

Energy Cost of Inflammation in Dairy Cows

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Abstract

Metabolic maladaptation to lactation (ketosis) and heat stress are two economically devastating hurdles to profitability. Heat stress and ketosis affect herds of all sizes and almost every dairy region in the world. The biology of ketosis and heat stress has been studied for almost a half century, but the negative impacts of both are as evident today as they were 30 years ago. Our recent discoveries suggest that endotoxin is the common culprit in both disorders and the intestine appears to be the etiological origin of both metabolic disorders. Endotoxin stimulates the immune system and activated leukocytes switch their metabolism away from oxidative phosphorylation to rely more on aerobic glycolysis. In multiple species, we estimate that immune activation consumes about 1 g glucose/kg BW^{0.75} or about 2 kg glucose/day in an adult lactating dairy cow. Thus, an activated immune system reprioritizes nutrient partitioning away from the synthesis of economically valuable products.

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 situations in a cow's production cycle when nutrient

utilization is reprioritized from milk synthesis towards agriculturally unproductive purposes. Two well-known examples that markedly reduce milk production are heat stress (**HS**) and the metabolic maladaptation to lactation (i.e., ketosis) following calving. Heat stress negatively impacts a variety of dairy production parameters including milk yield, milk quality and composition, rumen health, growth and reproduction, and is a significant financial burden (~\$1 billion/year for dairy the U.S. alone; St. Pierre et al., 2003). Similarly, ketosis is a costly disorder (estimated at ~\$300 per case; McArt et al., 2015) and also represents a major obstacle to farm profitability. While the metabolism of ketosis and HS has been studied for more than 40 years, the actual pathologies of both remain poorly understood. Suboptimal feed intake, experienced during both metabolic disorders, is unable to fully explain the decrease in productivity. In other words, the initial insult in the cascade of events ultimately reducing milk synthesis in both HS and ketotic cows has not been identified.

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

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and Rhoads, 2011, 2013; Belhadj Slimen et al., 2015). Heat stress interferes with animal comfort and suppresses productive efficiency (Fuquay, 1981; Strong et al., 2015). Furthermore, it is well-known that 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). During periods of HS, animals initiate major thermo-regulatory adaptations in order to maintain euthermy. The result of HS is underachievement of an animal's full genetic potential. 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 to 50% of the decreased milk yield during environmental-induced hyperthermia (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). Therefore, HS affects many production parameters either directly (i.e., decreased milk yield, increased mortality) or indirectly (i.e., via decreased feed intake; Collier et al., 2006; Adin et al., 2009; Hansen 2009; Baumgard and Rhoads, 2011, 2013; Mahjoubi et al., 2014). The remaining "direct" effects of HS are explained by the fact that heat-stressed animals exploit novel homeorhetic strategies to direct metabolic and fuel selection priorities independent of nutrient intake or energy balance.

Ketosis

The periparturient period is associated with substantial metabolic changes involving normal homeorhetic adaptations to support milk production. Early lactation dairy cattle

enter a normal physiological state during which they are unable to consume enough nutrients to meet maintenance and milk production costs and animals typically enter into negative energy balance (**NEB**; Drackley, 1999). During NEB, cows mobilize non-esterified fatty acids (**NEFA**) in order to partition glucose for milk production in a homeorhetic strategy known as the "glucose sparing effect." These NEFA can undergo one of three fates: 1) energy production via complete oxidation through the TCA cycle; 2) partial oxidation to produce ketone bodies (acetone, acetoacetic acid, and β -hydroxybutyric acid [**BHBA**]; and 3) re-esterification to form triglycerides (**TAG**), which are either exported as very low density lipoprotein to deliver fatty acids to extra-hepatic tissue or "stored" in the liver (Ingvarsen, 2006; Ingvarsen and Moyes, 2013; McArt et al., 2013). Mitochondria available oxaloacetate is needed for fatty acid derived acetate to enter the TCA cycle; however, oxaloacetate exits the TCA cycle because it is a key gluconeogenic precursor during NEB and therefore full NEFA oxidation is limited. The ruminant liver has limited ability to export the large amount of NEFA mobilized from adipose tissue during NEB, resulting in hepatic TAG accumulation (Grummer, 1993; Drackley, 1999; Gross et al., 2013). Consequently, ketone body production is a mechanism by which fatty acids can be partially oxidized in the liver and exported into the bloodstream as a water-soluble, transportable form of acetyl units to peripheral tissues. In dairy cattle, ketosis is arbitrarily 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 ($\text{BHBA} > 3.0 \text{ mM}$; Gillund et al., 2001) while the incidence of subclinical ketosis ($> 1.2 \text{ mM BHBA}$) is thought to be much higher ($> 40\%$; McArt et al., 2012).

Therefore, ketosis is thought to result from an imbalance in energy demand, excessive adipose tissue mobilization, and increased ketone body production in hepatic tissue (Drackley et al., 2001; Garro et al., 2014).

Heat Stress Etiology

Mechanisms responsible for altered nutrient partitioning during HS are not clear; however, they might be mediated by HS effects on gastrointestinal health and function as we and others have demonstrated HS compromised intestinal barrier function (Lambert et al., 2002; Dokladny et al., 2006; Yang et al., 2007; Pearce et al., 2013; Sanz-Fernandez et al., 2014). During HS, blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat (Lambert et al., 2002), 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, HS 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 is 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 during HS into the bloodstream 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). 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 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 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.

Transition Period Inflammation

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 numerous investigations. Increased

inflammatory markers following parturition have been reported in cows (Ametaj et al., 2005; Humblet et al., 2006; Bertoni et al., 2008; 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 TNF α infusion decreased productivity (albeit without overt changes in metabolism; Yuan et al., 2013; Martel et al., 2014). Additionally, in late-lactation cows, injecting TNF α 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 LPS-binding protein, 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 transition dairy cows include the uterus (metritis), mammary gland (mastitis), and the gastrointestinal tract (Mani et al., 2012). However, we believe intestinal permeability may be a prime contributor to inflammation in the transition dairy cow. Post-calving, dairy cows undergo a dietary shift from a high-forage to a high concentrate ration, and these grains can be rapidly fermented at a rate exceeding removal of volatile fatty acids, resulting in depressed ruminal pH (Owens et al., 1998). Consequently, rumen acidosis may be induced and this can compromise the gastrointestinal tract barrier (Khafipour et al., 2009). In order to further investigate the effects of intestinal permeability on production and inflammation, we intentionally induced intestinal permeability in 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. In agreement with characteristics of leaky gut, treatment with GSI decreased feed intake and altered jejunum morphology (shortened crypt depth, decreased villus height, and decreased villus height to crypt depth ratio). Furthermore, circulating insulin and LBP increased in GSI cows relative to controls. Interestingly, circulating serum amyloid A and haptoglobin of pair-fed controls increased similarly to GSI treated cows, indicating inflammation was occurring in both treatments (Kvidera et al., 2017b). 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) and we have also observed this in pigs (Pearce et al., 2013; Sanz-Fernandez et al., 2014). Recently, we confirmed the detrimental effects of feed restriction in mid-lactation cows 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). In summary, inflammation is present during the transition period and likely contributes to changes in whole-animal energetics.

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, Figures 2,3,4) and thus compromises

productivity. Upon activation, immune cells become obligate glucose utilizers via a metabolic shift from oxidative phosphorylation to aerobic glycolysis, a process known as the Warburg effect. 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 (Maitra et al., 2000; 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 (Wannemacher et al., 1980), and hypertriglyceridemia (Filkins, 1978; Wannemacher et al., 1980; Lanza-Jacoby et al., 1998; McGuinness, 2005) occur. Interestingly, despite hypertriglyceridemia, circulating BHBA 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 BHBA 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.

Adequately fueling immune cells is a critical component in successfully mounting

an effective immune response (MacIver et al., 2008). Improving glucose availability can increase longevity and function of activated leukocytes (Sagone et al., 1974; Furukawa et al., 2000; Healy et al., 2002; Garcia et al., 2015). Thus, mitigation strategies which may help divert glucose towards immune cells have the potential to improve function. Interestingly, Lee et al., (2000), observed increased glucose uptake and improved macrophage function with chromium supplementation, likely due to chromium's role in improving insulin sensitivity. We have also demonstrated increased circulating neutrophils in both pigs (Mayorga et al., 2016) and cows (Horst et al., 2018) with chromium supplementation.

Energetic Cost of Immune Activation

The energetic cost of immunoactivation is substantial, but the ubiquitous nature of the immune system makes quantifying the energetic demand difficult. Therefore, 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 the 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 lactating cows, growing steers, and growing pigs were 0.64, 1.0, and 1.1 g glucose/kg BW^{0.75}/h, respectively; Kvidera et al., 2016, 2017 a,c). 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, and wool).

We and others have now demonstrated that both HS and ketotic animals have

increased circulating markers of endotoxin and inflammation. We believe that the circulating LPS in both maladies originates from the intestine and thus both likely have an activated immune system. This activated systemic immune response reprioritizes the hierarchy of glucose utilization, and milk synthesis is consequently deemphasized.

Conclusion

Altogether, our studies suggest that ketosis and HS may share the same etiology (i.e., decreased gut integrity) as indicated by altered intestinal morphology and increased plasma inflammatory biomarkers. This inflammation can redirect resources normally used for growth, milk production, 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 during HS and the transition period.

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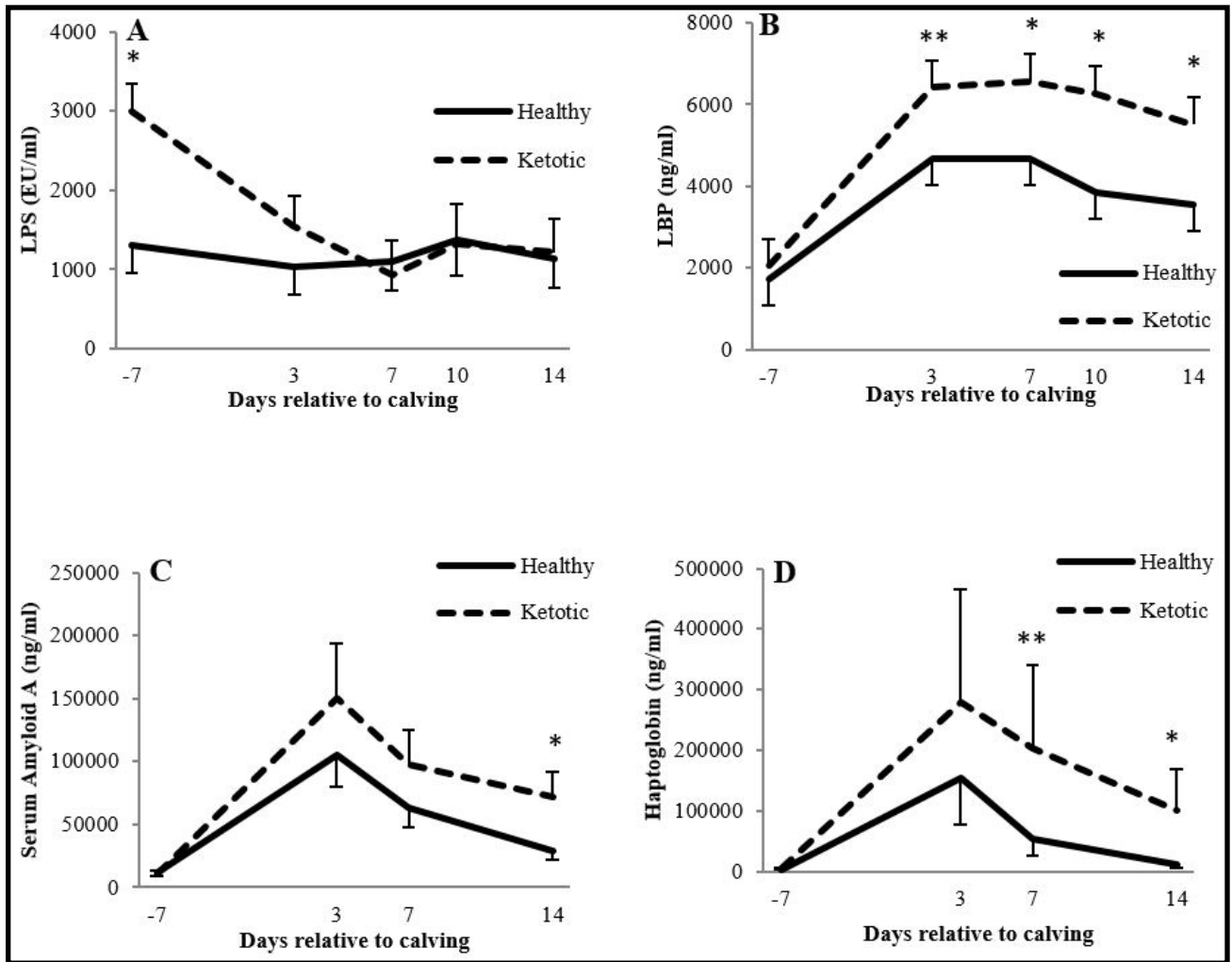


Figure 1. Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows (LPS = lipopolysaccharide and LBP = LPS binding protein; Abuajamieh et al., 2016).



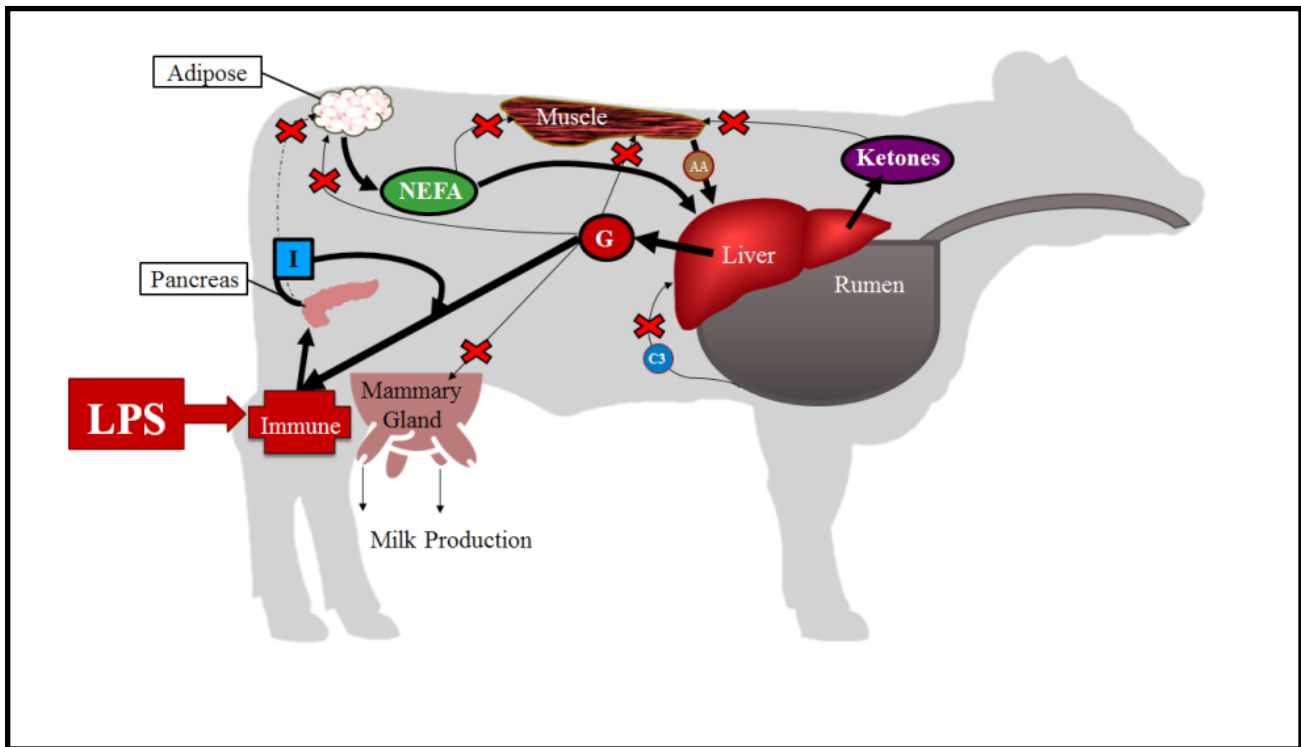


Figure 2. Lipopolysaccharide (LPS) induced alterations in glucose metabolism and insulin sensitivity.

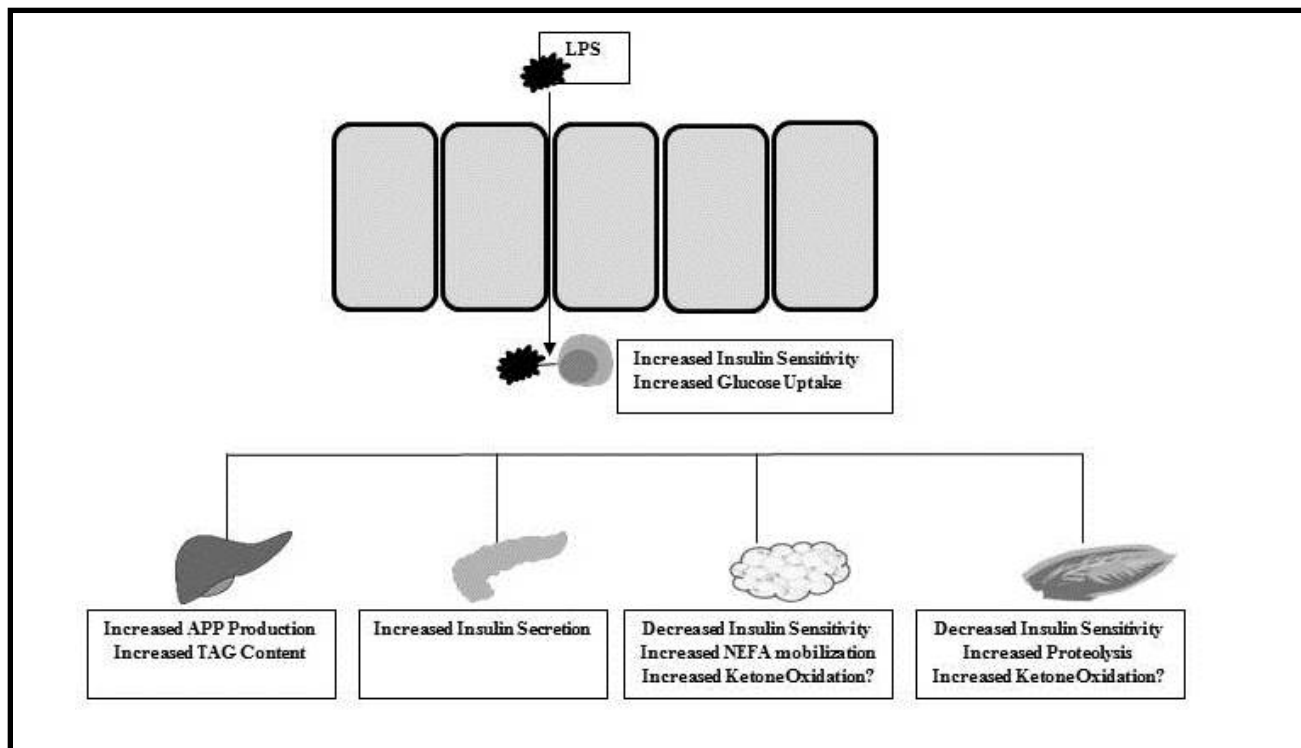


Figure 3. Lipopolysaccharide (LPS) induced alterations in peripheral metabolism

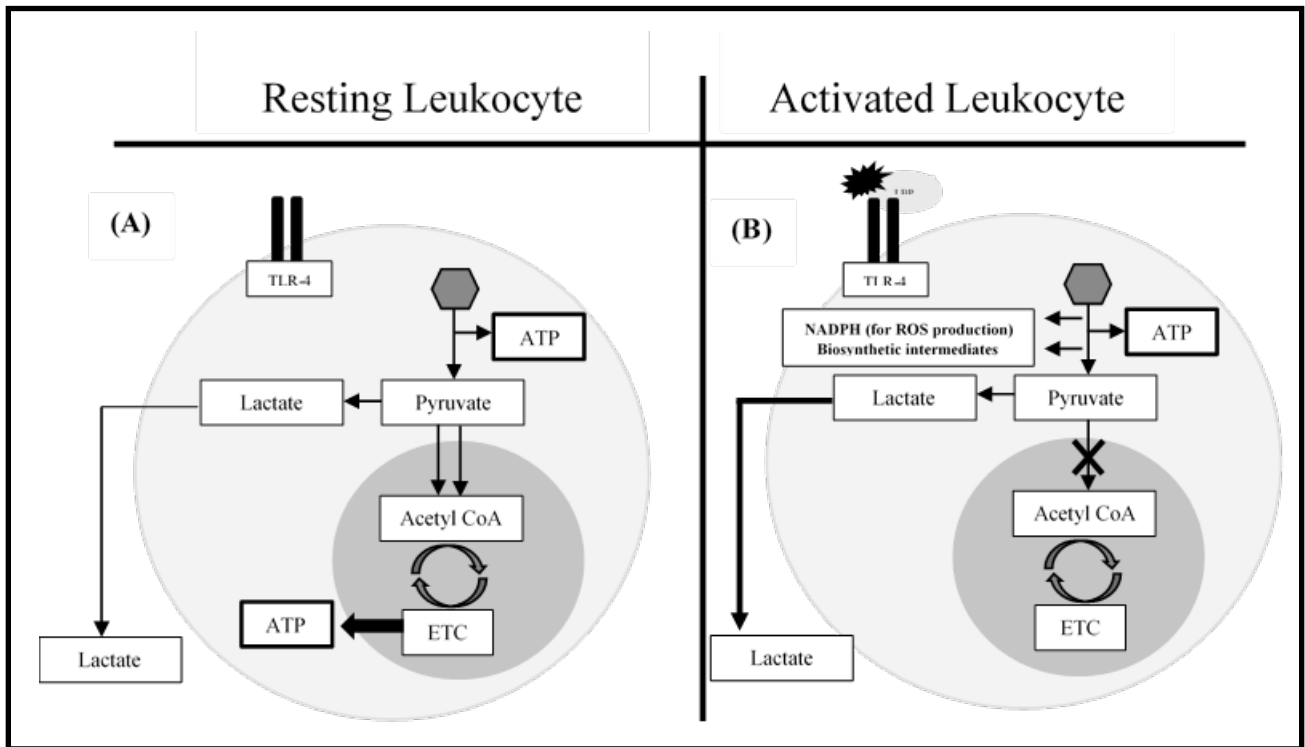


Figure 4. Metabolic pathway of a resting (A) vs. activated (B) leukocyte.

Amino Acid Nutrition During the Transition Period for Dairy Cows

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Introduction

During the transition period from late gestation through early lactation, the dairy cow undergoes tremendous metabolic adaptations (Bell, 1995). The endocrine changes during the transition period are necessary to prepare the dairy cow for parturition and lactogenesis. As peak milk yield increases, the transition period for dairy cows becomes much more challenging with most infectious diseases and metabolic disorders occurring during this time (Grummer, 1995; Drackley, 1999). Decreased dry matter intake (**DMI**) during late gestation influences metabolism, leading to fat mobilization from adipose tissue and glycogen from liver.

Nutrient demand for milk synthesis is increased in early lactation; if no compensatory intake of nutrients is achieved to cope with the requirement, reproductive functions (i.e., synthesis and secretion of hormones, follicle ovulation, and embryo development) may be depressed. Milk production increases faster than energy intake in the first 4 to 6 weeks after calving, and thus, high yielding cows will experience negative energy balance (**NEB**). Nutritional strategies and feeding management during pre-calving and post-calving periods impact health, productivity, and fertility of high producing dairy cows. Formulating diets to meet requirements of the cows but avoid over-consumption of energy may improve outcomes

of the transition period and lead to improved fertility. Management to improve cow comfort and ensure good intake of the ration is pivotal for success. Impacts of the transition program should be evaluated in a holistic way that considers disease occurrence, productivity, and fertility.

Studies over the last 2 decades clearly established the link between nutrition and fertility in ruminants (Robinson et al., 2006; Wiltbank et al., 2006; Grummer et al., 2010; Santos et al., 2010; Cardoso et al., 2013; Drackley and Cardoso, 2014). Dietary changes can cause an immediate and rapid alteration in a range of humoral factors that can alter endocrine and metabolic signaling pathways crucial for reproductive function (Boland et al., 2001; Diskin et al., 2003). Moreover, periconceptual nutritional environment in humans and other animals is critical for the long-term setting of postnatal phenotype (Fleming et al., 2015). Restricting the supply of B-vitamins and methionine during the periconceptual period in sheep, e.g., resulted in adverse cardiometabolic health in postnatal offspring (Sinclair et al., 2007). Feeding female mice a low-protein diet during the preimplantation period of pregnancy resulted in a reduction in amino acid (**AA**) concentration in uterine fluid and serum and attendant changes in the AA profile of the blastocyst (Eckert et al., 2012).

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Strategies have been used to improve the reproductive performance of dairy cows through alteration of nutritional status (Santos et al., 2001; Santos et al., 2008). In other species, dietary supplementation with specific AA (e.g., arginine, glutamine, leucine, glycine, and methionine) had beneficial effects on embryonic and fetal survival and growth through regulation of key signaling and metabolic pathways (Wang et al., 2012; Del Curto et al., 2013). Methionine and lysine are the most limiting AA in lactating cows (NRC, 2001), but supplementation of diets with crystalline methionine and lysine has been excluded because free methionine and lysine are quickly and almost totally degraded by the microorganisms in the rumen (NRC, 2001). In contrast, supplementing rumen-protected methionine (**RPM**) and rumen-protected lysine (**RPL**) has a positive effect on milk protein synthesis in dairy cows (Pisulewski et al., 1996; NRC, 2001; Ordway, et al., 2009; Osorio et al., 2013). Although the role of methionine in bovine embryonic development is unknown, there is evidence that methionine availability alters the follicular dynamics of the first dominant follicle (Acosta et al., 2017), the transcriptome of bovine preimplantation embryos in vivo (Penagaricano et al., 2013), and its contents (Acosta et al., 2016).

Reproduction, Nutrition, and Health

A widespread assumption is that fertility of modern dairy cows is decreasing, particularly for Holstein-Friesen genetics, at least in part because of unintended consequences of continued selection for high milk production. This assumption has been challenged recently (LeBlanc, 2010; Bello et al., 2012). There is a wide distribution of reproductive success both within and among herds. For example, within 5 California herds encompassing 6,396 cows, cows in the lowest quartile for milk yield in the first 90 days postpartum (32.1 kg/day) were less

likely to have resumed estrous cycles by 65 days postpartum than cows in quartiles two (39.1 kg/day), three (43.6 kg/day), or four (50.0 kg/day); milk production did not affect risk for pregnancy (Santos et al., 2009). Changes in management systems and inadequacies in management may be more limiting for fertility of modern dairy cows than their genetics per se.

Dairy cows are susceptible to production disorders and diseases during the periparturition period and early lactation, including milk fever, ketosis, fatty liver, retained placenta, displaced abomasum, metritis, mastitis, and lameness (Mulligan et al., 2006; Ingvarsen and Moyes, 2013; Roche et al., 2013). There is little evidence that milk yield per se contributes to greater disease occurrence. However, peak disease incidence (shortly after parturition) corresponds with the time of greatest NEB, the peak in blood concentrations of nonesterified fatty acids (**NEFA**), and the greatest acceleration of milk yield (Ingvarsen et al., 2003). Peak milk yield occurs several weeks later. Disorders associated with postpartum NEB also are related to impaired reproductive performance, including fatty liver (Rukkwamsuk et al., 1999; Jorritsma et al., 2003) and ketosis (Walsh et al., 2007; McArt et al., 2012). Cows that lost >1 body condition score (BCS) unit (1-5 scale) had greater incidence of metritis, retained placenta, and metabolic disorders (displaced abomasum, milk fever, and ketosis), as well as a longer interval to first breeding than cows that lost <1 BCS unit during the transition (Kim and Suh, 2003).

Indicators of NEB are highly correlated with lost milk production, increased disease, and decreased fertility (Ospina et al., 2010; Chapinal et al., 2012). However, the extent to which NEB is causative for periparturition health problems rather than just a correlated phenomenon must be examined critically (Roche et al., 2013).

For example, in transition cows, inflammatory responses may decrease DMI, cause alterations in metabolism, and predispose cows to greater NEB or increased disease (Bertoni et al., 2008; Graugnard et al., 2012 and 2013; Ingvarlsen and Moyes, 2013). Inducing a degree of calculated NEB in mid-lactation cows similar to what periparturient cows often encounter does not result in marked increases in ketogenesis or other processes associated with periparturient disease (Moyes et al., 2009). Nevertheless, early postpartal increases in NEFA and decreases in glucose concentrations were strongly associated with pregnancy at first insemination in a timed artificial insemination (TAI) program (Garverick et al., 2013). Although concentrations of NEFA and glucose were not different between cows that ovulated or did not before TAI, probability of pregnancy decreased with greater NEFA and increased with greater glucose concentrations at day 3 postpartum (Garverick et al., 2013). In support of these findings, early occurrence of subclinical ketosis is more likely to decrease milk yield and compromise fertility. McArt et al. (2012) reported that cows with subclinical ketosis detected between 3 to 7 days after calving were 0.7 times as likely to conceive to first service and 4.5 times more likely to be removed from the herd within the first 30 days in milk compared with cows that developed ketosis at 8 days or later.

Cows that successfully adapt to lactation (Jorritsma et al., 2003) and can avoid metabolic (Ingvarlsen et al., 2003) or physiological imbalance (Ingvarlsen and Moyes, 2013) are able to support both high milk production and successful reproduction while remaining healthy. Decreased fertility in the face of increasing milk production may be attributable to greater severity of postpartal NEB resulting from inadequate transition management or increased rates of disease. Competition for nutrients between the divergent outcomes of early

lactation and subsequent pregnancy will delay reproductive function. Because NEB interrupts reproduction in most species, including humans, inappropriate nutritional management may predispose cows to both metabolic disturbances and impaired reproduction. Cows must make “metabolic decisions” about where to direct scarce resources, and in early lactation, nutrients will be directed to milk production rather than to the next pregnancy (Friggens, 2003).

Different nutritional strategies have been proposed to improve reproduction of the dairy cow with no detrimental effect on lactation performance. Feeding high quality forages, controlled-energy (CE) diets, or adding supplemental fat to diets are some of the most common ways to improve energy intake in cows (Cardoso et al., 2013; Drackley and Cardoso, 2014; Mann et al., 2015). Reproduction of dairy cattle may be benefited by maximizing DMI during the transition period, minimizing the incidence of periparturient problems (Cardoso et al., 2013; Drackley and Cardoso, 2014).

Prepartum Dietary Considerations

Controlling energy intake during the dry period to near calculated requirements leads to better transition success (Grum et al., 1996; Dann et al., 2005 and 2006; Douglas et al., 2006; Janovick et al., 2011; Graugnard et al., 2012 and 2013; Ji et al., 2012). Research drew from earlier reports that limiting nutrient intakes to requirements of the cows was preferable to over-consumption of energy (e.g., Kunz et al., 1985). Cows fed even moderate-energy diets (1.50 to 1.60 Mcal NEL/kg DM) will easily consume 40 to 80% more NEL than required during both far-off and close-up periods (Dann et al., 2005 and 2006; Douglas et al., 2006; Janovick and Drackley, 2010). Cows in these studies were all less than 3.5 BCS (1-5 scale) at dry-off and were fed individually TMR based

on corn silage, alfalfa silage, and alfalfa hay with some concentrate supplementation. We have no evidence that the extra energy and nutrient intake was beneficial in any way. More importantly, our data indicate that allowing cows to over-consume energy even to this degree may predispose them to health problems during the transition period if they face stressors or challenges that limit DMI (Cardoso et al., 2013).

Prolonged over-consumption of energy during the dry period can decrease post-calving DMI (Dann et al., 2006; Douglas et al., 2006; Janovick and Drackley, 2010). Over-consuming energy results in negative responses of metabolic indicators, such as higher NEFA and betahydroxybutyrate (**BHB**) in blood and more triacylglycerol (**TAG**) in the liver after calving (Douglas et al., 2006; Janovick et al., 2011). Alterations in cellular and gene-level responses in liver (Lor et al., 2006 and 2007) and adipose tissue (Ji et al., 2012) potentially explain many of the changes at the cow level. Over-consumption of energy during the close-up period increases the enzymatic “machinery” in adipose tissue for TAG mobilization after calving, with transcriptional changes leading to decreased lipogenesis, increased lipolysis, and decreased ability of insulin to inhibit lipolysis (Ji et al., 2012). Controlling energy intake during the dry period also improved neutrophil function postpartum (Graugnard et al., 2012) and so may lead to better immune function.

Allowing dry cows to consume more energy than required, even if cows do not become noticeably over-conditioned, results in responses that would be typical of overly fat cows. Because energy that cows consume in excess of their requirements must either be dissipated as heat or stored as fat, we speculated that the excess is accumulated preferentially in internal adipose tissue depots in some cows. Moderate over-consumption of energy by non-lactating cows

for 57 days led to greater deposition of fat in abdominal adipose tissues (omental, mesenteric, and perirenal) than in cows fed a high-bulk diet to control energy intake to near requirements (Drackley et al., 2014). The NEFA and signaling molecules released by visceral adipose tissues travel directly to the liver, which may cause fatty liver, subclinical ketosis, and secondary problems with liver function.

Data from our studies support field observations that controlled-energy dry cow programs decrease health problems (Beever, 2006). Other research groups (Rukkwamsuk et al., 1998; Holcomb et al., 2001; Holtenius et al., 2003; Vickers et al., 2013) have reached similar conclusions about controlling energy intake during the dry period, although not all studies have shown benefits (Winkleman et al., 2008). Application of these principles can be through controlled limit-feeding of moderate energy diets or ad libitum feeding of high-bulk, low-energy rations (Janovick and Drackley, 2010; Janovick et al., 2011; Ji et al., 2012) as proposed by others (Beever, 2006).

Nutritionally complete diets must be fed and the TMR must be processed appropriately so that cows do not sort the bulkier ingredients (Janovick and Drackley, 2010). Feeding bulky forage separately from a partial TMR or improper forage processing will lead to variable intake among cows, with some consuming too much energy and some too little. Underfeeding relative to requirements, where nutrient balance also is likely limiting, leads to increased incidence of retained placenta and metritis (Mulligan et al., 2006). Merely adding a quantity of straw to a diet is not the key principle; rather, the diet must be formulated to limit the intake of energy (approximately 1.3 Mcal NE_L/kg DM, to limit intake to about 15 Mcal/day for typical Holstein cows) but meet the requirements for protein, minerals, and vitamins. Reports of

increased transition health problems or poor reproductive success (Whitaker et al., 1993) with “low energy” dry cow diets must be examined carefully to discern whether nutrient intakes were adequate.

Fresh Cow (postpartum) Dietary Considerations

Less is known about diet formulation for the immediate postpartum period to optimize transition success and subsequent reproduction. Increased research is needed in this area. Proper dietary formulation during the dry period or close-up period will maintain or enable rumen adaptation to higher grain diets after calving. Failure to do so may compromise early lactation productivity. For example, Silva-del-Rio et al. (2010) attempted to duplicate the dietary strategy of Dann et al. (2006) by feeding either a low-energy far-off diet for 5 weeks followed by a higher-energy diet for the last 3 weeks before parturition, or by feeding the higher-energy diet for the entire 8-week dry period. They found that cows fed the higher-energy diet for only 3 weeks before parturition produced less milk than cows fed the diet for 8 weeks (43.8 vs. 48.5 kg/day). However, the far-off dry period diet contained 55.1% alfalfa silage and 38.5% wheat straw but no corn silage. In comparison, the higher-energy dry period diet and the early lactation diet both contained 35% corn silage. Ruminal adaptation likely was insufficient for cows fed the higher energy diet for only 3 weeks.

A major area of concern in the fresh cow period is a sudden increase in dietary energy density, leading to subacute ruminal acidosis (**SARA**), which can decrease DMI and digestibility of nutrients (Mulligan and Doherty, 2008). Adequate physical form of the diet, derived either from ingredients or mixing strategy, must be present to stimulate ruminal activity and chewing behavior (Zabeli and

Metzler-Zabeli, 2012), although good methods to quantify “adequacy” remain elusive. Dietary starch content and fermentability likely interact with forage characteristics and ration physical form. Dann and Nelson (2011) compared three dietary starch contents (primarily from corn starch) in the fresh cow period for cows fed a controlled energy-type ration in the dry period. Milk production was greatest when starch content was moderate (23.2% of DM) or low (21.0% of DM) in the fresh cow diet compared with high (25.5% of DM). If SARA decreases DMI and nutrient availability to the cow, NEFA mobilization and increased ketogenesis may follow. In addition, rapid starch fermentation in the presence of NEFA mobilization leads to bursts of propionate reaching the liver, which may decrease feeding activity and DMI according to the hepatic oxidation theory (Allen et al., 2009). A moderate starch content (ca. 23 to 25% of DM) with starch of moderate fermentability (for example, ground dry corn rather than high-moisture corn or ground barley) along with adequate effective forage fiber may be the best strategy for fresh cows. Recent research also has demonstrated that high grain diets can lead to greater numbers of gram-negative bacteria such as *E. coli* with resulting increases in endotoxin present in the rumen, which may decrease barrier function and inflammatory responses in the cow (Zabeli and Metzler-Zabeli, 2012).

Supplemental fats have been widely investigated as a way to increase dietary energy intake and improve reproduction (Thatcher et al., 2011). A novel strategy to use polyunsaturated fatty acid (**PUFA**) supplements to improve reproduction has been reported (Silvestre et al., 2011). Cows fed calcium salts of safflower oil from 30 days before to 30 days after calving, followed by calcium salts of fish oil to 160 days postpartum, had greater pregnancy rates and higher milk production. The mechanism

is believed to be provision of greater amounts of linoleic acid (omega-6 PUFA) until early postpartum, which improves uterine health, followed by greater amounts of omega-3 PUFA from fish oil to decrease early embryonic loss (Thatcher et al., 2011). The effects of turbulent transitions on reproduction are established early postpartum, likely during the first 10 days to 2 weeks postpartum (Butler, 2003; McArt et al., 2012; Garverick et al., 2013). By 8 weeks postpartum, >95% of cows should be at or above energy balance (Sutter and Beever, 2000). Use of targeted prepartum and postpartum strategies may minimize health problems and lessen NEB, and thereby improve subsequent fertility.

Body Condition Score

The role of excessive BCS in contributing to transition problems and impaired subsequent reproduction is well established and has been discussed by many authors (Drackley et al., 2005; Garnsworthy et al., 2008; Roche et al., 2013). Cows with excessive body lipid reserves mobilize more of that lipid around calving, have poorer appetites and DMI before and after calving, have impaired immune function, have increased indicators of inflammation in blood and may be more subjected to oxidative stress (Contreras and Sordillo, 2011). What constitutes “excessive” BCS relative to the cow’s biological target remains controversial. Garnsworthy (2007) argued that the average optimal BCS has decreased over time with increased genetic selection for milk yield, perhaps related to correlated changes in body protein metabolism. Recommendations for optimal BCS at calving have trended downward over the last two decades, and in the author’s opinion, a score of about 3.0 (1-5 scale) represents a good goal at present. Adjustment of average BCS should be a longstanding project and should not be undertaken during the dry period.

Cows fed high-energy (1.58 Mcal NE_L/kg DM) diets during the last 4 weeks before calving lost more BCS in the first 6 weeks postpartum than those fed controlled energy (1.32 Mcal NE_L/kg DM) diets (−0.43 and −0.30, respectively) (Cardoso et al., 2013). The effect of BCS change on the cow’s fertility is clear. Carvalho et al. (2014) showed that cows that either gained or maintained BCS from calving to 21 days after calving had higher (38.2 and 83.5%, respectively) pregnancy per AI at 40 days than cows that lost BCS (25.1%) during that same period. Previously, Santos et al. (2009) had shown that cows that had > 1.0 BCS unit change from calving to AI at approximately 70 days postpartum had lower pregnancy per AI (28%) than cows that lost < 1.0 BCS unit change (37.3%) or did not have a BCS change (41.6%). In a grazing system, researchers from New Zealand suggested that BCS at calving should be targeted at 2.75 to 3.0 to optimize production, while reducing liver lipid accumulation and the negative effects of inflammation on liver function (Roche et al., 2013; Akbar et al., 2015).

The Importance of Amino Acids

Some AA are limiting for optimal milk production as evidenced by an increase in milk yield, percentage of milk protein, and milk protein yield after supplementation with specific, rumen-protected amino acids. The first 3 limiting amino acids for milk production are considered to be methionine, lysine (NRC, 2001), and histidine (Hutannen, et al., 2002). In addition, many amino acids can have positive effects on physiological processes that are independent of their effects on synthesis of proteins (Wu, et al., 2013). Fertilization and the first few days of embryo development occur in the oviduct. By about 5 days after estrus, the embryo arrives in the uterine horn. The embryo reaches the blastocyst stage by 6 to 7 days after estrus. The embryo hatches from the zona pellucida by

about day 9 after estrus and then elongates on days 14 to 19. The elongating embryo secretes the protein interferon-tau that is essential for rescue of the corpus luteum and continuation of the pregnancy. By days 25 to 28, the embryo attaches to the caruncles of the uterus and begins to establish a vascular relationship with the dam through the placenta. During all the time prior to embryo attachment, the embryo is free-floating and is dependent upon uterine secretions for energy and the building blocks for development, including amino acids. Thus, it is critical to understand the changes in amino acid concentrations in the uterus that accompany these different stages of embryo development.

