heat stress, dry cows have lower bodyweight relative to cooled herdmates as well. Despite the lower nutrient intake and lack of gain, there is no evidence that heat stressed dry cows experience any impact metabolically during heat stress. Indeed, there is no difference in basal or stimulated insulin, glucose or free fatty acids between cooled and heat stressed dry cows. After calving there are some transient affects of dry period cooling, but they are all consistent with the observed increases in milk yield in those cows, and it is important to note that all cows are cooled during lactation so those metabolic effects could not be due to continued heat stress.

4) Is cow health affected?

During the dry period, heat stress reduces antibody response to vaccination, and lymphocyte (i.e. white blood cell) proliferation is also lower. Thus, heat stress has direct negative impacts on the cows ability to respond to pathogens during the dry period. Interestingly, there are carry-over effects of dry period heat stress on immune function, with those cows having lower innate immune responses in early lactation relative to their cooled herdmates, even though they are at a lower level of milk production. The improved immune status in cooled dry cows resulted in better responses to *S. uberis* challenge in early

lactation. Consistent with the improved immune status, cows that are dry during cooler months have fewer cases of mastitis, respiratory illness, and retained fetal membranes relative to cows dry during the hottest months of the year. It is important to remember that the cows are more productive when they are dry when it is cool, yet they are also healthier.

5) What about reproductive performance?

The strongest indication that dry cow cooling does not negatively impact subsequent reproduction comes from a study that compared cows that were dry in the coolest months of the year (i.e. December to February) to those dry in the hottest months of the year (i.e. June to August). Cows dry in the coolest months produced more milk and were less likely to contract disease compared with those dry in the Summer. Cows dry in the cool months had fewer services to pregnancy, fewer days to pregnancy and thus fewer days open versus those dry in the hot months; all indications that despite higher milk yield, and being bred during the hottest months of the year, a dry period during the coolest months improves reproductive performance.

6) Is calf health and growth altered?

Calves born to heat stressed dams are lighter at birth, remain lighter at weaning and even through 12 months of age, relative to calves from cooled dams. Calves that are heat stressed in utero are also shorter through a year of age. Passive transfer is also compromised in calves from heat stressed dams, with lower apparent efficiency of immunoglobulin (IgG) absorption translating to lower circulating concentrations of IgG through the first month of life. This is not due to a reduction in colostrum quality from the dam, but rather a limitation of IgG uptake. We have tracked calf health through the first lactation and found that more in utero heat stressed calves leave the herd due to sickness or illness before puberty, and thus fewer complete the first lactation.

7) Is heifer reproductive and first lactation performance affected?

Heifers born to heat stressed dams achieve puberty at the same age as those from cooled dams, but they require more services to achieve pregnancy. Most importantly, heifers born to heat stressed dams produce about 10 lbs/d less milk in their first lactation compared to the heifers from cooled dams; this effect is apparent from the beginning of lactation and extends to at least 250 DIM, and likely through the

entire lactation. This response is not associated with differences in growth during the first lactation, as both groups of animals calved at the same bodyweight (BW) and had identical BW through the first lactation. More recently, we have determined that those calves that were heat stressed in utero remain less productive in the second and third lactations, and pass on that poorer performance to their offspring. Thus, in utero heat stress generates a phenotype that never achieves its genetic potential.

8) What are the economic impacts of heat stress for dry cows?

In a recent analysis we considered the economic losses associated with a lack of dry cow cooling across the US. Potential days during the year that a cow would experience heat stress were estimated for each state and the total number of cows in each state was used to estimate the total potential milk loss. The total potential loss from a lack of dry cow cooling is at least $810 million annually. However, that estimate only considers milk losses, and does not include any impact on cow health or on the calf. Thus, the total negative impact is likely much greater. But prevention of the milk loss alone is enough to yield significant positive return on any cooling system improvements.

9) How do I assess heat stress?

Because temperature and humidity both influence the ability of a cow to lose heat to the environment, it is best to use the temperature-humidity index (THI) to assess the relative heat load on an animal. Rectal temperature (RT) is the gold standard to determine heat stress, and RT increases at a THI of 68, so abatement should begin before that THI is reached. In addition to RT, respiration rate (RR) will indicate the relative heat stress a cow is experiencing, and can be used effectively in a barn to determine if animals are heat stressed. For example, measuring RR by observation of flank movements of a group of sentinel cows within a pen should provide an indication of heat load; an average RR of 60 or greater suggests that heat stress is occurring and abatement strategies need to be employed to actively reduce the heat load on cows.

10) How are dry cows best cooled?

Methods of cooling are no different from those used on lactating cows. In a hot, humid environment such as we have in Florida, soakers, fans and shade are effective abatement strategies for heat stress, whereas misters may be effective in more arid locations. However, shade alone will not provide complete cooling for cows during high heat and humidity, although it is better than no heat abatement. Sand bedded stalls may also provide additional relief via conductive heat transfer to the sand. Overcrowding will exacerbate heat stress so be sure that dry cows pens are not above 100% stocking rate. In general, the choice should be the most effective system available in the location, and the effectiveness of the system should be tested through RT or RR monitoring.

11) Where can I get more information?

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Metabolic Consequences of Leaky Gut

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Introduction

Suboptimal milk yield limits the U.S. dairy industry's productive competitiveness, marginalizes efforts to reduce inputs into food production, and increases animal agriculture's carbon footprint. There are a variety of circumstances in a cow's life which result in hindered productivity including heat stress, ketosis, rumen and hindgut acidosis, feed restriction, and psychological stress associated with normal animal practices (i.e., pen changes, weaning, shipping). Although these insults have different origins, a commonality among them is increased production of inflammatory biomarkers and markedly altered nutrient partitioning. We and others have generated preliminary data strongly implicating intestinally derived lipopolysaccharide (LPS) as a culprit in these situations.

Heat stress

During heat stress (HS), blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat leading to intestinal hypoxia (Hall et al., 1999). Enterocytes are particularly sensitive to hypoxia and nutrient restriction (Rollwagen et al., 2006), resulting in ATP depletion and increased oxidative and nitrosative stress (Hall et al., 2001). This contributes to tight junction dysfunction and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013), resulting in increased passage of luminal content into portal and systemic blood (Hall et al., 2001; Pearce et al., 2013). Endotoxin, otherwise referred to as LPS, is a glycolipid embedded in the outer membrane of Gram-negative bacteria, which are abundant and prolific in luminal content, and is a well-characterized potent immune stimulator in multiple species (Berczi et al., 1966; Giri et al., 1990; Tough et al., 1997). Immune system activation occurs when LPS binding protein (LBP) initially binds LPS and together with CD14 and TLR4 delivers LPS for removal and detoxification, thus LBP is frequently used as a biomarker for LPS infiltration (Ceciliani et al., 2012). For a detailed description of how livestock and other species detoxify LPS see our recent review (Mani et al., 2012). Endotoxin infiltration into the bloodstream during HS, which was first observed by Graber et al. (1971), is common among heat stroke patients (Leon, 2007) and is thought to play a central role in heat stroke pathophysiology as survival increases when intestinal bacterial load is reduced or when plasma LPS is neutralized (Bynum et al., 1979; Gathiram et al., 1987). It is remarkable how animals suffering from heat stroke or severe endotoxemia share many physiological and metabolic similarities to HS, such as

an increase in circulating insulin (Lim et al., 2007). Intramammary LPS infusion increased (~2 fold) circulating insulin in lactating cows (Waldron et al., 2006). In addition, we intravenously infused LPS into growing calves and pigs and demonstrated >10 fold increase in circulating insulin (Rhoads et al., 2009; Kvidera et al., 2016, 2017c). Interestingly, increased insulin occurs prior to increased inflammation and the temporal pattern agrees with our previous *in vivo* data and a recent *in vitro* report (Bhat et al., 2014) suggesting LPS stimulates insulin secretion, either directly or via GLP-1 (Kahles et al., 2014). The possibility that LPS increases insulin secretion likely explains the hyperinsulinemia we have repeatedly reported in a variety of HS agriculture models (Baumgard and Rhoads, 2013). Again, the increase in insulin in both models is energetically difficult to explain as feed intake was severely depressed in both experiments.

Ketosis and the transition period

Recently, the concept that LPS impacts normal nutrient partitioning and potentially contributes to metabolic maladaptation to lactation has started to receive attention. Although LPS itself has not been the primary causative focus, general inflammation has been the topic of investigations. Increased inflammatory markers following parturition have been reported in cows (Ametaj et al., 2005; Bertoni et al., 2008; Humblet et al., 2006; Mullins et al., 2012). Presumably, the inflammatory state following calving disrupts normal nutrient partitioning and is detrimental to productivity (Loor et al., 2005; Bertoni et al., 2008), and this assumption was recently reinforced when TNFα infusion decreased productivity (albeit without overt changes in metabolism; Yuan et al., 2013; Martel et al., 2014). Additionally, in late-lactation cows, injecting TNFα increased (>100%) liver TAG content without a change in circulating NEFA (Bradford et al., 2009). Our recent data demonstrates increased inflammatory markers in cows diagnosed with ketosis only and no other health disorders. In comparison with healthy controls, ketotic cows had increased circulating LPS prior to calving and post-partum acute phase proteins such as LBP, serum amyloid A, and haptoglobin were also increased (Figure 1; Abuajamieh et al., 2016). Endotoxin can originate from a variety of locations, and obvious sources in transitioning dairy cows include the uterus (metritis) and mammary gland (mastitis) (Mani et al., 2012). Additionally, we believe intestinal hyperpermeability may also be responsible for periparturient inflammation in dairy cows as many of the characteristic responses (rumen acidosis, decreased feed intake, and psychological stress) occurring during this time can compromise gut barrier function.

Rumen and hindgut acidosis

A transitioning dairy cow undergoes a post-calving diet shift from a mainly forage based to a high concentrate ration. This has the potential to induce rumen acidosis (RA) as increases in fermentable carbohydrates and DMI stimulate the buildup of short chain fatty acids and lactic acid (Nocek, 1997; Enemark, 2008). Rumen acidosis has direct and ancillary consequences accompanied by various production issues (decreased DMI, reduced milk yield, milk fat depression) and health challenges such as laminitis, liver abscesses, and potentially death (Nocek, 1997; Kleen, 2003). The mechanisms linking RA and the development of health disorders are not entirely clear, however, recent literature has indicated that inflammation associated with

epithelial damage and consequential LPS translocation are at least partially responsible for production losses associated with RA (Gozho, et al., 2005; Khafipour, et al., 2009). Although many hypothesize LPS translocation occurs at the rumen epithelium directly (Guo et al., 2017; Minuti et al., 2014), others point towards LPS translocation in the hindgut to be a potential source of peripheral inflammation (Li et al., 2012). Interestingly, when RA was induced using either alfalfa pellets or high-grain diets, increased peripheral inflammation was only observed in the high-grain group, irrespective of rumen acidotic conditions being similar between the two treatments (Khafipour et al., 2009a,b). It was hypothesized that the grain supplemented group likely had increased starch flow to the hindgut, and therefore, increased fermentation that could potentially lead to hindgut acidosis and LPS translocation across the large intestine. However, we were unable to recreate production losses and systemic inflammation when we abomasally infused 500 g/d of resistant starch (Piantoni et al., 2018) or even 4 kg/d of purified corn starch (Abeyta and Baumgard, unpublished). Both of our aforementioned experiments were accompanied with marked reductions in fecal pH so it is unlikely that large intestinal acidosis per se is the specific reason for systemic inflammation described in the previous reports (Li et al., 2012, Khafipour et al., 2009a,b).

Feed restriction and psychological stress

Stress associated with feed restriction along with several other regular production practices (e.g., heat stress, weaning, transportation, overcrowding, restraint, social isolation/mixing) is frequently encountered in animal agriculture (Chen et al., 2015) and is associated with gastrointestinal permeability. In fact, we have repeatedly reported reduced intestinal barrier integrity in pigs pair-fed to their HS counterparts (Pearce et al., 2013; Sanz-Fernandez et al., 2014). Furthermore, we recently demonstrated shortened ileum villous height and crypt depth, indicating reduced intestinal health in cows fed 40% of ad libitum intake (Kvidera et al., 2017d). Recent literature indicates that the corticotropin releasing factor (CRF) system may be the mechanism involved in stress-induced leaky gut (Wallon et al., 2008, Vanuytsel et al., 2014). The CRF and other members of the CRF signaling family including urocortin (1, 2, and 3) and their G-protein couple receptors CRF_1 and CRF_2, have been identified as the main mediators of the stress-induced intestinal changes including inflammation, altered intestinal motility and permeability, as well as shifts in ion, water, and mucus secretion and absorption (as reviewed by Rodiño-Janeiro et al., 2015). These alterations appeared to be regulated in large part by intestinal mast cells (Santos et al., 2000). Mast cells are important mediators of both innate and adaptive immunity and express receptors for the neuropeptides CRF1 and CRF2, which may in part explain the association between emotional stress and intestinal dysfunction (Smith et al., 2010; Ayyadurai et al., 2017). Furthermore, mast cells synthesize a variety of pro-inflammatory mediators (i.e., IFN-γ and TNF-α) that are released upon activation, mainly via degranulation (de Punder and Pruimboom, 2015). Excessive mast cell degranulation plays an important role in the pathogenesis of different intestinal inflammatory disorders (Santos et al., 2000; Smith et al., 2010). A better understanding of the role psychosocial stress plays on the initiation of different intestinal disorders in livestock is of great interest for animal agriculture systems.

Metabolism of inflammation

LPS-induced inflammation has an energetic cost which redirects nutrients away from anabolic processes that support milk and muscle synthesis (see review by Johnson 1997, 1998) and thus compromises productivity. Upon activation, immune cells become obligate glucose utilizers via a metabolic shift from oxidative phosphorylation to aerobic glycolysis (not anaerobic glycolysis typically learned about in biochemistry classes), a process known as the Warburg effect (Figure 2). This metabolic shift allows for rapid ATP production and synthesis of important intermediates which support proliferation and production of reactive oxygen species (Calder et al., 2007; Palsson-McDermott and O'Neill, 2013). In an effort to facilitate glucose uptake, immune cells become more insulin sensitive and increase expression of GLUT3 and GLUT4 transporters (Maratou et al., 2007; O'Boyle et al., 2012), whereas peripheral tissues become insulin resistant (Poggi et al., 2007; Liang et al., 2013). Furthermore, metabolic adjustments including hyperglycemia or hypoglycemia (depending upon the stage and severity of infection), increased circulating insulin and glucagon, skeletal muscle catabolism and subsequent nitrogen loss (Figure 3; Wannemacher et al., 1980), and hypertriglyceridemia (Filkins, 1978; Wannemacher et al., 1980; Lanza-Jacoby et al., 1998; McGuinness, 2005) occur. Interestingly, despite hypertriglyceridemia, circulating BHB often decreases following LPS administration (Waldron et al., 2003a,b; Graugnard et al., 2013; Kvidera et al., 2017a). The mechanism of LPS-induced decreases in BHB has not been fully elucidated, but may be explained by increased ketone oxidation by peripheral tissues (Zarrin et al., 2014). In addition to changes in circulating metabolites, LPS has been shown to increase liver lipid accumulation both directly through changes in lipid oxidation and transport enzymes and indirectly through increases in circulating NEFA (Bradford et al., 2009). Collectively, these metabolic alterations are presumably employed to ensure adequate glucose delivery to activated leukocytes.