The lipid profile of oocytes and early embryos can be influenced by the environment of the cow. Our group ran a trial with the objective to determine the effect of supplementing rumen-protected methionine on DNA methylation and lipid accumulation in preimplantation embryos of dairy cows (Acosta et al., 2016). Lactating Holstein cows entering their 2nd or greater lactation were randomly assigned to 2 treatments from 30 ± 2 DIM to 72 ± 2 DIM; Control (CON; n = 5, fed a basal diet with a 3.4:1 Lys:Met) and Methionine (MET; n = 5, fed the basal diet plus Smartamine M to a 2.9:1 Lys:Met). Embryos were flushed 6.5 days after artificial insemination. Embryos with stage of development 4 or greater were used for analysis. For lipids, fluorescence intensity of Nile Red staining was compared against a negative control embryo (subtraction of background). A total of 37 embryos were harvested from cows (MET = 16; CON = 21). Cows receiving MET had greater lipid accumulation (7.3 arbitrary units) when compared with cows receiving CON (3.7 arbitrary units). There were no treatment effects on number of cells or stage of development. In conclusion, cows supplemented with methionine produced embryos with higher lipid concentration when compared to CON,

which could potentially serve as an important source of energy for the early developing embryo.

The requirements for complete development of bovine embryos have not yet been determined. Current culture conditions allow development of bovine embryos to the blastocyst stage (day 7 to 8) and even allow hatching of a percentage of embryos (day 9); however, conditions have not been developed in vitro that allow elongation of embryos. The methionine requirements for cultured pre-implantation bovine embryos (day 7 to 8) was determined in studies from University of Florida (Bonilla et al., 2010). There was a surprisingly low methionine requirement ($7 \mu\text{M}$) for development of embryos to the blastocyst stage by day 7; however, development to the advanced blastocyst stage by day 7 appeared to be optimized at around $21 \mu\text{M}$ (Bonilla et al., 2010). Thus, the results of these studies indicated that development of morphologically normal bovine embryos did not require elevated methionine concentrations ($>21 \mu\text{M}$), at least during the first week after fertilization. Stella (2017) reported the plasma concentration of cows fed RPM or not (CON) (Figure 1). It seems that cows when fed RPM have plasma methionine concentration greater than $20 \mu\text{M}$.

Researchers at the Univ. of Wisconsin (Toledo et al., 2015) conducted a trial with a total of 309 cows (138 primiparous and 171 multiparous) that were blocked by parity and randomly assigned to 2 treatments: 1) CON: Cows fed a ration formulated to deliver 2500 g of metabolizable protein (MP) with 6.9% Lys (% of MP) and 1.9 Met (% of MP), and 2) RPM: Cows fed a ration formulated to deliver 2500 g of MP with 6.9% Lys, % of MP) and 2.3 % Met (% of MP). Cows were randomly assigned to 3 pens with head-locks and fed a single basal TMR twice daily. From 28 to 128 DIM, after the AM

milking, cows were head-locked for 30 minutes and the TMR of CON and RPM cows were individually top dressed with 50 g of DDG or 50 g of a mix of DDG (29 g) and Smartamine M (21 g), respectively. Following a double ovsynch protocol, cows were inseminated and pregnancy checked at 28 (plasma Pregnancy Specific Protein-B concentration), 32, 47, and 61 days (ultrasound). Individual milk samples were taken once a month and analyzed for composition. There were no statistical differences in milk production, but RPM cows had a higher milk protein concentration. Cows fed the methionine enriched diet had a lower pregnancy loss from 21 to 61 after AI (16.7% RPM cows vs. 10.0% from CON cows). Pregnancy losses between days 28 and 61 were not different in the primiparous cows (12.8% CON and 14.6% RPM), however, pregnancy losses between treatments were significant for the multiparous cows (19.6% CON vs. 6.1% RPM; Toledo et al., 2015).

Perhaps the most detrimental impact of NEB on reproductive performance is delayed return to cyclicity (Jorritsma et al., 2003). The dominant follicle (DF) growth and estradiol (E_2) production are key factors for a successful conception, and their impairment can be attributed to reduced luteinizing hormone (LH) pulses (Grainger et al., 1982), as well as decreased circulating insulin and IGF-I concentrations (Canfield and Butler, 1990; Komaragiri and Erdman, 1997). Furthermore, immune function is also suppressed along the periparturient period (Butler 2003; Kehrl et al., 1999); NEB and fatty liver syndrome were demonstrated to impair peripheral blood neutrophil function (Zerbe et al., 2000; Hammon et al., 2006). Acosta et al. (2017) reported that methionine and choline supplementation induced a down regulation of pro-inflammatory genes, possibly indicating lower inflammatory processes in follicular cells of the first DF postpartum. Also, supplementing methionine, during the

transition period increased 3β -hydroxysteroid dehydrogenase expression in the follicular cells of the first DF postpartum. It is important to highlight that higher methionine concentrations in the follicular fluid of supplemented cows can potentially affect oocyte quality. The understanding on how this finding may affect reproductive performance in commercial farms needs to be further investigated. Batistel et al. (2017) reported that that studies with non-ruminant species argue for the potential relevance of the maternal methionine supply during late gestation in enhancing utero-placental uptake and transport of nutrients. The authors hypothesized that the greater newborn body weight from cows fed RPM compared to control (42 vs. 44 kg) could have been a direct response to the greater nutrient supply from the feed intake response induced by methionine, the fact that certain amino acids and glucose are known to induce mTOR signaling to different degrees is highly suggestive of “nutrient specific” mechanistic responses.

Conclusions

Formulation and delivery of appropriate diets that limit total energy intake to requirements but also provide proper intakes of all other nutrients before calving can help lessen the extent of NEB after calving. Effects of such diets on indicators of metabolic health are generally positive, suggesting the potential to lessen effects of periparturient disease on fertility. Supplementation of cows with methionine during the final stages of follicular development and early embryo development, until day 7 after breeding, lead to lipid accumulation changes in the embryos and resulted in differences in gene expression in the embryo. Methionine supplementation seems to impact the preimplantation embryo in a way that enhances its capacity for survival because there is strong evidence that endogenous lipid reserves serve

as an energy substrate. The lower pregnancy losses from cows fed a methionine enriched diet suggest that methionine favors the embryo survival, at least in multiparous cows.

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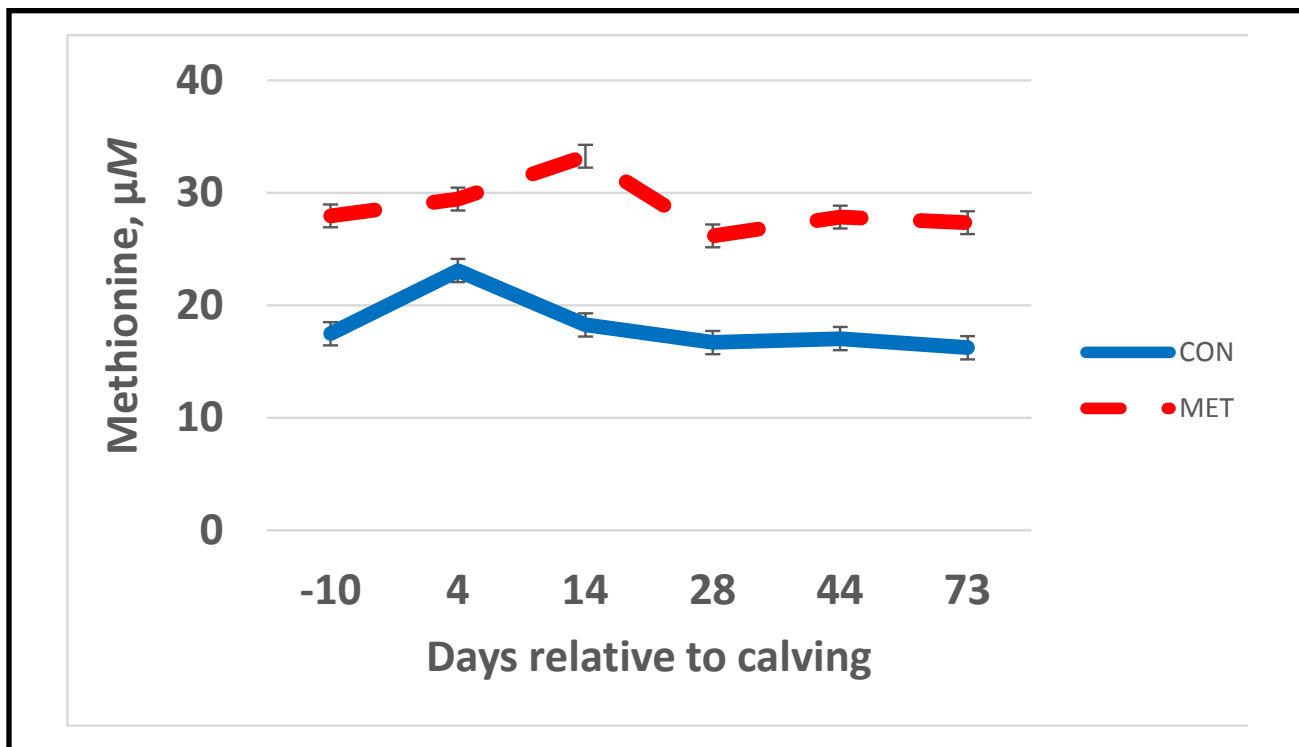


Figure 1. Serum methionine concentration was greater ($P < 0.05$) in cows fed rumen-protected methionine (MET; $n = 10$) than cows not fed rumen-protected methionine (CON; $n = 7$)(Stella, 2017).

Seasonal Variation in Milk Composition

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Abstract

Most dairy producers and consultants in the United States are familiar with the consistent decline in milk yield and fat and protein tests during the summer. Oftentimes, these declines in production are ascribed as strictly a consequence of environmental factors, such as heat stress. While heat abatement strategies are incredibly important for maintaining health and productivity of dairy cows in the summer months, evidence suggests that summer declines in production may also be due to cows' inherent annual rhythms. It is important to consider these rhythms while setting goals and evaluating herd production. It is not entirely clear how to overcome these rhythms, but appropriately managing photoperiod is recommended.

Introduction

Rather than simply *responding* to an environmental stimulus, endogenous 'calendars' in the hypothalamus allow the animal to *anticipate* yearly environmental changes before they occur. This adaptation is especially useful for timing reproduction, allowing animals to synchronize parturition to the season with the greatest available resources for the offspring.

Annual rhythms are present in nearly all studied organisms as a mechanism to perceive and adapt to seasonal environmental changes.

For example, many mammals in northern climates hibernate over the winter. Bears, while not true hibernators, experience winter *torpor*, which is characterized by dramatically reduced body temperature and basal metabolic rate. Migrating birds undergo astonishing changes in metabolism prior to spring and fall migration, including initiation of nocturnal activity and accretion of body fat reserves. For example, rufous hummingbirds (*Selasphorus rufus*) gain 50 to 67% of their prior body weight (**BW**) in fat prior to migration (Carpenter et al., 1983). In production livestock, sheep display yearly rhythms of estrous behavior, leading to spring lambing (Malpaux et al., 1997). In most species that exhibit seasonality, annual changes in physiological persist even after animals are placed in constant day length (photoperiod).

Measuring Biological Rhythms

The analysis of biological rhythms, including annual rhythms, is typically performed using a technique called cosinor rhythmometry (Figure 1). This technique fits the linear form of a cosine function with a defined period length (for annual rhythms, this period is 12 months) to time course data. Comparing the fit of the linearized cosine function to a standard linear model is used to determine whether the response variable follows a biological rhythm. This approach is called a zero-amplitude test because it tests if the amplitude of a cosine function is statistically

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greater than zero, and therefore is rhythmic. Our lab has applied this approach using mixed models to separate random effects, such as animal, herd, or year, from the effects of the rhythm, and to compare rhythms between different treatment groups. In addition to determining rhythm fit, cosinor rhymometry is used to calculate the time that a rhythm peaks, termed acrophase, and the difference from peak to mean of the rhythm, called the amplitude. The acrophase of an annual rhythm indicates the time of the year when response is greatest, and differences in acrophase between groups indicate that they are entrained to a different cycle. The amplitude is a measure of the robustness of a rhythm, and twice the amplitude (double amplitude) is a measure of the difference between maximal and minimal response.

Annual Rhythms in the Dairy Cow

Yearly patterns of milk production have been recognized for over 40 years (Wood, 1970). Producers are familiar with summer declines in milk production, and recovery during the winter. When examining average monthly bulk tank records from U.S. Federal Milk Marketing Orders, the presence of an annual rhythm is apparent. Fat and protein concentration from the years 2000 to 2017 display repeating 12-month cycles that are remarkably consistent between years (Figure 2). These yearly patterns fit a robust cosine function, suggesting that they represent a biological rhythm ($P < 0.001$; Salfer et al., 2016). The rhythms of fat concentration peak between December 29 and January 18 in all regions except for Florida, which peaks on December 4. Protein concentration is even more consistent among regions, with a maximum between December 27 and January 6. The variation in milk fat concentration due to the annual rhythm is between 0.15% and 0.30%, depending on the region. Notably, annual fat concentration rhythms of regions in the southern

U.S., mainly Florida and Arizona-Las Vegas, seemed to have lower amplitude rhythms than further north regions, which may be related to their lower latitude and smaller change in photoperiod across the year. The amplitudes of milk protein concentration were more consistent among regions, with peak to trough difference being 0.16% to 0.20% (2x the amplitude).

The presence of yearly production rhythms was confirmed using 10 years of DHIA data from individual herds in Minnesota, Pennsylvania, Texas, and Florida (Salfer et al., 2017). Similar to the U.S. milk markets, milk fat and protein concentrations peak around January 1 and reach a nadir on July 1 in Minnesota, Pennsylvania, and Texas. Florida, on the other hand, had the greatest fat concentration in November and greatest protein concentration in October. These data provide further evidence to suggest that annual rhythms of production vary by geographical location. States in the northern U.S. have markedly greater amplitude rhythms of fat and protein concentration. For example, in Pennsylvania and Minnesota, the difference between peak and trough for fat concentration was 0.32% and 0.28%, respectively, while Texas was 0.16% and Florida's was 0.08%. To put this in perspective, a farm in Pennsylvania with a 3.6% butterfat test in July, should expect their fat test to increase to 3.92% in January merely due to the animal's annual rhythm. A farm in Florida with the same fat test in July, however, should only expect their January fat percent to equal 3.68%. In agreement with milk market data, DHIA herd-level data shows that the amount of oscillation in the annual protein concentration rhythm is generally less variable among regions of the U.S. (peak to trough 0.16 to 0.18% in Minnesota, Pennsylvania and Texas), but was distinctly lower in Florida (0.06%).

While fat and protein concentration both peak near the first of the year, the annual

rhythm of milk yield peaks between late March and early April, right around the vernal equinox (Salfer et al., 2017). Fat and protein yields peak between late February and early March. Contrary to the rhythms of fat and protein concentration, amplitudes of annual milk yield rhythms are greater in the southern U.S. compared to the north. Cosinor rhythmometry revealed that the peak to trough in Pennsylvania and Minnesota was 2.5 and 2.2 kg, respectively, while that of Texas and Florida were 6.3 and 7.4 kg, respectively. Fat and protein yields also oscillated more in the southern U.S. than the northern U.S. The mechanism causing the amplitudes of milk, fat, and protein yields to be greater in southern climates is unclear. Data from DHIA also revealed slight differences in annual production rhythms between breeds. The rhythm of milk peaks on April 1 in Holstein, while it peaks on May 11 for Jersey. While it is difficult to discern if this effect is actually due to genetic differences between breeds or simply an artifact of the analysis, it should be considered by producers.

Data from 11 individual herds in Pennsylvania has also been examined to determine which cow-level effects influence annual rhythms of milk production (Salfer et al., 2016). The diacylglycerol o-acyltransferase 1 (**DGAT1**) gene, responsible for 40% of the genetic variation in fat percentage, does not influence annual rhythms of fat concentration or fat yield (Winter et al., 2002). Similarly, rhythms of milk, fat, and protein yields, and fat and protein concentrations are not affected by parity. Data from these 11 herds also confirmed that fat and protein concentrations peak in late December and early January, milk yield peaks in late March and early April, and fat and protein yields peak in late February and early March. While rhythms of fat and protein concentrations were incredibly consistent among herds, 2 herds had very low amplitude rhythms of milk, fat, and

protein yields. There were not, however, any detectable differences among these herds and the rests, so the reason for the low-amplitude rhythms is unclear.

Naturally, environmental temperature is often blamed for causing the seasonal changes in milk production. While it is certainly a factor, our results suggest that an annual rhythm exists independent of temperature (Salfer et al., 2017). We compared the fit of a model containing daily maximum temperature within each state, to a model containing the linearized cosine function. Results suggest that the cosine function fits the data dramatically better than temperature, implying that the effect is not simply a function of temperature (P value of F -test <0.0001). Furthermore, a decline in fat and protein concentrations is observed *below* the fitted cosine function in July and August, especially in Pennsylvania and Minnesota (Figure 3). This phenomenon appears to suggest that heat stress is an additive effect, separate from the annual rhythm that causes additional production declines in the summer. A final piece of evidence to support the suggestion that the annual rhythm is independent of temperature is that milk yield reaches a minimum in late September, instead of during the middle of the summer when temperatures are lowest.

Additional support suggesting that dairy cows are affected by annual rhythms is provided by yearly patterns of circulating metabolites. Piccione et al. (2012) determined that plasma concentrations of bilirubin, creatinine, triglycerides, and β -hydroxybutyrate (**BHBA**) fit 12-month rhythms. They observed that BHBA peaks on April 1, total bilirubin peaks on July 14, creatinine peaks on June 12, and triglycerides peak June 16. The circulating concentrations of prolactin vary by season in cattle, with drastically greater concentrations in summer (46 ng/mL) than winter (7 ng/mL) (Petitclerc et al.,

1983). These effects persisted even after animals were blinded or pinealectomized, suggesting that the effect is endogenous. Furthermore, the season of the year affects the diurnal rhythm of body temperature in cows. Body temperature has greater daily fluctuations in the summer compared to the winter (Kendall and Webster, 2009). While cattle are not generally considered to be seasonal breeders, there are modest effects of season and photoperiod length on reproduction in cows (Hansen, 1985). While the common assumption is that domestication has removed the evolutionary drive for annual rhythms in cattle, there is ample evidence to suggest that they are still influenced by seasonal physiology.

Potential Mechanisms of Seasonality

As discussed above, a primary role of annual rhythms is to coordinate reproduction with resource availability to maximize the likelihood of survival of the offspring. As an important component of reproduction, it is not implausible to expect that lactation is controlled through similar mechanisms. It stands to reason that producing more energy-dense milk with greater concentrations of fat and protein in the winter when energetic demands are greater would increase the likelihood of calf survival. In all mammalian species characterized, annual rhythms are controlled by a photoperiodic timer based on the duration of melatonin release (Lincoln and Hazlerigg, 2011). The synthesis of prolactin is also under the control of the photoperiod-based timing mechanism. Prolactin is released from the pituitary and is involved in feed intake and initiation of lactation in many mammalian species (Bauman and Bruce Currie, 1980; Lawrence et al., 2000).

Besides the melatonin-controlled day length timer, many species have evolved an endogenous timekeeping system that keeps

track of time in constant photoperiods. This system allow animals to anticipate seasonal changes and prepare for the upcoming climate. Furthermore, it allows migrating animals to continue to have a record of the time of year, even after moving to a geographical location with a different photoperiod (Lincoln and Hazlerigg, 2011). The combination of output from this endogenous timer and melatonin-based photoperiod signaling lead to the ultimate seasonal phenotype.

Effects of Photoperiod on Milk Production

Extensive research has examined the impact of altering photoperiod length on milk synthesis of the dairy cow. The first report of increased milk production after 16 h light: 8 h dark (16L:8D) photoperiod was made by H. Allen Tucker's lab at Michigan State (Peters et al., 1978). Since this initial discovery, several subsequent experiments have confirmed these findings (Stanisiewski et al., 1985; Dahl et al., 1997; Miller et al., 1999). Reksen et al. (1999) demonstrated that the effect occurs after implementation of any photoperiod greater than 12L: 12D; however, response is greatest at 16L: 8D.

The increase in milk production during long-day lighting has been associated with several hormonal changes that may be responsible for the observed effect. The duration of melatonin secretion is limited during artificial long-day lighting. Insulin like-growth factor-1 (**IGF-1**) is an effector molecule in the somatotropic axis. While the direct role of IGF-1 on milk synthesis is unclear, its concentration is increased after exogenous treatment with recombinant bovine somatotropin (Bauman and Vernon, 1993). Increased milk synthesis due to artificial 18L:6D photoperiod is associated with increased circulating IGF-1 concentrations (Dahl et al., 1997). Another hormone targeted

for its potential role in the lactational response to photoperiod is prolactin. Plasma prolactin concentrations increase during long days and decrease during short days (Tucker et al., 1984; Lacasse et al., 2014). Feeding melatonin to mimic the dark phase also decreases the concentrations of plasma prolactin (Buchanan et al., 1993). However, effects of prolactin on milk synthesis do not appear to be direct because no effects on milk production are observed after administration of exogenous prolactin (Plaut et al., 1987).

The results of photoperiod experiments and annual rhythms of production are seemingly paradoxical. While long-day lighting consistently increases milk synthesis, the cow's natural annual rhythm dictates that milk production increases when the duration of the light cycle is shorter than 12 hr. This anomaly is difficult to explain using current knowledge of annual rhythms in dairy cattle. One potential explanation is that long-day lighting may induce *photorefractoriness* to the annual rhythm of milk synthesis. Photorefractoriness is a phenomenon observed in other mammalian species, through which long-term exposure to a constant photoperiod leads to spontaneous reversion of a seasonal physiological response to the state expected in the opposite photoperiod (Lincoln et al., 2005). In other species, a fixed photoperiod must be applied for a long period of time (4 to 12 weeks) before switching of the physiological response occurs. In cows, the increase in milk yield after long days typically does not manifest until after 4 weeks of administration (Dahl et al., 2000). While this mechanism seems promising as a possible explanation for the observed effects of long-day lighting, it has not yet been studied in cows and further research must be done to test if it is related to the milk yield response.

Implications for Producers

While a greater understanding of the mechanisms responsible for the annual rhythm of milk production must still be developed, there are practical considerations dairy producers can make now to better manage for seasonal changes in production. An acceptance of the annual rhythm as a biological phenomenon that cannot be altered by nutrition or management can help producers adjust expectations across the year. As discussed above, the annual rhythm of milk production is responsible for a large amount of the variation in milk production and components. Using parameters derived from the linearized cosine function, we have calculated variables to adjust monthly milk, fat and protein yields and fat and protein concentrations based on their annual rhythms (Table 1). These adjustment factors can be added to monthly production to remove the effect of the annual rhythm and standardize production across the year.

Conclusions

Seasonal rhythms are controlled by timekeeping systems within an animal and allow adaptations before environmental weather and feed availability changes occur. The cow is not as seasonal as small ruminants that will only breed at certain times of the year, but a modest seasonal rhythm in milk and milk component yields is observed. The amplitude of the rhythm appears to be decreased in more southern regions of the US. Predicting the seasonal rhythm will allow a more precise evaluation of herd production. Long photoperiods are a well demonstrated method to increase milk and milk component yields and may actually work to reverse some of the negative effects of lengthening days, but more research is needed to clearly understand this phenomenon.

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Table 1. Values to normalize milk, fat, and protein yields, and fat and protein concentrations to account for the annual rhythm of these variables. Monthly production should be added to the appropriate value in the table.

	Milk lb	Fat, %	Protein, %	Fat, lb	Protein, lb
Jan	0.0	-0.13	-0.10	-0.09	-0.04
Feb	-1.5	-0.12	-0.08	-0.14	-0.08
Mar	-2.6	-0.07	-0.04	-0.15	-0.09
Apr	-3.1	0.00	0.00	-0.13	-0.08
May	-2.7	0.08	0.04	-0.07	-0.05
Jun	-1.7	0.14	0.06	0.00	0.00
Jul	-0.2	0.17	0.07	0.07	0.05
Aug	1.3	0.15	0.05	0.12	0.08
Sep	2.4	0.11	0.01	0.13	0.09
Oct	2.8	0.03	-0.03	0.11	0.08
Nov	2.5	-0.04	-0.07	0.05	0.05
Dec	1.5	-0.10	-0.10	-0.02	0.00

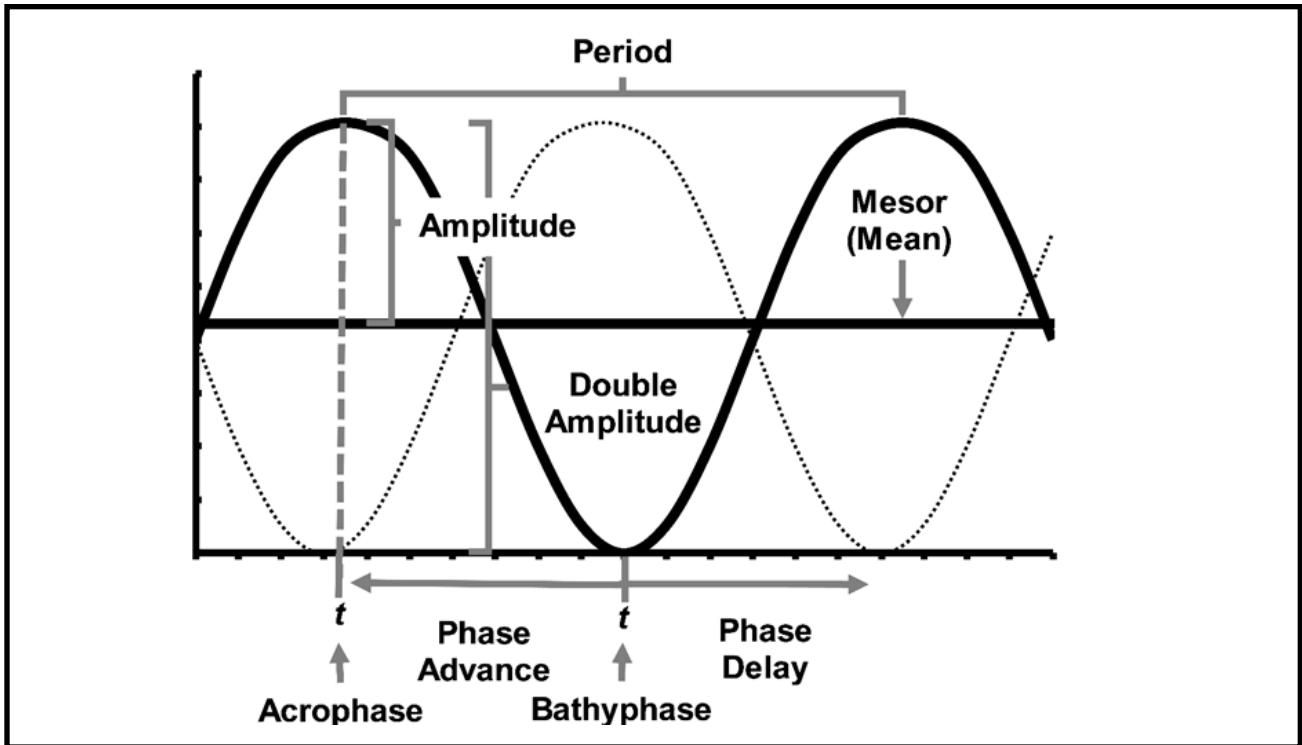


Figure 1. Parameters relevant to characterization of biological rhythms. Period refers to the length of time to complete one cycle of the rhythm. Amplitude is the difference between peak and mean. Double amplitude is the difference between peak and trough. Acrophase is the time at peak. Bathyphase is the time at trough. Phase advance refers to shifting the rhythm curve so that the acrophase occurs earlier than it was previously. Phase delay refers to shifting the rhythm curve so that acrophase occurs later than it was previously.

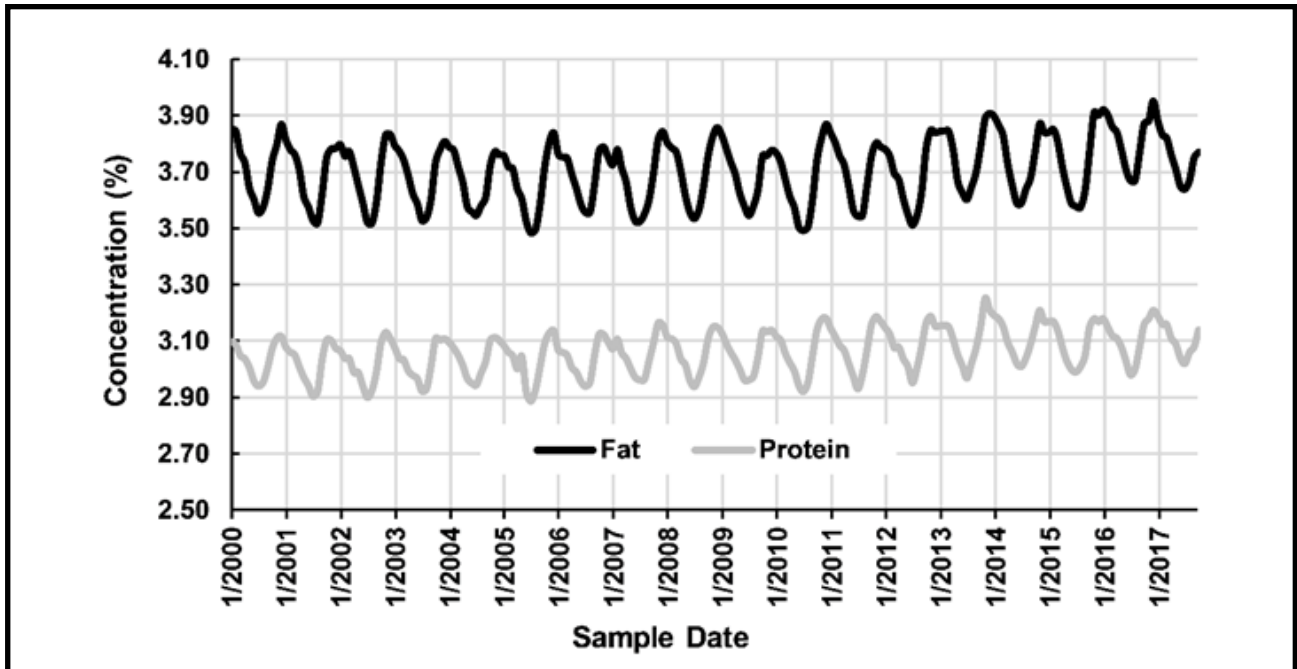


Figure 2. Yearly patterns of milk fat and protein concentration in the Mideast Milk Market. Rhythms are very repeatable between years.

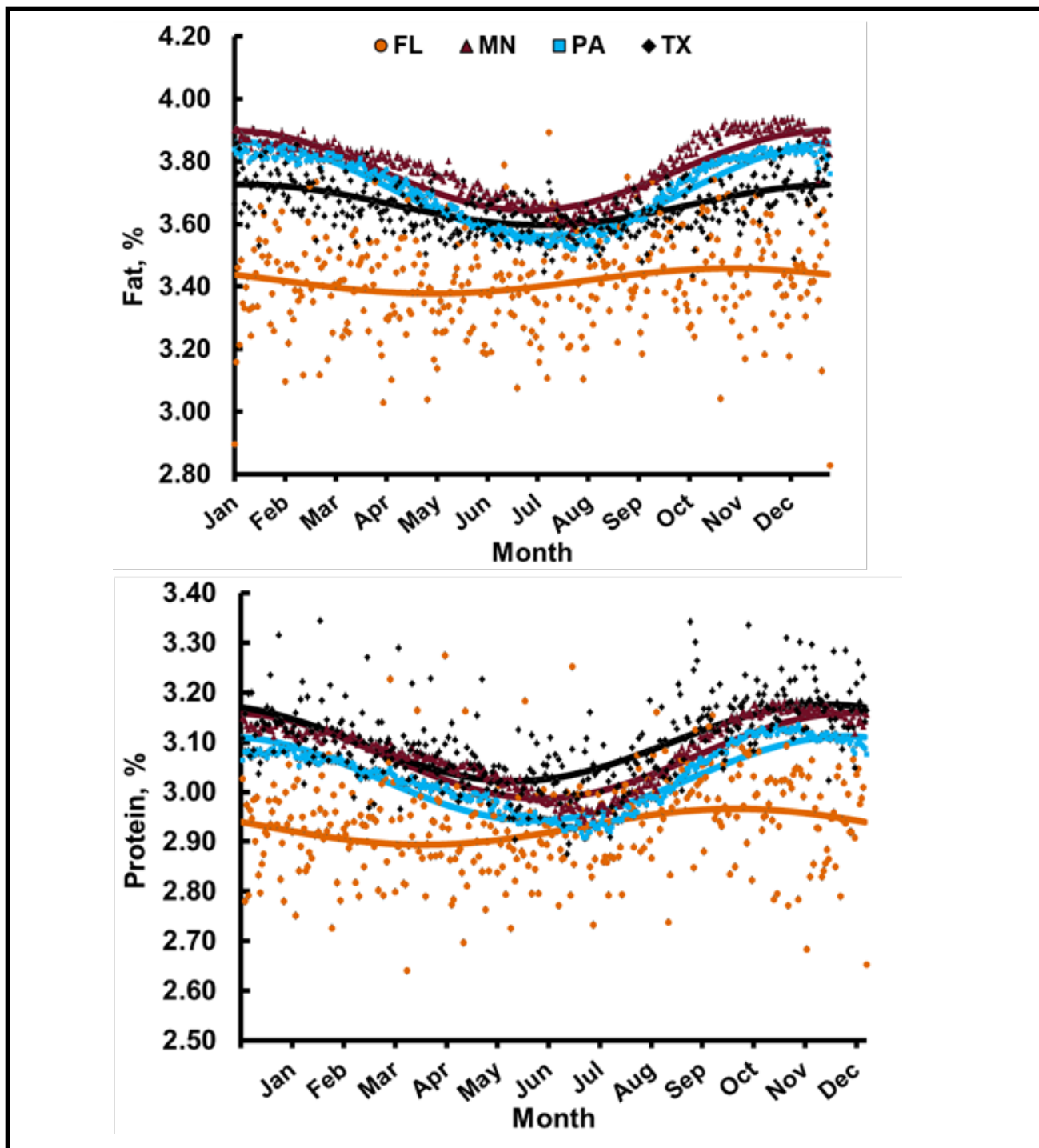


Figure 3. Yearly rhythms of fat and protein concentration from DHIA herd records in Minnesota, Pennsylvania, Florida and Texas from 2004 to 2016 (Salfer et al., 2017).

Using Physically Adjusted NDF in Formulating Rations for Dairy Cows

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Summary

Physically effective fiber (**peNDF**) is defined as that fraction of NDF that stimulates chewing activity and contributes to the floating mat of large particles in the rumen. A limitation of using peNDF is that it does not account for differences in rumen fermentability of nutrients, most notably rumen-degraded starch. The physically adjusted fiber (**paNDF**) system can be used to estimate TMR particle size and diet compositions needed to maintain target rumen conditions. The system is based on equations derived from a meta-analysis and estimates dietary physical and chemical characteristics required to maintain desired rumen conditions in lactating dairy cows. Effective fiber feeding recommendations are based upon diet ADF, NDF, forage NDF (**fNDF**), starch, and proportion of the ration as forage or cottonseed, as well as particle size measures.

Introduction

The physical nature of fiber consumed by the dairy cow is known to affect feed intake, chewing activities, rumen fermentation, and ultimately milk production and composition. In fact, because dairy cattle are grass and roughage eaters (Hofmann, 1989), and it is generally well understood that cows require coarse roughage and that this is “effective” in maintaining normal rumen fermentation, function, and

overall health (Clark and Armentano, 1993). With this being established, a number of investigators have sought to develop methods to quantitatively measure coarseness of roughage and integrate these measures into general feeding recommendations (Santini et al., 1983; Mertens, 1997). Probably the most well-known measure is peNDF, which is defined as that fraction of NDF that stimulates chewing activity and contributes to the floating mat of large particles in the rumen (Mertens, 1997). It was proposed that peNDF of individual feedstuffs could be estimated by multiplying a chemical measure of fiber in a feed by a physical measure. Over the last 20 years, the Penn State Particle Separator (**PSPS**) has been widely used on-farm to measure the particle size of TMR (Lammers et al., 1996; Heinrichs and Kononoff, 2002). Additionally, researchers have used the PSPS to report the physical characteristics of both forages and TMR in peer review scientific publications. Although it has been proposed that particle size measures using the PSPS could be used to estimate peNDF (Zebeli et al., 2012), such application is not widespread. Recently, the concept of peNDF has been re-evaluated by quantitatively summarizing available literature reporting physical and chemical characteristics of total diets and deriving equations that relate these to feed intake, chewing behavior, and ruminal fermentation (White et al., 2017a). This physically adjusted fiber (paNDF) system can be used to estimate TMR particle size and diet

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compositions needed to maintain target rumen conditions. The objective of this paper is to provide an update on findings related to effective fiber and to also outline the paNDF system for on-farm application.

New Method to Assess Effective Fiber

Feeding diets low in effective fiber may precipitate and contribute to the cascade of factors associated with ruminal acidosis, but the interactive effects of dry matter intake, digestibility, and nonstructural carbohydrate levels should also be considered. Unfortunately, in many studies, it is difficult to draw a clear link between peNDF and rumen pH. This is often the case when peNDF is decreased as grain is added to the diet. In this case, particle size is reduced but the portion of readily digestible carbohydrate is also increased. Here rumen pH is almost always reduced, but this may be a function of reduced saliva flow and increased organic acid production, with the latter often having the greatest effect. Thus, a limitation of using peNDF is that it does not account for differences in rumen fermentability of nutrients, most notably rumen-degraded starch (Dijkstra et al., 2012). Feeding recommendations of carbohydrates of NRC (2001) were summarized in a simple table (see Table 4-3, Page 37 from that source). In this table, minimum concentrations of forage NDF (fNDF), NDF, and non-fiber carbohydrates could be determined through interpolation. This table has proven to be extremely useful, but it did not include starch and also did not offer any recommendations regarding the particle size of a TMR. In fact, the table caption specified that these recommendations assumed that the forage particle size was adequate. In addressing this void, it has been suggested that the peNDF index is an oversimplification (Plaizier et al., 2008) of a complex phenomenon. With this in mind, we evaluated different peNDF representations as some particle size measure multiplied by diet

NDF content and concluded, that despite the fact that this product does account for some variation in ruminal pH, these dietary factors should be separated as core components and this will allow for consideration of other dietary components that influence pH (White et al., 2017a). We further hypothesized that the utility of peNDF could be expanded and improved by dissociating NDF and particle size and considering other dietary factors, all integrated into a system that can be used to estimate minimum particle sizes of TMR and diet compositions needed to maintain ruminal pH targets (White et al., 2017b). The system is based on equations derived from a meta-analysis (White et al., 2017a) and estimates dietary physical and chemical characteristics required to maintain desired rumen conditions in lactating dairy cows. In practice, the paNDF system generates feeding recommendations for diet characteristics that are based upon computation. All particle size measures used in the paNDF system are determined with PSPS and on a DM basis.

Modeling “Rumen Conditions” with Ensemble Models

Accurately modeling the rumen environment is challenging for several reasons. First, rumen conditions are difficult to measure and report, and this leads to uncertainty (Sarhan and Beauchemin, 2015). Second, it is difficult to identify or build datasets that possess sufficient independent variation within independent variables. This may make derivation of useful parameters estimates somewhat problematic. In practice, no single study can possibly evaluate all of dimensions simultaneously. The challenge of accurately modeling and predicting “*conditions*” is also existent in the field of weather forecasting (Meier et al., 2014). To overcome these challenges, some climatologists employ what is known as “*ensemble modeling*” and use the approach to generate predictions of weather

patterns as affected by various driving forces (Meier et al., 2014). We chose to use this multi-dimensional approach to predict dietary physical and chemical characteristics required to maintain desired rumen conditions (White et al., 2017a). Our target for prediction of the desired “rumen conditions” was mean ruminal pH. Ruminal pH was chosen because it was frequently reported in many of the studies included in our dataset, but it should be noted that other measures that were rarely reported, such as minimum or maximum pH and time under some specific pH, may better represent risk of acidosis. As already mentioned, this paNDF system can be used to estimate TMR particle size and diet compositions needed to maintain target rumen conditions.