Energetic cost of immune activation

The energetic costs of immunoactivation are substantial, but the ubiquitous nature of the immune system makes quantifying the energetic demand difficult. Our group recently employed a series of LPS-euglycemic clamps to quantify the energetic cost of an activated immune system. Using this model, we estimated approximately 1 kg of glucose is used by an intensely activated immune system during a 12 hour period in lactating dairy cows. Interestingly, on a metabolic body weight basis the amount of glucose utilized by LPS-activated immune system in mid- and late-lactation cows, growing steers and growing pigs were 0.64, 1.0, 1.0, and 1.1 g glucose/kg $BW^{0.75}$/h, respectively; Kvidera et al., 2016, 2017b,c, Horst et al., 2018a). A limitation to our model is the inability to account for liver's contribution to the circulating glucose pool (i.e., glycogenolysis and gluconeogenesis). However, both glycogenolytic and gluconeogenic rates have been shown to be increased during infection (Spitzer et a., 1985; Waldron et al., 2003). Furthermore, we have observed both increased circulating glucagon and cortisol (indirect markers of hepatic glucose output) following LPS administration (Horst et al., 2018b,c) suggesting we are underestimating the energetic cost of immunoactivation. The reprioritization of glucose trafficking during immunoactivation has particular consequences during lactation as it requires ~72 g of glucose for synthesizing 1 kg milk (Kronfeld, 1982).

Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake: this coupling of enhanced nutrient requirements with hypophagia obviously decrease the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus, wool). We and others have now demonstrated that HS, rumen acidosis, and psychological stress increase circulating markers of endotoxin and inflammation. We believe that the circulating LPS originates from the intestine and initiates an immune response. This activated systemic immune response reprioritizes the hierarchy of glucose utilization and milk synthesis is consequently deemphasized.

Calcium and Inflammation

Circulating Ca is markedly reduced during infection and the response is conserved across species (Tennant et al., 1973; Carlstedt et al., 2000; Toribio et al., 2005). Yet even though hypocalcemia is commonly observed, the biological reason for the decrease remains largely unexplained. Interestingly, Ca repletion during infection increases the incidence of organ failure and mortality (Malcolm et al., 1989). Therefore, sepsis induced hypocalcemia is hypothesized to serve as a protective strategy. Transition period hypocalcemia has long been considered a negative consequence of increased secretion of Ca in colostrum and milk coupled poor pre-calving dietary Ca strategies; which disrupt hormonal regulation of Ca homeostasis (Goff et al., 2014). However, despite successfully implementing pre-calving dietary Ca approaches subclinical hypocalcemia remains a common pathology. Therefore, it is possible that inflammation, at least to some extent, contributes to hypocalcemia in the transition cow, and the decrease in circulating Ca may serve some beneficial effect.

Calcium uptake is a key initial step of leukocyte activation and function (Lewis, 2001) and several investigators have proposed hypocalcemia is the underlying cause of periparturient immunosuppression (Kimura et al., 2006). However, Skarnes and Chedid (1964) demonstrated disrupted LPS detoxification via non-inflammatory mechanisms in the presence of Ca (Figure 4; reviewed by Eckel and Ametaj et al., 2016). To better understand Ca's role during infection we investigated the effects of maintaining eucalcemia following an LPS challenge in lactating cows. Interestingly, we observed an increase in inflammatory biomarkers and a decrease in productivity (i.e., milk yield) in cows maintained at eucalcemia compared to those allowed to develop hypocalcemia (Horst et al., 2018d). Our results are in agreement with previous studies that hypocalcemia may be a protective strategy. Therefore, it brings to question whether preventive strategies for subclinical hypocalcemia are necessary for optimal cow performance.

Conclusion

There are various situations in an animal's life that hinder production performance (i.e., heat stress, feed restriction, rumen acidosis, etc.) and we suggest, based upon the literature and on our supporting evidence, that LPS (of intestinal origin) may be the common culprit in these circumstances. Immune activation in response to LPS markedly alters nutrient partitioning as a

means of fueling the immune response. More research is still needed to understand the mechanisms and consequences of intestinal permeability and associated inflammation in order to provide foundational information for developing strategies aimed at maintaining productivity. Furthermore, it is of interest to further elucidate the contribution of inflammation to subclinical hypocalcemia frequently observed in postpartum cows.

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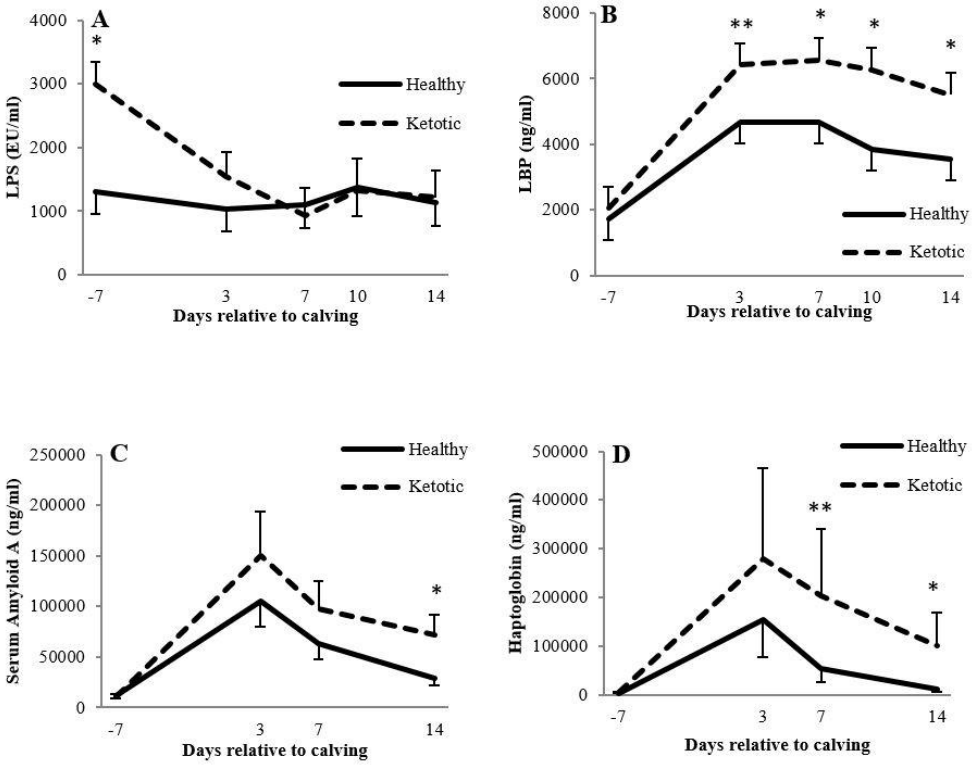

Figure 1. Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows.

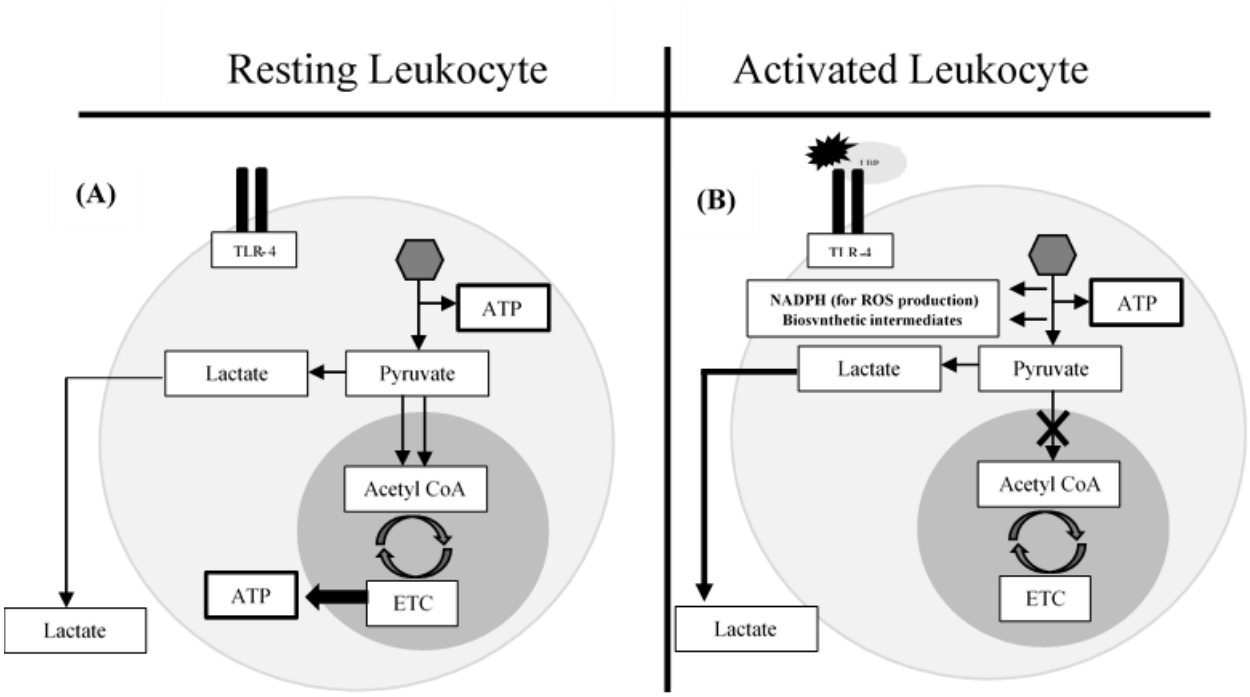

Figure 2. Metabolic pathway of a resting (A) vs. activated (B) leukocyte.

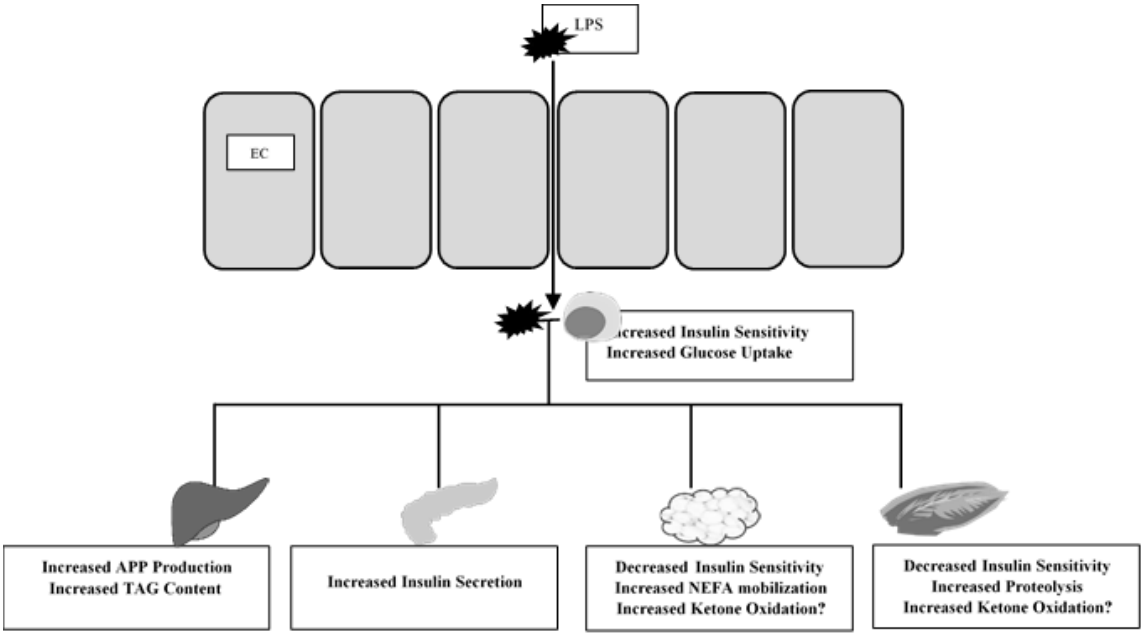

Figure 3. LPS induced alterations in peripheral metabolism

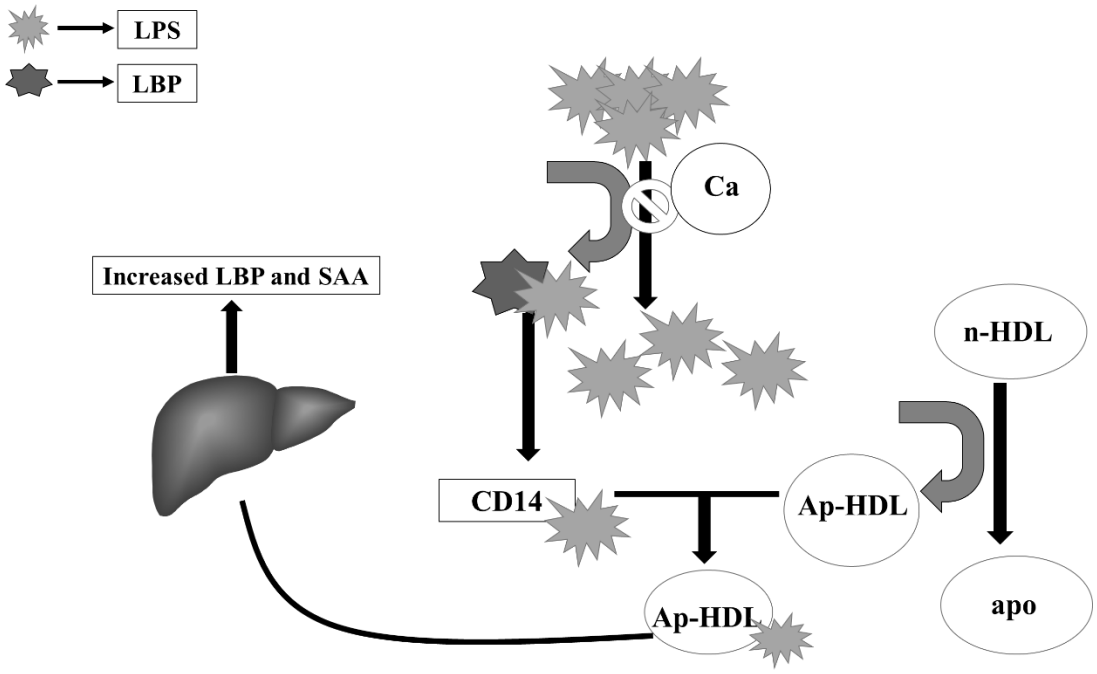

Figure 4. Calcium's role in LPS detoxification

Feeding and Lameness Relationships

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Laminitis is an inflammation in the hoof area causing disrupted blood flow to the corium. Inflammation of the laminae can compromise blood supply and nutrient delivery to the keratin-producing cells that affects horn tissue quality. Several potential causes of laminitis can be found in research reports and field observations.

- Excessive rumen fermentable carbohydrates can lead to volatile fatty acids that can lead to higher levels of lactic acid (strong acid dropping rumen pH) and an increase in rumen fluid osmolality.

- Blood histamine is increased after the death of gram-negative bacteria releasing endotoxin-causing blood pooling in the claw. Coliform bacteria may thrive releasing endotoxins and amides. Protein degradation in the rumen also could contribute histamine production.

- Rumen acidosis produces a toxin with activates a metalloproteinase (MMP) that breakdown bonds between the epidermis of the hoof wall and soft tissue in the corium leading to sole ulcers and white line abscesses.

- A lack of fat cushion that is a complex structure composed of adipose tissue located under the distal phalanx. This structure reduces compression of the corium tissue.