Structure of the Ensemble

An ensemble modeling approach is used to generate means and confidence intervals to describe the need for particle size, fiber, and other dietary components in diets for lactating cows. In this approach, a "mixture of expert" (ME_x) models from a range of dietary scenarios, such as high or low starch diets, are identified and rumen pH is then predicted with each expert model individually (Figure 1). The mean of the predicted pH is estimated based on dietary composition using expert algorithms. A confidence range is then estimated based on the minimum and maximum predictions of the ensemble. In practice, an ensemble of models aggregates predictions from multiple different models (Table 1) to yield a mean and range of responses. Compared with individual models, ensembles may provide more reliable predictions of events, estimate confidence in the reliability of those predictions, and are less likely to generate systematic errors. For example, rather than forcing integration of all models over an entire range of conditions such that the full range has areas of instability, the ensemble approach integrates equations

with varying weighting factors over the entire range of conditions. Compared with individual models, an ensemble approach has improved utility, particularly in situations where minimal data is available for equation development. As illustrated in Figure 1, the individual expert models correspond to “model 1, model 2, model 3,” with each model being selected as an “expert” based on its performance against subsets of the data. In our case, the available input data were then run through each model, resulting in 6 predictions of pH. An algorithm was then used to consolidate those 6 predictions into a single pH prediction. The predicted pH was then back-calculated and a recommendation of material to be retained on the 8 mm sieve of the PSPS is generated.

Rumination Activity and Rumen pH

A general concept related to physically effective fiber is that coarse fiber particles stimulate chewing activity, and this in turn stimulates saliva production that buffers the ruminal environment (Beauchemin et al., 2008). Although total chewing time, as the sum of time eating and ruminating, is commonly reported in studies which have evaluated effective fiber, in our study the effects of total chewing time, as well as eating time and ruminating time (and these factors divided by DMI) on rumen pH was evaluated. Interestingly, of all of the chewing activities tested, only rumination time per unit of DMI was observed to significantly affect rumen pH (Table 1). In general compared to time spent eating, the time spent ruminating likely has a greater influence on rumen pH (Zebeli et al., 2010) and has been observed experimentally (Beauchemin et al., 2003). This may be because cows spend as much as twice the amount of time ruminating than eating and more saliva is produced from rumination activities (Maekawa et al., 2002a,b). The importance of rumination is not only limited to saliva production and rumen

pH, as the act of ruminating is also believed to be closely integrated with reticulo-ruminal motility and consequently overall gut health (Van Soest, 1994). The equation used to predict rumination time is listed in Table 1 and factors observed to affect it included particle size measures of the TMR, NDF and starch contents of the TMR, and DMI.

The objective of creating the pNDF system was NOT to develop a predictive equation of rumen pH but to use it as a target for desirable rumen conditions. Ruminal pH is known as a key physicochemical measure of rumen fermentation (Aschenbach et al., 2011a; Penner et al., 2011). If too low, it can negatively affect rumen microbes and inhibit fiber digestion (Krajcarski-Hunt et al., 2002) and also the flow of microbial CP out of the rumen (Firkins, 1996, 2010; Russell and Wilson, 1996). In our ensemble approach, two different models were used to predict rumen pH (Table 1). As the consumption of starch leads to the increased production of organic acids (Firkins, 1996), it was not surprising that starch was used in both models to predict pH. Currently, there is not agreement for the “best or optimal ruminal pH” for lactating dairy cows, but White et al. (2017b) used 6.1 as an example. Overall, our quantitative findings provide a comprehensive approach to estimating the effective fiber needs of dairy cattle as both TMR particle size and diet NDF influence both DMI and rumination time and this is in agreement with mechanistic expectations, and these factors in turn were integrated into a system that could be robustly related to observed ruminal pH in dairy cattle.

Forage Fragility, a New Consideration

Fragility of a feed has been defined as the rate at which plant tissues contained in a feed particle are further fragmented into small particles (Grant, 2010). Compared to fiber in

grasses, the fiber in legumes is thought to be more fragile and can be more easily fragmented (Kammes and Allen, 2012). Consequently, legumes stimulate less rumination and in turn, salivary buffer production. Grasses also have a higher content of hemicellulose (Van Soest, 1994), which crosslinks with lignin, may be less fragile, and might be more effective in stimulating chewing activity (Mertens, 1997). In an attempt to account for this, we included ADF/NDF as an indirect measure of forage fragility. A laboratory method to measure forage fragility has been developed (Farmer et al., 2014), but it is not widely used either in the field or in published studies.

The Importance of fNDF and Inclusion of Article Size

Time and application has proven recommendations of minimum fiber and maximum nonfiber carbohydrates outlined in Table 4-3 of the Dairy NRC (2001) to be extremely robust and applicable. This is in part because of its simplicity, but as already mentioned, the table does not account for feed particle size. The influence of effectiveness of fiber using the ensemble approach without any measure of particle size was also evaluated using something called Lin’s concordance correlation coefficient (CCC). This value ranges from 0 to 1 like a regular correlation coefficient but is more robust at comparing across different models of diverging structures. When using only forage NDF, the unadjusted CCC was only moderately lower (worse) than the CCC from the more complicated ensemble model (0.52 vs 0.59), but the ensemble model still is much more robust and flexible. It explored broader sources of variation affecting animal chewing and ruminal pH, which cannot readily be measured on farms, while also controlling multicollinearity (the latter term refers to trends which tend to follow each other; for example, various protein sources

rising and falling in price on average). Of course, just because we can generalize doesn't mean we always should. In the above example, when protein sources are out of synch, that is when you can lower feed costs, but of course you would need to do this consistently because relative prices change. Similarly, we think expanding the fNDF model allows more robustness and flexibility when assessing rumen health.

For the current discussion, Table 4-3 in the NRC (2001) documents the need to decrease non-structural carbohydrate (now better measured as starch plus sugars) simultaneously as fNDF decreases. Some studies in the literature have followed these or similar recommendations just like some farms have. In that case, decreasing fNDF would be statistically associated with decreasing starch in diets; however, what about the flexibility of using nonforage NDF to replace part of both forage and grain? Let's look at two contrasting examples. First, consider that forage price is relatively high in a certain region. In some herds with excellent management, would a nutrition advisor be willing to take more risk of lower ruminal pH and its associated responses (lower NDF digestibility or depressed milkfat) to lower diet cost? Balancing for fNDF and starch certainly is a good place to start. However, in addition, the ensemble model embeds dietary components associated with ruminal NDF and starch digestibilities; these components are combined with fNDF and other dietary factors while also adding the dimension of increasing chop size of forage. A diet can be formulated along with directions on how coarse to chop hay while subsequently assessing TMR sieve data. Second, what if forage price is relatively low in certain regions; wouldn't a nutrition advisor now be willing to assess how to optimize that forage's inclusion level while potentially shortening chop size to help prevent depressed dry matter intake? What if corn silage is chopped very coarsely on one farm but not on another in the same region?

The ensemble model allows these types of varying conditions to be assessed in diets with less trial feeding to cows.

The take-home message is that using fNDF and starch alone is good but can be better. In most diets assessed, TMR particle size in our dataset was near recommendations more of the time than it was not (i.e., fNDF and starch are ok), but the divergence of diets that were very short or very coarse under different fNDF and starch concentrations also allowed opportunity. Shouldn't a Penn State shaker box be routinely used, anyway? With minimal extra data, then the ensemble approach allows more information to be integrated and provided in a user-friendly format for nutrition advisors to think "outside of the box". Interestingly, models developed to predict rumen pH did not include any measure of particle size; however, the relationship of particle size to rumen conditions appears by way of its effects on feed intake and rumination time (Table 1). Driving factors that influence rumen pH include rumen degradation of carbohydrate, fNDF, and rumination activities. Given the inter-relatedness of these factors, it is impossible to determine which is more "important" or which has more influence on rumen conditions and the ensemble approach considers them all.

Towards On-Farm Application of paNDF

Figure 2 illustrates how inputs are used to generate feeding recommendations for target rumen conditions. The proportion of TMR on the top screen (19-mm) varied on the top axis by 6, 12 or 18%, while fNDF varied on the bottom axis, starch varied on the right axis, and the model solves for the left axis which is the proportion of TMR on the second screen (8-mm). In the top left graph of this figure, depicting 6% of TMR DM retained on a 19-mm screen and 15% TMR DM starch, two inflection points are visible. One occurs at approximately 16.0%

fNDF and the other at approximately 26.5% fNDF. This figure can be interpreted to suggest that ruminal pH can be maintained in a diet low in fNDF (16.0 %) by increasing the proportion of TMR (between 40 and 60%) retained on the 8-mm sieve. Alternatively, when feeding a diet high in fNDF (26.6%), a lower proportion of TMR (< 20 %) retained on the 8-mm sieve is needed. In practice, a true recommendation for the percentage of DM material on the 8-mm sieve should be based on the diet target fNDF and likely lies somewhere between these 2 inflection points. An additional example can be found in the figure depicting 6% of TMR DM retained on a 19-mm screen and 25% TMR DM starch, in which one inflection point at approximately 22% fNDF is visible. This figure can be interpreted to suggest that longer TMR particles plays a lesser of a role in maintaining pH when fNDF is greater than 22 %.

For deriving solutions or feeding recommendations with the pNDF system, a mobile phone application will be available free of charge early in 2018. To use the application, users will simply key in desired rumen conditions; diet ADF, NDF, fNDF, starch, proportion of the ration as forage; and cottonseed, as well as particle size measures. Users can then use the application to determine the proportion of TMR that should be retained on the second sieve (8-mm) of the PSPS to maintain a defined rumen pH. The application will also provide a confidence interval for all recommendations. It should be stressed that meeting the derived feeding recommendations will not guarantee a specific average rumen pH in the herd. The application was designed to generally predict rumen conditions as affected by major diet factors. Other factors are known to affect rumen pH and could not be included in the system. These include the concentration of other carbohydrates, such as water-soluble carbohydrates and soluble fiber (Hall et al.,

1999); chemical or physical processing of feed; use of ionophores (Firkins and Yu, 2015); feeding management and behavior (Miller-Cushon and DeVries, 2010), associative rumen effects, such as volatile fatty acid and ammonia absorption and urea secretion in rumen (Aschenbach et al., 2011b); and dietary cation-anion difference (Iwaniuk and Erdman, 2015).

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Table 1. Models developed by White et al. (2017a) through during ensemble model training (units of all parameters on a DM basis) and used to generate feeding recommendations for effective fiber (adapted from White et al., 2017b).

Response	Equation ¹
DMI, kg/day	$-0.889 - 0.460 \times \text{MPS} + 0.0203 \times \text{BW} + 0.110 \times \text{Forage} + 0.794 \times \text{NDF} - 0.0117 \times (\text{NDF} \times \text{NDF})$ $-1.74 - 0.432 \times \text{MPS} + 0.0218 \times \text{BW} + 0.163 \times \text{Cottonseed} + 0.117 \times \text{Forage} - 0.238 \times \text{fNDF} + 0.771 \times \text{NDF} - 0.0116 \times (\text{NDF} \times \text{NDF})$
Rumination Time, min/day	$-357 - 16.7 \times \text{MPS} + 4.34 \times 19 \text{ mm} + 2.49 \times 8 \text{ mm} + 71.5 \times \text{DMI} - 1.54 \times (\text{DMI} \times \text{DMI}) + 4.78 \times \text{NDF} - 1.68 \times \text{dNDF} - 2.35 \times \text{dStarch}$
pH	$12.0 + 0.0112 \times \text{fNDF} - 0.0190 \times \text{Starch} + 0.0003448 \times (\text{Starch} \times \text{Starch}) - 0.679 \times \text{CP} + 0.0186 \times (\text{CP} \times \text{CP}) + 0.01052 \times (\text{Rumination Time/DMI})$ $6.72 + 0.0137 \times \text{fNDF} + 0.00798 \times \text{Starch} - 0.0456 \times \text{CP} - 0.00835 \times \text{dStarch} + 0.0204 \times (\text{Rumination Time/DMI})$

¹MPS, estimated mean particle size from PSPS data in mm; BW, body weight in kg; All dietary concentrations are on a DM basis: Forage, % of forage in the TMR; NDF, % NDF in the TMR; Cottonseed, % of cottonseed in the TMR; fNDF, % of forage NDF in the TMR; 19-mm, % of TMR retained on the 19-mm sieve of the PSPS; 8-mm, % of TMR retained on the 8-mm sieve of the PSPS; DMI, dry matter intake, kg/d; dNDF, rumen degraded NDF as estimated by White et al., 2017a; dStarch, rumen degraded starch as estimated by (White et al., 2016); Starch, % of starch in the TMR; CP, % of CP in the TMR; Rumination time, time spend ruminating, min/day.

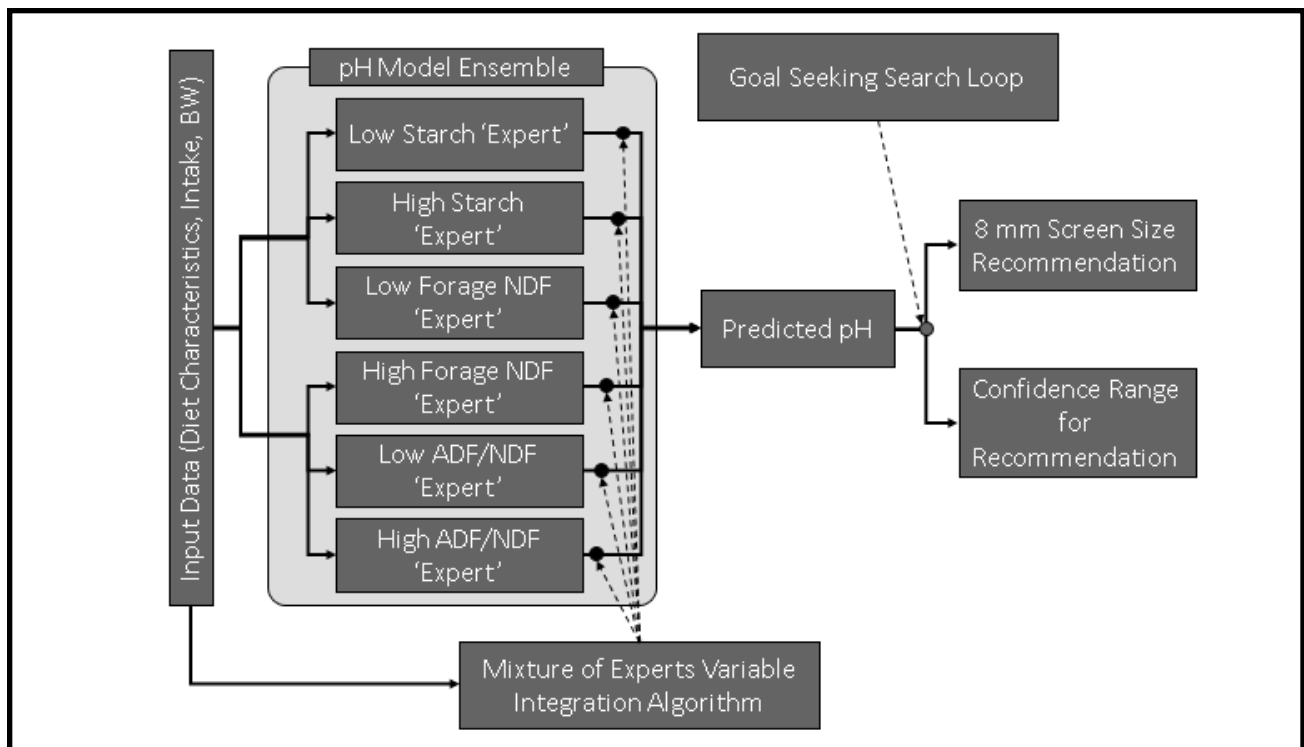


Figure 1. Depiction of strategy to estimate mean and confidence range of pH responses estimated by the model ensemble. Various "expert" models are identified (high starch vs. low starch) and pH is estimated with all expert models individually. The weighted mean of the predicted pH from 6 equations is estimated based on dietary composition using the variable mixture of experts integration algorithm. The confidence range is estimated based on the minimum and maximum predictions of the ensemble (adapted from White et al., 2017b).

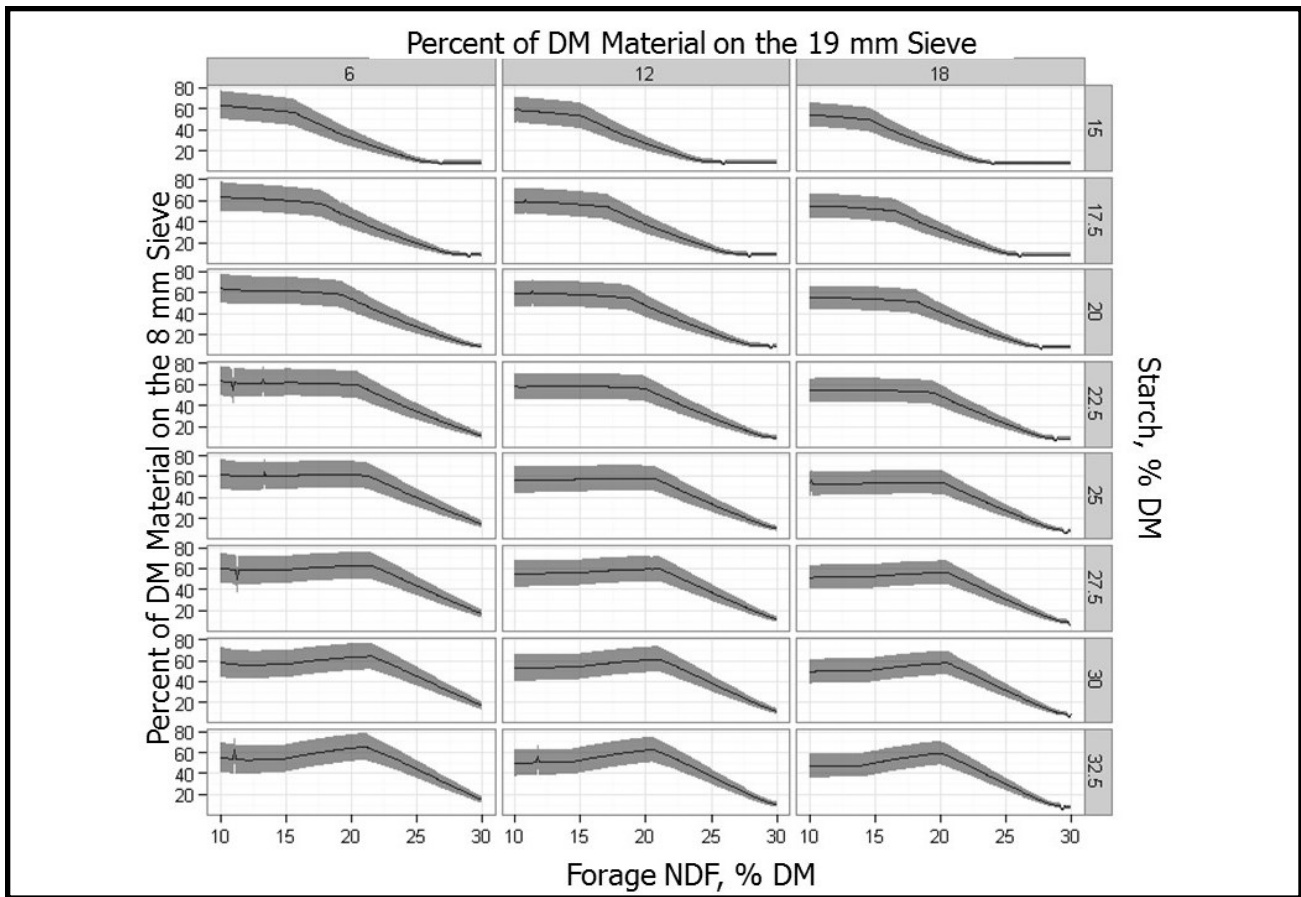


Figure 2. Response surfaces generated by the multi-model ensemble for a target pH of 6.1. Curves were generated by iterating through the system of equations (adapted from White et al., 2017b).

Understanding the Regulation of Milk Fat Synthesis and Its Potential Application in Herd Management

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Introduction

There is growing interest in nutrition strategies focused on increasing the yield of milk components. The yields of milk fat and protein are the major contributors to the price that producers receive for milk. In an economic analysis assessing the value of milk components, a 5% increase in fat yield, protein yield, and milk yield increased net farm income by 13%, 15%, and 2%, respectively (St-Pierre, 2017). This reinforces the importance of focusing on increasing the yield of milk components and not milk yield per se to maximize milk price and income. Synthesis of milk fat in the mammary gland is a highly-coordinated process involving de novo synthesis of fatty acids (FA) and incorporation of preformed FA. Importantly, several factors can influence milk fat synthesis, including genetics (breed and selection), stage of lactation, parity, mastitis, dietary forage and grain levels, diet fermentability, dietary FA level and profile, and seasonal and regional effects. Furthermore, the potential utilization of milk FA as a management tool has recently received attention. We will briefly review the biological processes for milk fat synthesis, the interrelationship between different FA in the regulation of milk fat synthesis, and the potential for nutrition and management decisions based on milk FA analysis.

Milk Fat Synthesis

Milk FA originate from 1 sources: < 16 carbon FA are synthesized de novo in the mammary gland and > 16 carbon FA are extracted from plasma as preformed FA. Mixed FA (16-carbon FA) can be derived from either de novo or preformed sources. Acetate and β -hydroxybutyrate, formed by rumen fermentation of carbohydrates, represent the major carbon sources for FA synthesized de novo in the mammary gland (Bauman and Griinari, 2003). In plasma, FA absorbed from the intestine are transported in lipoproteins and FA mobilized from body tissues are transported as NEFA (Bauman and Griinari, 2003). Microbial synthesis of odd and branch chain FA in the rumen and absorption of biohydrogenation intermediates also contribute to the diversity of FA secreted in milk fat. A diagram representing the major metabolic pathways involved in milk fat synthesis is presented at Figure 1.

De novo FA synthesis: To produce milk FA from 4 to 16 carbons in length in the mammary gland, the main pathway involves acetate being converted to acetyl CoA by acetyl CoA synthetase. Next, acetyl-CoA carboxylase (ACC) converts acetyl-CoA to malonyl-CoA in an irreversible reaction (Bauman and Davis, 1974). The production of malonyl-CoA is considered the rate-limiting step for de novo synthesis of milk FA and the activity of ACC

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is considerably lower than the activity of other FA synthesis enzymes. β -hydroxybutyrate can also contribute carbons for initiating milk FA synthesis. In fact, Lin and Kumar (1972) indicated that the lactating mammary gland utilizes butyryl-CoA more efficiently than acetyl-CoA as a “primer” for FA synthesis. As shown by Palmquist et al. (1969), up to 50% of FA synthesized de novo by the lactating mammary gland utilizes β -hydroxybutyrate as the initial C4 methyl primer for FA synthesis. Propionate and branch chain volatile FA can be used as a primer for milk FA synthesis, leading to the synthesis of odd and branch FA (Palmquist, 2006). Acetate and β -hydroxybutyrate account for all carbons in C4:0-C12:0 milk FA, 75% of C14:0 and 50% of C16:0 (Smith et al., 1974). It is important to point out that although several precursors can initiate FA synthesis, acetyl-CoA is the principal building block that is used by the FA synthase (**FAS**) complex generating palmitate.

Besides a carbon source, FA synthesis requires NADPH. The activities of both citrate lyase and malic enzyme increase with high carbohydrate diets in non-ruminants. The activities of these latter enzymes are low in ruminants (Bauman et al., 1970), probably reflecting the greater availability of acetate as a lipogenic precursor in these species or the absence of the need to transport these units from the mitochondrion to the cytosol, or both. In ruminants, most of the glucose is derived from gluconeogenesis, while acetate, and other principal fuel molecules produced in the rumen provide the precursors for the initiation of lipogenesis in adipose tissue and the mammary gland.

Preformed FA: A second source of FA in the mammary gland is long chain FA from the diet and other tissues. The triglycerides (**TAG**) contained within chylomicrons and very low

density lipoproteins (**VLDL**) in plasma are the primary source of milk FA >16 carbons in length taken up by the mammary gland (Palmquist, 2006) with NEFA also contributing FA to milk fat when concentrations of plasma NEFA are high, usually occurring during periods of negative energy balance in early lactation. Dietary FA, and FA formed during rumen biohydrogenation, are absorbed in the small intestine, esterified to glycerol forming relatively inert TAG and then packaged into TAG-rich lipoproteins that usually comprise chylomicrons or VLDL (Smith et al., 2006). Due to the large size of chylomicron and VLDL particles, they have little capacity to move across capillaries. Therefore, the movement of FA into the mammary gland depends on hydrolysis of the TAG within these particles, a process that is carried out by lipoprotein lipase (**LPL**) along the luminal surface of capillary endothelial cells (Smith et al., 2006). This process removes around 90% of the TAG from the particles, generating remnant lipoproteins that are largely taken up and removed by the liver (Drackley, 2000). Therefore, FA enter the cells either as FA released from the TAG-rich lipoproteins or FA within the albumin-FA pool. Free FA and diacylglycerol are taken up by mammary epithelial cells and used for TAG synthesis in the mammary gland.

Triglyceride synthesis: Milk fat is composed of 95 to 98% (**TAG**), 0.30 to 2.0% diacylglycerol, and small concentrations of phospholipids, cholesterol esters, and free FA (Jensen, 2002). The primary pathway used for synthesis of TAG in the mammary gland is the glycerol-3 phosphate pathway where both de novo and preformed FA are incorporated onto the glycerol-3 phosphate backbone. Glycerol phosphate acyl transferase (**GPAT**) is responsible for adding a fatty acyl-CoA to the sn-1 position of glycerol-3 phosphate and acyl glycerol phosphate acyl transferase (**AGPAT**)

adds the second fatty acyl-CoA to the sn-2 position. The final fatty acyl-CoA is added to the sn-3 position by diglyceride acyl transferase (DGAT) forming the TAG.

The location of FA along the glycerol backbone is not random with individual FA being preferentially located at different positions (Jensen, 2002). Interestingly, saturated FA are predominantly esterified at the sn-1 position and unsaturated FA at the sn-2 position (Jensen, 2002). Since C16:0 is the end product of de novo synthesis, it is potentially a key FA in this process. A higher preference (8- to 10-fold) was shown for C16:0 as a substrate for GPAT than C18:0 and *cis*-9 C18:1 in the mammary gland of dairy cows (Kinsella and Gross, 1973). Also, short- and medium-chain FA are preferentially esterified to the sn-3 position. Over 98% of C4:0 and 93% of C6:0 are esterified on the sn-3 position (Table 1; Jensen, 2002). The sn-2 position contains greater than 50% of all C10:0 to C14:0 milk FA. Distribution of C16:0 is uniform between the sn-1 and sn-2 position, while C18:0 is primarily esterified to sn-1 with a smaller proportion esterified to sn-3. *cis*-9 C18:1 is esterified to either the sn-1 or sn-3 position of TAG (Jensen, 2002).

Importantly, this control of FA placement within TAG provides the mammary gland with plasticity to secrete TAG into droplets that can be incorporated into milk and be fluid at body temperature (Dils, 1986; Jensen, 2002). Therefore, the control of melting point of milk fat is relatively constant even with large variations in the availability of FA with different melting points. The mechanisms by which the mammary gland controls the melting point of TAG include: increasing unsaturated FA by desaturation, the synthesis of short-chain FA, and preferentially positioning short-chain FA at the sn-3 position of the glycerol backbone.

Interdependence Between De Novo and Preformed FA During Milk Fat Synthesis

The concept of interdependence suggest that de novo synthesis may to a certain extent drive milk preformed FA yield, and vice versa, indicating a positive relationship between de novo and preformed milk FA. In a meta-analysis, Glasser et al. (2008) suggested that in low-fat diets, milk 18-carbon yield is probably limited by 18-carbon supply. Low 18-carbon availability may limit the incorporation of short- and medium-chain FA into milk TAG. The explanation for this likely lies at the mammary FA esterification steps, which involves both de novo synthesized FA and long-chain FA taken up from plasma. The production of diacylglycerols (mainly composed of long-chain FA), which are a substrate for DGAT, would remain low and would thus limit the incorporation of short- and medium-chain FA in milk TAG. In this context, increased dietary 18-carbon could act as primers for TAG synthesis and increase both de novo synthesis and 18-carbon incorporation into milk fat (Glasser et al., 2008).

We have recently investigated the relationship between the omasal flow of different FA and their effects on milk FA synthesis (de Souza et al., 2018a). Our analysis used individual observations (n=132) in lactating Nordic Red dairy cows from 9 Latin square or switch-back design studies. We observed a positive relationship between the omasal flow of C16:0 and total milk FA driven by an increase in the yield of de novo and mixed FA (Figure 2). Increasing C18:0 omasal flow did not affect the yield of de novo FA, but quadratically increased the yield of preformed and total FA in milk (Figure 3). For the flow of C18:0, maximum preformed and total FA yields were achieved when 18:0 flow was 1065 and 943 g/day respectively. Therefore, our results agree with the previous findings of Glasser et

al. (2008) demonstrating the interdependence between de novo synthesized FA and preformed FA and highlight that effects on de novo, mixed, and preformed milk FA is dependent upon the amount and profile of absorbed FA. Importantly, this interdependence between FA sources appears to mostly occur in dietary situations with low-risk for milk fat depression (MFD).

Substitution of Different Sources of Milk FA

In some instances, changes in milk fat yield to alterations in the supply of dietary FA may result in the substitution of different sources of milk FA, in which an increase in milk preformed FA yield coincides with a decrease in de novo FA yield. Grummer (1991) reported that an inverse relationship exists between the amount of fat supplemented in the diet and the concentration of de novo FA in milk fat. As more dietary fat is added, the proportion of de novo synthesized milk FA decreases, whereas the proportion of preformed milk FA increases. On a FA yield basis, the substitution effect of preformed de novo milk FA was recently reported by He and Armentano (2011) and He et al. (2012), who noted that the reduction in the yield of de novo milk FA was often compensated for by an increase in the yield of preformed milk FA when fat supplements were fed.

Similarly, Leonardi et al. (2005) indicated that increasing the fat content of distillers' grains with added solubles reduced de novo milk FA synthesis. However, the decreased yield of short-chain FA coincided with an increased yield of long-chain FA, resulting in no differences across treatments in total milk fat yield (Figure 4). Dorea and Armentano (2017) summarized the effects of five common dietary FA (C16:0, C18:0, *cis*-9 C18:1, *cis*-9, *cis*-12 C18:2, and *cis*-9, *cis*-12, *cis*-15 C18:3) on milk FA sources (de novo, mixed, and preformed). The results

indicated that supplements rich in unsaturated FA decreased milk FA or caused substitution by inhibiting secretion of de novo milk FA with dietary *cis*-9, *cis*-12 C18:2 being the most inhibitory. Therefore, the substitution effect seems to occur when dietary interventions or management induce a 'mild MFD situation' and likely represents a lost opportunity since the substitution of different milk FA sources does not usually result in an increase in milk fat yield.

This is different to a 'classical' MFD situation which is characterized by a decrease in milk fat yield of up to 50%, with no change in milk yield or in the yield of other milk components (Bauman et al., 2011). During MFD, the profile of milk FA is markedly altered, and this is a characteristic of the biohydrogenation theory. We recently utilized a random regression model to analyze available individual cow data from 3 studies that induced MFD in dairy cows (unpublished results). We observed that as the degree of MFD increased, the concentration of de novo milk FA markedly decreased, while the concentration of preformed milk FA increased (Figure 4). However, on a yield basis, as the degree of MFD increased, the yield of de novo, mixed and preformed FA all decreased (Figure 5). Therefore, when evaluating the effects of nutrition and management strategies on milk FA profile, it is important to consider the unit that milk FA is reported in (concentration or yield basis), since this may impact the interpretation of results.

C16:0 Supplementation and Milk FA

We have recently carried out a series of studies examining the effect of individual saturated FA on production and metabolic responses of lactating cows. Most of our short-term studies involving C16:0 supplements (fed at 1.5 to 2.0% diet DM) have indicated increases in milk fat yield of ~100 g/day (Piantoni et

al., 2013; Lock et al., 2013; de Souza et al., 2018b). In long-term feeding, Mathews et al. (2016) observed that feeding a C16:0-enriched supplement (3.9% diet DM) over a 7-wk period also increased milk fat yield by ~200 g/day. Similarly, we recently observed that C16:0 supplementation consistently increased milk fat yield by 155 g/day compared with a non-fat control diet over a 10-wk supplementation period (de Souza and Lock, 2018). Although Rico et al. (2017) observed that maximum milk fat yield response occurred when C16:0 was fed at 1.5% of diet DM, the incorporation of C16:0 into milk fat increased linearly as C16:0 dose increased.

We recently utilized a random regression model to analyze available individual cow data from 13 studies that fed C16:0 supplements to dairy cows (unpublished results). We observed that C16:0 supplementation increased the concentration of mixed milk FA and reduced the concentration of de novo and preformed milk FA (Figure 6). On a yield basis, we observed that C16:0 supplementation increased the yield of mixed and total milk FA and did not change the yield of de novo and preformed milk FA (Figure 7). More importantly, we observed that C16:0 supplementation affected the yield of individual FA differently. The yield of milk C4:0 and C6:0 were positively associated with C16:0 supplementation while the yield of milk C12:0 and C14:0 were negatively associated with C16:0 supply. Dorea and Armentano (2017) summarized the effects of common dietary FA (C16:0, C18:0, *cis*-9 C18:1, *cis*-9, *cis*-12 C18:2, and *cis*-9, *cis*-12, *cis*-15 C18:3) on milk FA sources (de novo, mixed, and preformed). The results indicated that C16:0 supplementation increased total milk FA, mainly by increasing milk C16:0 yield, without affecting milk de novo and preformed yield. According to their regression of milk C16:0 yield on dietary FA, endogenous C16:0 contributes ~80% of total

milk C16:0, but this proportion varies with the level and type of dietary FA fed.

Tzompa-Sosa et al. (2014) reported that the proportion of other FA at sn-2 was correlated with the total amount of C16:0 in the TAG. They suggested that an increase in availability of C16:0 for lipid synthesis in mammary epithelial cells will increase the activity of both isoforms of GPAT in the mammary gland. This increase in activity will then increase the proportion of C16:0 and other long-chain SFA acylated at sn-1 at the expense of sn-2. A decrease in the amount of long-chain saturated FA at sn-2 would be counterbalanced by other FA. Overall, this hypothesis could explain our finding that C16:0 increased the yield of mixed-source FA without reducing the yield of de novo and preformed FA, not only by increasing TAG synthesis but also by changing the FA interpositional distribution in the TAG.

Milk FA as a Herd Management Tool

Recently, the use of milk FA as a potential herd management tool has been proposed. In bulk tank milk samples from 430 commercial farms, Barbano et al. (2014) identified a positive correlation between de novo milk FA concentration and milk fat and true protein content. Similarly, Dorea and Armentano (2017) suggested that since milk mid-infrared analysis can be used to routinely measure the profile of milk FA, the concentration of different classes of milk FA may provide a good indication of inhibition of milk FA secretion.

Woolpert et al. (2016) investigated the relationship between de novo FA concentrations in bulk tank milk with management practices, dietary characteristics, milk composition, and lactation performance on commercial dairy farms. Farms were categorized as high (HDN; 26.18 ± 0.94 g/100 g of FA) or low de novo

(LDN; 24.19 ± 1.22 g/100 g of FA) FA in bulk tank milk. The authors reported that the yield of milk fat, true protein, and de novo FA per cow per day were higher for HDN versus LDN farms. The HDN farms had lower freestall stocking density (cows/stall) and higher feeding frequency than LDN farms. No differences between HDN and LDN farms were detected for dietary dry matter, crude protein, neutral detergent fiber, starch, or percentage of forage in the diet. However, dietary ether extract was lower for HDN than LDN farms.

In a subsequent study with a similar characterization of commercial dairy farms with HDN and LDN, Woolpert et al. (2017) detected no differences between HDN and LDN farms in the yield of milk fat or true protein. HDN farms tended to be more likely to deliver fresh feed twice versus once per day, have a freestall stocking density $\leq 110\%$, and provide ≥ 46 cm of feed bunk space per cow. There were no differences in forage quality or ration dry matter, crude protein, or starch content between HDN and LDN farms; however, ether extract was lower and physically effective fiber was higher for HDN farms.

Although the aforementioned results suggest that milk FA analysis may have potential as a management and nutritional tool, further research is needed to evaluate whether changes in management or nutritional strategies are related to milk FA and in which specific conditions. Importantly, it needs to be determined how well changes in milk FA on a concentration basis is related to changes in milk component yields.

Conclusions

The synthesis of milk fat in the mammary gland is a highly-coordinated process involving de novo synthesis of FA and incorporation of

preformed FA. Importantly, milk FA interact by competitive and complementary mechanisms under different situations. We presented different scenarios in which changes in the supply of milk FA precursors can affect milk FA sources and the yield of milk fat. The interdependence between de novo synthesis and long-chain FA may to a certain extent drive milk preformed FA yield, indicating a positive relationship between de novo and preformed milk FA. The substitution effect seems to occur when dietary or management changes induce a 'mild MFD situation' and the substitution of different milk FA usually does not result in increases in milk fat yield. A 'classical' MFD situation when there is a decrease in milk fat yield is associated with a decrease in both de novo and preformed milk FA yields. Finally, when C16:0 supplements are fed, increased total milk FA yield is mainly driven by increasing milk C16:0 yield, without affecting the yields of de novo or preformed milk FA. Potentially, milk FA can be used to help monitor herd performance and farm decisions, but careful considerations should be given to other dietary factors, feed management, production level, and stage of lactation. Further research should be carried out to determine the utility of this analysis versus the use of more traditional dietary factors and milk component measures as important management benchmarks.

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Table 1. Positional distribution of different FA in milk TAG. Adapted from Jensen, (2002).

	sn-1	sn-2	sn-3
Milk FA mol/100 mol FA			
C4:0	1.6	0.30	98.1
C6:0	3.1	3.9	93.0
C8:0	10.3	55.2	34.5
C10:0	15.2	56.6	28.2
C12:0	23.7	62.9	13.4
C14:0	27.3	65.6	7.1
C16:0	44.1	45.2	10.5
C18:0	54.0	16.2	29.8
<i>cis</i> -9 C18:1	37.3	21.2	41.5

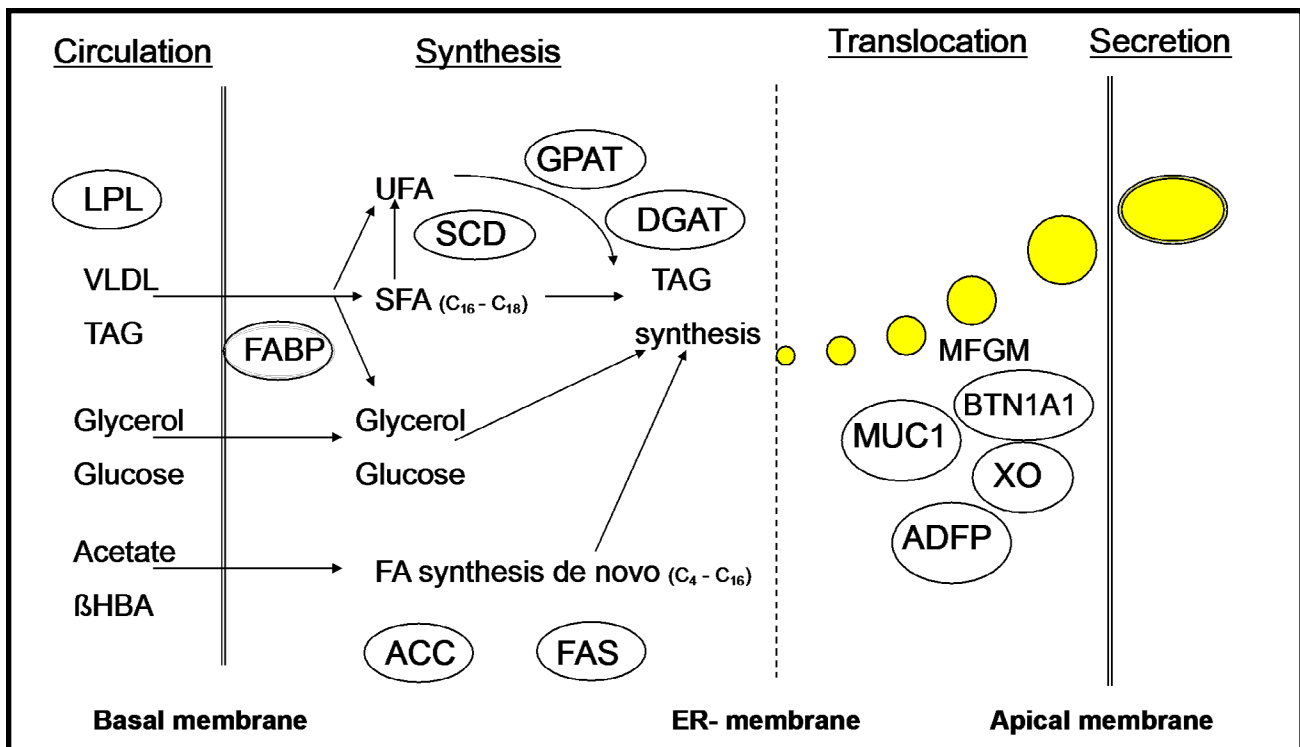


Figure 1. Diagram demonstrating the pathways involved in the synthesis and secretion of milk fat. Adapted from Bauman Lab (Cornell University). lipoprotein lipase (LPL); stearoyl-CoA desaturase (SCD); fatty acid transport proteins (FATP); glucose transporters (GLUT); glycerol phosphate acyltransferase (GPAT); diacylglycerol acyltransferase (DGAT); lipin (LPIN); fatty acid binding proteins (FABP); acetyl-CoA carboxylase (ACC); fatty acid synthase (FAS); mucin 1 (MUC1); butyrophilin (BTN1A1); xanthine oxidoreductase (XO) and adipophilin (ADPH).