Subacute rumen acidosis (SARA) continues to be a factor in hoof disorders as it can lead to laminitis (Hutjens, 2008). Factors that can lower rumen pH below 6 include high levels of rumen fermentable starch and sugars , unsaturated fatty acids (PUFA), high dry matter intakes shifting rate of passage and availability of nutrients, slug feeding of grain (over 2.2 to 3.2 kg of dry matter per meal), forages lower in natural buffering capacity (such as corn silage), forages that are processed too short reducing cud chewing (less than 18 mm), wet rations (over 55 percent moisture), high quality pasture (lack of functional fiber), empty feed bunks (over two hours resulting in engorgement of feed), and feed sorting (allows dairy cows to consume high levels of fermentable carbohydrates). A suboptimal transition program can lead to rumen acidosis and risks

as dry cows consuming high fiber rations are moved to rations containing higher levels of grain and less forage. This can lead to hoof disorders observed 100 days after calving.

Related feeding factors, feed related factors, and recommended nutrient levels to minimize the hoof risk are summarized are listed below. More than one feeding factor may contribute to a herd hoof problem.

Starch and sugar leads to greater dry matter intake and increase in VFA production. These carbohydrates can shift fermentation from fiber digestion and increase levels of propionic and lactic acids. Grain particle size (finely ground less than 500 microns), grain processing (steam flaking or high moisture grain over 28 percent moisture), and starch source (wheat grain vs. corn grain) impact the rate of rumen fermentation. Sugars have faster rates of rumen fermentation (found in high quality pasture for example). Suggested starch levels in the total ration dry matter is 24 to 28 percent starch and 5 to 7 percent sugar in the total ration dry matter.

Protein quality and quantity can affect hoof hardness. High levels of degradable protein and total protein may lead to rumen fermentation products that can affect foot health. Balance rations based on metabolizable protein requirements using a rumen modelling program to avoid excessive nitrogen while meeting amino acid needs for milk yield.

Physically effective fiber maintains a rumen forage raft to optimize rate of passage and normal rumination (over 450 to 600 minutes of cud chewing activity per day). Rumen pH should be maintained above 6.0 related to saliva production rich in sodium bicarbonate (rumen buffer at a pka 6.25). Two kilograms of forage particles over 25 millimeters in length, 21 percent forage NDF, or 19 to 21 percent effective NDF are suggested minimum levels. Rumination collars can measure on-farm cow cud chewing time and Penn State particle box to evaluate feed particle size can be effective tools to use on dairy farms to evaluate effective fiber. Milk fat test may not be an effective tool to evaluate physically effective fiber.

Unsaturated fats and oils can reduce fiber digestion, shift VFA patterns, or lower rumen pH depressing fiber digesting bacteria. Unsaturated fatty acid can be changed to CLA (conjugated linoleic acids) forms of the fatty acid lowering milk fat test. Limit added vegetable oil to 2.5 percent as oilseeds, free oil (not contained in the oil seed cell) to 225

gram per cow per day, and/or fish oil to 50 grams per day. Feeding rumen inert fat sources reduce rumen fermentation changes. Total levels of PUFA should be under 500 grams per cow per day. Keep total ration ether extract below six percent of the ration dry matter.

Copper is needed for synthesis and maintenance of elastic tissue such as tendons. Copper can affect the claw by increasing the production of a copper enzyme, thiol oxidase, increasing hoof hardness through disulfate bonds in keratin. Immunity and antioxidant activity by superoxide dismutase need copper for cell membrane protection. Cattle deficient in copper were more susceptible to heel, foot rot, and sole abscesses. Suggested level of total copper in the ration dry matter is 10 to 15 ppm (one third from organic copper sources and two thirds from inorganic copper sources). If molybdenum is over 1 ppm, higher levels of supplemental copper will be needed.

Manganese is needed for normal bone density and joint structure. It is required for chondroitin sulfate synthesis in its role in joint cartilage. Manganese has a role as a superoxide scavenger decreasing free radical leading to oxidative damage. Suggested level of manganese is 40 to 60 ppm with one third from organic sources.

Sulfur is needed for sulfur containing amino acids synthesized by rumen bacteria (requires a ratio of 10 to 12 parts nitrogen to one part sulfur), vitamins (biotin and thiamine), and chondroitin sulfate. Harder hooves have been reported with added sulfur by strengthening associated protein bond. Suggested levels of total sulfur is 0.25 to 0.28 percent in the total ration dry matter.

Zinc is a component of over 300 enzyme systems and improves claw integrity through wound healing, epithelium maintenance, and keratin synthesis and maturation. Synthesis of collagen, keratin, and related protein-keratin compound require zinc for enzyme function. Pasture zinc levels vary with lowest levels in the spring lush growth period. Recommended zinc levels in the total ration dry matter is 40 to 60 ppm (one-third organic zinc sources).

Calcium, phosphorous, and vitamin D contribute to bone formation and skeleton soundness. Suggested ration levels for calcium vary from 0.65 to 0.80 percent, phosphorous guidelines are 0.38 percent and 25000 to 35,000 IU of added vitamin D in the total ration dry matter.

Vitamin A is important for epithelial skin and bone health. A deficiency can result in inflammation of the coronary band of the hoof. Suggested supplemental levels of vitamin A range from 75,000 to 100,000 IU per cow per day.

Biotin is needed for keratin formation and claw horn development leading to foot disorders during deficiencies in cattle and horses. Biotin can increase milk yield by 2.0 to 2.5 kg. Milk production increases were not related to hoof improvement due to the immediate milk response. The mechanism for higher milk yield may be related to its metabolic vitamin B function while added biotin requires 6 to 12 months to observe a hoof response. The recommended level of biotin is 20 mg per day.

Feed additives

Sodium bicarbonate, sodium sesquicarbonate, and/or potassium carbonate are rumen buffers that maintain an optimal rumen pH. Potassium carbonate is used during heat stress to maintain feed intake and replace lost potassium. Suggested levels of sodium bicarbonate or sodium sesquicarbonate are 0.75 to 1 percent of total ration dry matter. Potassium and sodium buffers are added to raise the dietary cation-anion balance to a positive 400+ meq/kg during heat stress.

Direct fed microbial (DFM) or probiotics include yeast products and live bacteria that can reduce lactic acid levels in the rumen, increase fiber digestion, and/or stabilize the rumen environment. Check DFM products for controlled research results as numerous commercial products are available with different bacterial combinations.

Monensin reduces lactic acid levels in the rumen while lowering ketosis risk in transition cows that can maintain feed intake and meet nutrient needs. Monensin can also lead to smaller and more meals per day that reduces shifts in the rumen environment based on feedlot data.

Body condition scores are positive associated with digital cushion thickness (DCT) which can provide cushion to the hoof support structure. Cows with the highest DCT score/thickness had 15 percent lower lameness score compared to the lowest DCT scored cows. The DCT continued to drop after parturition reaching a nadir at 120 days after calving. Dairy managers need to monitor changes in BCS after calving minimizing BCS losses to less than 0.5 point (on a 1 to 5 scale).

Transition ration feeding program can cause a buildup of VFA and lactic acid as rations are shifted to higher fermentable carbohydrates. Changing rumen bacteria and reduced rumen VFA absorption can be risks. Stepping up ration nutrient concentration from far off to close up ration to fresh cow ration and to high group rations may allow rumen environment adaptation. Greater risk occurs when higher dry matter intakes occur after calving.

Heat stress can lead to a drop in rumen pH values by 0.2 point (for example from 6.0 to 5.8 units) as experience respiratory acidosis and a drop in blood carbon dioxide and bicarbonate. Cow related factors include lower and variable dry matter intake due to heat stress, a decrease in rumination as cows may be panting, more sorting of feed, and shift of blood flow to the surface area of the cow for heat transfer. Dairy managers may also shift ration ingredients increasing concentrate feeding, lower forage and fiber levels, and added fats/oils that can affect rumen fermentation. Increased the DCAD to over +400 meq/kg of ration dry matter can be effective.

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Table 1. Illinois nutrient recommendations for dairy cows in different stages of lactation and gestation.

	Dry Cow		Fresh	Milk Cows		
	Early	Close-up	0 to 21d	Early 22 to 80d	Middle 80 to 200d	Late >200d
DMI (lbs)	30	22	>35	53	48	44
Crude Protein(CP)%	12	Cows 12 – 13 Heifers 14 – 15	19	18	16	14
Metabolizable Protein (%)	6.0	8.0	13.8	11.6	10.2	9.2
*RDP:% of CP (DM)	70 (8.4)	60 (10)	60 (11.4)	62 (11.2)	64 (10.2)	68 (9.5)
RUP:% of CP (DM)	30 (3.6)	40 (5)	40 (7.6)	38 (6.8)	36 (5.8)	32 (4.5)
SIP:% of CP(DM)	35 (4.2)	30 (4.5)	30 (5.7)	31 (5.60	32 (5.10	34 (4.8)
TDN%	60	67	75	77	75	67
NE_L(Mcal/lb)	0.63	0.69	0.78	0.80	0.78	0.69
Ether Extract %	2	3	4	5.0	5	3
ADF%	30	24	21	19	21	24
NDF%	40	35	30	28	30	32
Starch%	12	15	24	25	22	19
Major Minerals in % of DM						
Calcium (Ca)	0.60	0.7 (*1.0)	1. 0	0.90	0.70	0.60
Phosphorous (P)	0.26	0.30	0.40	0.38	0.36	0.32
Magnesium (Mg)	0.16	0.4	0.33	0.30	0.25	0.20
Potassium (K)	0.65	0.65	1.00	1.00	0.90	0.90
Sodium (Na)	0.10	0.05	0.33	0.30	0.20	0.20
Chlorine (Cl)	0.15	0.15 (*0.8)	0.28	0.25	0.25	0.25
Sulfur (S)	0.16	0.2 (*0.4)	0.25	0.25	0.22	0.22
**When anionic salts are used: mineral/anionic salts (%)*						
Vitamins in IU per Day						
Vitamin A	100,000	100,000	100,000	100,000	50,000	50,000
Vitamin D	25,000	30,000	30,000	25,000	20,000	20,000
Vitamin E	1,000	2,000	2,000	1,000	600	400

a. Trace minerals: iron (150 ppm), cobalt (0.1 ppm), copper (15 ppm), manganese (60 ppm), zinc (60 ppm), iodine (0.6 ppm), and selenium (0.3 ppm).
b. Ratio of minerals in total ration: zinc to copper 4:1, iron to copper 40:1, potassium to magnesium 4.5:1, copper to molybdenum 6:1, potassium to sodium 3:1, nitrogen to sulfur 11:1

Title: Interactions between the stage of maturity of *Eragrostis tef* hay and supplemental energy source on forage utilization in beef heifers.

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Eragrostis tef (teff) grass can be an excellent source of forage for beef cattle. However, its nutritional quality changes with advancing maturity, which could necessitate supplementation to enhance animal performance. Although grains such as corn can be used as energy supplements, their fast rate of ruminal fermentation could result in acidosis and compromise digestive function. Therefore, the use of non-forage fiber sources (NFFS), such as beet pulp, which are highly digestible has appeal in some instances. However, there is still limited information on the ideal energy supplement for beef cattle fed teff hay harvested at different stages of maturity. Therefore, our objective was to evaluate the effects of feeding teff hay harvested at either the early- (EH) or late heading (LH) stage of maturity and providing either corn grain [corn] or beet pulp pellets [BP]) as energy supplements on feed intake and rumen fermentation characteristics in beef heifers. Six ruminally-cannulated continental cross-bred beef heifers were used in a 3 × 3 split-plot design with three 21 d periods. The whole plot factor was stage of maturity of teff hay (EH or LH), and the subplot factor was type of energy supplement (no supplement/control [CON], Corn or BP). Heifers were fed once a day (0600 h) and intake was recorded daily. Corn and BP were fed at an inclusion level of 0.5% of BW and BW was measured on two consecutive days at the beginning of each period to determine the amount to feed. Supplements were offered separate from the hay at feeding and the remaining amount was introduced into the rumen via the cannula 1 h post-feeding. Rumen fluid samples were collected on d 19 (0900, 1500, and 2100 h), 20 (0300, 1200, and 1800 h), and 21 (0000 and 0600 h) to determine fermentation characteristics. In addition, rumen pH was recorded every minute, from d 14 to 21 using indwelling pH loggers. Hay intake tended ($P \leq 0.07$) to be greater for heifers fed the EH than LH hay (9.86 vs 8.58 kg); however, there was no supplement effect on hay intake ($P = 0.88$). Except for ruminal isovalerate concentration ($P \leq 0.08$), there were no stage of maturity × energy supplement interaction ($P \leq 0.90$) for all measurements. Ruminal acetate concentration tended to be greater ($P = 0.10$) for heifers fed EH than LH hay whereas butyrate concentration was greater ($P \leq 0.01$) for heifers supplemented with corn compared to CON and BP. Ruminal isobutyrate concentration tended to be lower ($P = 0.05$) and total branch-chain fatty acid concentration was lower ($P < 0.01$) for heifers supplemented with BP compared with CON and Corn. However, ruminal propionate and total VFA concentrations did not differ across diets ($P \geq 0.12$). There was no diet effect ($P > 0.05$) on mean, minimum and maximum rumen pH, or on the duration and area rumen pH was below 6.2 and 5.8. In summary, hay intake tended to be greater for heifers fed teff hay harvested at the early compared to late heading stage of maturity. In addition, despite changes in the rumen VFA profile, both stage of maturity and type of energy supplement had no detectable effect on rumen pH.

Feed quality and fermentation characteristics of barley and triticale grown for forage under irrigation in the Pacific Northwest

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An experiment was conducted near Moses Lake, Washington to evaluate the utility of growing spring forage barley (*Hordeum vulgare*) and triticale (*Triticale hexaploide Lart.*) in monocultures and blends to maximize yield and forage quality. We hypothesize that blending spring barley and triticale will maintain the exceptional forage yields of triticale and gain forage quality by including the barley. A field study was initiated in April of 2017 planting eight mixtures of Pronto barley and Merlin triticale at a rate of 112.1 kg/ha in a randomized complete block design with four replications: Treatment 1 - 100% Pronto; Treatment 2 - Pronto 90%/Merlin 10%; Treatment 3 - Pronto 70%/Merlin 30%; Treatment 4 - Pronto 50%/Merlin 50%; Treatment 5- Pronto 30%/Merlin 70%; Treatment 6 - Pronto 10%/Merlin 90%; Treatment 7 - 100% Merlin; and Treatment 8 - Pronto 20%/Merlin 100% (seeding rate increased to 120% of monoculture). Forage yields were determined using a forage harvester in July (harvested plots size = 0.9 meters x 4.3 meters). After harvest, the forage was further processed using a Bear Cat chopper before collection of pre-ensiled samples. Forages were packed (4.54 kg) into mini PVC research silos (four replications) and allowed to ensile for 88 days. Following ensiling, silages were extracted and subsampled for determination of dry matter recovery, forage quality, and fermentation chemistry. Treatment 6 had the highest DM forage yield of 10.67 Mg/ha but was not significantly different from other treatments except Treatment 1and Treatment 2 (8.45Mg/ha and 8.92 Mg/ha, respectively; $p < 0.05$). The fresh forage quality analysis assessed the blends potential as a hay crop. Crude protein did not differ across treatment groups, ranging from 98.5 g/kg to 108.3 g/kg. Treatments 1 and 2 had the greatest starch concentration, 72.3 g/kg and 72.0 g/kg respectively, but were not different from Treatment 3 (58.0 g/kg). Acid Detergent Fiber was highest in Treatment 6 (407.4 g/kg) but was not different from Treatment 1, 5, and 8 (389.9 g/kg, 404.4 g/kg, and 397.3 g/kg, respectively). Neutral Detergent Fiber was highest in Treatment 5 (592.0 g/kg) but was not different from Treatment 1, 3, 6, and 8 (578.2 g/kg, 584.0 g/kg, 584.3 g/kg, and 585.5 g/kg, respectively). The post-ensiled product was also analyzed for feed quality. Crude protein did not differ across treatment groups, ranging from 96.1 g/kg to 105.8 g/kg with only minimal losses in the ensiling process. Starch and fiber concentrations were affected by the ensiling process. Starch concentration was highest in Treatment 1 after ensiling (137.3 g/kg) but was not different from Treatment 1 pre-ensiled (121.8 g/kg). Treatment 2 post-ensiled starch (103.5 g/kg) was not different from Treatment 1 pre-ensiled but was higher than all other treatments ($p < 0.05$). Acid Detergent Fiber was lowest in Treatment 1 (381.5 g/kg) and Treatment 2 (322.7 g/kg). Neutral Detergent Fiber was also lowest in Treatment 1 (480.0 g/kg) and Treatment 2 (502.7 g/kg). In conclusion, blends with a higher proportion of Pronto barley have a lower fiber content and a higher starch content indicating the potential to supply greater energy per unit of feed to livestock. Blends with a higher proportion of Merlin triticale have a higher DM yield. Blending forages did not affect crude protein when harvested at mid-maturity growth. It was demonstrated that spring barley/triticale blends display good forage quality and only small differences in DM yield when compared to their respective monocultures. The forage quality is sufficient to meet the protein requirements of dry beef cows in late gestation and the measures of fiber indicate support of optimum intake and digestion. For beef and dairy