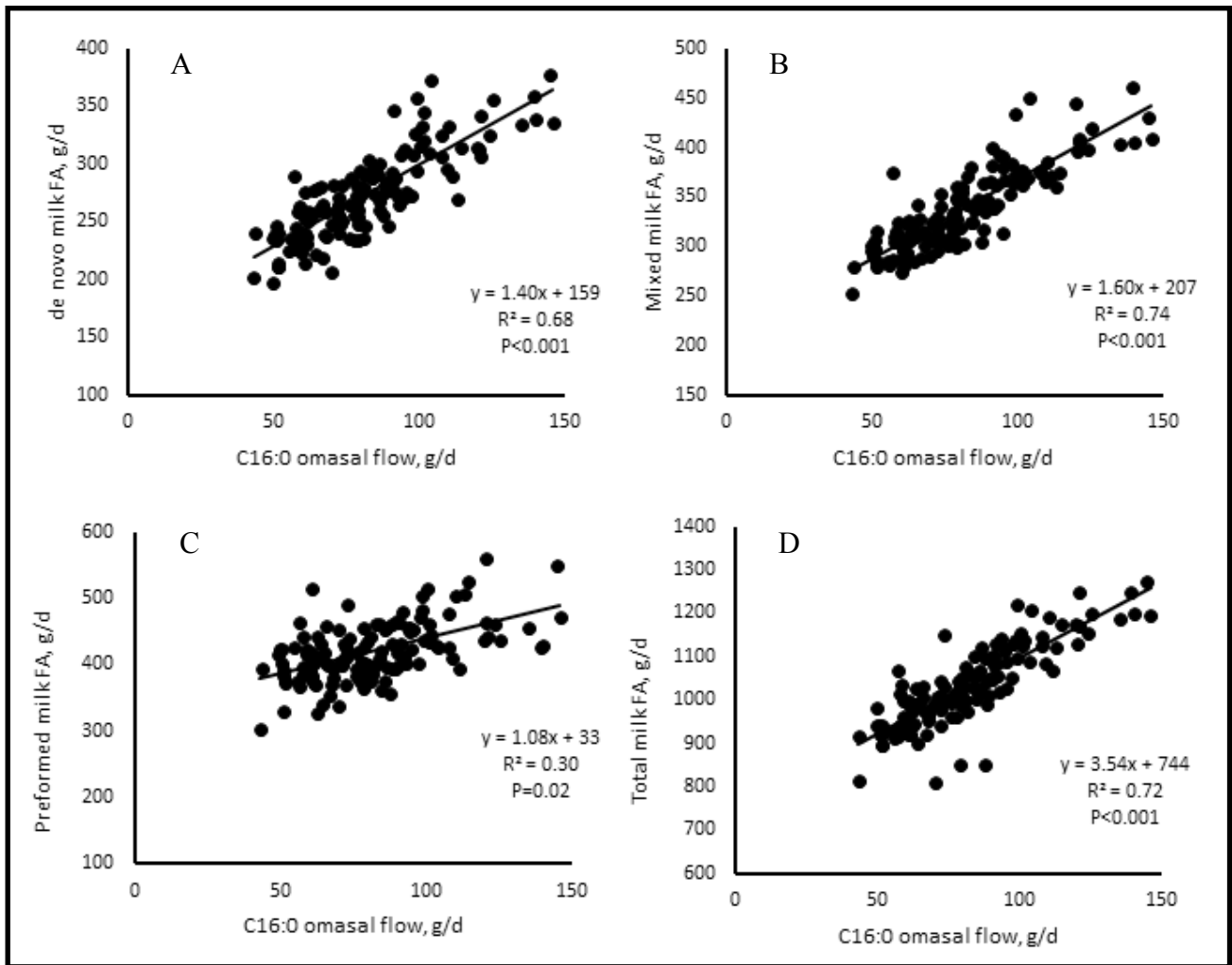


Figure 2. Relationship between C16:0 omasal flow and de novo milk FA (Panel A), mixed milk FA (Panel B), preformed milk FA (Panel C), and total milk FA (Panel D). Our analysis used individual observations ($n=132$) in lactating Nordic Red dairy cows from 9 Latin square or switch-back design studies. Mixed model regressions were developed between variables of interest taking into account experiment, period within experiment, and cow within experiment as random factors (de Souza et al., 2018a).

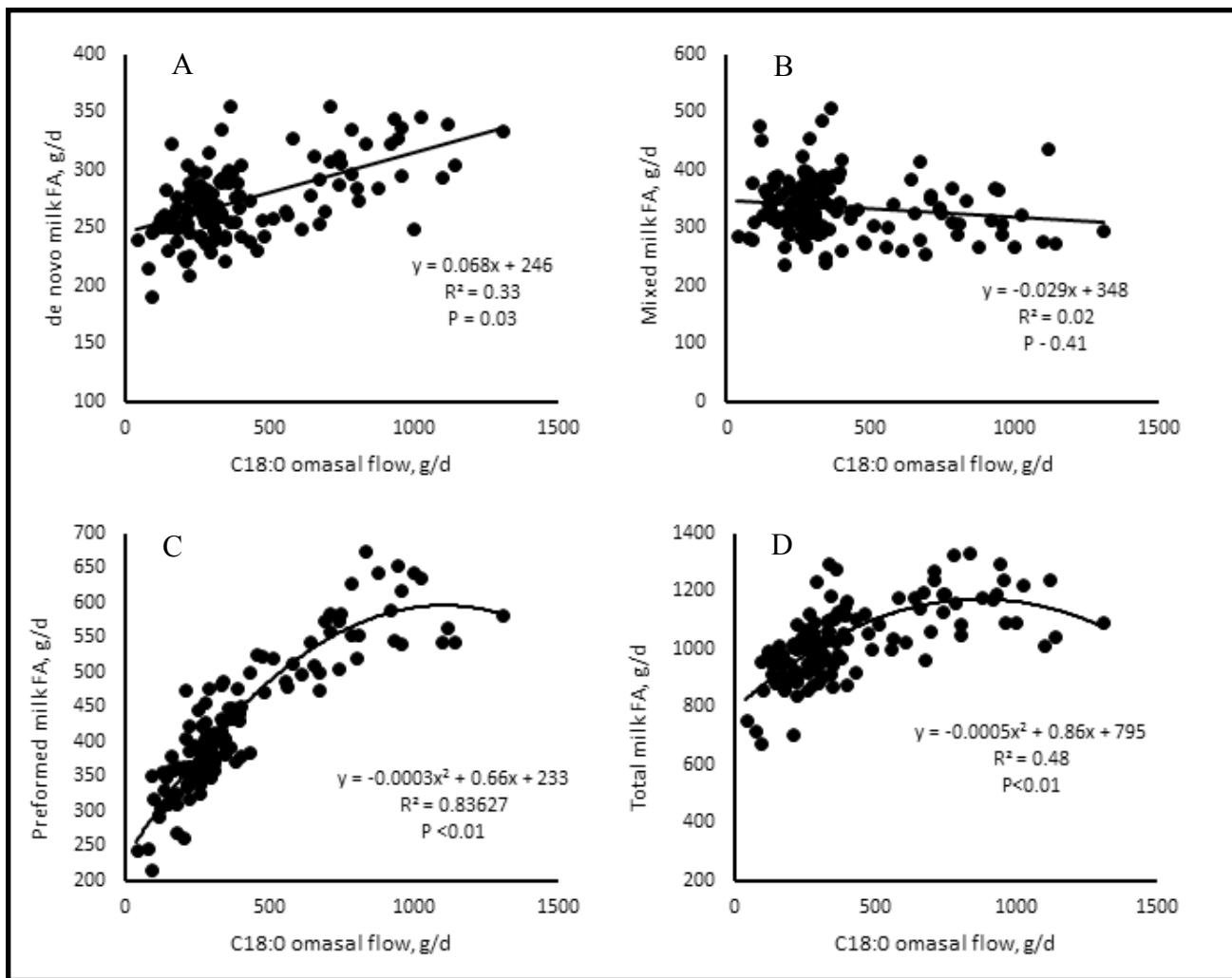


Figure 3. Relationship between C18:0 omasal flow and de novo milk FA (Panel A), mixed milk FA (Panel B), preformed milk FA (Panel C), and total milk FA (Panel D). Our analysis used individual observations ($n=132$) in lactating Nordic Red dairy cows from 9 Latin square or switch-back design studies. Mixed model regressions were developed between variables of interest taking into account experiment, period within experiment, and cow within experiment as random factors (de Souza et al., 2018a).

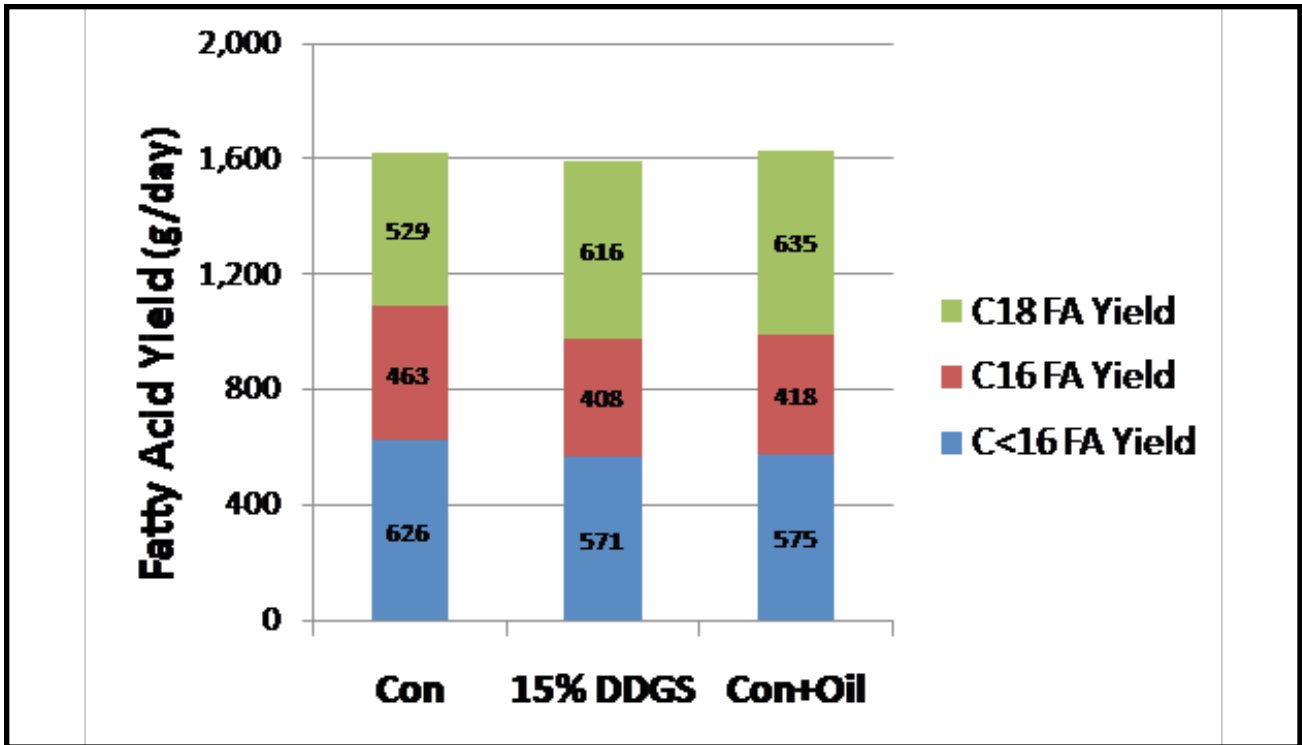


Figure 4. Effects of dry distillers grain (DDGS) and corn oil on the yield of de novo, mixed and performed milk FA in mid-lactation cows. Twenty multiparous lactating Holstein cows were assigned to a replicated, 5 × 5 Latin Square design with periods of 21 days (Leonardi et al., 2005). Figure from Lou Armentano (University of Wisconsin).

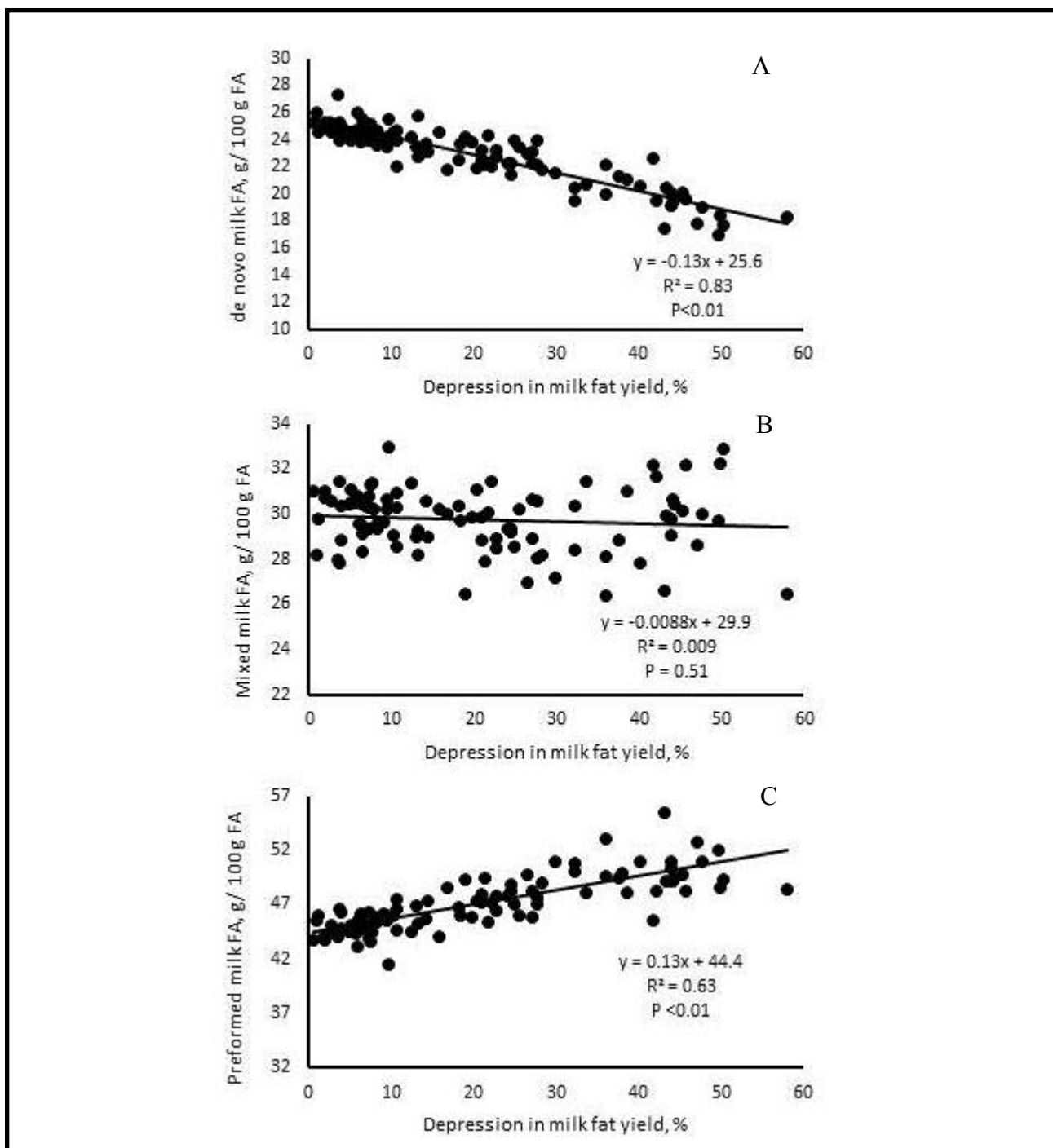


Figure 5. Relationship between the degree of diet-induced milk fat depression on the concentration of de novo milk FA (Panel A), mixed milk FA (Panel B), and preformed milk FA (Panel C). Our analysis used individual observations (n=134) in lactating dairy cows from 3 studies. Mixed model regressions were developed between variables of interest taking into account experiment, period within experiment, and cow within experiment as random factors (unpublished results).

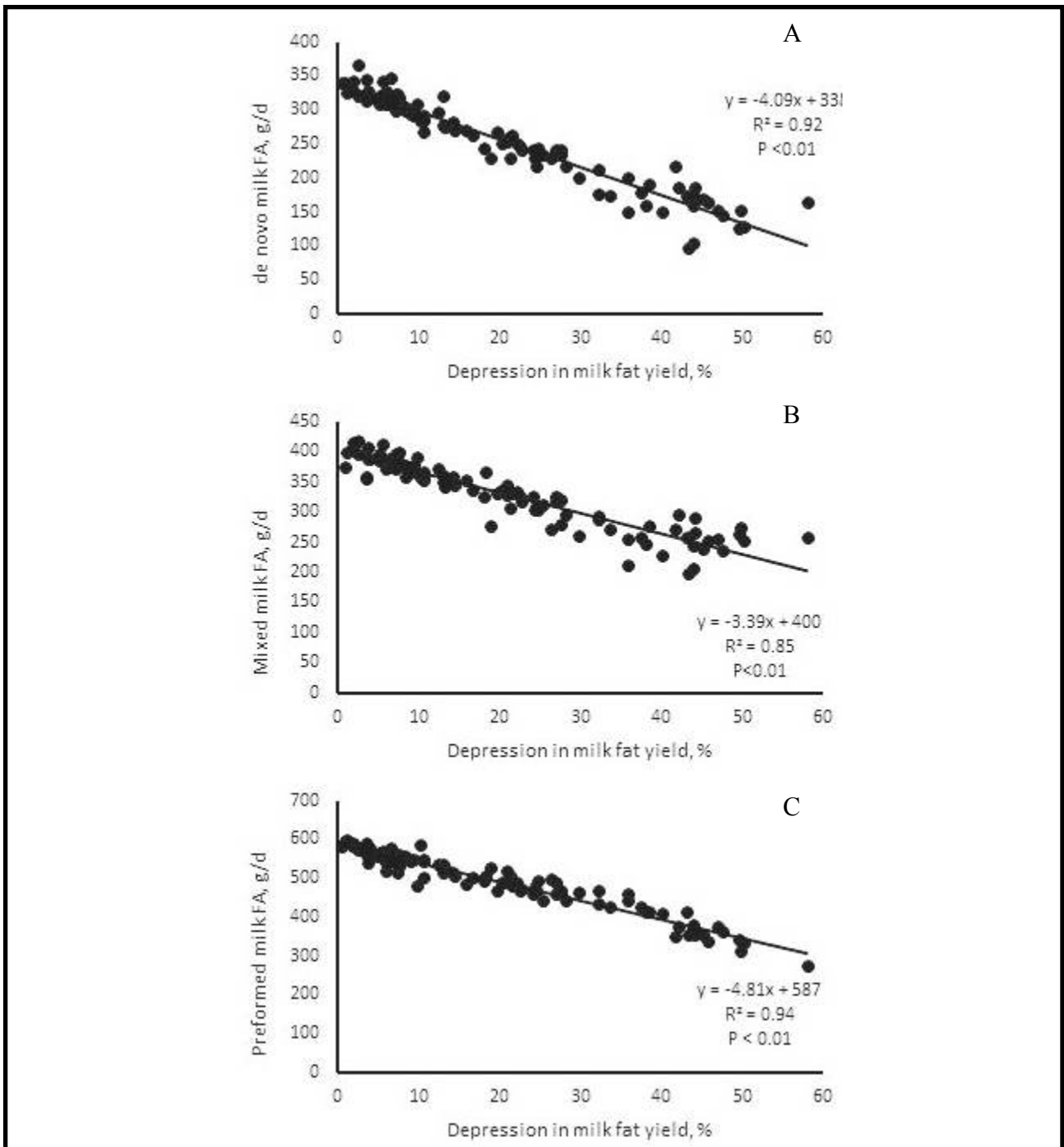


Figure 6. Relationship between the degree of diet-induced milk fat depression on the yield of de novo milk FA (Panel A), mixed milk FA (Panel B), and preformed milk FA (Panel C). Our analysis used individual observations ($n=134$) in lactating dairy cows from 3 studies. Mixed model regressions were developed between variables of interest taking into account experiment, period within experiment, and cow within experiment as random factors (unpublished results).

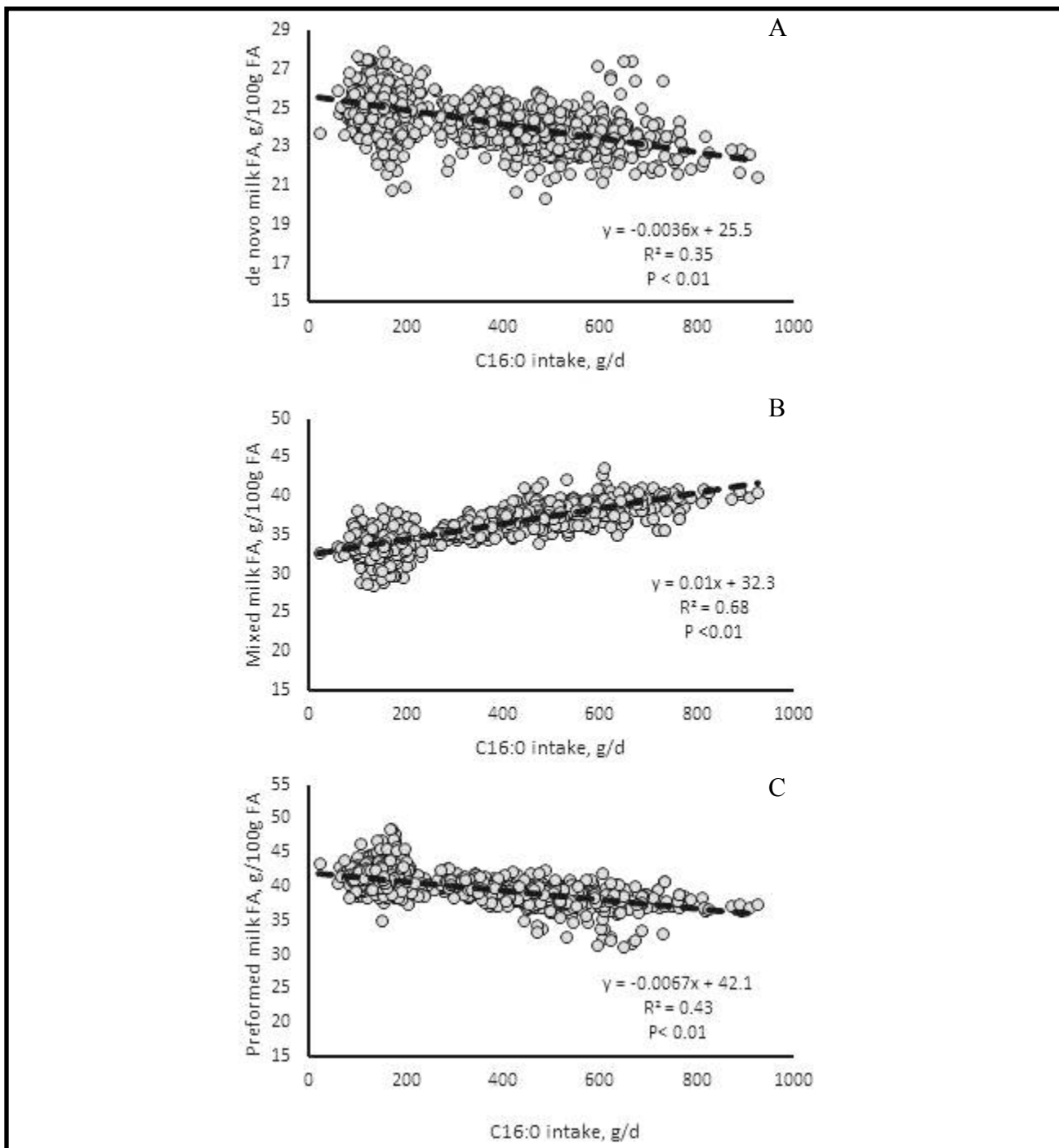


Figure 7. Relationship between C16:0 intake and the concentration of de novo milk FA (Panel A), mixed milk FA (Panel B), and preformed milk FA (Panel C). Our analysis used individual observations (n=1200) in lactating dairy cows from 13 studies. Mixed model regressions were developed between variables of interest taking into account experiment, period within experiment, and cow within experiment as random factors (unpublished results).

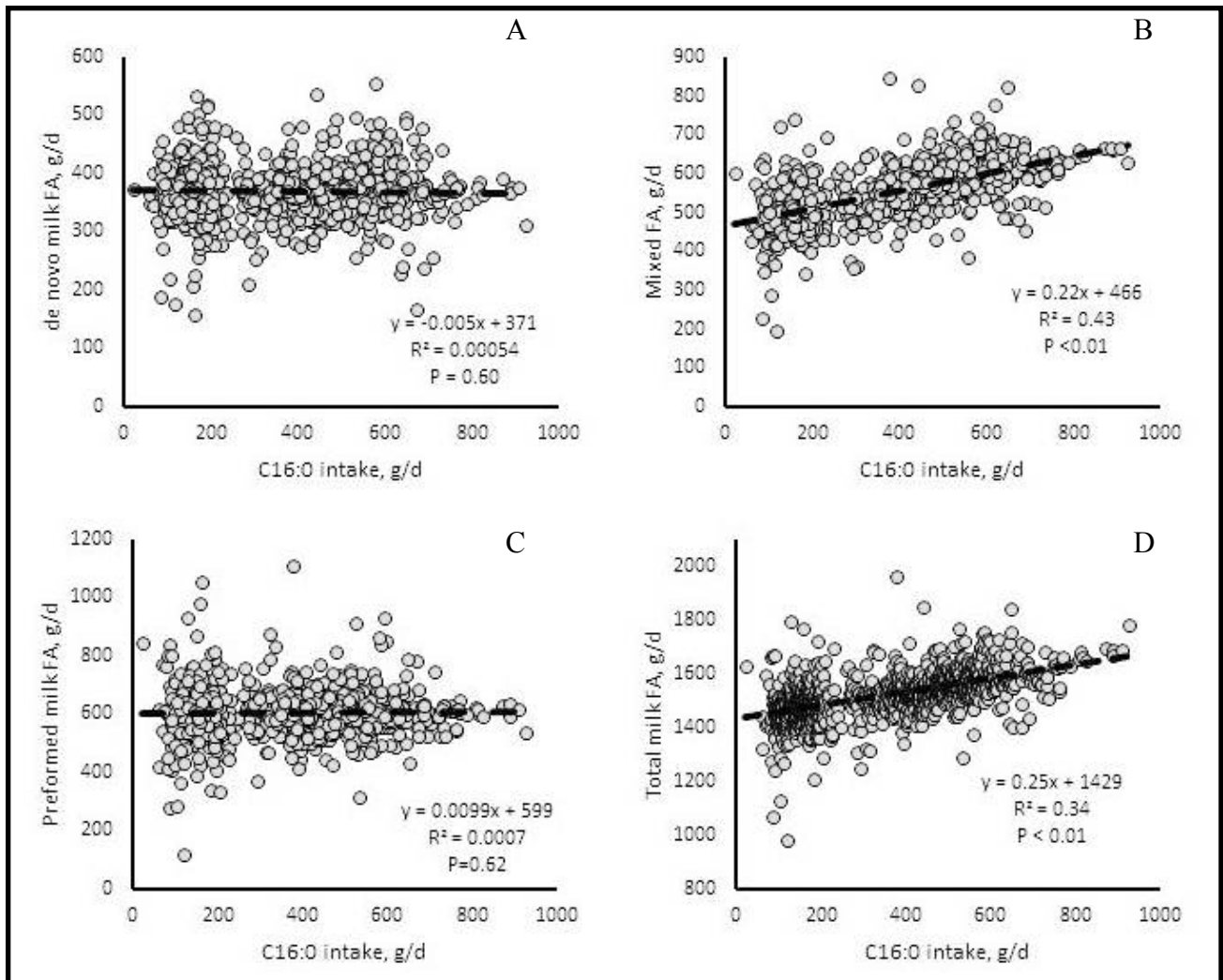


Figure 8. Relationship between C16:0 intake and the yield of de novo milk FA (Panel A), mixed milk FA (Panel B), preformed milk FA (Panel C), and total milk FA (Panel D). Our analysis used individual observations ($n=1200$) in lactating dairy cows from 13 studies. Mixed model regressions were developed between variables of interest taking into account experiment, period within experiment, and cow within experiment as random factors (unpublished results).

Environmental Sustainability of Dairy Farm Systems - Facts about Cows and Climate Change

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Abstract

The carbon footprint of food production is under discussion at the regional, national, and international levels. For example, some European fastfood chains now offer information to their customers including not only nutritional facts, but also the carbon footprint of its tofu-, turkey-, or beef burgers. Furthermore, in the recent past, resolutions were passed to encourage a Meatless Monday or “Veg Day” at restaurants, schools, and grocery stores in an effort to promote a “green diet”. While some scientists (especially in agriculture) remain climate change skeptics, it should be clear to everyone that animal agriculture is in midst of a considerable societal debate with far-reaching consequences.

Facts and Fiction about Cows and Climate Change

Much of the discussion about livestock agriculture’s contribution to climate change stems from a United Nations Food and Agriculture Organization (FAO) report titled “Livestock’s Long Shadow” (Steinfeld et al., 2006; from here on referred to as LLS). This report determined the climate change impact of global livestock production using a method called Life Cycle Assessment, which sums up greenhouse gas emissions from the entire production chain. Included in the LLS’s calculations were crop production, land-use change (e.g., clearing

rainforest to establish pastures and cropland), the animals themselves, and the transportation of final products. The LLS report concluded that *globally* 18% of human-caused greenhouse gas emissions could be attributed to livestock agriculture, and this was a larger share than transportation. However, the authors of LLS made this claim without actually conducting a similarly comprehensive Life Cycle Assessment for the global transportation sector.

Here at UC Davis, we have published a peer-reviewed paper titled “Clearing the Air: Livestock’s Contribution to Climate Change” (Pitesky et al., 2009), which pointed out the flawed comparison between the livestock and transportation sectors, and the FAO has since admitted their mistake. Additionally, “Clearing the Air” highlighted that the global percentage is not accurate at the regional, national, or state level because in highly developed nations, such as the U.S., greenhouse gas emissions from the energy and transportation sectors of the economy dwarf emissions from the livestock agriculture sector. For example, according to U.S. EPA data from 2009, transportation and electricity production account for 26 and 31% of emissions, respectively, while livestock agriculture accounts for approximately 3%. However, in developing countries like Paraguay, the trend is likely reversed because of the small transportation and energy sectors, and a relatively large livestock sector (that has relied on clearing forests to

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establish pastures), which might contribute to more than 50% of that county's carbon footprint. Furthermore, while land-use change contributed to over one-third of the FAO's total carbon footprint for the global livestock sector, these changes are largely occurring in developing nations and not developed nations, like the U.S., where changes in land-use occurred decades ago and are now reversing. These differences in percentages clearly emphasize the need to separate emission estimates by region and also by livestock species – a step recently undertaken by the FAO and other organizations.

While we differed with the authors of LLS on their carbon footprint comparison of livestock versus transportation, as well as with the usefulness and correctness of their 18% figure, we do agree with their overall concern that satisfying upcoming animal protein demands will pose a challenge to the environment. Global animal protein production is projected to double from its year 2000 levels by 2050 and the majority of this livestock production growth will occur in the developing world. Much of the growth in the global livestock sector will occur in areas that are currently forested (i.e., parts of South America and South East Asia), which will create pressure to rely on deforestation to facilitate increased livestock production. It has been well established that significant reductions of carbon sequestering forests will have large effects on global climate change; therefore, avoiding deforestation is paramount.

By examining the historical trends in livestock production in the developed world, it becomes clear that there has been a marked improvement in efficiency, leading to reductions in numbers of animals required to produce a given amount product that satisfies the nutritional demands of society. For example, researchers at Cornell University [Capper, et al., 2009] found that compared to

1944, the 2007 U.S. dairy industry reduced its greenhouse gas emissions per unit of milk by 63%. This reduction was achieved through improved nutrition, management, genetics, etc. born through scientific research that has led to dramatic improvements in milk production per cow. According to LLS, this type of intensification of livestock production provides large opportunities for climate change mitigation and can reduce deforestation to establish pastures, thus becoming a long-term solution to more sustainable livestock production. Indeed, the authors of LLS are currently working on a follow-up paper titled “Shrinking the Shadow”, which will focus on how advanced biotechnologies, improved genetics, nutrition, and comprehensive waste management already utilized in most parts of the developed world can be applied effectively worldwide.

While the extraordinary reduction in the U.S. dairy industry's carbon footprint may be viewed by some as a vindication of modern production practices, attention should be given to the areas of opportunity that still exist, including transition cow management, lameness, and reproductive failure. Improving these and other areas on U.S. dairy farms should allow for further reductions in carbon footprint per unit of milk, and these areas often intersect with another hot issue that livestock industries face: animal welfare.

Summary

Ultimately, ignoring the carbon footprint debate will not make this issue go away for those involved in the livestock industries. The actual science behind many of the current claims has been incomplete or lacking, and it is in the best interest of producers and consumers to have environmental claims made on solid, peer-reviewed scientific data. What is needed is a global green revolution in animal agriculture,

coupled with technology transfers, to supply a growing demand for animal protein while providing environmental stewardship by using sustainable and modern production practices.

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The Changing Nature of Mastitis and Mastitis Treatments

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Introduction

Reducing mastitis has always been an important priority of dairy farmers; management of udder health has been influenced by continued evolution of dairy herd structure. In the U.S., the majority of milk is produced on farms that contain greater than 500 milking cows (http://www.nass.usda.gov/Quick_Stats/) and the presentation of mastitis on larger dairy farms has changed. As housing and management have become more intensive, the distribution of mastitis pathogens has changed. Larger farms have greater adoption of modern management practices that reduce transmission of subclinical infections (Rodrigues et al., 2005, Rowbotham and Ruegg, 2015). These improvements have contributed to control of *Staph. aureus* and near eradication of *Strep. agalactiae* and resulted in considerable decreases in bulk tank somatic cell counts (SCC; Figure 1). While intensification has resulted in reduced bulk tank SCC, mastitis remains a significant challenge for many dairy farms. Increased animal densities and changes in dairy housing (Ericsson Unnerstad et al., 2009) have increased potential exposure to opportunistic intramammary pathogens that often present with mild clinical signs, and national surveys have indicated that the rate of clinical mastitis has consistently increased (Figure 1). In most larger herds, the majority of clinical cases are caused by opportunistic pathogens that originate from the environment

(Oliveira et al., 2013). These trends are especially evident when reviewing microbiological results of milk samples obtained from cows with cases of clinical mastitis and one study reported that only about 35 of 741 cases of clinical mastitis occurring on 52 larger Wisconsin dairy farms were caused by *Staph aureus* (Oliveira et al., 2013). Recovery of “traditional” pathogens, such as *Strep. agalactiae* or *Staph aureus*, tends to more frequent in regions that are populated by a greater proportion of smaller herds that utilize tie stall facilities (Olde Riekerink et al., 2008) or herds that have failed to use well-known preventive strategies, such as comprehensive use of intramammary antimicrobials at dry off (Olde Riekerink et al., 2010). Understanding the changing nature of mastitis is necessary to manage it and the purpose of this paper is provide an update on current concepts of preventing and managing bovine mastitis.

Detection of Mastitis

Management of mastitis requires use of accurate detection and recording systems for both subclinical and clinical presentations of the disease. Without the use of routine SCC testing, effective management strategies for control of subclinical mastitis are extremely limited. On many farms, subtle signs of clinical mastitis are overlooked or disregarded by both humans and automated detection systems. Clinical mastitis is usually defined as the production of abnormal

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milk (with or without secondary symptoms), but the working definition of clinical mastitis varies greatly among farm workers. On large farms, detection of mastitis is usually dependent on the training and observational skills of the milking technicians.

Use of standardized case definitions for clinical mastitis is helpful for monitoring detection intensity and accuracy. Use of a 3-point scale based on clinical symptoms is practical, intuitive, simply recorded and can be an important way to assess detection intensity (Pinzon-Sanchez et al., 2011). In this system, a mastitis severity score of 1(mild) is assigned when abnormal milk is the only symptom, 2 (moderate) is assigned when abnormal milk is accompanied by localized udder symptoms (such as swelling or redness), and 3 (severe) is assigned when systemic symptoms, such as fever, anorexia, rumen stasis, or a large decrease in milk production are observed. Research has consistently indicated that severity of clinical mastitis is about 50% mild, 35% moderate, and 15% severe (Pinzon-Sanchez et al., 2011, Oliveira et al., 2013). When foremilk is not examined, approximately 50% of cases will not be detected and if the proportion of severe cases exceeds about 15%, it is a signal that detection intensity and case definition should be investigated.

Diagnosis of Etiology

Mastitis is caused by intramammary infection (**IMI**) and the agents are usually bacteria, thus appropriate treatment and control programs are based on understanding the etiological agent. On many modern dairy farms, subclinical mastitis is primarily caused by Gram-positive organisms [such as environmental *Streptococci* or coagulase negative staph (**CNS**)] while the greatest proportion of milk samples from clinical cases are culture negative or Gram-

negative. Of 741 cases of clinical mastitis that were cultured from 52 Wisconsin dairy herds, the most common bacteriological diagnoses were no bacterial growth (27% of cases) and *E coli* (23% of cases); *Streptococci* were isolated from about 13% of clinical cases and a further 17% of cases were caused by a variety of opportunistic organisms (Oliveira et al., 2013). While clinical signs may be suggestive of some pathogens, detection of mastitis is based on observation of non-specific signs of inflammation, and it is impossible to diagnose the cause based on observation of the milk, gland or animal. Thus, on modern dairy farms, increased use of milk culturing to direct mastitis control programs is recommended. When results of milk cultures are closely linked to treatment decisions, the value of culturing milk from cows with clinical mastitis is clearly evident to owners of larger herds. Of 325 large Wisconsin dairy herds recently surveyed, use of culture of milk from most or all clinical cases was 20%, 22%, 52%, and 75% of herds containing 200 to 499, 500 to 999, 1000 to 2000, and >2000 cows, respectively (Rowbotham and Ruegg, 2015 unpublished).

On a typical farm, about 50% of milk samples obtained from cows with subclinical mastitis and 25 to 40% of milk samples obtained from cases of clinical mastitis are microbiologically negative. The reason that cases are culture negative likely varies based on case presentation. Chronic subclinical cases have strong evidence of on-going inflammation that is most likely the result of persistent IMI. These cases are often false negatives because the inflammatory response has successfully reduced the number of organisms to below the normal detection limit (about 100 cfu/mL in most mastitis laboratories). Repeated culturing of ¼ milk samples may help arrive at a diagnosis for some of these cases. In contrast, in herds that have environmental mastitis problems, many mild and moderate clinical cases are caused

by opportunistic pathogens that have been successfully eliminated by a localized immune response. The clinical symptoms are observed after the immune system has responded and about 75 to 80% of these cases may actually be spontaneously cured of the IMI before detection of the symptoms. The only way to determine if the symptoms of mild and moderate mastitis are accompanied by active infection (and thus will benefit from antimicrobial therapy) is to perform microbiological analysis.

Epidemiology of Mastitis on Modern Dairy Farms

Bulk tank SCC have declined throughout the world, but the ability to maintain a low bulk tank SCC does not always indicate that mastitis is controlled. Even in regions that are known for producing high quality milk, about 15 to 20% of cows have monthly SCC values that indicate at least one mammary gland quarter is affected with subclinical mastitis. Accurate detection and recording of clinical mastitis and a review of management strategies used to reduce bulk tank SCC (such as use of quarter milkers or drying off of chronically affected quarters) are necessary to determine the magnitude of mastitis challenges on dairy farms. A study of large Wisconsin dairy herds with bulk tank SCC around 220,000 cells/mL indicated that there were approximately 40 clinical mastitis treatments per 100 lactating cows per year, with a range of 6 to 90 treatments per 100 cows per year (Oliveira et al., 2013). In spite of low bulk milk SCC, some of these herds had evidence of considerable mastitis problems. While the average herd withheld from sale about 1.8% of daily milk (from treatments), the maximum amount of daily milk withheld was 6.7%. Likewise, the average proportion of cows milking with <4 quarters was 4.7%; however, one herd reported that 30% of the cows had at least one non-functioning quarter. The shift to clinical mastitis (rather than subclinical disease)

is primarily a response to changing exposures to pathogens.