producers, blending barley and triticale has the potential to supply high quality and affordable feed.

Title: The effects of altering ruminal fermentable energy supply on rumen function, nutrient supply, and nitrogen utilization in finishing cattle fed diets containing distillers grains

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Because they are cost-effective compared to traditional feedstuffs, there is widespread use of corn dried distillers grains with solubles (CDDGS) in finishing cattle diets. However, as a result of its high crude protein (CP) content, dietary inclusion of CDDGS leads to nitrogen (N) intake exceeding requirements and, thus, wastage. Ruminal fermentable energy supply is a major determinant of the efficiency of N use. However, there is limited information on whether altering ruminal fermentable supply when feeding CDDGS could enhance ruminal N use efficiency, which improves production performance, and limits nitrogen (N) excretion. Therefore, the objective of this study was to examine the effects of feeding different types and amounts of grain (corn vs wheat) on ruminal fermentation characteristics and nitrogen utilization in finishing cattle fed diets containing 15% CDDGS (DM basis). Six ruminally-cannulated beef continental crossbred heifers were used a replicated 3 × 3 Latin square design with 28 d periods (first 21 d for adaptation and last 7 d for measurements). The three dietary treatments were either corn (73% of diet DM; CON), 53:20 corn/wheat blend (20W) or 33:40 corn/wheat blend (40W) as the major fermentable energy source. All diets contained 15% CDDGS (DM basis), 10% grass hay, and 1.155% mineral supplement. Heifers were also fed Melengesterol acetate (MGA) at 227 g each d. Animals were fed once daily (0700 h) and DMI was measured during the last 6 d of each period. Ruminal pH was also measured using an indwelling pH logger over the last 6 d of each period. To determine fermentation characteristics, rumen fluid was collected on d 26 (0900 h, 1500 h, 2100), 27 (0300 h, 1200 h, 1800 h) and 28 (0000 h, 0600 h) and samples were composited by cow and period. Blood samples were collected from the jugular vein 3.5 h after feeding on d 28 to determine blood glucose concentration. Data were analyzed using SAS. Dry matter intake tended to be lower ($P = 0.06$) for heifers fed the 40W compared with the CON and 20W diet. Mean and minimum pH were lower ($P \leq 0.03$) for heifers fed the 20W and 40W diets compared with heifers fed the CON diet. However, there was no diet effect ($P = 0.84$) on maximum pH. The molar proportions of acetate, propionate, butyrate, total volatile fatty acid, and branched chain fatty acid concentrations did not differ ($P \geq 0.15$) across treatments. However, heifers fed the wheat-containing diets experienced a longer duration ($P \leq 0.04$) with rumen pH less than 5.8 and 5.5; and tended ($P \leq 0.10$) to have a greater area when pH was less than 5.5. There was no diet effect ($P = 0.36$) on blood glucose concentration. In summary, increasing ruminal fermentable energy supply by feeding increasing amounts of wheat resulted in a decrease in ruminal pH that possibly led to the decrease in DMI.

Title: Bitter or Better Taste Buds? Aversion to Phenylthiocarbamide in Mature Rams

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Shrub encroachment on grasslands is a worldwide issue. Sheep are a potential tool for mitigating shrub encroachment. However, many shrubs contain bitterness attributes that serve as a mechanism to detour grazers. With human interactions limiting rangeland fire interval and little to no reduction from herbivores, these shrubs grow in size and population, thereby decreasing plant community diversity and reducing wildlife habitat. Sheep with a greater tolerance for bitter compounds would be expected to consume more bitter-tasting vegetation. We hypothesize that sheep can detect bitter-tasting compounds and the sensitivity to bitter compounds will vary from animal to animal. The objective of this study was to determine whether sheep could detect the bitter tasting compound, phenylthiocarbamide (PTC), and if so, what PTC concentration would elicit an avoidance response. Using a crossover study design, mature Rambouillet and Targhee rams (n = 30) were subjected in randomized order to various PTC concentrations mixed in the drinking water (PTC-solution). In trial 1 and 2 (n = 15/trial), 0.20, 0.56, 1.57, 4.39, and 12.39 mM and 0.20, 0.43, 0.94, 2.03, and 4.39 mM of PTC were tested, respectively. On test days, PTC-solution (trial 1: 1.5 kg; trial 2: 3.0 kg) and water (same amounts) were offered for *ad libitum* intake in a side-by-side presentation for 1 hour in trial 1 and 2 hours in trial 2. Test days were followed by a rest day where similar amounts of water and PTC-solution replaced with water were offered to limit potential carry over effects into the next test day. Consumption of PTC-solution for each PTC concentration was expressed as the percentage of PTC-solution consumed of total morning fluid intake [(PTC-solution / (PTC-solution + water without PTC)) × 100]. There was no effect ($P > 0.74$) of sequence that rams received PTC-solutions on PTC consumption during either trial. As PTC concentration increased, percentage of PTC-solution consumed decreased. The greatest decrease in percentage of PTC-solution consumed occurred between 1.57 – 4.39 mM (58%), and between 2.03 – 4.39 mM (72%) for trials 1 and 2, respectively. In trial 2, the least percentage of PTC consumed was the 4.39 mM PTC concentration that was different ($P \leq 0.05$) than all other PTC concentrations, which did not differ ($P > 0.05$) from each other in percentage consumed. For both trials, total fluid intake on rest days was not affected ($P > 0.05$) by PTC treatment or sequence from the previous test day. A high degree of variation in avoidance of PTC was observed between individuals in trials 1 ($R^2 = 0.28$) and 2 ($R^2 = 0.11$). This research suggests rams could taste the PTC, and the concentration at which PTC-treated water was avoided varied across rams. To our knowledge, this is the first study to test PTC as a tool to identify variation in bitter avoidance among sheep. Therefore, this study suggests that it may be possible to select sheep, based on demonstrated avoidance of PTC, for targeted grazing applications to manipulate vegetation towards range management goals.