Based on their primary reservoir and most likely mode of transmission, mastitis pathogens have typically been characterized as either “contagious” or “environmental.” Using this traditional classification, the udder of cows with subclinical infections serves as the primary reservoir of contagious pathogens. Transmission of contagious pathogens occurs when teats of healthy cows are exposed to organisms in milk that originated from infected udders. The most common point of exposure is usually bacteria present in milk droplets on teat contact surfaces (such as milking inflations or milked leaked onto bedding surfaces). In the US, contagious mastitis pathogens commonly include *Staph aureus* and *Mycoplasma bovis*. However, transmission via a “contagious route” is possible for any microorganism that can cause persistent subclinical mastitis and shed sufficient colonies in milk to establish an infective dose. Thus, chronic subclinical infections with organisms such as *Prototheca zopfii* or *Klebsiella spp.*, can also result in contagious transmission among cows.

In most developed dairy regions, udder health programs are increasingly focused on mastitis caused by environmental pathogens. The term “environmental pathogen” refers to mastitis caused by opportunistic bacteria that often reside in the housing area of cows. Common pathogens include both Gram-negative bacteria (such as *E. coli* and *Klebsiella spp.*) and Gram-positive bacteria (such as *Strep. uberis* and other *Streptococcal* like organisms). Opportunistic pathogens tend to be less adapted to survival in the udder and IMI often triggers sufficient inflammation to result in visually apparent mild or moderate clinical signs. Bedding materials, and moisture or manure in animal walkways are common reservoirs and

controlling environmental mastitis is based on reducing exposure of teats of the most susceptible cows.

The duration of infection with environmental pathogens varies among pathogens (Smith et al., 1985) and can be associated with the degree of host adaptation of the pathogen. Some environmental pathogens (such as most *E. coli*), are truly opportunistic and the immune response is usually successful in eliminating these pathogens after a brief period of mild clinical disease. Other environmental pathogens (such as many IMI caused by *Streptococci* or *Klebsiella spp.*) seem to have become more host adapted and may present as mild clinical cases that appear to resolve when in actuality the case has returned to a subclinical state. Control of mastitis caused by environmental pathogens can be more complex than control of mastitis caused by bacteria usually considered to be contagious. Bedding materials, moisture, mud, and manure in housing areas of cows are common reservoirs for these pathogens and controlling them requires reducing exposure of teats of the most susceptible cows.

Risk Factors for Intramammary Infection

Environmental Risk Factors

Manure handling, type of bedding, and stall maintenance all have significant impacts on exposure of teats to mastitis pathogens. While many bedding materials initially have relatively low bacterial populations, organic matter in some bedding contains nutrients that support bacterial growth and results in exposure of teats to a great variety of potential mastitis pathogens. This is especially true of recycled manure which is very rich in nutrients that support growth of fecal organisms. A recent observational study of large Wisconsin dairy farms demonstrated lower rolling herd average (RHA), greater SCC, more

treated cows, and a greater proportion of cows with non-functional quarters in herds that used manure based bedding as compared to herds that used sand (Rowbotham and Ruegg, 2015). In general, the number of Gram-negative bacteria (often associated with shorter duration infections and occurrence of clinical mastitis) is greater in organic bedding materials (such as recycled manure solids) as compared to new sand bedding. However, the number of opportunistic Gram-positive bacteria (often associated with longer duration subclinical infections) can be quite significant in recycled sand, and IMI with these organisms may contribute to increased bulk tank SCC (BTSCC).

The number of bacteria recovered from teat skin is typically 2 to 3 log units (100 to 1000 times) less than that found in bedding, indicating potentially greater risk of IMI for quarters exposed to bedding that contains greater quantities of bacteria. A linear relationship between exposure to bacteria in bedding and rate of Gram-negative clinical mastitis has been demonstrated but that association was relatively weak and the authors of the study cautioned that <16% of variation in clinical mastitis rate could be attributed to differences in bedding bacterial count (Hogan et al., 1989). Exposure to bacteria alone doesn't necessarily result in IMI. For all infectious diseases, exposure to a pathogen is necessary for infection, but mastitis is a multifactorial disease and other risk factors are needed for exposure to result in mastitis. Factors that influence the risk of infection with opportunistic pathogens include management factors, such as design and usage of stalls, management of bedding (including particle size and content of moisture and organic matter), adequacy of milking procedures, and gentleness of milking. Important cow-level factors include anatomical characteristics of the udder and teats. While exposure is important, risk of IMI is also influenced by the ability of the cow to mount

an effective and rapid immune response after bacteria have penetrated the teat orifice.

Cow Level Risk Factors

Reducing risk of opportunistic IMI is based on reducing exposure to potential pathogens, but risk of developing mastitis is not equal among animals because different groups of cattle have differing abilities to withstand environmental challenges. The ability to resist and respond to infection is influenced by both stage of lactation and parity. As compared to older animals, cows in first and second lactation have reduced risk of developing clinical mastitis caused by opportunistic pathogens (Zadoks et al., 2001; Pantoja et al., 2009; Pinzon-Sanchez and Ruegg, 2011). Stage of lactation is also a risk factor for development of clinical mastitis and the disproportionate occurrence of clinical mastitis in early lactation is a hallmark of mastitis caused by environmental pathogens (Oliveira et al., 2013). It is well documented that leaking milk, high daily milk yield, and reduced immunological capabilities are associated with increased risk of clinical mastitis (Schukken et al., 1990) and all of these characteristics are more common in early lactation. While exposure to opportunistic environmental pathogens can occur throughout the lactation cycle, cows initiating lactation are less able to withstand exposure to microorganisms because of innate immune suppression.

Anatomical characteristics of the udder and teat are known risk factors for IMI. Cows with larger udders are at increased risk of IMI as are cows with udder hygiene scores (UHS) that indicate dirtier udders (scores 3 or 4 on a 4-pt. scale) (Barkema et al., 1999, Schreiner and Ruegg, 2003). Udders become dirty as a consequence of a number of routine management decisions. Risk factors for “dirty udders” were evaluated on 79 commercial

Wisconsin dairy farms (Salgado and Ruegg, data unpublished). The farms included 11,200 lactating cows housed in both freestalls (n = 51 herds) and tie stall barns (n = 28). There was no difference in the proportion of clean UHS (77%) based on type of facility. For animals housed in tie stalls, the risk of dirty udders was increased 1.5 times when stalls were cleaned <2 times per day, 4.5 times when stall beds were scored as dirty, and >10 times when a large proportion of the cows had loose manure. For animals housed in freestalls, the risk of dirty udders was increased 1.8 times when organic bedding materials were replenished less than daily, 4 times when stall beds were scored as “dirty,” >10 times when a large proportion of the cows had loose manure, 2.5 times when cows had access to outdoors, and >10 times as barns were increasingly overstocked. This data reinforces the role of facility management and cow comfort in reducing risk of environmental mastitis.

Changing Concepts of Mastitis Control

On modern dairy cattle farms, most antibiotics are administered to treat sick animals but some are used for prevention of disease during high risk periods. Almost all conventional dairy farms report some regular usage of antibiotics (Zwald et al.; 2004 Oliveira, 2013). Nationally, in 2007, about 7, 3, 7, and 16% of adult dairy cows were treated for foot infections, pneumonia, metritis, or mastitis, respectively (USDA, 2009). When antibiotic treatments are summed up, research has demonstrated that each adult cow receives about 5 defined daily doses per year (Pol and Ruegg, 2007; Saini et al., 2012). In a recent study of large dairy farms in Wisconsin (Oliveira and Ruegg, 2014), a dramatically greater proportion of animals were treated for mastitis (40 treatments/100 cows/yr) as compared to reproductive disorders (13 treatments/100 cows/yr), respiratory disease (4 treatments/100 cows/yr),

yr), lameness (5 treatments/100 cows/year), or digestive problems (2 treatment/100 cows/yr) (data not presented in original study). This data indicates that efforts to reduce antibiotic usage on dairy farm must be targeted on prevention and appropriate treatment of mastitis. Several important principles should be considered before using antibiotics for treatment of mastitis.

Antibiotics should not be used for cows that are unlikely to benefit. Cows that have a previous diagnosis of mastitis caused by a refractory pathogen (*Mycoplasma bovis*, *Staph aureus*, *Prototheca*, *Serratia*, etc.) should not receive antibiotics as they are unlikely to be effective. Likewise, it is unusual for antibiotic therapy to be effective for cows that have chronic symptoms of mastitis (>3 cases of clinical mastitis (CM) during the current lactation or >4 months of SCC > 200,000 cells/mL). In these instances, abnormal milk should be discarded until it returns to normal (usually about 4 to 6 days) and “watchful waiting” should be performed (frequent observation of the cows behavior and symptoms) to detect the rare instances where the severity of the case progresses.

Abnormal milk is a visible indication that the cow’s immune system has responded to an infection. Much antibiotic usage associated with mastitis cannot be justified because the infective bacteria is often gone before the inflammation is detected or the mastitis is caused by a type of bacteria that is unlikely to respond to the types of drugs that are available. In most modern dairy herds, clinical mastitis is caused by a diverse group of opportunistic pathogens and at least 20 to 25% of milk samples are culture negative at the time that the case is detected. Depending on specific virulence factors, organisms infect different locations in the udder, have differing abilities to cause illness in the cow, and differ in the expected rate of spontaneous bacteriological

cure. Thus, identification of type of bacteria causing the infection is important to properly select an antibiotic (if needed) and to determine the best duration of therapy. Additionally, many characteristics of the cow are known to influence the probability of successful immune responses and cure after intramammary infections (Burvenich et al., 2003; Pinzon-Sanchez and Ruegg, 2011). Parity, stage of lactation, and history of previous clinical or subclinical mastitis cases are all factors that should be considered before using an antibiotic to treat a case of clinical mastitis.

Criteria for justifiable antibiotic usage

1. *Antibiotic usage should involve veterinary guidance.* On most farms, many mastitis treatments involve extralabel use of drugs. Extralabel drug usage must be supervised by a local veterinarian that has a proper veterinary client patient relationship (VCPR). Criteria for establishing a proper VCPR are codified by state and federal regulations, but the American Association of Bovine Practitioners (AABP) has guidelines that identify critical components of that relationship (http://www.aabp.org/resources/aabp_guidelines/vcprguidelinefinal11-2013.2.pdf). To maintain consumer confidence, farmers should work closely with their local veterinarians to deliver treatment protocols that are compliant with FDA regulations and meet general principles of appropriate antibiotic usage (Weese et al., 2013).
2. *Antibiotics should only be used when there is a reasonable likelihood that a bacterial infection is present.* This criteria cannot be met for most of the 25 to 40% of CM cases that are culture negative when detected and alternative ways to manage these cases should be considered. When possible, use of

rapid culturing methodologies is encouraged to identify active IMI.

3. *Narrow spectrum antibiotics that are less critical for treating human illnesses should be used as a first choice.* The World Health Organization has classified antibiotics based on their importance for treating human illnesses (Anonymous, 2012). Most intramammary (IMM) products available in the US, are not high priority drugs for treatment of human illnesses and only ceftiofur (a third generation cephalosporin) is listed as high priority and critically important for human use. Depending on the intrinsic susceptibility of bacteria, antibiotics are classified as either narrow or broad spectrum. Narrow spectrum drugs are usually active against either Gram-positive or Gram-negative bacteria, whereas broad spectrum drugs have activity against both types of organisms. When possible, narrow spectrum drugs are preferred as they have less potential for selection for resistance and are usually less critical for human health needs (Weese et al., 2013). Most approved IMM products are considered narrow spectrum and use of the broader spectrum IMI drugs should be reserved for cases that will benefit.
4. *Antibiotics should be used for as short a duration as possible.* In general, duration of antibiotic treatment should be kept as short as possible to minimize economic losses associated with milk discard while maximizing the probability of achieving bacteriological cure (Pinzon-Sanchez and Ruegg, 2011). The appropriate duration of antibiotic treatment for CM is not well-defined and varies depending on etiology. Different pathogens have varying abilities to infect mammary gland tissue. Some pathogens preferentially infect superficial mucosal surfaces, while other pathogens have the ability to deeply infiltrate mammary gland secretory tissue. There is considerable evidence that extended duration antibiotic therapy increases bacterial cure of invasive pathogens (such as *Staph aureus* and some environmental *Streptococci spp.*) (Oliver et al., 2004a; Oliver et al., 2004b). However, no research has indicated that extended duration therapy improves clinical outcomes of mastitis caused by non-invasive pathogens (such as CNS or most *E. coli*). Use of extended duration therapy to treat these types of pathogens significantly increases costs without improving economic outcomes (Pinzon-Sanchez and Ruegg, 2011). For mastitis caused by invasive pathogens, the duration of therapy should be extended, but for other etiologies, short duration is recommended. When extended therapy is considered, veterinarians should assess the ability of farm personnel to perform aseptic infusions as extended IMI treatment is associated with an increased risk of infection from opportunistic pathogens.
5. *Characteristics of affected cows should be reviewed before antibiotics are administered.* The purpose of antibiotics is to help the cow's immune response successfully eliminate IMI and many characteristics of the cow are known to influence the probability of successful immune response (Burvenich et al., 2003). Thus, an assessment of the cow's ability to mount an immune response should be performed as part of the medical exam. Characteristics related to a healthy immune response include age, stage of lactation, negative energy balance, history of previous treatments, and environmental factors (such as heat-stress). Older cattle (>3rd parity) often have poorer responses to treatment as compared to younger cattle (Hektoen et al., 2004; McDougall et al., 2007a; McDougall

et al., 2007b). A history of chronically increased SCC is also associated with poorer prognosis after mastitis therapy (Bradley and Green, 2009; Pinzon-Sanchez and Ruegg, 2011). Cows in the immediate post-partum period are known to be immunosuppressed and heat stress can reduce the ability of the cow to respond to an IMI (do Amaral et al., 2011). Before administration of antibiotics, farmers should review the medical history of the cow and assess if she has risk factors that indicate antibiotics may be beneficial. For example, short-duration IMI antibiotics may be considered for CM occurring in valuable older cows that have non-severe Gram-negative mastitis in the immediate post-partum period. Conversely, “watchful waiting” may be considered for CM occurring in older cows that have a long history of repeated non-severe cases.

6. *Extralabel use should be avoided when on-label use is a possibility.* Extralabel use of intramammary products includes use for durations or dosing intervals that are not explicitly listed on the product label. These deviations from label guidelines are common for mastitis treatment and may be justifiable for some drugs but must be done under veterinary supervision. Extralabel use of parenteral antibiotics to treat mastitis is not unusual (Raymond et al., 2006; Pol and Ruegg, 2007; USDA, 2009; Oliveira and Ruegg, 2014) but should be restricted to justifiable cases, such as cows affected with severe mastitis.

Conclusion

Mastitis remains the most frequent and costly disease of dairy cows and is the most common reason that antimicrobials are administered to adult dairy cows. On modern dairy farms, mastitis is caused by an increasingly

diverse group of pathogens. The separation between “contagious” and “environmental” organisms is not complete and many organisms can be transmitted in either manner. Detection systems for mastitis must include methods to detect both subclinical and clinical disease and should include severity scoring of clinical cases. It is not possible to determine etiology without microbiological examination of aseptically collected milk samples.

To control mastitis and use appropriate treatments, etiology must be determined. The worldwide dairy industry is continuing to rapidly change and to ensure the continued production of high quality milk mastitis control strategies must also progress.

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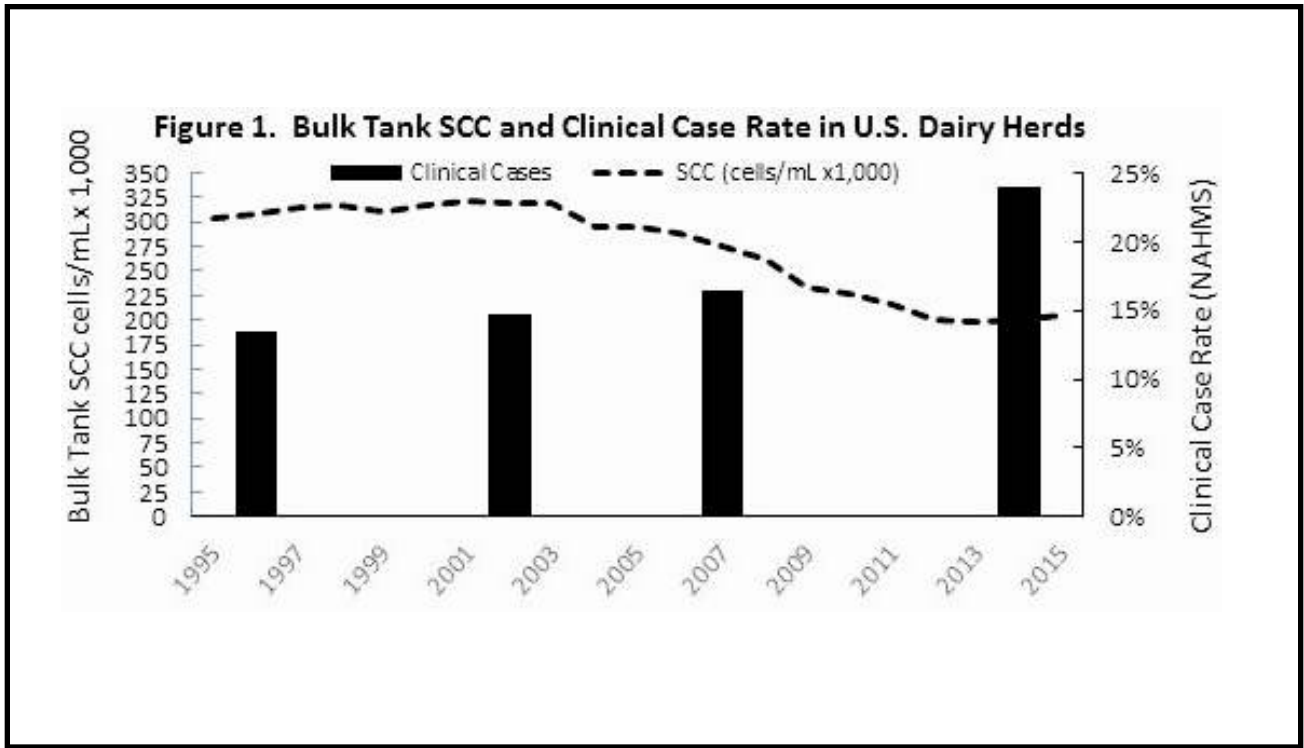


Figure 1. Data from: <https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/nahms>.



Nutrient Digestibility for High-Producing Dairy Cows: How Much Milk Can You Get from a Ration?

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Introduction

Dietary energy comes from four main feed compound classes: fiber, starch, protein, and fat. Although fiber and starch are both carbohydrates, we will separate them in this paper as if they are different “nutrients”, and we will define fiber as neutral-detergent fiber (**NDF**). There are other feed compounds (such as sugars, soluble fiber, and organic acids) that do not fit into one of these four categories, but we will ignore them in this discussion. The typical Gross Energy (**GE**), Digestible Energy (**DE**), Metabolizable Energy (**ME**), and Net Energy for Lactation (**NE_L**) values for these nutrients are shown in Table 1. Because the content of protein and fat is relatively constant, the major determinant of the energy available from a diet is the amount of starch and fiber and the digestibility of each. Starch is generally about 90% digested, whereas the digestibility of fiber (as NDF) can vary widely among feeds but is usually 40 to 50%. Fiber could be subdivided into that from forage and that from nonforage sources. The fiber from some nonforage sources can be quite digestible.

In the 6th edition of the Nutrient Requirements for Dairy Cattle by the National Research Council (NRC, 1989), and previous versions, feeds were each given fixed **NE_L** values that could be used to balance the energy supply of feeds with the energy requirements of a cow.

Because protein and starch had roughly the same **NE_L** value, and because fat is only a minor part of a dairy diet, balancing diets was largely a matter of altering the amount of individual feed ingredients of varying energy intake based on their starch and fiber contents. The **NE_L** value of starch is considerably greater than that of fiber, so to achieve high energy intake, high starch feeds were added in place of high fiber feeds to increase the **NE_L** density of the ration. If the forage had more digestible fiber, less starch from grain was needed. Based on these fixed **NE_L** values, nutritionists frequently talked about ration targets of 0.76 to 0.80 Mcal/lb **NE_L** of dry matter intake (**DMI**) when feeding high-producing cows.

The 7th edition of the Dairy NRC (NRC, 2001) introduced a new concept: feed energy values are not constants but instead depend on composition of the total diet and on the animal being fed. The fact that feed **NE_L** values were not constant was frustrating for many nutritionists, especially at first. Feed labs still predict the **NE_L** value of feeds, but the values are not used in a system where **NE_L** values are not constants.

In the 2001 NRC, the total possible **DE** (**DE_{1X}**) content of a diet is first calculated based on feed ingredients, nutrients within feeds, and the expected digestion coefficients for each nutrient when a nonlactating animal is eating just enough feed to maintain life. This **DE_{1X}**

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value is then adjusted based on the level of milk production and the diet composition to give a DE value at production level (**DE_p**). The DE_p of the total diet is then used to predict the ME and NE_L values of the ration at production levels.

As cows eat more, the DE_p value of a diet decreases, and so its ME and NE_L values also decrease. Intake should be considered relative to an animal's body weight (**BW**), as a 2 lb increase in intake is biologically more important in a small cow than a large cow. One way to consider level of intake relative to a cow's BW is by calculating her "multiple of maintenance" (**MM**), with 1 MM being a level of intake that sustains life with no gain or loss of body mass and no milk production. Each MM above 1 is used for milk production, activity, or body tissue gain. The 2001 NRC used this MM concept to estimate the digestibility depression of diets, or the calculation of DE_p from DE_{1x}. For the typical high starch diet fed to a high-producing cow in the midwest, the digestibility depression in the 2001 NRC was 3 to 4% per MM.

In the 2001 NRC, the digestibility of individual feed components was not altered per se, but the total possible DE_{1x} of a diet was adjusted based on the level of milk production (see Figure 1). This decrease in DE_p was greater for diets that contained a greater content of non-fat DE, which would be highly correlated with the content of non-fiber carbohydrate (mostly starch). This interaction of non-fat DE content, level of intake, and digestibility was commonly called an "associative effect", because the digestibility depression for the diet was dependent on how much nonfiber carbohydrate was associated with it.

New Equations Based on Dietary Starch

Because starch was not commonly measured before 2001, the 2001 NRC committee

had insufficient data to quantify the effect of starch on fiber digestion. Since 2001, several studies have reported new digestibility values along with animal and diet characteristics. Most of these newer studies reported dietary starch content. Using newer data from individual cows also enables us to get a better estimate for the effect of high feed intake. Finally, statistical tools have become more sophisticated, and we are better able to unravel the multitude of factors that influence nutrient digestion.

Using new data and new tools, we recently published a study (de Souza et al., 2018) with the goal of developing new equations for predicting nutrient digestibility in high producing cows, using data from individual cows to get a better estimate for how variation in intake alters digestibility. Coauthors were Mike Allen and Rob Tempelman (Michigan State), Bill Weiss (Ohio State), and John Bernard (University of Georgia). First, we compiled a database of 1900 observations from 660 cows in 54 studies from Michigan, Ohio, and Georgia to determine the effects of DMI, BW, and diet characteristics on total tract digestibilities of DM, NDF, and starch in high-producing dairy cows. On average, cows ate 51 lb/day of feed DM (3.5% of BW), weighed 1470 lb, and produced 84 lb/day of milk. Cows near the top ate 68 lb/day of feed DM (4.6% of BW) and produced 130 lb/day of milk. Diets averaged 31% NDF, 27% starch, 2.6% fatty acids, and 17% crude protein. The average digestibility values were 66% for DM, 42% for NDF, and 93% for starch. Data from individual cows were analyzed using mixed models including diet composition (chemical composition, forage source, and corn source), DMI as percentage of BW (**DMI%BW**); location; and 2-way interactions as fixed effects, and cow, block, period, treatment, and study as random effects. Best fitting candidate models were generated, as well as a simple model using only DMI and location as fixed effects and all

random effects. Candidate models were cross-validated across studies. For each nutrient, the digestibility model that resulted in the highest predictive correlation coefficient and lowest root mean square error of prediction was determined to be the best fitting model. Coefficients for factors were averaged across locations. After averaging for location effects, the overall best fitting prediction equations were determined (Table 2).

Our results confirm that digestibility is reduced as DMI increases, albeit at a lower rate than that reported in NRC (2001), or more recently by Huhtanen et al. (2009). Our decrease in DMD of 0.83 percentage units per unit DMI%BW is a 1.0 percentage unit depression in DMD per MM. Using the diets in our database, the expected decreases in DMD would have been 2.4 and 1.9 percentage units per MM in NRC (2001) and Huhtanen et al. (2009). The studies used in our analysis had much higher average milk production than in NRC or Huhtanen. In addition, the diets in Huhtanen et al. (2009) were mostly high in grass and averaged only 14% starch. Thus, we believe the data from our study are more relevant for modern dairy cows fed diets typical of most US cows today.

Whereas DMD can be predicted based only on DMI, the best predictions for NDFD and StarchD required DMI and diet characteristics. Some feed characteristics used in the NDFD and StarchD equations are likely due directly to characteristics of the NDF or starch. For example, if the diet contains more starch that is highly fermentable (**HFERM**), StarchD will be greater, or if the diet contains more NDF from grass, NDFD will be greater. This effect of grass is not so much an effect of grass on the digestibility of NDF in general, but simply reflects the fact that the NDF of grass is more digestible than the NDF of alfalfa in the total tract at the range of intakes in the studies. However,

NDFD was also altered by dietary starch, and this general effect of starch is presumably an effect on all the NDF in the diet.

The effects of starch and DMI on NDFD are shown in Figure 2. In this figure, we show the original prediction of Souza et al. (2018) along with estimates for linear relationships based on the original prediction. Souza's original data included a study with very low intakes and very low digestibilities, and the 95% confidence interval around the prediction at low intakes was broad. Thus, we developed another response that was linear. The linear relationship was set to match the Souza curves at DMI > 3.5% of BW and to be consistent with predictions based on the previous NRC. Note that even with this change, the effect of starch is still much greater than the effect of DMI within the range of normal intakes expected for high producing cows (>3% of BW). The resulting change in NDFD with changes in intake and starch is:

Change in NDFD as %NDF = -0.59 (change in % starch) - 1.1 (change in DMI%BW)

Our equation presents a middle ground on predicting NDFD between two other recent meta-analyses. Ferraretto et al. (2013) reported a similar drop in NDFD from starch but no change due to feed intake, whereas White et al. (2017) reported no change in NDFD due to starch but a greater effect of intake. One problem with analyzing the effects of DMI and starch on NDFD is that seldom does one change without a change in the other. Level of intake is strongly associated with starch content of a diet. The depression in NDFD as dietary starch increases is reflective of the "associative effect" described in the 2001 NRC. In NRC 2001, diets with a greater %TDN from nonfiber carbohydrate had greater depressions in digestibility at high intakes. This was complicated by the fact that diets with more starch (higher %TDN) are less filling, and thus

enable greater intake; and conversely, that cows on low starch diet (low %TDN) cannot eat as much. Thus, the digestibility depression caused by high feed intake was overestimated in NRC 2001. Greater intake is associated with lower NDFD for two reasons: 1) greater intake might directly increase passage rate and so decrease NDFD, and 2) greater intake is often the result of a greater %starch, which also decreases NDFD. In the new equation, we account for these two factors (%starch and DMI) separately, although changes in one are almost always concurrent with changes in the other. NRC 2001 also accounted for these separately, with starch accounted for as basal TDN. However, NRC 2001 only predicted changes in digestibility for DE, not individual nutrients. If we assume that much of the change in DE digestibility in NRC was due to changes in NDFD and that the effect of basal non-fat TDN in NRC was due to starch, then NRC 2001 predicted an interaction on NDFD between DMI and starch content. We saw no evidence for this interaction. In addition, the effect of starch was much greater than the direct of intake. All cows were fed ad lib in de Souza et al. (2018), so we are not sure these equations are relevant for cows fed at restricted feed intake.

The effect of intake on starch digestion is less than that of Ferraretto et al. (2103). They found a drop in total tract starch digestibility of 0.24% units per kg of DMI, which would be 1.7% for a 1 unit of DMI per BW in a 700 kg cow (1540 lb).

In the Souza et al. (2017) study, the level of intake was described as DMI as a % of BW, rather than as MM. Multiples of Maintenance can be a problem to quantify intake because it presumes that we accurately know maintenance requirements and because it can cause circular arguments (level of MM alters digestibility, which alters the amount of feed needed for

maintenance, which alters level of MM at any given intake). A more direct way to consider level of intake is to simply avoid estimating maintenance and instead divide daily intake by BW. Because maintenance is considered a function of BW to the 0.75 power, these two methods differ (Figure 3), but for a cow at 1500-1600 lb body weight (**BW**), 1 MM is about 1 lb/day of feed DM per 100 lb of BW. Most high-producing lactating cows eat between 3 and 5% of BW per day during lactation.

Implications

So how would these new equations affect the energy value of feeds? In Table 3, we show the implications of changing DMI and starch content on the predicted NE_L of the diet and expected milk production if energy is the limiting factor for milk.

Using the new equations, increasing intake depresses digestibility of fiber and starch a little and decreases the NE_L of the total diet. As expected, increasing DMI can greatly increase the energy available to make milk, regardless of this small depression in digestibility.

Starch is about twice as digestible as fiber, and feed laboratory reports typically give NE_L values for grains that are considerably greater than those of forages. Thus, one would expect that increasing starch would increase the energy available for milk, as shown in the second tier of rows in Table 3. If starch had no effect on NDFD, then the increase in milk would be 7.4 lb/day for every increase of 8% units of starch (not shown in table). Instead, because starch depresses fiber digestibility, the increase is only 5 lb, and financial advantage from replacing fiber with starch may be lost. The NE_L available would increase just as much by increasing the base NDFD of the diet by 8% units as by increasing starch content 8 % units.

As an example, if intake were held constant, the addition of soyhulls to a diet in place of forage with low NDFD would increase diet NE_L supply as much as would corn grain, because the soyhulls are high fiber with a high basal NDFD and contain no starch to depress NDFD as does the corn grain.

Finally, the table demonstrates that if greater dietary starch enables cows to eat more, then milk yield can increase dramatically with the higher starch diet. The values in the table are for purposes of illustration only and do not necessarily reflect the expected changes in DMI with different starch concentrations. In the end, the predicted changes in NE_L supply for milk using these new equations is similar to the predictions based on NRC 2001. However, the direct effect of starch (or % basal non-fat TDN in NRC) is greater and the direct effect of DMI is less in the new equations than in the NRC 2001.

The changes in expected NE_L values in Table 3 may still be unrealistic. When balancing or evaluating diets, we typically calculate the NE_L value of a diet based on its nutrients, digestibility and expected losses in urine and gas energy and heat, as was done for Table 3. We could also calculate the apparent NE_L value of the diet if we know how much NE_L she apparently consumed based on her response to a diet. Apparent NE_L supply can be calculated as the sum of NE_L for maintenance ($0.08 \times BW^{0.75}$) + NE_L for BW change (~ 6 Mcal/kg) + milk energy output. In recent studies at MSU, where we had accurate measures of BW and BCS change, cows have been fed diets with varying amounts of forage NDF, nonforage NDF, and starch. Replacing NDF with starch causes even less difference in the apparent NE_L value of a diet than expected based on diet calculations, such as those in Table 3 (Carrasquillo-Mangual et al., 2017; Potts et al., 2017). The major benefit of replacing forage fiber with starch was that it increased feed intake in high-producing cows.

Limitations

In this study of de Souza et al. (2018), we had insufficient data to account for the ruminal digestibility of starch, as was previously shown to be important in Ferraretto et al. (2013). We recognize that dietary starch content alone is inadequate to describe the mechanisms for the effect of starch on NDFD. Future studies should further examine the impact of ruminally-available starch. In addition, we expected to find that the NDF from grass would be more digestible than alfalfa NDF at low intakes but then become less digestible relative to alfalfa as intake increased. The studies included in Souza et al. had insufficient diets containing grass to accurately assess the interactions of DMI for NDFD of grass and alfalfa.

One reason to predict energy values of feeds is to choose feeds that will give the most profit; this requires having some knowledge of the available energy from a feed relative to its cost. Various systems have been developed to account for the additional value of protein or other nutrients within a feed. More sophisticated methods might even assign feeds a cost related to nutrient excesses (such as for phosphorus) and try to account for all of the other feeds that are actually available for use on a farm. Implicit in any least-cost or profit-maximization balancer is the assumption that we can accurately model how feeds alter energy availability from the feed, intake of the diet, and partitioning of available energy; none of these are true.

Our proposed system clearly shows that NE_L can only be predicted for a complete diet, not for individual feeds (NRC 2001 also showed this). The idea that a feed has one energy value (as feed labs indicate) is just not true. If adding more corn to a diet decreases the digestibility of the alfalfa, then single NE_L values for feeds are meaningless. Should we give alfalfa a lower NE_L

value because it might be fed with corn? Should we give corn a lower NE_L value because it can decrease the digestibility of alfalfa? There is no way to accurately compare the price of feeds that vary in starch without first determining what their effect will be in the total diet. The idea that individual feeds have their own NE_L values is clearly not the way that the real world works.

Not only can one feed alter the digestibility of another, but feeds can alter appetite and nutrient partitioning. Unless we can accurately predict nutrient digestibility, intake, and partitioning in cows fed ad libitum, we cannot use models to accurately formulate diets, as we cannot accurately predict many of the intermediates needed in ration formulation, such as microbial protein yield and mammary amino acid requirements (if a diet increases intake, both will likely increase). New equations have been developed that seem to do a reasonably good job of predicting feed intake based on feed factors (Sousa et al., 2017), but these have not yet been implemented in ration balancing models, and how they should be implemented is not a simple decision. Equations that work in peak lactation may not work in later lactation because cow nutrient demand, which is the cumulative effect of stage of lactation, milk production, milk composition, body condition score, and maturity, alters how a cow responds to dietary changes. Low producers with heavier body condition scores will not respond to dietary starch the same way as a high producing cow (Boerman et al., 2015).

Until we can accurately predict responses in the voluntary feed intake, digestion, and partitioning of nutrients in response to dietary changes, we cannot predict how diets will alter milk income and profitability. More than ever, we need to pay attention to cows, not just computers, when formulating diets for high production (Allen and VandeHaar, 2016).

Summary

NE_L values of individual feeds, whether from feed tables or from feed analyses, are largely irrelevant, and even worse, they can be misleading. Energy availability must be considered on a total diet basis because nutrients interact with each other. Both level of intake and dietary starch content alter fiber digestibility, and starch content seems more important than level of intake. Increasing dietary starch decreases fiber digestibility so that the predicted increase in NE_L density of a diet is less than expected and may not change very much. The real value of feeding grain to a high producing cow whose intake is limited by gut fill is that the grain enables greater feed intake per day. With greater intake, more energy is available to produce milk, and feed efficiency and profitability will generally increase. This can and should be monitored on farms so that starch is used most effectively.

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Table 1. Energy values of nutrient based on average conversions.

	Fiber	Starch	Protein	Fat
Gross Energy (GE), kcal/g	4.2	4.2	5.7	9.4
Conversion of GE to DE	50%	90%	90%	75%
Digestible Energy (DE), kcal/g	2.1	3.8	5.1	7.1
Conversion of DE to ME ¹	81%	86%	70%	100%
Metabolizable Energy (ME), kcal/g	1.7	3.3	3.6	7.1
Conversion of ME to NE _L ²	66%	66%	66%	80%
Net Energy for Lactation (NE _L), kcal/g	1.1	2.1	2.3	5.6
Net Energy for Lactation, Mcal/lb	0.5	1.0	1.1	2.6

¹Conversions of DE to ME are based on Appuhamy et al. (2016) and Ermias Kebreab (personal communication).

²Conversions of ME to NE_L are based on Moraes et al. (2015), except fat is based on NRC (2001).

Table 2. Total tract digestibility equations for DM, NDF, and Starch (de Souza et al., 2018).

DM Digestibility (DMD) = $69 - 0.83 \times \text{DMI}\% \text{BW}$ where DMI%BW is DMI as a % of BW.

NDF Digestibility (NDFD) = $53 + 0.26 \times \text{Grass}\% \text{DM} - 0.59 \times \text{Starch}\% \text{DM} + 3.06 \times \text{DMI}\% \text{BW} - 0.46 \times \text{DMI}\% \text{BW}^2$

where Grass%DM is the DM of grass in the diet as percentage of total diet DM, and Starch%DM is the starch DM in the diet as a % of total diet DM.

Starch Digestibility (StarchD) = $96 + 0.19 \times \text{HFERM}\% \text{DM} - 0.12 \times \text{Starch}\% \text{DM} - 1.13 \times \text{DMI}\% \text{BW}$ where HFERM%DM is highly-fermentable starch as percentage of DM.

Table 3. Predicted total tract digestibilities for starch and NDF, dietary NEL content, and energy-available milk at various intakes and dietary starch contents.

DMI % of BW	Dietary Starch	Dietary NDF	Predicted StarchD ¹	Predicted NDFD ¹	Predicted Diet NE _L Mcal/lb ²	NE _L -available 3.5% Fat-Milk lb/day ³
Effect of increasing intake with 26% starch diet						
2.0%	26%	36%	94%	48%	0.750	40
3.5%	26%	36%	92%	46%	0.739	94
5.0%	26%	36%	91%	44%	0.729	146
Effect of increasing starch at DMI of 3.5% of BW						
3.5%	18%	44%	92%	51%	0.718	90
3.5%	26%	36%	92%	46%	0.739	94
3.5%	34%	28%	92%	41%	0.768	99
Effect of increasing intake with diets that increase in starch						
2.0%	8%	54%	94%	58%	0.713	36
2.5%	14%	48%	93%	54%	0.718	54
3.0%	20%	42%	93%	50%	0.726	73
3.5%	26%	36%	92%	46%	0.739	94
4.0%	30%	32%	92%	43%	0.749	114
4.5%	34%	28%	91%	40%	0.761	135
5.0%	36%	26%	91%	38%	0.766	156

¹In this example, base NDFD at 26% starch and DMI of 3.5% of BW is considered to be 46% and the NDF quality of the diet is not altered with different scenarios. Base starchD is 92%. In real life, higher NDF diets are frequently associated with greater inclusions of more digestible NDF sources.

²Predicted NE_L assumes the diet also contains 5% ash, 2% fatty acids, 17% CP, and 14% other organic material (such as sugars, soluble fiber, and silage acids). The DE to ME and ME to NE_L conversions were those used in Table 1.

³NE_L-available milk was calculated by subtracting 10.9 Mcal/day for maintenance from the NE_L supply and assuming no change in BW.

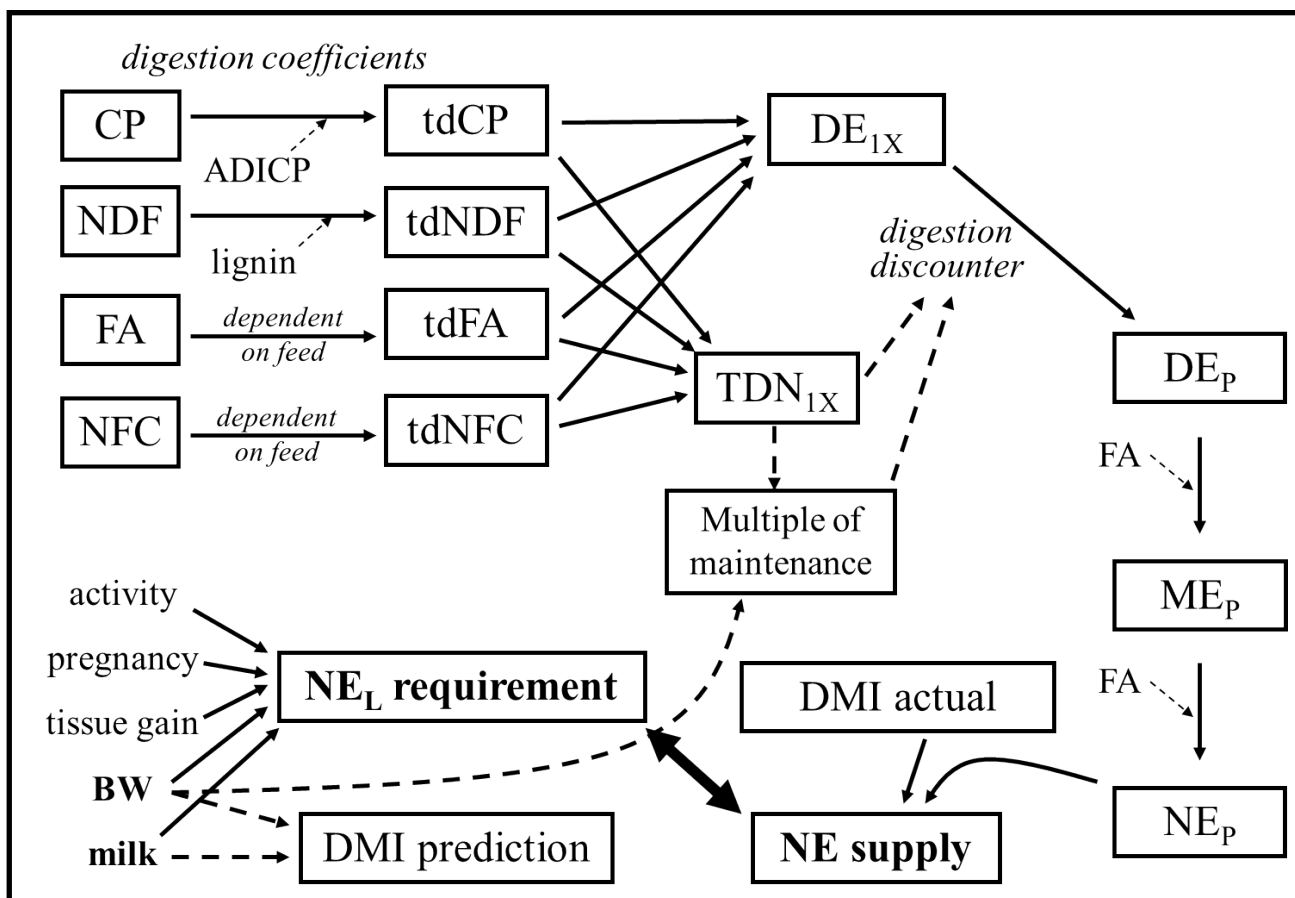


Figure 1. Energy calculations in the 2001 Dairy NRC. DE_{1X} is calculated for each feed based on its nutrients and digestion coefficients, and then DE at production level is determined by the multiple of maintenance and base TDN value of the total diet. Finally, ME and NE_L values for each feed are predicted, and the total NE_L supply is a function of the amount of each feed and its NE_L content.