Title: Effect of Irrigation on Fiber Concentration and In-Vitro Fiber Digestibility of Corn Plant Tissues

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Abstract

Water stress may be detrimental to corn plant growth due to the evident reduction in yield. While corn cell wall composition is directly correlated with digestibility in dairy cattle, the effects of water stress on corn plant cell wall composition is still unknown. The objective of this study was to determine the effect of irrigation on fiber concentration and in vitro fiber digestibility on stems, leaf-sheaths, and leaf-blades of corn for silage. Five commercial corn hybrids for corn silage (one of them showing the Brown Mid-Rib phenotype) were planted in a split-plot within a randomized complete block design with four replicates. Experimental treatments consisted of a control treatment (watered) with furrow irrigation at planting and three more times during crop growth, and a non-irrigated treatment (non-watered) with furrow irrigation only at planting. When the corn was between ¼ and ¾ milk-line, 10 corn plants from each plot were cut by hand at 15 cm above the ground, weighed, and chopped using a wood chipper. A 400-g sample of chopped material was dried to determine biomass dry matter (DM) concentration. A composite sample of the stems, sheaths and blades were collected from each of the plots. Samples were also collected from the upper and lower internodes. The samples were then dried at 55ºC and ground, first through a 5 mm Wiley Mill then through a 1 mm screen. Concentrations of in vitro DM, cell wall, neutral detergent fiber, and lignin were determined for each sample. Data were analyzed using Proc Mixed of SAS (version 9.4), and the model included the effects of block (random, df = 3), treatment (fixed, df = 1), block by treatment interaction or whole-plot error (random, df = 3), hybrid (fixed, df = 4), treatment by hybrid interaction (fixed, df = 4), and the residual or split-plot error (random, df = 25).

Watered plots contained less neutral detergent fiber concentrations than non-watered plots (64.6 vs. 67.6% NDF; $P < 0.01$). Watered plots had a greater in-vitro apparent DM digestibility than non-watered plots (56.7 vs. 54.8% IVDMD; $P < 0.05$). The in-vitro neutral detergent fiber digestibility tended to be greater for the watered plots than for the non-watered plots (51.7 vs. 50.1% IVNDFD, $P < 0.10$). This observation is contrary to the general industry belief that water-stress increases fiber digestibility in forages. The concentration of lignin within the cell wall was not affected ($P > 12$) by irrigation and averaged 19.3%. In summary, based on the data of this controlled study, limited water supply does not affect lignin concentration of the cell wall and does not increase the in-vitro digestibility of fiber in corn for silage.

Title: Effects of weaning and supplemental butyrate on calf performance and rumen fermentation in dairy calves

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This study examined the effect of the weaning transition and supplemental butyrate on feed intake and rumen fermentation in dairy calves. Holstein bull calves (n=36; age= 10.7 ± 4.1d) were assigned to one of four treatment groups: two pre-weaning groups, animals fed milk only (PRE-M) and those fed milk, calf starter, and hay (PRE-S); and two post-weaning groups, animals fed milk, calf starter, and hay without supplemental butyrate (POST-S) or with supplemental butyrate at a rate of 1% w/w during the weaning transition (POST-B). Milk was provided at 1200 g/d; starter, water, and hay were provided ad libitum. Weaning transition occurred in POST-S and POST-B by reducing milk replacer to 800 g/d in week 7 and 400 g/d in week 8, and complete weaning at week 9. Rumen pH was measured continuously for seven days prior to terminal sampling. At harvest, rumen fluid was sampled and analyzed for volatile fatty acids (VFA). Starter intake was measured daily; weights were measured weekly to calculate average daily gain (ADG). Between PRE-M and PRE-S, total VFA concentrations increased (11.8 ± 5.8 vs. 35.6 ± 5.6 mM, $P < 0.01$), but mean pH was unaffected (6.16 ± 0.83 vs. 7.44 ± 0.79, $P = 0.28$), suggesting the rumen can manage rumen pH despite increases in fermentation prior to weaning. Between PRE-S (age = 42 d) and POST-S (age = 63 d), calf starter intake increased (250 ± 219 vs. 2239 ± 219 g/d, $P < 0.01$), total VFA concentrations increased (35.6 ± 5.6 vs. 154.3 ± 15.0 mM, $P < 0.01$), but mean rumen pH was unaffected (7.44 ± 0.79 vs. 6.39 ± 0.19, $P = 0.48$), suggesting the rumen can also manage rumen pH during the weaning transition. Between POST-S and POST-B, starter intake increased (2239 ± 219 vs. 3094 ± 219 g/d; $P = 0.01$), total VFA concentrations were unaffected (154.3 ± 15 vs. 131.0 ± 15.8 mM, $P = 0.23$), and mean rumen pH decreased (6.39 ± 0.19 vs. 5.83 ± 0.18, $P = 0.05$), even though ADG increased (0.77 ± 0.04 vs. 0.92 ± 0.04 kg/d; $P = 0.03$), suggesting rumen pH is sensitive to dietary changes post-weaning. In all, these data suggest the rumen's ability to manage rumen pH changes fundamentally post-weaning. Additionally, the supplementation of calf starter with butyrate during the weaning transition helps to sustain greater calf starter intakes and ADG. Why calves with lower rumen pH can achieve greater calf starter intakes is unclear; these data suggest the impact of rumen pH on feed intake differs between calves and cows

Title: Effect of agronomic selenium biofortified alfalfa hay on selenium status and glutathione peroxidase activity in transition dairy cows and their calves

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Abstract

Selenium (Se) is an important trace mineral for human and livestock. Selenium is an essential micronutrient for the antioxidant glutathione peroxidase (GPx) which is important to protect the cell from free radicals. Oxidative stress is particularly acute during early post-partum in dairy cows. Supplementation of selenium in pregnant cows can improve the Se status of their offspring, bursting theirs antioxidant capability and, thus, fostering a better health and growth performance. Se supplementation in ruminants is particularly important in region of low Se in soil, such as Oregon; however, inorganic Se supplementation is limited by FDA. Thus, feeding agronomic Se biofortified alfalfa hay to dairy heifers during pregnancy can improve their Se status, antioxidant activity, and the amount of Se transfer into the calves. To test the hypothesis, we used 18 primiparous cows (8 Holsteins and 10 Jerseys) fed ad libitum with a TMR based on grass silage (0.14 mg Se/kg DM). Cows were blocked by breed and randomly assigned to two groups. One group received from 40 days prior parturition to 2 weeks post-partum 1 kg agronomic Se biofortified alfalfa hay (3.2 mg/kg DM)/100 kg of BW mixed with the TMR (TRT). A group received alfalfa with low Se (0.4 mg/kg DM; CTR). Whole blood in cows and their calves and liver and milk samples of cows were used to determine Se and other trace minerals by ICP-MS. Plasma of cows and their calves, erythrocyte, and milk samples of cows were used to measure GPx activity by using a commercial assay kit. Dry matter intake and milk yielded were measured daily. Data were analyzed by GLIMMIX of SAS with the fixed effect of treatment, breed, time and their interactions and cows as random effect. PROC CORR was used to find the correlation between the variances.

After 4 weeks into the trial, Se concentration in blood increased 2-fold (P<0. 0001) in TRT vs. CTR (204.49±19.19 vs. 95.01±20.90 ng/ml) that results in greater (P<0.0001) Se in liver (1137.09±68.70 vs. 619.10±73.45 mg/g), Se in milk (48.3415±3.40 vs. 22.52±3.3562 ng/ml), and Se in blood of calves (215.50±10.92 vs. 161.22±10.92). GPx activity increased in plasma (92.84±3.44 vs. 77.88±3.36 nmol/min/mL) and erythrocyte of cows (549.23± 35.32 vs. 260.02± 34.15 nmol/min/mL) by Se biofortified hay, while only a numerical increase was detected in their calves and no effect was detected in milk GPx. Our results point out that feeding pregnant dairy heifers with a relative low amount of Se-fortified alfalfa hay is an effective way to increase Se in blood, liver, and milk, which is in turn improved antioxidant activity and the amount of Se that transferred into the calves.

Keywords: Agronomic Se; Selenium concentration blood; GPx; liver; Milk; dairy heifers; Calves