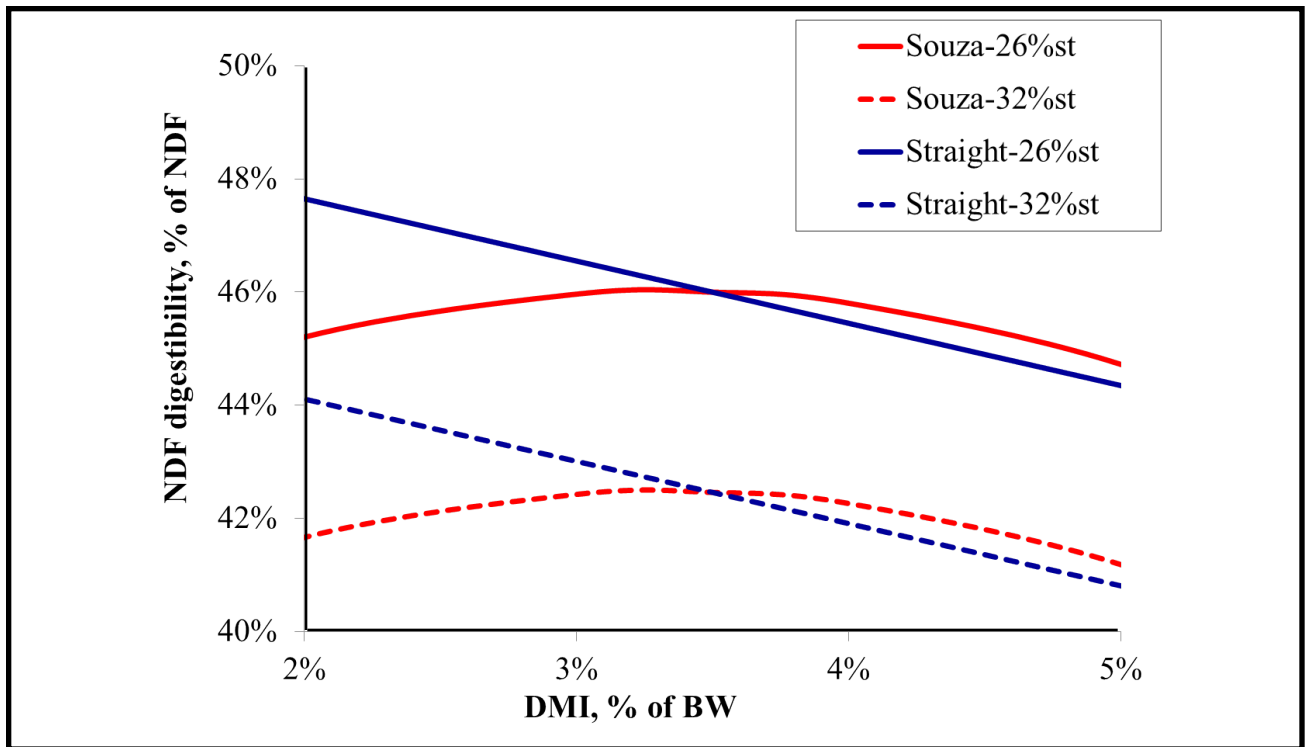


Figure 2. Effects of % starch in the diet and DMI as % of BW on the digestibility of NDF in a typical dairy diet. The response of NDFD to 26 (solid) and 32% (dashed) starch diets is shown using the original equation of Souza et al. (2018) or a derivation that includes only a linear relationship between intake and NDFD. Note that the effect of starch is greater than the effect of DMI.

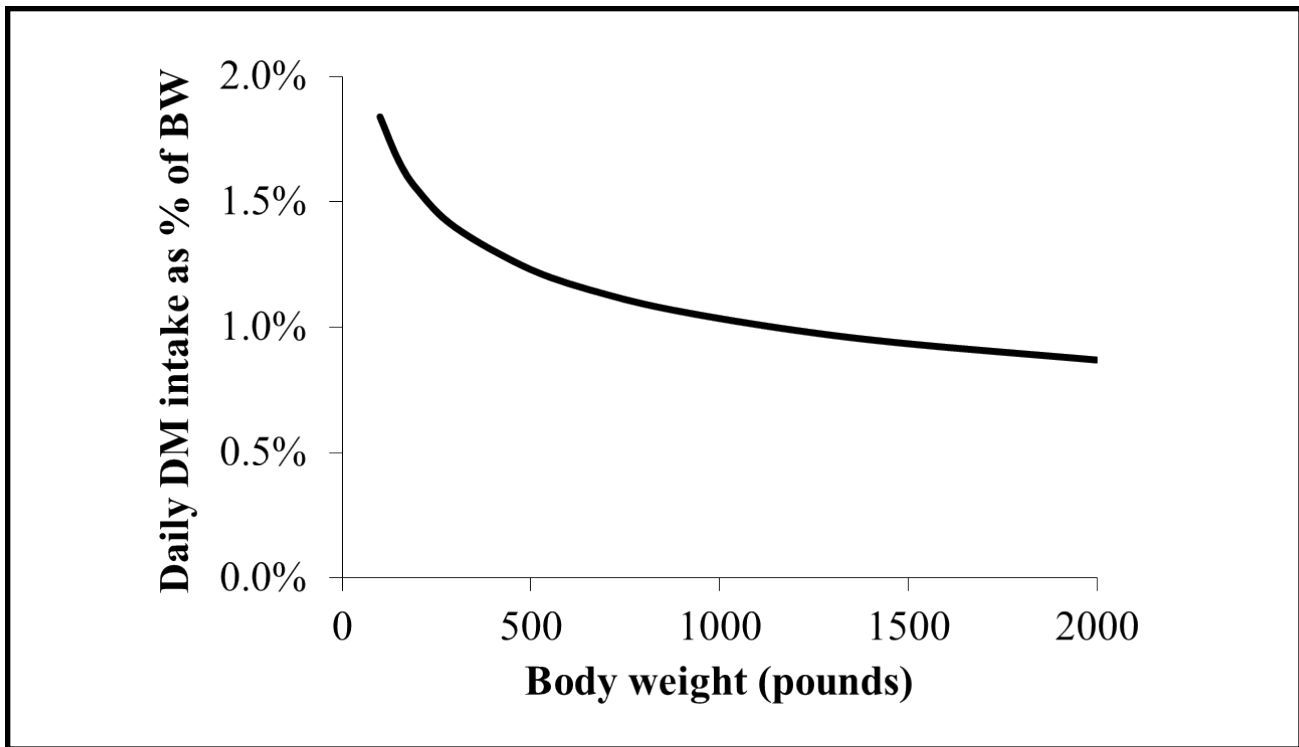


Figure 3. Amount of DM intake as a % of BW to meet the maintenance requirement of an animal if the diet contains 0.76 Mcal of NE for maintenance per pound. For a 1500-lb cow, an intake of 1 multiple of maintenance is equal to a DMI of 1% of BW.

How to Achieve the Dairy Calf and Heifer Association's Gold Standards for Calves - Your Guide For Success

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Abstract/Summary

Lifetime performance of heifer calves is greatly influenced by proper care and colostrum feeding within the first few hours after birth and growth rate over the first 6 to 8 weeks of life. Specific written goals and mindful efforts among all stakeholders can result in systematic improvements in calf performance. The Dairy Calf and Heifer Association (**DCHA**) has developed Gold Standards which are achievable benchmarks for heifer production. Dairy men are encouraged to obtain these standards from and participate with DCHA. To maximize calf performance, feeding high quality milk replacer at a level to achieve high growth rate is essential. Protein deposition during growth requires high protein ($\geq 28\%$) and medium levels of fat (10 to 15%) fed to minimize body fat deposition. Increased energy demands of cold weather should be met by feeding 1 to 2 bottles of additional milk replacer per day, depending on ambient temperature. To minimize death losses due to dehydration, a systematic effort must be made as early as possible to maintain hydration, especially in calves with scours. Fluid should be provided in levels commensurate with level of dehydration to keep calves alive.

Introduction

More than 20 years ago, Israeli researchers (Bar-Peled et al., 1997) showed

that Holstein heifer calves responded to a higher plane of nutrition with more rapid growth rate in the first 42 days and subsequently had an earlier age at calving (669 vs 700 days, respectively, $P = 0.05$) and greater milk production (21,217 vs 20,218 lb, respectively, $P = 0.08$) in their first lactation (300 days) than their traditionally fed, slower-growing counterparts. This remarkable discovery has led to subsequent research which has confirmed that growth rate of heifer calves during the first 6 weeks of life has a profound influence on promoting early fertility and lifetime milk production. Further, it has been shown that the total cost of producing a heifer from birth to freshening is essentially the same for calves managed with a traditional program or with an intensified program, but the return-on-investment is higher for the intensified program due to the increased lifetime milk production (Overton et al., 2014). The past 20 years have witnessed a revolution in calf nutrition and management based on an understanding of the impact of neonatal growth on lifetime milk production.

DCHA recently revised their Gold Standards (2016) benchmarks of performance standards for dairy heifers. The new standards clearly define objectives with regards to health status, survival rate, growth rate, reproduction, and production standards. While it is not the intent of this paper to discuss the Gold Standards in detail, it is my intention to present nutritional

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needs and management practices to achieve or exceed the Gold Standards.

DCHA Gold Standards

The DCHA Gold Standards are available from its website: www.Calfandheifer.org. More than being a list of goals, the Standards are an opportunity to create open interaction with dairy farmers, calf care givers, veterinary support, milk replacer and starter feed suppliers, and other stakeholders who then set measurable, meaningful, and objective guidance for their own calf performance. This paper will focus on technical and practical guides to meet or exceed the DCHA Gold Standards with a strong recommendation to become involved with DCHA and implement the recommendations herein to improve your calf growing operation.

Define the Destination and Get Started

To paraphrase Lewis Carroll: “If you don’t know where you are going, any road will take you there.” The Gold Standards represent a clear destination, so it is then possible to map the best route to reach the destination. The starting point for making improvements must be an honest evaluation of the current status of the operation, defining goals for various production variables, and time frames for milestones that indicate progress. An operation with 14% death loss in calves younger than 60 days cannot realistically set a goal of <2% death loss within 4 months, but it is realistic to reduce death loss to 2% within 24 months. By reducing death loss by 1% within each 60 day period, we could reach the goal of 2% within 2 years. Having specific targets also means that changes or adjustments must be made in many different areas of the operation: dry cow management, neonatal calf management, milk replacer and starter feed formulation and management, and upgrading the scours management program.

Calf Nutrition: What the Calf Needs and How to Meet Those Needs

The Latin phrase *finis origine pendet* means “the end depends upon the beginning.” While it usually is used in reference to having good early education as a basis for lifetime achievement, it clearly applies to heifer (and also bull) calves. Bar-Peled et al. (1997) showed that faster growth rate in heifer calves during the first six weeks of life resulted in greater milk production when those calves joined the milk string later in life. The faster-growing calves in the Bar-Peled et al. (1997) study weighed 195% of birth weight at six weeks of age compared with 162% of birth weight for the control calves fed a traditional milk replacer program. Van Amburgh et al. (2014) summarized milk production differences from 12 published studies in which calves were fed approximately 50% more nutrients prior to weaning. The increase in milk yield ranged from 0 (no difference in calf growth) to 3,092 lb per lactation, but all other values were in the range of 1000 to 2200 lb (Table 1). Drackley (2005) showed that higher milk replacer protein level (22 vs 18%) resulted in increased milk yield [26,096 vs 24,979, not significant (NS)] but higher starter protein level had a negative impact (22 vs 18%, 24,944 vs 26,132, NS) so decisions must be science-based.

Maintenance energy: live weight and metabolic weight

Requirements for energy and protein depend on the calf’s metabolic body weight (**BW**), ambient temperature, and rate of gain. Metabolic BW is the live weight (kg) to the 0.75 power and compensates for the fact that smaller animals have a greater surface area relative to BW than larger animals. Therefore, smaller animals have greater heat loss and higher energy requirements per unit of live BW. A 60 lb calf needs 1192 kCal Metabolizable Energy

(ME) for maintenance which is 19.87 kCal/lb of BW, while a 90 lb calf needs 1748 kCal ME for maintenance which is 17.48 kCal ME/lb of BW. Jersey calves have a relatively higher energy need than Holstein primarily because Jersey calves weigh less, not because of some inherent metabolic differences between the 2 breeds. The maintenance energy requirement of a 75 lb calf is approximately 1400 kCal/day, regardless of whether the calf is a large Jersey or a small Holstein (Van Amburgh and Drackley, 2005).

Beyond maintenance...growth!

Additional energy can be used for growth after maintenance energy requirements have been met. For growth, the need for additional protein increases more rapidly than the need for additional energy. At maintenance, in which a calf is gaining 0 lb/day, milk replacer would only need to contain about 9.3% crude protein to meet the maintenance requirement for protein. The amount of protein required in milk replacer increases rapidly as feed intake is increased because the ME intake above maintenance enables growth. Protein requirement in milk replacer plateaus around 28%. If adequate protein is available, the calf grows muscle and bone, but if protein is inadequate, the rate of gain decreases and composition of gain is higher in fat and lower in muscle and bone (Donnelly and Hutton, 1976 ab; Bartlett et al., 2006).

Milk replacer composition and body composition

Daily intake of total calories (determined by DM intake) as well as protein and fat intakes will determine both the rate of gain and the composition of gain. Dairy farmers claim that they do not want fat heifers, but feeding a low protein (20%), high fat (20%) milk replacer, which is still a common practice throughout the United States, is a sure way to have a slow rate

of gain and a high amount of fat deposited on the body of heifer calves. On a dry basis, cow's milk contains approximately 28% protein, and this is a protein level that satisfies the protein requirement for most calves within a wide range of BW and intake levels. From a philosophical standpoint, we can choose to accommodate calves with the highest nutrient requirements and "overfeed" calves with lower requirements, or we can choose to accommodate calves with the lowest nutrient requirements and "underfeed" calves with higher requirements. My personal philosophy is to meet the needs of the calves with the highest needs. Feeding milk replacer with 28% protein satisfies this condition most of the time. In special cases where calves are uniform in weight and fed a lower total daily milk replacer intake, such as a dairy beef operation, 26% protein is generally adequate. But, in a large calf ranch or any dairy operation where calves arrive weighing from 45 to 105 lb, the small calf with high intake requires higher protein to meet its needs and 28% protein is recommended.

Calves do not utilize fat very well, and as fat level of milk replacer increases, daily starter feed intake generally decreases. Digestibility of DM, organic matter, fat, nonfiber carbohydrates, and Ca and P may also be reduced. Fat is preferentially used by the body to deposit body fat, so calves are fatter when fed higher amounts of fat in milk replacer (Tikofsky et al. 2001). In most milk replacer applications, a protein:fat ratio of 2:1 is preferred, such as milk replacers with 28% protein and 14 to 15% fat. Hill et al. (2006 ab, 2009) conducted several studies examining daily feeding rates as well as protein and fat levels in milk replacers and the reader is directed to these studies for additional information.

Cold weather dramatically increases maintenance energy requirement and slows growth

In simplest terms, the potential to gain weight depends upon the amount of energy available after maintenance needs have been met. Think of it like our ability to go shopping at the end of the month depending on having money left over for shopping after paying the mortgage, utility bills and food bills (maintenance). Cold ambient temperatures increase the calf's need for energy to stay warm, so there is less energy available for growth. To maintain rate of gain, calves must consume more feed to provide more energy during cold weather.

The lower limit of the thermoneutral zone for young calves is 68°F which means that young calves begin to feel cold stress on a cool night, even in the summertime. We generally do not think in terms of cold stress until winter is approaching, but maintenance energy requirement increases by approximately 50% at freezing and by 100% at 0°F. Feeding an extra bottle of milk replacer per day to calves when temperatures drop to 32°F and 2 extra bottles when temperatures drop to 0°F will provide additional energy to keep calves gaining weight even when cold.

There is an often repeated myth that to compensate for cold weather, increase fat content in milk replacer. The fact is the only way to compensate for increased maintenance energy due to cold weather is to increase the daily amount of milk replacer ounces fed to calves. In round numbers, a medium fat milk replacer contains approximately 2000 kCal ME/lb and a 95 lb calf needs about 1700 kCal ME/day for maintenance at 68°F and about 1350 kCal ME/lb gain. A calf gaining 1 lb per day needs about 1.5 lb milk replacer per day $((1700+1350)/2000=1.525 \text{ lb})$. When the

temperature falls to 0°F, maintenance energy increases by an additional 1700 kCal to a total of 2900 kCal/day. To meet additional maintenance requirements and maintain average daily gain (ADG), milk replacer would have to contain 70% fat if feeding rate remains at 1.5 lb/day. Obviously, nobody is neither going to feed 70% fat nor should feed that high amount of fat to a calf. Figure 1 shows the change in ME intake with increasing fat content in milk replacer at a feeding rate of 2 bottles (24 oz) per day. Additionally, it shows ME intake in calves fed 3 bottles (36 oz, 32°F) and 4 bottles (48 oz, 0°F). It is obvious that increasing daily intake of milk replacer is the best way to compensate for cold weather increases in maintenance requirements.

Ash content of milk replacers

Ask a dairy nutritionist what the effect would be on cows with an addition of 5% salt to the TMR and the nutritionist immediately reacts with concern about toxic salt levels. Yet, many milk replacers have extremely high levels of Na, K, and Cl due to the use of delactosed whey and/or whey permeate as milk replacer ingredients. Ash content in whey and whey protein concentrate are primarily composed of Na, K, and Cl, whereas non-fat dried milk is primarily composed of Ca and P. While veal milk replacers are normally 6.0 to 7.5% ash, poor quality herd milk replacers can have ash content $\geq 10\%$ with the additional ash being Na, K, and Cl. Strayer et al. (2014) showed analysis of 2 herd milk replacers with ash contents of 11.72%. Since ash and lactose are not listed in the guaranteed analysis, the only indication of high ash content is the listing of "dried whey product" as an ingredient. Use of dried whey product replaces lactose in milk replacer with Na, K, and Cl, which lowers ME and increases risks for Na toxicity when feeding milk replacers $\geq 150 \text{ g/L}$ and/or using water with high Na levels. Quality herd and veal milk replacers

usually have ash contents <7.5% and do not include whey permeate or delactosed whey as ingredients, so “dried whey product” is not listed as an ingredient.

Water

Research shows that calves given free access to water consume more starter feed and gain more weight than calves without access to water (Kertz et al., 1984). My experience is that most calf water buckets have 3 to 4 inches of water remaining and if we add water, calves will drink. Calves don't like to put their head into buckets past their eyes. They don't drink water from the bottom inches of the bucket unless they are forced to drink it and reduce starter feed consumption and rate of gain without adequate water, especially in hot weather.

Calf starter feed and weaning

Heinrichs and Lesmeister (2005) have a very good review of rumen development in calves and the reader is directed to this review for more detailed information and references to appropriate literature. In brief, calves are born with a prototypic reticulo-rumen which grows rapidly due to the volatile fatty acids which result from fermentation of starch. Logically, one might believe that since calves are cattle, and cattle are ruminants, and ruminants have a bacterial population capable of digesting fiber, that feeding fiber to calves would be an appropriate practice. But, the reality is that calves are pre-ruminants and as such are incapable of digesting fiber. In fact, feeding fiber to milk-fed calves is detrimental, causing abomasal ulcers among other problems. Mattiello et al. (2002) tested different solid feeds in milk-fed veal calves and examined abomasal damage and concluded, “a solid feed able to satisfy calves' behavioral needs and improve digestive processes without

damaging the digestive apparatus still has to be identified.” Feeding roughage to older calves, post-weaning, may have some beneficial effect, depending on the buffer capacity of the starter feed and form of grain in the feed because it affects rate of fermentation, but in young milk fed calves, high levels of fermentable starch are required for the rumen to develop, but roughage should not be fed.

Does an increase in feed intake cause an increase in rumen development or does increased rumen development result in an increase in feed intake? Hodgson (1971) concluded that both conditions are true. Calves that consume more starter feed have greater rumen development due to increased fermentation of starch and calves with greater rumen development consume more starter feed as a result of greater rumen capacity. A key finding from Hodgson's research is that starter feed intake is an excellent indicator of rumen development, so weaning should be dependent on amount of daily dry starter feed intake.

Greenwood et al. (1997) compared 3 levels of feed intake as a percentage of initial BW as the initiation point for weaning and concluded that daily feed intake equal to 1.0% of initial BW is adequate to begin the weaning process. To complete the weaning process, calves should be at least 21 days old, have a daily starter intake of at least 1% of initial BW, have a cumulative total starter intake of at least 9% of calf's initial BW, and have gained at least 12% of its initial BW. This weaning strategy means that calves can be weaned earlier than most dairy farms currently wean calves, but is similar to most dairy beef operations where the objective is to minimize the cost of production from day 1 to 300 to 400 lb live weight. For heifer calves, where the objective is to maximize lifetime milk production, longer milk feeding period and higher amounts of daily

milk replacer is recommended. Table 2 shows recommended milk replacer feeding schedules for bull and heifer calves to achieve their respective objectives. The schedule assumes 12 ounces of milk replacer powder (28% CP/14% fat) in 2 quart bottles. Ziegler et al. (2005) fed heifer calves 12 ounces of milk replacer (28% protein/16% fat) in either 2 quarts or 3 quarts of total solution. Even though both groups of calves were given the same amount of milk replacer per day, calves given 2 quarts weighed more on day 56 (180.09 vs 169.42 lb ($P < 0.05$), respectively), than calves given 3 quarts.

The Practical Side of Caring for Calves

The key to success with calves is doing many little things consistently well every day, with achieving your benchmarks in mind. Milk replacer should be mixed the same way, at the same temperature, for the same amount of time, the same quantity of powder, and delivered at the same time and at the same temperature every feeding and every day. I call this the “precise, boring, sameness that leads to success.”

To ensure consistency, develop written protocols for on-farm procedures and review with people caring for calves and cows. Develop a simple system to indicate status of animals that does not require looking in a book or checking a computer. Colored clothes pins, golf tees, or chalk marks on the outside of the pen, or position of pails or bottles can readily indicate calves that have been treated or need treatment that every worker can quickly and easily recognize without “checking the book.” As soon as possible might not be for 12 hours, so written procedures should be specific such as “feed colostrum within 2 hours of birth” rather than ambiguous such as “feed colostrum as soon as possible.”

Navel dipping

Infection of the navel, called “navel ill,” leads to infection of joints, called “joint ill,” and/or infection in other parts of the body and septicemia. Even in ancient times, the connection between navel infection and joint infection was recognized. Proverbs 3:8 states “it shall be health to thy navel and marrow to thy bones,” according to the King James Version Bible. Preventing navel infections and associated joint and systemic problems is perhaps the most cost-effective practice on the dairy, costing less than 40 cents per calf.

Within 30 minutes of birth, dip the navel using 7% tincture of iodine solution or another product specifically designed for dipping navels. Do not use teat dips and do not spray the navel but dip the entire navel using about 1 fluid ounce of solution per calf using a disposable paper Dixie cup and pressing the cup against the body wall to ensure complete immersion of the navel in disinfectant solution. Throw away the cup and disinfectant solution after each use and use a fresh dose of iodine for each calf. Check the navel of each calf on day 2, 4, and 6 after birth. Navel should be the size of a pencil or smaller. Mark calves with navel the size of your thumb as “suspect” and re-check the next day. If the navel is the size of a walnut or larger, treat navel using penicillin injected into navel, under veterinary supervision.

Preventing scours: Cow vaccination and colostrum

Immunity to calftod diseases in newborn calves is obtained through feeding colostrum within hours of birth. To assure that colostrum will provide protection against *Rotavirus*, *Coronavirus*, *Clostridium perfringens* Type C, and K99 *E. coli*, cows should be vaccinated 6 to 9 weeks and given a booster 3-6 weeks before

calving. Giving cows vaccinations improves the quality of colostrum, but calves need to consume colostrum to receive the benefit. In addition to colostrum, dairy farmers should consider blood-derived IgG (non-specific), colostrum-derived antibodies (*Coronavirus* and K99+ *E. coli*), and/or egg-derived antibodies (*Rotavirus*, *Coronavirus*, *E. coli*, and *Salmonella*) which can be effective in reducing morbidity and mortality.

Colostrum harvest, storage, and feeding

The importance of colostrum for calves cannot be overstated. In addition to immunoglobulins, colostrum contains many bioactive substances which are critical for optimum growth and well-being. Not every dairy can successfully collect and feed colostrum. Those dairy farms should consider feeding a high-quality dried colostrum or high quality colostrum replacer to provide calves with >150 g IgG instead of feeding poor quality colostrum to newborn calves. Sick or dead calves have a higher cost than buying high quality dried colostrum or a high quality colostrum replacer. For dairy farms feeding colostrum, investing in an improved colostrum program yields immediate rewards.

Not every cow produces colostrum that should be fed to calves. Only feed colostrum from cows that have a “negative” *Johnes* ELISA test. Cows should be healthy, free of mastitis, and should not have leaked milk, and should not have blood in milk. Cows should have been dry at least 45 days prior to calving and in the transition group for a minimum of 14 days. The right cow will have had an appropriate vaccination program based on consultation with the herd veterinarian.

Colostrum should be harvested within 2 hours of calving. Fresh cows should be milked before sick or treated cows to avoid transferring

disease organisms and cow preparation should be identical to routine parlor practices, including equipment service and sanitation between cows and between milking. Save a sample of colostrum for future reference.

After testing colostrum quality with a refractometer or colostrometer, colostrum should be fed or chilled and properly stored within 30 minutes of collection. What’s proper storage? Pour colostrum into milk bottles, plastic food storage bags, or other clean containers with the cow ID and date of collection clearly marked. The container should be immediately placed into ice water. Do not put hot colostrum into a refrigerator because it takes >8 hours to cool in a refrigerator. During this time, bacteria are growing rapidly in the colostrum and any vaccines or medications stored in the refrigerator are being subjected to temperatures higher than their ideal storage temperatures. Freeze plastic storage containers of water in the freezer section of the refrigerator to keep an adequate supply of ice available to rapidly chill harvested colostrum. After colostrum has been cooled in ice water, put the chilled colostrum in the refrigerator. While potassium sorbate can be added to colostrum to extend shelf-life, colostrum should be fed or frozen within 7 days or the colostrum should be discarded.

Calves should be fed colostrum equal to 10% of their BW within the first 2 hours of life and an additional 5% of BW before 12 hours of life. For a 90 lb calf, this is equal to 4 quarts within the first 2 hours of life and an additional 2 quarts within the next 10 hours. Test serum using a refractometer. Well-managed farms may have >90% of calves with serum total protein ≥ 5.2 g/dL. Dairy farmers should set a goal of having >80% of calves with serum total protein of ≥ 5.0 g/dL and 50% of calves with ≥ 5.5 g/dL. Serum protein values should be interpreted to evaluate the overall colostrum program and

not necessarily to evaluate the colostrum status of any individual calf because dehydration can increase the serum protein value. The best use of these data is to look at serum total protein values for calves on a monthly basis and determine the percent of calves above 5.0, 5.2, and 5.5 g/dL as an indication of whether or not the colostrum collection, storage, and feeding program is working well or if it is in need of improvement.

Bull calves need colostrum, too!

Shields (1994, unpublished) showed that veal calves with adequate colostrum had 10% higher gain (349.6 vs 319.2 lb, respectively) and half as many calves treated in the first 28 days (25% vs 50%, respectively) versus calves which had not received adequate colostrum. My testing routinely shows that >70% of bull calves received in veal and dairy beef barns have not received any colostrum! Dairy farmers who would never think of withholding milk replacer routinely send bull calves to a sale barn without first giving them the superfood and immunity protection of colostrum and think nothing of it. Sending a calf to a sale barn without colostrum puts the calf at risk. Bull calves go from a sale barn to an order buyer's station and are then transported to a farm where a veal producer or dairy beef producer struggles to care for them and keep them alive. Such high numbers of calves sent to sales barns without colostrum is a shameful failure on the part of dairy farmers. Every calf, both bulls and heifers, need to be fed colostrum equal to 10% of their BW within the first 2 hours of life and an additional 5% of BW before 12 hours of life. Send your customer the best calf possible and give colostrum first!

Milk replacer mixing, temperature, and feeding

Buying a diesel pickup truck means using a different fuel than used in the gasoline

pickup truck it replaced. So too, a different milk replacer might require a very different mixing procedure than the one it replaced. The method used by various milk replacer manufacturers to transform liquid fat into dry powders determines the optimum mixing temperature and conditions. So-called easy mix formulas usually have a dry fat ingredient in which the fat has been encapsulated in a protein matrix with heat. This type of fat can be mixed in water at 125 to 135° F with a wire whisk. Another process is just the opposite in which milk replacer powders are encapsulated in fat which is crystallized with extreme cold. For this type of milk replacer, water should be 155 to 165°F and mixed with a power mixer. There are other processes, but the important lesson is to always read and follow manufacturer's directions for mixing milk replacer.

A small farm mixing a small quantity of milk replacer might use a 5 gallon bucket with a wire whisk or a plastic drum and an electric drill with a mixer to make milk replacer. Large operations generally use stainless steel mixers designed to mix milk replacer. In both small and large operations, the preferred milk replacer mixing procedure is very simple and should be consistent every day. Remember "TWA" to mix correctly. TWA stands for Time, Water (temperature and quality), and Agitation. As an example, imagine that we need to mix milk replacer for 50 calves and we're feeding 12 ounces of milk replacer powder per calf in 2-quart bottles. We need $(50 \times 12 \text{ ounces} \div 16 \text{ oz/lb} = 37.5)$ lb of milk replacer powder and $(50 \times 2 \text{ quarts} \div 4 \text{ qt/gallon} = 25)$ gallons of total milk replacer solution. Step 1 is to add 50% of the total water needed into the mixer which is 12.5 gallons. The water should be at or slightly higher than the mixing temperature according to manufacturer's directions which we will assume is 135°F for our example. Next, we add all the milk replacer powder to the mixer and

mix for the amount of time recommended by the manufacturer which we will assume is 3 minutes for this example. Use a thermometer to check the temperature of the mix after all the milk replacer powder is added to the mixer. Adding hot water and cold milk replacer powder to a cold mixer in January will result in colder milk replacer than the same mixer in July. Make sure that the water temperature is within the prescribed range. After mixing, add additional water to bring the volume to 25 gallons and bring the temperature down to approximately 118°F. Mix for 1 minute and then fill the bottles, put on nipples, and deliver bottles to feed the calves. Check the temperature of the milk in the last bottle fed. It should be minimum 113°F. If it is too cold, increase filling temperature for the next batch, and if it is too hot, decrease filling temperature for the next batch. Repeat this process for every feeding, every day. My recommended feeding temperature is higher than most recommendations and is based on the digestibility and melting temperatures of fats used to make milk replacers, ingredient composition of modern milk replacers, and medication cost and performance of thousands of calves. My preferred feeding temperature for milk replacer is 113°F for the last calf.

Cleanliness is next to...

The old saying is “cleanliness is next to Godliness,” but in many operations, cleanliness is next to impossible! Proper cleaning of equipment used to mix, deliver, and feed milk replacer to calves has many benefits to the calf and calf producer. Reducing the risk of sickness, use of antibiotics, and risk of respiratory and digestive problems are immediate benefits from proper cleaning procedures. For best results, rinse all equipment with warm (80 to 110°F) water to remove manure, dirt, and all milk residues. Rinsing with lukewarm water allows milk residues to rinse off without becoming permanently attached. Use a thermometer to

adjust rinse temperature every time you rinse. Clean using a mixture of chlorinated alkaline soap and hot water (165°F) to wash the mixing and feeding equipment. Chlorine is a powerful disinfectant and alkaline soap dissolves fat. Wear gloves and scrub all surfaces to remove protein, fat, and foreign materials that adhere to surfaces. Special brushes may be needed to clean bottle nipples, bottles, esophageal feeders, floating nipples, feed buckets, etc. Use a thermometer to adjust wash temperature to 165°F every time you wash. Chlorine solutions must be at least 150 parts per million to effectively kill bacteria. Liquid chlorine products generally have shelf-lives in the range of a few weeks, and should be purchased in small quantities frequently rather than large quantities infrequently. Suspended milk solids can re-deposit on equipment if the temperature of the wash water falls below 120°F. For this reason, the temperature of the final wash water should be above 130°F. Use a thermometer to check final temperature. Finally, rinse with an acid-sanitizing solution in warm water (70°F) per manufacturer’s directions and allow to completely dry. Acid final rinses reduce surface pH to <4 for up to 12 hours which reduces bacterial growth. This cleaning ritual should be performed every time calves are fed.

Electrolytes and scours

This is a topic area in which I am very passionate, and I have developed several electrolyte products over the past 30 years. Oral rehydration solutions were developed for humans in the 1960’s by Drs. Norbert Hirschorn and William Greenough, working with the World Health Organization (**WHO**). The original formula used sodium bicarbonate, dextrose, sodium chloride and potassium chloride. In the 1970’s, sodium citrate replaced sodium bicarbonate to reduce reinfection because bicarbonate neutralizes stomach acid while citrate has no effect on pH of the stomach.

Acid in the stomach provides natural protection against pathogens. In the 1990's, the WHO adopted a formulation in which dextrose had also been replaced with complex carbohydrates.

Regardless of the cause of scours, calves can suffer from Dr. Drew's Four D's: **D**iarrhea leads to **D**ehydration, **D**epression (outward signs of acidosis), and **D**eath occurs when dehydration reaches 12 to 14%. We cannot usually see changes, such as sunken eyes or droopy ears, until dehydration reaches 6%, meaning that the calf is "halfway dead" when we first see signs of dehydration! Reducing death losses due to scours requires changing the old way of how we treat calves with scours to a new and different way of doing things. The simplest change is to systematically feed electrolytes to calves based on their level of dehydration. I call it the 1-2-3 plan: feed **1** bottle of electrolyte to calves with scours (1 to 5% dehydrated). Feed **2** bottles to calves with signs of dehydration (6 to 8% dehydrated). Feed **3** bottles to calves with severe dehydration (9 to 12% dehydration). If your electrolyte contains dextrose, give 2 liters of Lactated Ringer's solution (intravenous) and give oral electrolyte when calves have recovered to moderate dehydration levels. Dextrose can be deadly to calves with severe dehydration because of the negative impact of high osmotic pressure. Continue to offer milk or milk replacer to calves with scours because they still need and benefit from the energy and nutrients in milk replacer, and use products that contain sodium citrate and not sodium bicarbonate because bicarbonate interferes with milk digestion because it neutralizes pH of the abomasum. Calves that show signs of dehydration, such as sunken eyes, droopy ears, or skin tenting >1 second, require immediate attention! Keep calves hydrated and you will keep calves alive, regardless of the cause of scours.

Conclusions

Successful calf raising is doing 100 little things right every day, over and over. We no longer have to live in a world of starving calves with 8 ounces of milk replacer containing 20% protein and 20% fat. Modern heifer raising involves proper pre- and post-natal care and feeding higher (intensified) daily amounts of milk replacers containing 26 to 28% protein and lower levels of fat. Calves raised under these conditions routinely double their birth weight in 50 to 60 days and produce 1000 to 2000 lb more milk than their slower growing counterparts raised on traditional diets of yesteryear. Establishing written protocols and clear production goals can help dairy farmers become more productive, gauge their progress as they achieve each benchmark, and become more profitable.

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Table 1. Milk production differences among treatments where calves were allowed to consume approximately 50% more nutrients than the standard feeding rate prior to weaning from liquid feed.

Study	Milk yield, lb
Foldager and Krohn, 1991	3,092
Bar-Peled et al., 1997	998
Foldager et al., 1997	1,143
Ballard et al., 2005 (@200 DIM)	1,543
Shamay et al., 2005 (post-weaning protein)	2,162
Rinker et al., 2006 (projected 305 @ 150 DIM)	1,100
Drackley et al., 2007	1,841
Raith-Knight et al., 2009	1,582
Terre et al., 2009	1,375
Morrison et al., 2009 (no difference in calf growth)	0
Moallem et al., 2010	1,600
Soberon et al., 2011	1,217

Source: Reprinted from Van Amburgh et al. (2014)

Table 2. Milk replacer feeding schedules for bull and heifer calves¹.

Days	Bull Calves,	Cumulative milk replacer fed, lb	Heifer Calves,	Cumulative milk replacer fed, lb
	Bottles/day 2x4+1 Schedule		Bottles/Day 2-3-2-1 Schedule	
1-7	2	10.50	2	10.50
8-14	2	21.00	2	21.00
15-21	2	31.50	3	36.75
22-28	2	42.00	3	52.50
29-35	1	47.25	3	68.25
36-42	0		2	78.75
43-49			2	89.25
50-56			1	94.50
57+			0	

¹Assumes 12 oz milk replacer powder per bottle. Increase 1 to 2 bottles/day based on cold weather.

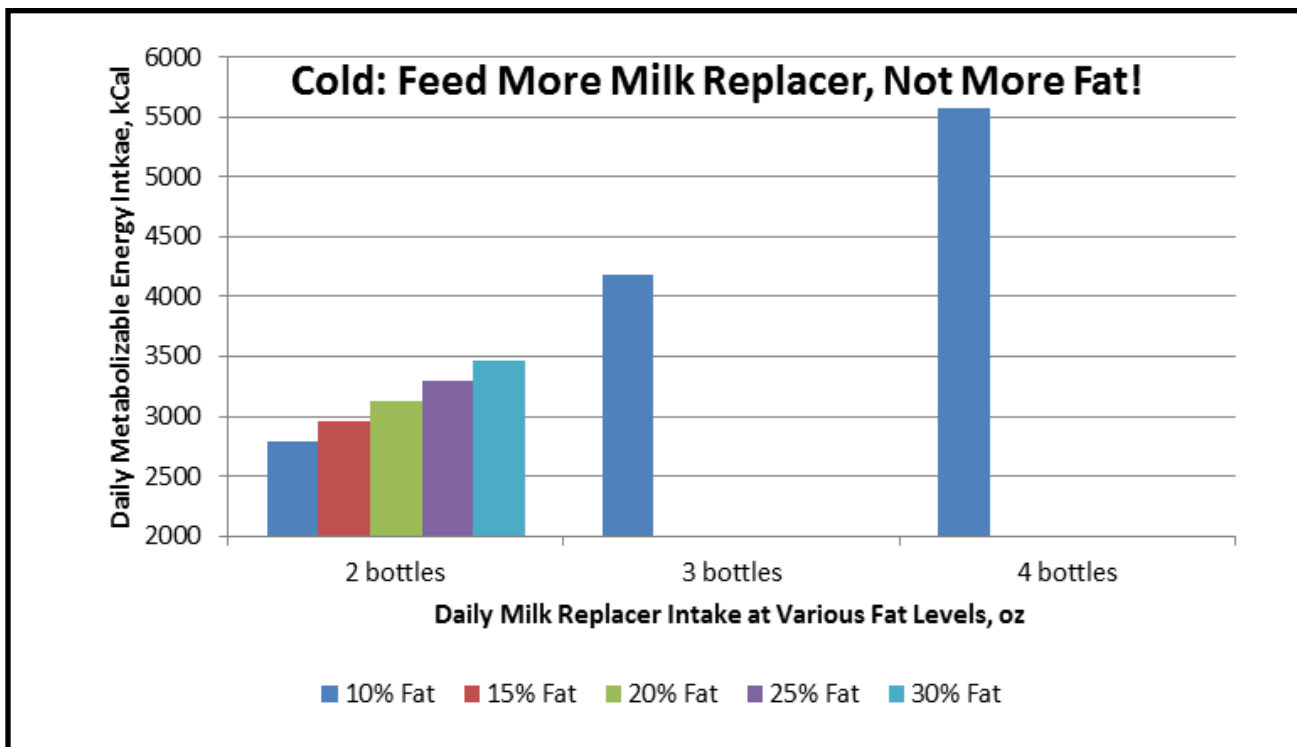


Figure 1. Daily metabolizable energy intake with increasing levels of intake versus percentage of fat.

The Resilience of the Rumen Microbial Population

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Abstract

In healthy dairy cows, rumen microbial community composition is highly individualized by host animal; displays modest inertia when host diet is altered; and displays impressive resilience when perturbations are relaxed. The mechanisms underlying this resilience are poorly understood, but appear to involve intrinsic properties of the microbial community that work in concert with metabolic, immunological, and behavioral contributions from the host. Attempts to modify the ruminal community by strain inoculation or whole-community exchange generally achieve only transient shifts in community composition. By contrast, dysbiotic cows appear to be more amenable to manipulation of their communities to restore their function, suggesting a natural tendency of the rumen to achieve a stable functional community. While microbial community composition appears to affect performance metrics, such as milk production efficiency and milk composition, manipulating the communities to improve overall performance remains elusive, although analysis of community composition may provide a tool to inform management strategies and culling decisions. Owing to the difficulty of manipulating rumen microbial community composition in adult animals, there has been much interest in early-life (pre-weaning) interventions to direct the development of the community prior to maturity.

Introduction

The ruminant animal is defined by the presence of a specific gastrointestinal organ, the rumen, in which a complex and highly adapted microbial community carries out an anaerobic conversion of feed materials to VFA and microbial cell mass that respectively provide the main energy and protein sources to nourish the host animal. Establishment and evolution of this complex community occurs gradually as the organ itself develops within a juvenile, originally monogastric host. Once established, this community drives the ability of the host to utilize a wide variety of feed components, including fibrous plant materials that cannot be significantly digested by non-ruminants. Owing to these spectacular and irreversible benefits exchanged between the animal and its microbiome, the ruminant is unsurpassed as an example of host/microbe mutualism.

From a microbial ecology standpoint, the rumen can be considered as its own ecosystem, in which fairly stable environmental conditions (temperature, pressure, and water content) interact with additional variables – particularly the chemical composition of inputs (diet) and the rate of passage of materials – to set the conditions that regulate the microbial metabolic processes. Early studies in rumen microbiology were facilitated by Hungate's development of anaerobic culture methods, which permitted

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isolation and characterization of a limited number of individual microbial species. These species could readily be isolated from almost all ruminants tested. And because, taken together, they appeared to encompass most of the substrate conversions known to occur in the rumen, it was long thought that the ruminal community was similar across all individual ruminants within a species, and even across multiple species within the ruminant order.

The development in the 1990s of more sophisticated, culture-independent methods for characterizing microbial communities revealed that all microbial communities in nature, including those of the rumen, were far more complex and diverse than were indicated by culture-dependent methods. We now know that, although the rumen contains a “core microbiome” (i.e., a collection of species that are present in most individual ruminants), there are a large number of other species present as well (Jami and Mizrahi, 2012; Henderson et al., 2015). Moreover, the abundance of individual microbial species – both core and non-core -- varies considerably both within and across individuals over time. Although much of the variation in community composition is driven by diet (some examples of which will be given below), there are substantial differences in community composition among animals fed the same diet. This has led to the concept of host-specificity, i.e., microbiomes individualized to their specific host. This concept has recently been noted, with great fanfare, in the human gastro intestinal (**GI**) tract (Lozupone et al., 2012; Lynch and Pederson, 2015), but interestingly was first demonstrated in the rumen way back in the 1930s for protozoa (Kofoid and MacLennan, 1933), and in the 1990s for bacteria (Weimer, 1998), and only recently for methanogens (Zhou et al., 2012).

Central to the concept of host individuality is the notion that the microbial community is relatively stable when environmental conditions and inputs (e.g., feed composition) are stable, and displays some resistance to change when conditions are changed. In other words, the community would display the ecological property of *inertia* (Table 1; sometimes termed *resistance* [Allison and Martiny, 2008]). The inertia of the community would allow its composition to be maintained within a reasonable range, even as the community undergoes some changes in its composition over time (i.e., during a feeding cycle, or across months). Moreover, host individuality would also imply that the community, once perturbed (for example, by a substantial change in diet), would be able to re-stabilize itself once the perturbation was removed. In other words, the community would display the ecological property of *resilience* (Table 1). We can employ a simple metaphor to characterize these properties: If the microbial community can be regarded as a rubber band, inertia describes the deformation of the band. How far can it be stretched, and how far does it stretch for a particular input of effort? By contrast, resilience describes the relaxation of the band. Once the stress is removed, does the band return to its original conformation (i.e., display *elasticity* [Table 1]), and how rapidly does the return occur? We will consider these two properties in turn.

Relevant Properties of Microbial Communities

Inertia

Diet appears to be the major force that overcomes the natural inertia of the rumen microbial community (Henderson et al., 2015). Numerous studies have shown that, within individual animals, changing the diet results in changes in prokaryotic (bacterial and archaeal)

communities; changes are much less detectable in the protozoal communities (deMenezes et al., 2011). In fact, diet-induced changes in prokaryotic community composition appear to be much stronger, and occur much more rapidly, in ruminants than in humans, whose bacterial communities generally fall into one of three “enterotypes”, within which diet-induced differences are “small compared to baseline interpersonal variations” (Lozupone et al., 2012).

In ruminants, changes in microbial community composition might be expected during the course of the feeding cycle, owing to the different rates of utilization of different feed components, which would lead to time-dependent changes in the composition of the remaining, undegraded feed. In fact, bacterial community composition (**BCC**) was shown to change both within and across feeding cycles in cows fed the same TMR at 12 h intervals (Welkie et al., 2010). Interestingly, BCC returned to a different end-point at the end of each of 4 successive feeding cycles, suggesting that BCC is actually in a continual state of flux (i.e., is not completely elastic). Nevertheless, several studies with cows fed once-daily have shown that BCC measured in individual cows at the same time after feeding over the last 3 days of a 28-day experimental period showed much greater similarity to one another than to the BCC of other cows on the same diet and that displayed similar production metrics.

One interesting aspect of dietary effects is that even a modest change in diet can have a major effect on specific taxa. For example, Mohammed et al. (2014) used automated ribosomal intergenic spacer analysis (**ARISA**) and next-generation sequencing (**NGS**) to characterize the bacterial community in heifers that were either grazed fresh orchardgrass pasture (**OP**) for 3 consecutive 28-day periods,

or were alternated from OP to orchardgrass hay (**OH**) and then returned to OP over successive 28-day periods. Heifers whose diets were switched to OH showed significant decreases in the relative population size of genus *Butyrivibrio* (one of the most abundant genera in the rumen) along with a decrease in the molar proportion of ruminal butyrate and an increase in the molar proportion of ruminal acetate. Such changes may have resulted from OH’s lower level of water-soluble carbohydrates, a preferred substrate for *Butyrivibrio*. Both the molar proportion of butyrate and the relative abundance of *Butyrivibrio* returned to their previous levels when the heifers were returned to OP, providing an indication of the elasticity of this particular genus within the rumen bacterial community.

One of the more interesting principles that has emerged from theoretical studies in microbial ecology relates to the effects of positive and negative interactions among community members on community stability. Surprisingly, while cooperative interactions among species can improve overall efficiency of the community, they tend to destabilize rather than stabilize communities (Coyte et al., 2015). Consequently, interspecific competition actually makes the community more stable, i.e., display greater inertia, and the effects of this competition become more important as community diversity increases. This conclusion is a bit counterintuitive, as it goes against our general notion that one of the hallmarks of the ruminal community is its complex network of cooperative interactions (such as interspecies hydrogen transfer and cross-feeding of nutrients among different metabolic classes of microbes). However, when one considers the large number of closely-related species within the rumen, which presumably have substantial overlap of function, it is likely that competition for substrate is intense, whether it be for colonizable surfaces of feed particles, or soluble substrates

present at concentrations similar to those of the transport coefficients of microbes that use these substrates.

What forces, then, drive resilience of the ruminal community? In the case of a dietary shift followed by return to the original diet, competition is likely a major driver. If competition was the dominant interaction on the original diet, new competitive interactions would result from a change in the availability of a new group of substrates, and a return to the original diet should again favor the original competitions that led to the establishment of the original community.

Resilience

The resilience of the rumen microbial community is further (and more dramatically) demonstrated by ruminal contents exchange experiments. Near-total (~95%) exchange of ruminal contents between multiparous ruminally cannulated Holstein cows resulted in gradual return, over the course of several weeks, to a community composition similar to that in the recipient host, even though the donor inoculum was derived from a cow fed the adjacent diet and subjected to the same environmental conditions (i.e., housed in an adjacent tie stall; Weimer et al., 2010a; Weimer et al., 2017). Perhaps more surprising is the observation that differences in ruminal chemistry between donor and recipient cows were overcome in the recipient within a day of the contents exchange, suggesting that the cow has substantial control over her own ruminal chemistry, whether it be by controlling the rate of VFA absorption, rate of passage, or the volume and composition of salivary buffers. In some exchange experiments, the differences in ruminal chemistry may strongly influence microbial community composition, and thus partially explain community resilience. However, community resilience has also been

demonstrated in exchange experiments between cows that had similar ruminal chemistries (Weimer et al., 2017).

While resilience has been demonstrated experimentally, the underlying mechanisms have received little study. Presumably resilience is determined by the strengths of the interactions (positive and negative) among the different community members, and the degree to which the individual animal has strengthened its mutualism with its own community. While we may speculate on these various determinants of resilience (Table 2), at present we have little knowledge as to their relative contribution.

One would expect that in these exchange experiments, the donor community would also be highly competitive (because it had developed on the same diet, albeit in another host). But this new community is eventually displaced by the recipient's original community, which suggests that the primary determinant of community composition is the interactions between the host and her individualized host community. The likely complexity of these interactions may explain why a return to the original community composition following contents exchange is slower than the shift in community composition following dietary change without further addition of exogenous microbes.

Impacts of Host Individuality and Resilience on Dairy Production Traits

Where does resilience fit into the general concepts of how microbial communities behave? In what way does resilience of the community affect how we should be feeding cows? And how does it affect our ability to manipulate the ruminal community to improve animal performance?

The resilience of the rumen community appears to substantially exceed that of most other microbial habitats. Soil communities appear to recover only slowly from perturbation (Allison and Martiny, 2008) and anaerobic digesters are often subject to failure upon drastic changes in the type and rate of substrate loading (Chen et al., 2008). The ruminal community does not experience such failures, and as a result, our focus can shift to a more practical issue -- how community composition might affect, or even improve, animal performance. Resilience remains an important aspect of this relationship because conditions that perturb the community may affect performance, and recovery of performance may require re-establishment of the pre-disturbance community.

Feed efficiency

In beef cattle production, measurement of feed efficiency in the feedlot is relatively simple, owing to the continuous increase in weight over the grow-out period. Feed efficiency can be expressed as average daily gain per unit of DM intake. This can be a bit misleading because it does not necessarily reflect the variation in metabolic efficiency of different animals due to the effect of maintenance requirements, which varies with BW. An alternative way of expressing feed efficiency is residual feed intake (**RFI**), which is the difference between the amount of feed required to produce one unit of output in an individual animal versus that predicted from regression data obtained from a cohort of animals of the same age, fed the same diet, and housed under the same conditions (Koch et al., 1963). By this measure, steers with a positive RFI require more feed to produce the same weight gain (i.e., are less efficient), while those with a negative RFI require less feed to produce the same weight gain (i.e., are more efficient). The advantage of RFI is that it allows direct comparison of animals at the same level of

production. Five major physiological processes have been suggested to account for the variation in RFI among steers, 2 of which (digestibility and heat increment plus fermentation) have been suggested to account in aggregate for about 19% of the variation (Herd and Arthur, 2009).

In dairy cows, feed efficiency is more complicated because the output variable (energy corrected milk, **ECM**) is affected by the metabolic demands of pregnancy and by changes in body composition, particularly in the periparturient period. As a result, RFI changes continuously over the lactation cycle. Thus, when comparing feed efficiency among cows, it is important to obtain measurements at the same stage of lactation, preferably within the same range of days in milk (**DIM**). In contrast to beef cattle, there are no studies that have explicitly partitioned the relative contribution of different physiological processes to feed efficiency in dairy cows.

A key measure of feed efficiency in dairy cows, namely milk production efficiency (**MPE**, expressed as ECM/DMI) varies substantially among animals on the same diet at the same stage of lactation. RFI, which can also be used as a surrogate for feed efficiency in cows, is considered to be moderately heritable, although heritability (h^2) values have varied widely among studies (Connor, 2015). However, within cohorts of cows under the same management conditions, a substantial portion of the variation in RFI is not explained by genetics. Is some of this variation explained by inter-animal differences in their ruminal microbiomes? Two studies (Jami et al., 2014; Jewell et al., 2015) have shown that groups of cows divergent in MPE (as assessed by RFI) have different microbial communities. Substantial differences have been noted in the relative abundance of individual bacterial species (“operational taxonomic units”, or **OTU**, in microbial ecology parlance) between

high- and low-efficiency cows. Shabat et al. (2016) have further shown that the rumen fluid of cows of higher MPE contain a higher molar proportion of propionate, and elevated levels of 2 specific species, *Megasphaera elsdenii* and *Coproccoccus catus* (assessed by not only conventional 16S rRNA sequencing, but also a metagenomics analysis). However, both taxa represented only a tiny fraction of the bacterial community (<0.01%), casting some doubt on how they could have an outsized effect on the performance of the whole community. Further research aimed at establishing the potential relationships are clearly warranted.

In order to determine if microbial communities directly determine differences in MPE (rather than merely being associated with differences in MPE), we performed near-total exchange of ruminal contents between pairs of ruminally cannulated cows [using 3 pairs identified in the Jewell et al. (2015) study] that differed in DMI at the same level of ECM (Weimer et al., 2017). Detecting patterns of change in MPE following exchange was complicated by the general difficulty of accurately measuring MPE over short time periods, and by the fact that the cows were in different stages of lactation at the time of the contents exchange. Nevertheless, we did observe short-term trends in MPE following the exchange. For all 3 of the low-efficiency (LE) cows, MPE increased upon receipt of the ruminal contents of the high efficiency (HE) cows, and for 2 of the 3 HE cows, MPE decreased to a greater extent following receipt of the contents from the LE cows. Surprisingly, the other HE cow displayed an increase in MPE following receipt of the contents from her LE pair-mate. The effects on MPE were transient, however: by day 10 post-exchange, all the cows displayed MPE consistent with that expected had the exchange not taken place. Examination of BCC using next-generation sequencing revealed

that the BCC resembled that of the donor cow at the time of the exchange, but within ~10 days had returned toward that of the donor cow. This provides a further confirmation of community resilience, as well as more direct evidence of a microbial influence on MPE. However, it also points out that some cows may not have fully optimized their community composition, which may be amenable to manipulation.

The rumen microbial community has a high degree of species diversity, and as noted above, there is evidence from modeling studies that diversity has an unexpected destabilizing effect on the community. Diversity also seems to have a relationship with milk production efficiency. Both Shabat et al. (2016) and Weimer et al. (2017) have observed that species diversity is lower in cows that have a higher milk production efficiency. It can be argued that the high-efficiency communities are more “refined”, i.e., are less encumbered by low-abundance species that do not effectively contribute to the metabolic or energetic efficiency of the ruminal fermentation.

Milk composition

Milk composition, particularly the percentages of fat, protein and lactose, are major determinants of not only milk’s nutritional value, but also the price paid to producers. In most of the US, fat is the most valuable component. Fat levels below 3.2% in Holstein cows provide a common definition of milk fat depression (MFD), a costly condition that is often induced by certain dietary combinations. The primary mechanism for MFD is the ruminal accumulation, and translocation to the mammary gland, of certain unsaturated fatty acids, particularly *trans*-10, *cis*-12 linoleic acid, a potent repressor of milk fat synthesis. Because ruminal microbes are known to participate in isomerization and biohydrogenation of these

long-chain unsaturated acids, their involvement in MFD has long been suspected, but the role of specific taxa has remained elusive.

One intriguing aspect of MFD is that the fat test response to a given diet varies markedly among individual cows (Weimer et al., 2010b). When switched across TMR that contained corn oil but varied in the rate of starch fermentability and the presence of monensin, some cows showed no change in milk fat levels. Others showed MFD immediately upon substitution by the rapidly fermenting starch, while others displayed MFD only when the diet was further altered by inclusion of monensin. Moreover, after withdrawal of the monensin, some cows regained milk fat, while others remained fat depressed for several months. Examination of the bacterial communities using ARISA revealed shifts in BCC that were consistent with the fat test response: non-responding cows showed only slight shifts in BCC. Cows whose MFD was reversible showed a partial return of BCC to the pre-MFD community, while cows whose fat test remained low had BCC that were far removed from those of the original, pre-MFD BCC. Overall, the results indicate that cows vary in their resilience, or at least in the rate at which they recovered their milk fat production, and the rate at which their bacterial community ultimately returns the composition of the original, pre-disturbed community.

Recovery from diet-induced MFD has been investigated in more detail by Rico et al. (2014; 2015). Inoculation of MFD cows with ruminal contents from non-MFD cows did not improve overall fat yield, but did slightly accelerate recovery of de novo FA synthesis and normal ruminal FA biohydrogenation (Rico et al., 2014). In addition, recovery was accompanied by rapid changes (over a few days) in the relative abundance of particular taxa, in most cases to resemble their abundances prior to MFD induction (Rico et al., 2015).

What About Sick Cows?

Up to now, we have noted and documented the resilience of the ruminal community in healthy adult cows fed conventional diets. What about “dysbiotic” cows whose ruminal community has been compromised by illness (for example, metabolic disorders or a nutritional toxicosis) to the point that its function has been impaired? Can such cows restore their ruminal community composition on their own, or can producers or veterinarians assist in the restorative process?

In fact, the process of “transfaunation” (i.e., direct ruminal contents transfer from a healthy donor cow to a dysbiotic recipient) is widely practiced, and this topic has been recently reviewed (De Peters and George, 2014). Transfer of 8 to 16 L of rumen fluid from healthy cows on diets similar to that of the recipient sick animal has been recommended, although success may also hinge on prior partial removal, via stomach tube, of as much dysbiotic digesta as possible. This practice finds analogy to the currently faddish fecal microbiota transplants carried out to correct chronic intestinal dysbiosis in human subjects (Grehan et al., 2010). As pointed out by De Peters and George (2014), practical development of transfaunation methods in ruminants has outpaced our understanding of the mechanisms underlying its success. Clearly, transfaunation provides mechanical stimulation to a static (atonic) rumen, along with VFA and other nutrients to the dysbiotic host (which typically has gone off of feed and is thus likely to be metabolically stressed). Nevertheless, we can speculate on the nature of the transfaunation process from the standpoint of microbial ecology: the dysbiotic state is likely maintained by an unstable collection of ruminal microbes that interact ineffectively, resulting in poor metabolism of (and energy harvest from) feeds, and in ancillary disruptions

in host-microbe interactions (e.g., interkingdom signaling). This community can be supplanted, via transfaunation, by a more highly functional community whose members interact more effectively with each other (either by competition or cooperation) and with the host, and which may obtain a higher yield of energy (and thus faster and more complete microbial growth), with an eventual re-stabilization of host-microbe interactions.

Applying the Lessons of Host Individuality and Community Resilience to Ruminant Production

Implications for animal science research

The resilience of the ruminal community provides a lens through which both producers and consumers of animal science research can formulate and interpret animal feeding studies. Historically, feeding studies have been conducted with a view that adaptation of the rumen microbial community occurs by the time production and microbiological data are collected near the end of each time period within an experiment -- typically 14 to 8 days -- but the time periods selected have been based more on personal preference than on systematic analysis. We have observed that BCC stabilizes within the last few days of 28-day periods when dietary changes across period were modest (Weimer et al., 2010b). More recent studies (Machado et al., 2016) have revealed that in beef steers subjected to a switch from sugarcane to corn silage, adaptation of BCC at the phylum level, at least in the liquid phase of ruminal contents, was quite rapid (mean = 7.2 days, range = 3 to 9 days). Although finer-scale taxonomic measurements were not made in that study, it appears that the adaptation period of the community may generally be more rapid than previously suspected. Shorter experimental periods can greatly reduce the overall costs of

dairy trials and would allow experiments to be conducted over a narrower time range, thereby minimizing effects of stage of lactation.

A second consideration involves the common use of Latin squares for nutritional studies. Such designs are prized for their compactness (low animal numbers) and their statistical power, but they may not be appropriate for all studies. If a subset of cows within a study have microbial communities that display particularly strong inertia or poor resiliency, they could skew the results because their communities have not stabilized by the time the next dietary treatment is applied.

Modifying or redirecting microbial community composition

The potential of altering microbial community function through manipulation of its composition has long fascinated both animal scientists and rumen microbiologists. Several successes have been achieved in establishing inoculated strains (usually by direct dosing) to overcome nutritional toxicoses, such as poisoning by mimosine (Jones and Megaritty, 1986) or fluoroacetate (Gregg et al., 1998). By contrast, numerous attempts to improve fiber digestion or to decrease losses of feed to methane or ammonia, via inoculation of bacterial monocultures, have almost always resulted in failure, going all the way back the seminal work of Varel et al. (1995). Success appears to require the availability of an open niche that the inoculated strain can fill (Weimer, 1998). Ruminal contents exchange experiments (detailed above) have also resulted in only transient shifts in microbial community composition, apparently due to the lack of selective pressure to overcome a well-established indigenous community in mature host animals. This has led to proposals that early interventions (i.e., inoculating calves prior to weaning, or even at birth) may provide a means

of imprinting or directing the development of a unique and more functional community at maturity. These concepts, well-described by Yáñez-Ruiz et al. (2015), may hold some promise, but overcoming community inertia and resilience under any circumstance is not likely to be easy or straightforward (Figure 1).

Exploiting inter-animal variation in rumen microbial composition

Absent a clear pathway to overcoming rumen microbial community resilience to improve production, can we find a way to work variation in MCC among animals to our advantage? One strategy worth considering is using analysis of MCC as a tool to screen cows for predicted performance. Traits such as MPE are difficult to quantify, even under intensive testing (high-precision measurements over substantial time periods in tie stalls). If robust associations can be established between MPE (or the susceptibility to disorders, such as MFD or ruminal acidosis) and the abundance of specific taxa in easily collected samples (e.g., buccal swabs, Tapio et al., 2016), it would be possible to screen large numbers of animals and perhaps enable decisions of culling or group feeding that could improve overall productivity of a herd.

The relationship between resilience and functional redundancy

While rumen microbial community composition and its dynamics have received substantial attention of late, their importance must be kept in perspective. The community contains over a thousand OTU (a proxy for species), and their relative proportions vary greatly among cows. Yet, as pointed out by Taxis et al. (2015), the communities in different cows each work to convert a great variety of feeds to a remarkably similar suite of fermentation products that nourish the host. This is likely

due to the fact that there exists a relatively small number of “degradation points” (substrates and hydrolysable linkages in biopolymers) and a commonality of catabolic pathways that can be distributed among this large number of species (Weimer, 2015). Few studies have examined resilience at both the microbial community and metabolic functional level, but it appears that the two largely run in parallel (Machado et al, 2015).

Conclusions

Evidence is accumulating that important dairy production metrics, such as milk production efficiency and milk composition, are associated with specific microbial taxa, and thus might be of interest as targets for community composition manipulation. However, healthy adult dairy cows display considerable individuality in the species composition of their rumen microbiota, and these communities display strong resilience upon perturbation. This will make difficult any directed manipulation of community composition, except in cases where open niches are available for colonization. Nevertheless, basic studies of the mechanisms underlying resilience may yield strategies for future modification of these communities (e.g., interventions conducted prior to weaning). Additionally, community composition analysis may inform decisions on herd management, such as group feeding or culling. To guide further advances, it is important that community composition not be viewed in isolation but must be tied to community function and an appreciation for functional redundancy of the community.

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Table 1. Characteristics that describe the stability and adaptability of the ruminal microbial community.

Characteristic	Definition ¹	Likely status in the rumen
Inertia	Resistance to change	High, based on dosing studies
Resilience	Ability to restore its structure following acute or chronic disturbance	High, based on exchange studies
<i>Components of resilience:</i>		
Elasticity	Rapidity of restoration of a stable state following disturbance	Relatively high, based on exchange studies
Amplitude	Zone from which the system will return to a stable state	Very high, based on exchange studies
Hysteresis	Degree to which path of restoration is an exact reversal of path of degradation	Unknown
Malleability	Degree to which stable state established after disturbance differs from the original steady state	Low

¹Verbatim definitions of Westman (1978).

Table 2. Resilience of a rumen bacterial population following dietary change. Holstein heifers grazed orchardgrass pasture showed a shift in the abundance of *Butyrivibrio*, and the molar proportions of acetate and butyrate, when switched to orchardgrass hay; the effects were reversed when the heifers were returned to pasture. Heifers maintained on only pasture did not show these effects. Shifts in ruminal VFA profiles were consistent with observed shifts in *Butyrivibrio* abundance. Values are means from last 3 days of 28-day periods. Data from Mohammed et al. (2014).

Heifer	Diet ¹	Relative abundance of <i>Butyrivibrio</i> in period ²			Mol % Acetate in period			Mol % Butyrate in period		
		1	2	3	1	2	3	1	2	3
3274	PHP	15.3 ^a	6.9 ^b	10.8 ^{ab}	70.2 ^b	72.6 ^a	70.4 ^b	9.7 ^a	7.7 ^b	9.4 ^a
3292	PHP	13.5 ^a	6.1 ^b	10.2 ^a	63.8 ^b	71.7 ^a	67.7 ^b	11.1 ^a	7.9 ^b	10.5 ^a
3295	PHP	10.9 ^a	6.4 ^b	11.1 ^a	66.3 ^b	72.3 ^a	70.8 ^{ab}	11.6 ^a	7.7 ^b	9.4 ^{ab}
3298	PPP	13.6	14.8	15.7	69.1	70.9	70.4	10.6	9.6	9.8
3412	PPP	19.6 ^a	18.6 ^a	13.7 ^b	69.5	71.0	70.1	10.1	9.2	9.1

¹PHP=Heifers switched from pasture (period 1) to hay (period 2) then back to pasture (period 3).
 PPP=Heifers maintained on pasture throughout all 3 periods.

²Percent of 16S rRNA gene reads from next-generation sequencing (Roche 454). Data are averaged for liquid- and solids-associated communities, which were analyzed separately.

^{a,b}Different letters between periods within heifer differ ($P < 0.05$).

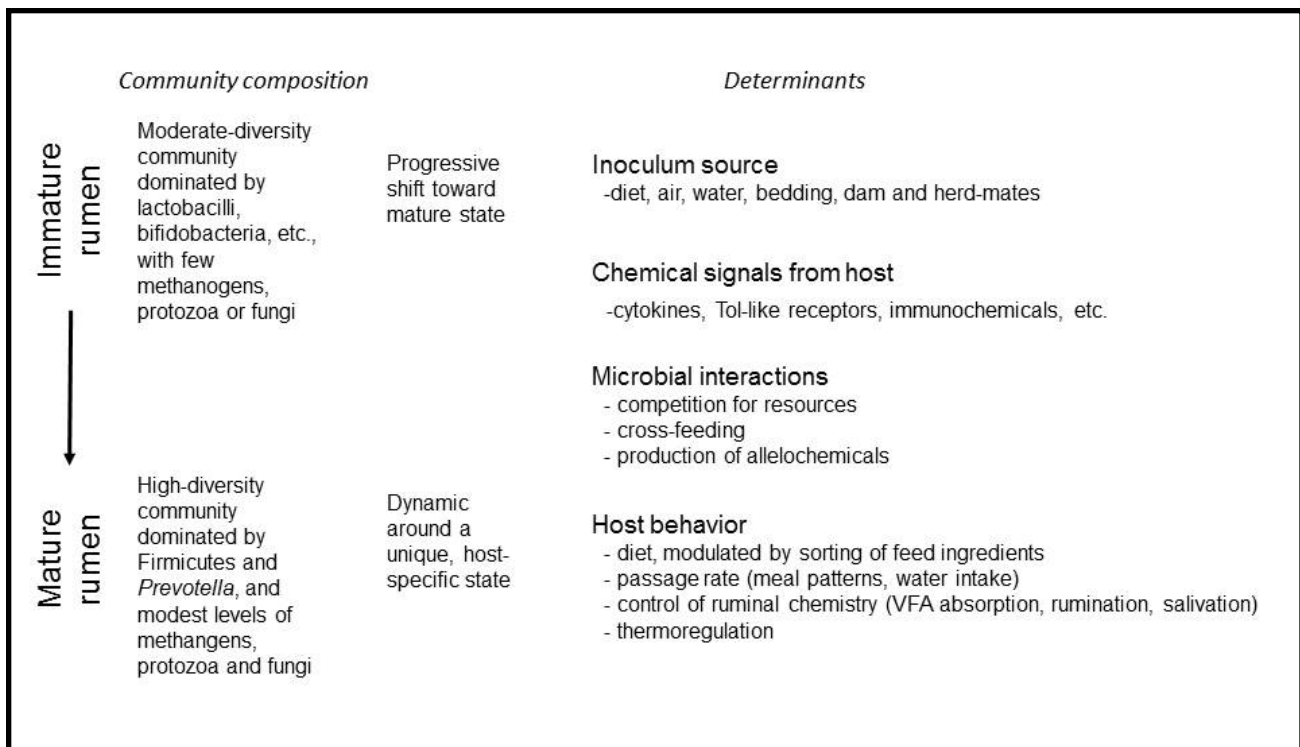


Figure 1. Comparison of the microbial community in the immature and mature rumen, along with factors that determine community composition. As the rumen matures, exogenous inoculation has less influence, and the adult community is shaped, and likely maintained, by a combination of microbial interactions and host behavioral adaptations.

How Much Supplemental Vitamins do Cows Really Need?

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Summary

Because of major production problems, vitamin A and to a lesser extent vitamin E are in very limited supply and prices have increased markedly. Because of price and scarcity, many nutritionists are re-evaluating vitamin supplementation strategies. Based on current information, the NRC (2001) requirements for vitamin A (approximately 75,000 IU/day for all cows) and vitamin E (500 IU/day for lactating cows and 1000 IU/day for dry cows) are adequate. However, feeding an additional 1000 IU of vitamin E per day during the prefresh period (2 or 3 weeks prepartum) can improve cow health post partum. More data are needed but limited information suggest that for lactating cows, supplementation rates for vitamin D should be increased to about 1.5 X NRC (about 30,000 IU/day). Because vitamin A is in very limited supply, supplementation may need to be prioritized. Because of low expected intakes of basal β -carotene and the high requirement of vitamin A for colostrum synthesis, prefresh cows should be fed at NRC rates at the expense of other cows. Next highest priority is far-off dry cows, followed by lactating cows. Some supplemental vitamin A should be provided to all types of cows if possible; however, if necessary, the liver can supply adequate vitamin for several weeks, and perhaps up to a few months, without adversely affecting lactating cow health or productivity.

Introduction

Historically, most nutritionists have given little consideration to the cost of vitamins A, D, and E. Cows needed them and even at high supplementation rates, cost per cow per day was reasonable. However because of a fire at a chemical factory in late 2017, worldwide production of feed grade vitamin A has been reduced by more than 40%. Production of vitamin E has also been reduced because an intermediate that was produced at the factory with the fire cannot be produced right now. Because of other production issues, vitamin D supply is also tighter than normal. These production problems have led to major increases in vitamin prices. Compared to historic norms, vitamin A price at wholesale level has increased about 10 times (local and spot markets may differ markedly), vitamin E price has increased 3 to 4 times, and vitamin D price has increased less than 2X. Approximate cost of supplementing vitamins A, D, and E at NRC recommended levels would cost about 10 to 12 cents per day (there will be a very wide range on this value because of margins and local markets). Using historically typical prices, it costs 3 or 4 cents per day to provide supplemental vitamins A, D, and E. Although this is a very substantial increase in cost, it is still a very small portion of the total feed bill (about 3% of total feed costs). A bigger problem than increased cost is limited supply. In some markets, vitamin A simply is

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not available at any price or supplies are being rationed. This paper will review current research and recommendations regarding vitamins A, D, and E and strategies to use when supplies are inadequate.

Vitamin A

The common form of supplemental vitamin A is all-trans retinyl palmitate with some retinyl acetate also being used. Based on current standards, 1000 IU of vitamin A is equal to 0.55 mg of retinyl palmitate or 0.35 mg of retinyl acetate. Based on a survey of nutritionists we conducted about 20 years ago (Weiss, 1998), average supplementation rates ranged from 100,000 to 150,000 IU/day depending on the type of cow. On a mass basis, that is only about 80 mg/day of supplemental vitamin. The current NRC recommendation for supplemental vitamin A (all NRC 2001 vitamin recommendations are for supplemental, not total vitamins) is 50 IU/lb of body weight (**BW**) or about 75,000 IU/day for an average Holstein cow (Table 1). That recommendation is for all classes of dairy cattle. Although vitamin A is not an active area of research, there is little data indicating that the NRC (2001) recommendation is inadequate for lactating cows fed a typical diet. A recent study evaluated feeding 2X NRC (95 IU/lb BW) and reported some small increases in various measures of immune function, but no effects on production and clinical responses (e.g., mastitis) were measured (Jin et al., 2014).

The NRC recommendation is based on several assumptions:

1. The diet is approximately 60% forage,
2. The cow is consuming little or no fresh forage,
3. Milk yield is approximately 75 lb/day, and
4. Basal diet provides typical amounts of β -carotene.

The conditions stated above either effect vitamin A supply or vitamin A requirements. Based on in vitro rumen studies (Rode et al., 1990; Weiss et al., 1995), a substantial amount of vitamin A is destroyed in the rumen and destruction rate increases with the amount of concentrate in the diet (in those studies the concentrate was predominantly starch-based). In vitro ruminal destruction of vitamin A was 20 to 25% when the substrate was 90 to 100% forage and 70 to 75% was destroyed with diets containing 50 or 30% forage, respectively. In studies evaluating responses to vitamin A, diets were around 60% forage; ruminal destruction was assumed to be about 50% via extrapolation. Therefore, a diet with 50% forage may need about 17% more vitamin A (~84,000 IU/day) than recommended by NRC (2001); however, a cow fed an 80% forage diet may need only 0.65 X NRC requirements (~47,000 IU/day).

Higher forage diets are also typically higher in β -carotene which can be converted into vitamin A by the cow. Once a forage plant is cut, β -carotene starts being oxidized (destroyed). Losses during silage making can be greater than 50%, and for hay, losses can exceed 80% as compared to fresh forage (Noziere et al., 2006). Corn silage is a poor source of β -carotene and usually has about 50% of the concentration found in haycrop silages. However since most of the experiments evaluating responses to vitamin A consisted of corn silage, this effect is already incorporated into requirements. Most concentrates are poor sources of β -carotene. Straw, a common ingredient for dry cows, has virtually no β -carotene. The take home from this is:

- Cows that are grazing fresh, green forage with pasture providing at least 40% of diet DM probably need very little, if any, supplemental vitamin A because pasture is probably providing 70,000 to 100,000 more

IU/day of vitamin A equivalents than a cow consuming silage.

- Hay-based diets will need more supplemental vitamin A than silage based diets. If you change from a diet in which the forage was 50% hay and 50% silage (similar to many of the studies) to a diet with all the forage as hay, intake of basal vitamin A equivalents would be reduced by 15,000 IU/day. Most diets in the Midwest do not have that much hay so the adjustment will be smaller.
- Straw-based dry cow diets will require more supplemental vitamin A than hay or silage based diets. Replacing 8 lb of haycrop silage DM with straw will reduce intake of basal vitamin A equivalents by about 45,000 IU/day. You probably do not need to increase supplementation that much because efficiency of conversion of β -carotene to vitamin A is likely lower than anticipated, but a substantial increase in supplementation is likely necessary with straw based diets

Milk contains about 7 mg of retinol/kg of fat or about 0.11 mg/lb of milk (assumed 3.7% fat). The average milk yield by cows in studies evaluating responses to vitamin A was about 75 lbs/day. Therefore, the current NRC recommendation should be adequate for cows producing 75 lb of milk. For every additional pound of milk above 75 lb, vitamin A supplementation should be increased by about 450 IU to cover losses in milk (for a Jersey cow it would be about 580 IU/lb of milk). In a pen situation, if the average cow is milking 75 lb and needs 75,000 IU of vitamin A, the diet needs to contain about 1400 IU/lb of DM. If a cow was milking 100 lb, she would need an additional 11,000 IU of vitamin A to cover milk losses but because she would be expected to eat about 10 lb more DM, her intake of A would be adequate. In other words, the concentration of vitamin A (IU/lb of DM)

does not have to be increased for high producing cows).

Substantial amounts of feed grade vitamin A (retinyl palmitate) can be destroyed during storage and this potential loss should be considered when developing formulating strategies. If vitamin A is blended in a premix with inorganic zinc and copper, vitamin A activity decreased by about 9% per month (compared to about 3% for other vitamins) (Shurson et al., 2011). Pelletting and excess heat, humidity and sun exposure during storage will greatly increase losses in activity. If feed mixes are stored for long periods of time, especially if it contains inorganic trace metal or is stored under poor conditions, supplementation should be increased to cover losses in activity.

How Low Can You Go

Cows are efficient storers of retinol when fed in excess or when large amounts are injected. Excess retinol is stored in the liver and liver retinol concentrations are a good indicator of status. It changes rapidly (days to weeks) in response to changes in supply. Hepatic retinol concentrations less than 30 mg/kg (dry basis; all liver concentrations in this paper are on a mg/kg dry weight basis) is considered indicative of a vitamin A deficiency and values less than about 100 mg/kg are considered suboptimal. Beef cattle fed a high concentrate diet (so ruminal destruction of vitamin A was likely high) and approximately 40 or 80 IU of vitamin A/lb of BW for 140 days (Figure 1) had liver concentrations ranging from about 500 mg/kg dry weight to more than 800 mg/kg (Bryant et al., 2010). It is very likely that dairy cows fed ~100,000 IU/day of vitamin A probably have liver concentrations in excess of 400 mg/kg. Liver retinol concentrations in beef heifers and steers fed no supplemental vitamin A and a basal diet void of β -carotene diet (Figure 2) dropped

from about 474 mg/kg (dry basis) to 210 mg/kg over 84 days (Alosilla et al., 2007). If fed the same diet, depletion will occur more rapidly in a dairy cow than a beef animal because of secretion of retinol in milk; however, typical dairy cow diets contain more β -carotene than feedlot diets. Liver depletion rates have not been determined in lactating cows fed typical diets, but based on beef data, liver retinol will remain in the adequate range for several weeks to a few months when all supplemental vitamin A is removed from the diet. I am not advocating removing all supplemental vitamin A from lactating cow diets; however, feeding no supplemental vitamin A for a month or so likely will have no negative impacts.

Dry cows and Prefresh Cows

The 2001 NRC has the same supplemental vitamin A requirements for all dairy cattle (50 IU/lb BW) and data generally support that. However, with the widespread application of straw-based dry cows diets (i.e., low β -carotene diets), increased supplemental vitamin A may be warranted (discussed above). Independent of basal diet, the prefresh cow may need increased vitamin A supplementation. As with vitamin E, plasma concentrations of retinol and β -carotene drop markedly starting about 2 weeks prepartum, even when cows are fed diets adequate in supplemental vitamin A (Goff and Stabel, 1990; Weiss et al., 1994). What is unusual is that plasma retinol concentration is a very poor indicator of vitamin A status or vitamin A intake. When fed deficient diets, animals mobilize retinol from the liver and plasma levels are maintained until liver concentration drops below about 30 mg/kg (clinical deficient state). But in the prepartum dairy cow, plasma concentrations decrease even though the liver likely has more than adequate stores. The decrease in plasma retinol is caused entirely by secretion of retinol into colostrum starting about 7 days before calving because

mastectomized cows experienced no decrease in serum retinol at calving (Goff et al., 2002). It is not known whether additional vitamin A during the prefresh period will prevent the decrease in plasma vitamin A or whether the decrease is even a problem. However, when supplemental vitamin E is added and the decrease in plasma tocopherol is prevented, improved mammary gland health is observed.

Prioritizing When Vitamin A Supplies are Limited

If vitamin A supplies are limited or price is a major factor, the first step is to feed supplemental vitamin A at NRC recommendations. Based on survey data, this will reduce supplementation by about 50% on average. If additional cuts are needed, the dry cow and prefresh cow should be fed at NRC levels if possible. They have low intakes of basal β -carotene, several studies have shown increased retained placenta and mastitis when dry cows are not fed adequate vitamin A, and the newborn calf will need retinol-rich colostrum since calves are born with almost no circulating retinol. The last priority is lactating cows. Intakes are very high and the basal diet generally has substantial β -carotene (all hay diets are an exception). In addition, most cows are probably in excellent vitamin A status (large liver stores of retinol), and it is acceptable for the cow to mobilize that as long as liver concentrations of retinol stay above 30 mg/kg and ideally above about 100 mg/kg.

Vitamin E

The standard form of supplemental vitamin E used in the feed industry is all-rac α -tocopheryl acetate. By definition, 1 IU of vitamin E equals 1 mg of all-rac α -tocopheryl acetate. Based largely on reduction in incidence of mastitis and retained placenta, the 2001 NRC

set the supplemental vitamin E requirement at 0.36 IU/lb BW for lactating cows and 0.73 IU/lb BW for dry cows. This equates to about 500 IU/day for lactating cows and 1000 IU/day for dry cows (Table 1). Basal diets can provide substantial amounts of tocopherol, but the same factors that affect β -carotene concentrations (discussed above) affect tocopherol concentrations. Diets used in the studies evaluating supplemental vitamin E were largely hay-based for dry cows and silage based for lactating cows. The only major adjustment to vitamin E supplementation needed because of basal diet is for grazing cows. Fresh pasture can have 2 to 10 times more tocopherol than silage or hay (respectively), and plasma concentration of tocopherol in grazing cattle (with no vitamin E supplementation) is usually much higher than what we observe in confinement cattle fed supplemental vitamin E per NRC. If the diet is composed of 50% or more of pasture DM, no supplemental vitamin E is needed. Based on average tocopherol concentrations in fresh pasture and corn silage and alfalfa silage and assuming pasture replaces silages, a diet with about 30% fresh pasture (DM basis) will need about 50% of NRC supplementation. Another type of basal diet that needs to be considered with respect to vitamin E supplementation is straw-based dry cow diets. Straw is essentially void of tocopherol, but it often replaces hay which is low in tocopherol. If 8 lb of straw replaced 8 lb of hay, basal intake of tocopherol likely did not decrease very much. However, if the straw replaced hay silage, intake of basal tocopherol could decrease by 100 to 150 IU/day.

Current data support the 2001 NRC requirement for dry and lactating cows. One study suggested that excess vitamin E during the dry period (3X NRC) may actually be detrimental to cow health (Bouwstra et al., 2010). Since 2001, several studies have evaluated the effect of additional vitamin E during the prefresh

period, and in general, positive response on immune function or clinical measures have been reported (Politis et al., 2001; Politis et al., 2004; Chandra et al., 2014). Supplementation rates during the last 2 to 3 weeks of gestation ranged from 2000 to 4000 IU/day. Because of cost, providing prefresh cows (not grazing) with about 2000 IU/day will likely improve immune function and cow health.

Vitamin E supplies have been reduced and prices have increased 3 to 4 times over historic prices, but true shortages have not been reported. Considering the benefits of adequate vitamin E relative to its cost, NRC supplementation rates should be maintained, and if a prefresh diet is fed, consider increasing vitamin E to 2000 IU/day.

Vitamin D

The primary form of supplemental vitamin D fed to livestock is vitamin D3. Vitamin D2 may be available, but it is vastly inferior to D3 and probably should not be fed. If it is used, supplementation rates should be about double those for vitamin D3. For this paper, recommendations are appropriate for vitamin D3. Cows and other animals can synthesize vitamin D when the skin obtains adequate UV irradiation from the sun. The amount of vitamin D synthesized depends on intensity of the sunlight which depends on season (summer >> winter) and time of day (noon > morning or evening), cloud cover, and duration of exposure. Cows exposed to 90 minutes of intense sun (centered around noon) maintained serum concentrations of 25-OH vitamin D in the adequate range (Hymoller and Jensen, 2012). Based on human synthesis rates, cows in winter in the tristate area cannot synthesize adequate vitamin D, regardless of how long they are outside, and during spring and fall may need more than 5 hours of sun exposure to synthesize adequate vitamin D.

After decades of almost no research on vitamin D for dairy cows, it is starting to receive substantial interest. This is probably caused by the data showing relationships between low vitamin D status and increased risk for numerous diseases in humans. Previously, vitamin D was considered only with respect to calcium metabolism and current requirements (14 IU/lb of BW or about 20,000 IU/day; Table 1) are adequate to maintain normal calcium metabolism. New data suggests a role of vitamin D in immune function and more general health responses (Lippolis, 2011) and supplementation rates may need to be higher to see this responses. Based more on data from human subjects than cattle, blood concentrations of 25-OH vitamin D (an excellent status indicator of vitamin D) below 30 ng/ml are associated with increased health problems. Concentrations of 8 to 10 ng/ml are probably adequate for Ca metabolism. From a survey of commercial and university dairy herds, feeding 30,000 to 50,000 IU/day (1.5 to 2.5 X current NRC recommendation) maintained serum 25-OH vitamin D well above 30 ng/ml. However, one herd was fed 20,000 IU/day (i.e., NRC requirement) and although the blood average was above 30 ng/ml, several individual cows had concentrations less than 30 ng/ml. This suggests that feeding 20,000 IU/day may not be adequate; however, data showing improved clinical or production responses with additional vitamin D supplementation are lacking. Based on the limited data available, supplementation rates of 1.5 X NRC are justified (i.e., about 30,000 IU/day for lactating cows). Because calcium metabolism is so important to transition cows, at this time, feeding at NRC (2001) rate is recommended.

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Table 1. Recommended daily intakes (IU/day) of supplemental vitamins A, D, and E for a Holstein cow (multiply values by 0.75 for Jersey cows).

Vitamin	Type of Cow			Adjustments
	Far-off Dry	Prefresh	Lactating	
A	75,000	75,000	75,000	<ul style="list-style-type: none"> • Increase when feeding straw-based diets and consider increasing when feeding hay-based diets. • For grazing cattle, these should be reduced substantially (sometimes to 0). • Prefresh cows may benefit from higher intakes because of colostrum synthesis • For lactating cows producing more than 75 lb of milk, increase by 450 IU/day per pound of milk greater than 75 lb
D	20,000	20,000	30,000	<ul style="list-style-type: none"> • Cows grazing at least 2 hours per day in the summer probably do not need supplemental D. • Increase substantially if using vitamin D2.
E	1,000	2,000	500	<ul style="list-style-type: none"> • Increase by about 100 IU/day with straw based diets. • Hay based diets may need slightly more vitamin E. • For grazing cows, reduce supplementation substantially (sometimes to 0)

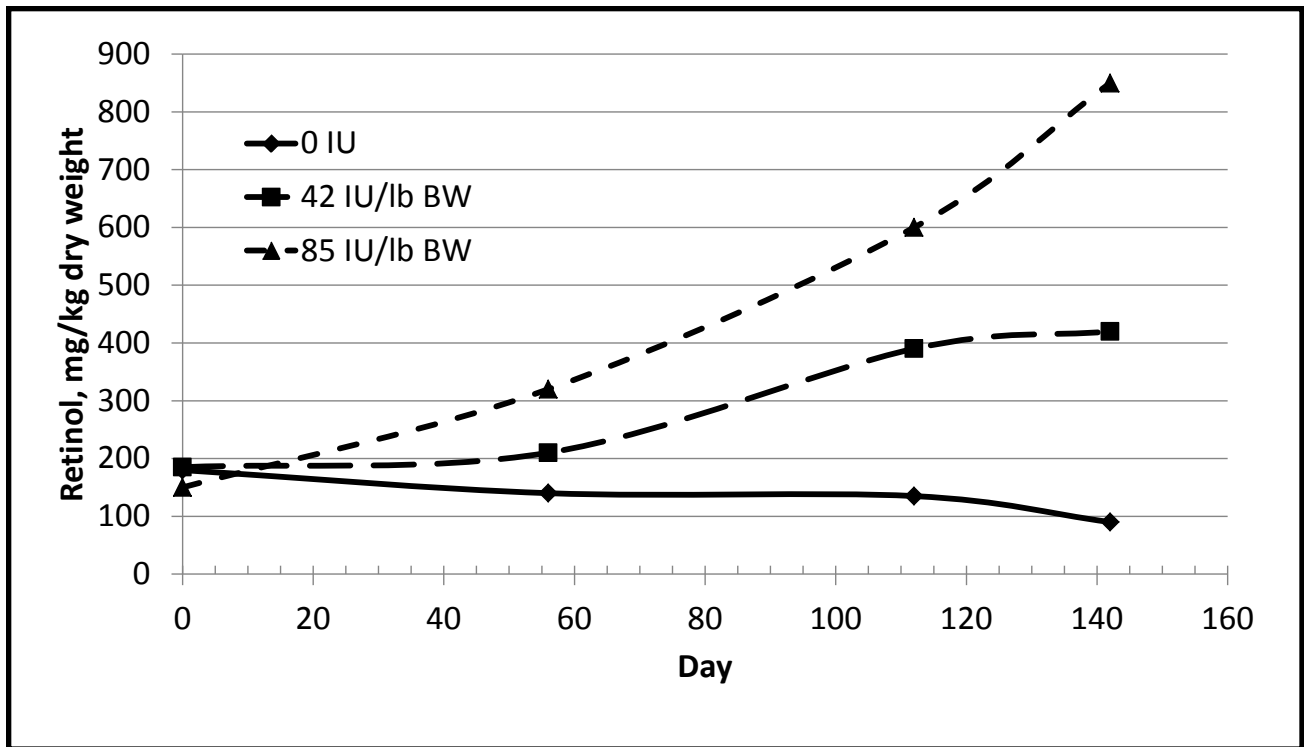


Figure 1. Concentrations of retinol (vitamin A) in liver of beef steers that were fed no supplemental vitamin A or approximately 40 or 80 IU/lb of BW (the NRC requirement for dairy cattle is 50 IU/lb BW). The basal diet likely provided some β -carotene (not measured). The black arrow marks the clinical deficient concentration and the grey arrow indicates marginal deficiency (Bryant et al., 2010).

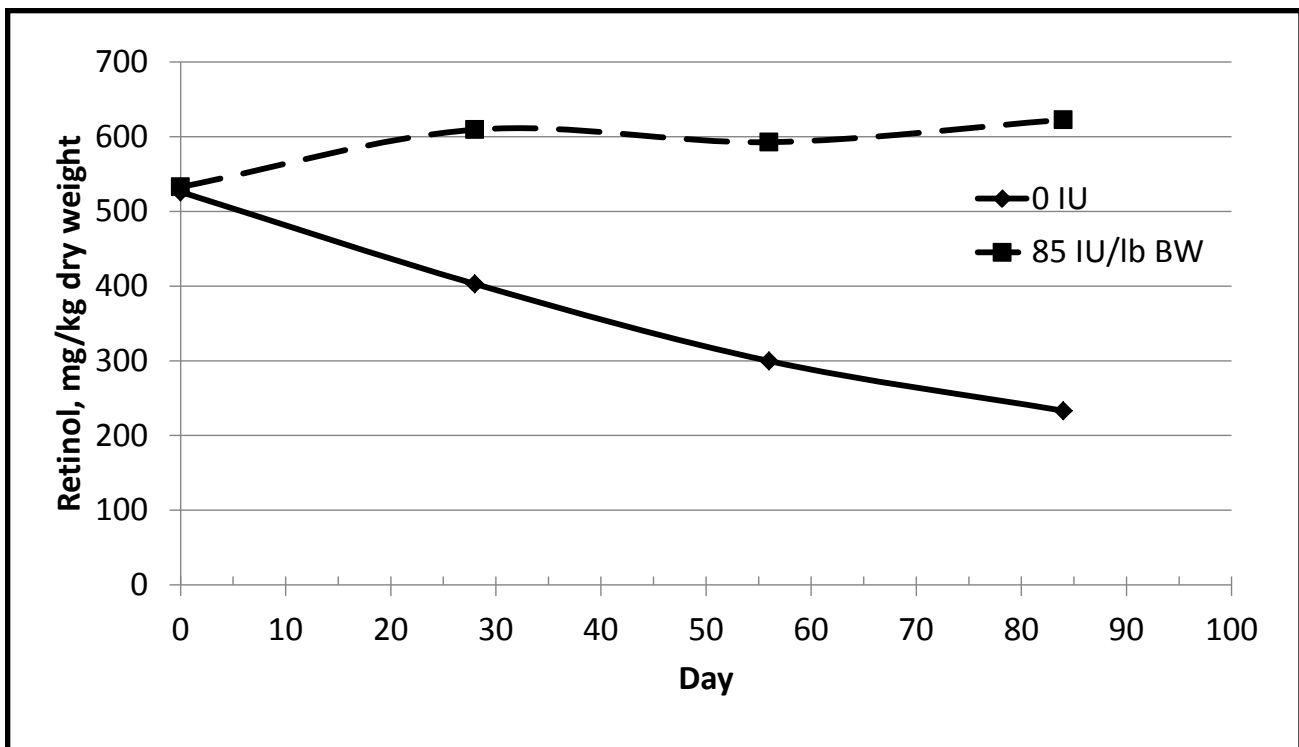


Figure 2. Concentrations of retinol (vitamin A) in liver of growing beef steers and heifers fed no supplemental vitamin A or approximately 85 IU/lb of BW (1.7 X NRC requirement for dairy cows). The basal diet likely provided no β -carotene. The black arrow marks the clinical deficient concentration and the grey arrow indicates marginal deficiency (Alosilla et al., 2007).

Feeding the Rumen to Maximize Milk Components

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Abstract

Current milk prices should cause reflection on methods to improve milk component production with minimal increase in feed costs. Improved milk components in this market still have positive return on investment. The market pressure provides an opportunity for dairy producers and nutritionists to review basic cow and diet management, and how these play into rumen fermentative efficiency. Rumen microbes are central to biohydrogenation induced milk fat depression – both in causing and preventing it. Improving fiber digestibility not only reduces risk to milk fat depression but will increase milk fat synthesis by the mammary gland from acetate and butyrate absorption. General mechanisms and relationships within the rumen are reviewed, as well as the influence of calf management, diet delivery, feeding frequency, and feed shrink on the rumen microbial population.

Introduction

We are all acutely aware of the current milk market challenges imposed on the dairy industry. Increased global production and weak exports from a strong U.S. dollar paired with gains in domestic supply have put strong downward pressure on farm-gate milk prices. With feed responsible for an excess of 50% of dairy production costs, now is a good time to reflect on the basics of ruminant nutrition

as well as recent research to refine nutrient formulation in order to lend value on the farm. Previously at this conference, St-Pierre and Weiss (2012) demonstrated the clear value of producing additional pounds of milk protein or fat by dilution of feed cost beyond maintenance. Other solids in milk are correlated with milk volume and contribute negligible value to monthly milk value. Water in the milk does not contribute to farm productivity and necessitates discussion on improvement in milk component yield over growing volume. Increasing milk volume without gains in components likely has a negative return on nutrient investment through much of 2018, whereas gains in milk fat or protein still pay dividends. One can easily calculate the value of component production between 2 herds typical of what we may see in this region (Table 1), with improved profitability in the higher component herd milking lesser volume (Table 2) (USDA, 2018).

A shift in consumer perspective towards reduced demonization of saturated fat dairy products has boosted butterfat value, while dairy products based in protein (cheese and nonfat dry milk) are in abundance and abnormally hamper current milk protein component value. As such, my current focus is in how to improve milk fat yield rather than milk protein via the rumen. Many factors contribute to overall farm profitability in today's down market, including calf rearing efficacy, replacement

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heifer development, reproductive efficiency, forage quality, feed ingredient value (nutrient deliverables for purchase price), feed storage shrink, and consistency of total mixed ration (TMR) delivered to the cow. Just as all roads “lead to Rome”, many of these considerations converge in the rumen and influence the microbial efficiency with which the dairy cow takes feed and converts it to volatile fatty acids (VFA) and metabolizable protein (MP) that serve as precursors for milk component biosynthesis.

Rumen Contribution to Milk Fat Synthesis

Milk fat within the mammary gland stems from 2 energy sources: long chain fatty acids (LCFA) that are provided from dietary intake of LCFA or mobilization of adipose stores of LCFA and also from rumen-derived acetate and butyrate. In the udder, LCFA and de novo synthesized short and medium chain FA are attached to glycerol to form milk triglycerides (Palmquist, 2006). While butyrate is considered important for gut health and rumen epithelia (Baldwin, 1999; Guilloteau et al., 2010; Laarman et al., 2013), it plays a smaller role in de novo fatty acid synthesis – estimated around 8% of VFA (Palmquist, 2006). This leaves the bulk of the responsibility for milk fat synthesis to acetate which is traditionally associated with fiber digestion in the rumen (Murphy et al., 1982) and recently demonstrated to directly increase milk fat synthesis (Urritia and Harvatine, 2017). In diets with low forage, variability in VFA composition can account for a large proportion of variation in milk composition (Sutton, 1989). Thus, while there are diverse pathways for substrate fermentation and conversion in the rumen, it is logical that improved fiber digestibility is a substantial target to increase milk fat yield.

What makes ruminants unique is their capacity to consume human indigestible cellulose- and hemicellulose-based forages and through rumen microbial symbiosis generate VFA as a primary energy source for the ruminant. The rumen is essentially a giant fermentation vat where the nutrients consumed by the cow are broken down at varying rates influenced by intake levels, chewing patterns, rumen buffering, microbial populations, and chemical structures. Unsaturated fatty acids have toxicity towards rumen microbes and highly degradable carbohydrates can lead to rapid fermentation and quickly increase acidity in the rumen. We have learned that some unsaturated fatty acids are more toxic to microbes than others. There is a hierarchy of microbes who biohydrogenate unsaturated fatty acids to whatever saturation level removes the risk of toxicity for that particular species (Jenkins et al., 2008). As fatty acids are biohydrogenated down particular pathways, the process can bottleneck if biohydrogenation specialist bacteria become inhibited by rumen conditions or if supply of unsaturated fatty acids exceeds these species’s capacity to biohydrogenate (Jenkins et al., 2008). Specific biohydrogenation intermediates have been identified as key indicators of milk fat depression, with trans-10, cis-12 C18:2 conjugated linoleic acid (CLA) known as a potent inhibitor of milk fat synthesis (Bauman and Griinari, 2003; Urrutia and Harvatine, 2017). Biohydrogenation-induced milk fat depression (MFD) should be considered separately from opportunities to increase milk fat synthesis in generally low-producing cows. To better understand the role of the rumen in supporting milk fat production, we must understand a bit about the diversity of the rumen microbiome.

Diversity Within the Rumen

The rumen hosts a vast consortium of microbial species in 3 main categories:

bacteria, protozoa, and fungi. Bacteria can be further classified by their function (fiber, starch, protein, and sugar digesters). Most microbes have a competitive niche to fill that justifies their survival, either specializing in a specific substrate or generalizing to permit flexibility between substrates (Hungate, 1966; Dehority, 2003). Culturing rumen microbes is fairly difficult – such that we can put a man on the moon, but 50 years later, we still have limited knowledge of the roles of many species within a cow’s stomach. Cultured bacterial species to date represent less than 7% of the genetic sequences recovered in the rumen, indicating the potential for several thousand uncharacterized species in the rumen (Kim et al., 2011). What we do know is that **diet** is the primary determinant of rumen microbial populations more than host species, with 30 top microbial groups represented in 90% of species across the globe (Henderson et al., 2015).

Generally speaking, cellulolytic bacteria are gram-positive and either mediate attachment to fiber or adhere purely based on local cation concentration (Dehority, 2003). Bacteria transition among fiber particles, first inoculating and then multiplying enzymatic degradation of fiber within the rumen. The gap between first inoculation and full degradation is commonly called “lag phase” and is modeled by *in vitro* lab digestibility assays. Some *Butyrivibrio* species have also been associated with fiber digestion (Dehority, 2003; Hackmann and Firkins, 2015a), as well as credited a key role in the final stage of biohydrogenation (Jenkins et al., 2008). Most cellulolytics are involved in biohydrogenation to some extent. Starch-digesting bacteria might be best exemplified by the infamous *Streptococcus bovis* (Russell, 2002) known for rapid fermentation in unadjusted cattle that leads to a downward spiral in clinical acidosis. Typically, starch digesters ferment readily fermentable substrates more rapidly at a lower

return of growth for substrate digested (i.e., they are less efficient). Starch-fermenting species fit in the balance of an adapted rumen on high starch inclusions for both beef and dairy cattle, but rapid transition away from forage towards a high starch diet shifts the rumen microbiome (Petri et al., 2013). Protein degraders in the rumen are a significant contribution to amino acid breakdown, and the rumen is also full of generalists with agile metabolisms capable of involvement in a buffet of substrates (Russell, 2002).

Rumen protozoa are commonly associated with consumption of bacteria, leading to degradation and deamination, contributing to inefficiencies in microbial utilization of ruminal degradable protein (**RDP**) and the waste of amino acids and peptides (Newbold et al., 2015). Often forgotten is protozoal contribution to rumen buffering and fiber digestibility (Newbold et al., 2015) by rapid consumption of starch and subsequent internal sequestration of it as glycogen (Denton et al., 2015), in essence pulling it from circulation and preventing rapid declines in rumen pH by opportunistic starch digesters, such as *S. bovis*. Protozoa are sensitive to pH (Dehority, 2005) and migrate the rumen in search of nutrients (Dehority, 2003); *in vitro* work has shown protozoa align cell division in response to feeding patterns (Sylvester et al., 2009).

Fungi are the most likely to be an underappreciated species in the rumen microbiome, mostly because they have been least studied. Rumen fungi have complex cellulolytic machinery akin to protozoa, and once embedded in lignified fiber, they can fracture it apart (Russell, 2002). Fungal digestive action on fiber that is resistant to degradation opens up surface area for cellulolytic bacteria, but it is a thankless task; bacteria release antifungal secretions (Russell, 2002). Fungi have

been implicated in biohydrogenation within the rumen, but their rate is such that they are likely a minor player (Nam and Garnsworthy, 2007).

Nutrient Contribution of Rumen Microbes

With the advent of bypass protein feed ingredients and rumen-protected amino acids (AA) inspired by limiting AA supplementation success of non-ruminant feeding operations, less attention has been paid to the value of microbial protein to the ruminant. Yet, commercial models in the industry still attribute microbial protein contribution to MP to be approximately 45 to 55% in a typical lactating diet (Sok et al., 2017). Rumen bacteria and protozoa have differential amino acid concentrations, with protozoa possessing 45% more lysine and 17% more isoleucine (Sok et al., 2017). However, protozoal contribution to total microbial biomass is in doubt and certainly does not approach early estimates of 50% (Fessenden, 2016; Wenner et al., 2017). Particle-associated bacteria also appear to differ in AA composition compared with fluid-associated bacteria, with 7% more leucine, 8% more phenylalanine, and 6% less threonine (Sok et al., 2017). Dry matter intake drives microbial protein production because increased passage rates force microbes to grow faster and greater microbial growth rates increase microbial protein outflow to the omasum (Dijkstra et al., 1998; Firkins et al., 2007). Small increases in microbial outflow in a lactating dairy cow can have significant effects on downstream AA supply (Table 3). For example, based on an estimated microbial N daily flow of 325 g (Hristov, 2007), an increase of only 3% microbial N flow to the duodenum would increase lysine flow by 5 g/day – an equivalent savings of \$0.08/cow/day in synthetic lysine supplementation.

Rumen microbes also contribute fatty acids to the ruminant. Bacteria range from 5 to

15% fatty acids on a DM basis (Vlaeminck et al., 2006a), and fatty acids are primarily associated with microbial membranes. Vlaeminck et al. (2006b) demonstrated large shifts in fatty acid composition of rumen bacteria when fed decreasing quantity of forage in the diet, but the primary 2 fatty acids, palmitic and stearic, remained fairly constant. Stearic acid is the primary fatty acid in bacteria, while protozoa more heavily favor palmitic (Harfoot and Hazlewood, 1997). Because microbial lipid composition is largely influenced by dietary conditions and cellulolytics have characteristic odd-chain fatty acids compared to non-cellulolytic bacteria, microbial-specific fatty acids that are incorporated into milk triglycerides can be an effective indicator of rumen fiber digesting activity with a detailed milk fatty acid analysis (Fievez et al., 2012). Ruminal contributions of both AA and fatty acids to ruminant absorption can be significant and are typically attained at much smaller cost than a purchased supplement. Thus, it is apparent that maximizing rumen microbial growth adds value to a dairy producer's bottom line.

Microbial Response to pH

Protozoa are most notoriously sensitive to rumen pH; protozoal viability declined sharply in vitro when culture pH was allowed to drop below 5.6 (Dehority, 2005). Rumen cellulolytics can also be generalized as pH sensitive, both decreased in cell quantities by low pH (Petri et al., 2013) and also decreased in cellulolytic activity with pH dropping below optimum levels for attachment, cellulase function, and cell growth (Russell, 2002). Declining pH has lesser effects on cellulolytic cell numbers but decreases fiber digestibility until pH returns to more desirable levels (Russell, 2002), where fiber digestibility has been shown to compensate for periods of low pH (Calsamiglia et al., 2002; Cerrato-Sanchez et al., 2008; Wenner et al.,

2017). If pH remains too low, then digestibility will suffer (Calsamiglia et al., 2002; Cerrato-Sanchez et al., 2008) as microbial populations are shifted (Fuentes et al., 2009).

Diet plays a role in pH decline and microbial shifts due to introduction of rapidly fermentable carbohydrates and/or lack of effective fiber. Lowering pH independent of diet will shift microbial fermentation activity (Calsamiglia et al., 2009; Fuentes et al., 2009), but recovery of pH to optimal cellulolytic conditions will encourage compensatory fermentation (Wenner et al., 2017) (Figure 1). Animal intake behavior and ruminal pH must be interrelated as cows with reversed rumen contents will revert to previous populations just weeks after a complete ruminal exchange (Weimer et al., 2010), and the effect of SARA-induction can be temporary if the insult is removed (Plazier et al., 2017). As rumen pH declines by any variety of dietary imbalances on the farm, cellulolytic species are inhibited in the process. These cellulolytic species inhibited by low pH or inconsistent pH are the same cellulolytic species implicated in complete biohydrogenation, and their inhibition limits ruminal biohydrogenation capability (Fievez et al., 2012). Loss of function can bottleneck biohydrogenation intermediates and increase the likelihood of omasal flow for undesirable unsaturated fatty acids, such as trans-10, cis-12 CLA (Jenkins et al., 2008). Microbial growth will also be limited by lower rumen pH and outflow of microbial MP could also be lowered.

Unfavorable Biohydrogenation Risk Factors

Trans-10, cis-12 linoleic acid is known to be a strong inhibitor of milk fat synthesis in the mammary gland and just 10 g/day passing to the small intestine triggered milk fat depression of 23% (Urritia and Harvatine, 2017). There is

typically no shortage of unsaturated fatty acids in corn- and corn silage-based diets (Baldin et al., 2018), but unsaturated fatty acid load in the rumen (**RUFAL**) merely provides the opportunity for MFD. Additional risk factors make cows more susceptible to incomplete biohydrogenation whether it be slug feeding depressing rumen pH, rapid starch fermentation, imbalance of carbohydrate and N pools and degradation rates, transition cow disruptions, or reduced intake of effective neutral detergent fiber (**NDF**). Knowing the fatty acid composition of feedstuffs can improve your understanding of how much unsaturated fatty acid risk you've provided in your diet. Prevention of rapid starch fermentation or slug feeding responsible for sharp declines in pH helps protect cellulolytics responsible for supporting biohydrogenation. Animals that are adapted to more highly fermentable diets may be more likely to absorb VFA from the rumen more efficiently and decrease acid load of the rumen (Bannink et al., 2008). Lastly, feed additives that disturb the rumen ecosystem may provide immediate gains in milk volume under most conditions but also leave less margin for error in a feeding program. Care should be taken to limit cumulative risk for fat depression by keeping an eye on the combination of rumen pH, unsaturated fatty acid load, and destabilization of the rumen ecosystem.

Improving Fiber Digestibility Through Management

Fiber digestibility is not only important in limiting MFD risk, but improved fiber digestibility translates well to greater milk fat concentration and improved overall diet fermentability provides an additional energy boost that can help improve milk fat yield. Improving diet fermentability provides additional energy for milk synthesis, including VFA for *de novo* fatty acid synthesis. Rather than debate

which pricey feed additives should be used to boost milk production, my preference is to focus on feed management issues that contribute to ruminal stability and maximized digestibility rather than pricey alternatives that can have situational efficacy. Primary areas of opportunity to evaluate at this time include calf growth/heifer development, feed ingredient quality, TMR delivery, shrink, and feed additives. All of these can support a healthy, consistent rumen.

Calves and heifers

Too many farms still operate on the “no news is good news” plan when it comes to raising calves, but these animals are the investment in your operation’s future 2 years down the road. The high producing cows you want when the milk prices turn around are being born in your barn today, and now is a perfect time to re-evaluate your calf program to capitalize on any management opportunities. While there is debate on how much milk replacer to provide calves, there is certainly a lot of data to support the concept that pre-weaning gain translates to first lactation milk (Van Amburgh, 2017) and faster maturing heifers with a more economical age at first calving (St-Pierre, 2002). Increased stress during weaning can erase gain so attention should be paid to transitioning calves and limiting lost time on feed. Calves generally learn more quickly if housed together pre-weaning and are more likely to return to feed post-weaning if previously raised in a larger social group (De Paula Veira et al., 2010; Gaillard et al., 2014) or led by example from older calves (De Paula Veira et al., 2012). Getting calves onto starter early is critical for rumen development (Laarman et al., 2012) and exposure to some long-stemmed forage also adds value (Khan et al., 2011). Early rumen microbial populations are diverse in calves and likely susceptible to volatility but develop into a core microbiome by adulthood (Jami et al., 2013). Learned

intake patterns may also translate to feeding behavior in mature cows (Miller-Cushon and DeVroes, 2016). Care in the development of calves improves rumen function and leads to easy-transitioning, early-maturing heifers with strong lifetime potential.

Feed ingredient quality

Given the high degree of variability in the market for some feed ingredients, there is money to be saved or wasted on ingredient sampling. If you’re spending money on feed sampling, you surely want to spend enough money to get details that you have a high degree of confidence in – starting with the quality of samples taken at the farm in the first place. True feed variability (St-Pierre and Weiss, 2015) and the value in paying for detailed analysis can best be illustrated by looking at blood meal. Valued for high bypass protein values (CP can exceed 100%) and high metabolizable lysine and histidine, blood meal prices have ranged from \$600 to \$1200/ton in the past calendar year and consistently vary \$300/ton across suppliers within any one week. Despite nearly a decade of knowing how variable the market can be (Boucher et al., 2011), we often ignore the importance of ingredient testing. Figure 2 illustrates the distribution of a group of blood meals analyzed in 2017. In panel A, the CP is seen to be fairly consistent and lysine as percent of CP would be similar if represented alongside. However, when subjected to the Ross assay for unavailable N (Ross, 2013), a discrepancy arises where the distribution of blood begins to widen first for RUP (%CP basis, panel B) and then for dRUP (%DM basis, panel C). If charted for unavailable N (%DM basis, panel D), we can see the actual quantity of protein purchased that is expected to be excreted from the ruminant. Values for unavailable N demonstrate product value excreted and wasted for the producer, averaging 45% and at \$800/ton would be a

loss of \$360/ton to the end user! I would much rather see poor rumen bypass numbers with some product available to rumen microbes than to have cows excreting expensive protein ingredients in the feed.

The quality of feed in a diet also relates to the source of N provided to rumen microbes. For a long time, we've known that cellulolytics respond favorably to rumen ammonia (NH₃) (Russell, 2002; Dehority, 2003) and so urea supplementation is used as a safety factor to ensure that daily fluctuations in rumen NH₃ concentration never leave microbes starved for N with access to degradable starch. Microbes do not adhere to a sharing policy; if they do not allocate energy into growth, they are more likely to burn the energy in wasteful cycles (Hackmann and Firkins, 2015b). Cellulolytic activity has been increased in vitro where non-NH₃ RDP sources are provided in replacement of some NH₃, indicating cellulolytics respond favorably to amino acids or peptides in addition to NH₃ (Gorosito et al., 1985; Atasoglu et al., 2001; Hackmann and Firkins, 2015b). Lowering dietary CP from urea and substituting in higher quality RDP sources should lead to improved fiber digestibility, more rapid growth, and greater stability of the cellulolytic niche in the rumen. Prioritizing RDP and rumen degradable starch over bypass will ensure that you are providing microbes all they need to thrive in the rumen.

Attention to TMR delivery details

A dairy producer may not currently have a lot of cash flow to invest in feed ingredients, but they still have time to invest in the accuracy and efficacy of ration delivery to the herd. Farm walk-throughs must note timing of TMR preparation, thoroughness of delivery to the bunk, weighbacks, frequency of TMR push-ups, degree of TMR sorting, and overcrowding at the bunk. All of these factors relate directly to DMI

and rumen pH in a group of cows, affecting fiber and overall TMR digestibility and influencing the milk fat production of that group. Whether or not cows are fed to a slick bunk can also influence rumen pH and diet fermentability. When feeding a ration that simulated ignoring heavy rain events, McBeth et al. (2013) demonstrated that constant ingredient weights (underformulated forage DM) had little effect on milk production or intake over the course of a few weeks when cows were fed *ad libitum*. Collings et al. (2011) demonstrated that cows on a restricted feed diet shifted intake patterns towards slug feeding more so than cows penned at a 200% stocking density measured in bunk space; this slug feeding would negatively impact rumen pH and instigate SARA in a proportion of the pen. Limit fed cows are much more susceptible to large fluctuations in DM within the TMR and feed shortages that lead to time without feed, representing opportunity for rumen bugs to be deficient in either readily degradable starch or ruminal ammonia concentrations.

Some research would indicate an advantage to delivering feed at an alternative time to when cows return from milking (DeVries and Von Keyserlingk, 2005; King et al., 2016) or more than once per day (DeVries et al., 2005; Bannink et al., 2016). What is most important is availability of TMR to cows throughout the day with the least exposure to sorting. Sova et al. (2013) reported that every 2% increase in sorting against longer particles represented a 2.2 lb decrease in milk production, and an improved milk fat yield attributed to stabilized rumen pH with increased fiber intake (DeVries et al., 2008). Unevenness in dietary intake or forage content can lead to larger decreases in pH post-feeding (Allen, 1997), partially due to decreased salivary secretion (Beauchemin et al., 2008). Increasing feeding frequency or push-up of feed to stimulate meal frequency also has the advantage of increasing passage rate in the

rumen and thereby increasing microbial growth efficiency (Le Liboux and Peyraud, 1999) for gains in microbial MP flow to the duodenum. The greatest gains to feed push up may be in the first couple hours post-feeding (Armstrong et al., 2008) when TMR can quickly be eaten out of reach for less dominant cows.

Feed shrink's real but unknown cost

As an industry, feed shrink is nebulous and often avoided because it is difficult to estimate. There is limited research for book values and feed tracking data is painstaking to sort through to determine cost savings estimates. Nevertheless, feed shrink has real cost and with tight margin between feed costs and milk production, now is a good time to explore strategies to decrease shrink loss starting with the most expensive ingredients, either by volume or cost per ton. For example, if a ration costs \$6.00/cow/day and shrink is reduced by just 2%, that equates to a savings of \$0.12/cow/day. It's probably worth taking a look at reducing your shrink before you cut feed additives that may be promoting milk component production. Tracking shrink becomes an issue of scale usage. Diet delivery weights are typically easy to estimate, but without weighbacks to know TMR intake, it is difficult to know how much TMR is left unfed and essentially wasted. Feeding a pen of cows to 3% weighback is much easier if the weights are tracked and a visual reference is established for the feeder; otherwise, you are just pretending to feed to a target DMI and hoping to get lucky often.

Fine particles in mixes are susceptible to loss by the wind, especially in loose storage, and shrink can be increased by 8 to 20% if wind exceeds 15 mph (Harner et al., 2011). Fine particles are also commonly the most expensive. Historical weather for 2018 indicates wind has exceeded 20 mph every week this year. Exposure

to rain can promote spoilage, even in commodity sheds built facing away from typical weather directions (Standaert et al., 1997; Harner et al., 2011). Fine particles, primarily starch and fat, are lost to rodents and birds. A recent paper (Carlson et al., 2018) estimated worst case scenarios of 100 birds/cow could reduce TMR energy concentration by 5%. You would have to be a regular Annie Oakley to continuously reduce pest depredation of TMR and ingredient storage across the farm, but a regimented pest control protocol can prevent populations from getting out of hand. Poor forage packing and covering can increase loss by spoilage and increased linear feed rates beyond 12 cm/day can decrease DM loss by 10% (Ruppel et al., 1995). It is generally better to remove spoilage than to try to feed your way through it; remember, you are trying to provide consistency in the rumen. All of this shrink can contribute to a variation in ration delivered compared to what is formulated, ultimately at the expense of rumen microbes for which you balanced the diet or at a loss of valuable rumen bypass fat or protein expected to deliver nutrients to the cow.

Feed additives and rumen fermentation

My approach to rations is fairly simplistic; I prefer to focus on high quality feed ingredients at best value purchases rather than a plethora of trendy feed additives. This especially includes the value of digestible forages that provide effective NDF to induce rumination, salivation, and a resilient rumen mat. The physical effectiveness of fiber can be influenced by the degradability of both NDF and starch in a given diet (White et al., 2017). In this paper, I want to avoid recommendations on feed additives but would rather focus giving 2 examples of how feed additives can interact to influence the rumen microbial population. A quick look will reveal the complexity of the rumen environment and how small changes can have large downstream effects.

Methionine analogs have been commercially available for ruminant diets for several decades. While they come in different forms with varying data on rumen bioavailability (Graulet et al., 2005; Nofstger et al., 2005; Fowler et al., 2015), it is becoming clear that they can have a stimulatory effect on cellulolytic activity (Martin et al., 2013; Fowler et al., 2015) that prevents milk fat depression in acidosis challenge diets (Baldin et al., 2018). It is interesting to note that cellulolytic bacterial numbers do not change (Martin et al., 2013), but their activity appears to increase with supplementation (Fowler et al., 2015). Metabolic activity is positively associated with biohydrogenation activity by cellulolytics (i.e., more biohydrogenation with growth) and it is likely the stimulatory effect of a methionine analog on cellulolytics that helps to limit risk of high producing dairy cows to MFD (Baldin et al., 2015). Further, supplementation with methionine analogs appears to improve microbial N flow (Fowler et al., 2015; Lee et al., 2015). If you think that you have milk fat on the table and want to pursue changes to the diet beyond management considerations, methionine in the rumen to stimulate fiber digestion might be a good place to look.

Mineral sources can also have complex interactions in the rumen. Copper, specifically, can be inhibitory to cellulolytics in the rumen (Martinez and Church, 1970). Recently, mineral source was found to have an effect on fiber digestibility *in vivo*, with greater effect in a forage fiber diet than a byproduct fiber diet (Faulkner and Weiss, 2017). Mineral sources can also affect microbial populations downstream in hindgut fermentation (Faulkner et al., 2017), potentially changing fecal excretion of bacteria that could be implicated in hoof infections plaguing the dairy industry (Klitgaard et al., 2014; Faulkner et al., 2017). Magnesium source was also recently shown to interact

with monensin in lactating dairy cows on NDF digestibility (Tebbe et al., 2018), possibly because of the countering stimulatory effects of available magnesium on NDF digestibility versus monensin's activity against gram-positive cellulolytics. We still understand very little about the interactive effects of minerals within the rumen on microbial activity, and it is best to remember for now that choices may have consequences.

Summary

The current milk market is challenging but provides us the motivation to clean up inefficiencies on the farm for cost savings. Milk fat production still provides value to the farm and can be boosted with the following strategies: 1) limiting risk for induced milk fat depression via biohydrogenation intermediates, and 2) improving fiber digestibility in the rumen. The rumen is a complex place with many interactions between microbes, the diet, and cow feeding behavior. While many feed additives promise improved milk fat production, proper diet management and TMR delivery will promote DMI and ruminal stability from weaned calves through multiparous high cows. Management practices can have a high return on investment during a time when producers would like to keep feed costs low. Proper TMR delivery, continual access to unsorted TMR, and diets balanced for N responses of cellulolytic microbes afford producers the opportunity to capitalize on greater stability in rumen pH, passage rate, and fermentable carbohydrate fractions. Attention to detail is practically free and has more value now than ever.

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Table 1. Two sample herds with varying component production.

	Dairy 1	Dairy 2
Cows	200	200
Milk (lb/day)	90	85
Fat (%)	3.6	3.9
Protein (%)	2.9	3.1
Other Solids (%)	5.7	5.7

Table 2. Value of milk from two sample herds with varying milk and component yields.¹

	Dairy 1		Dairy 2	
	2017	2018	2017	2018
Fat (\$/cow)	\$8.20	\$7.94	\$8.39	\$8.12
Protein (\$/cow)	\$5.69	\$4.33	\$5.74	\$4.37
Other Solids (\$/cow)	\$1.28	\$0.41	\$1.21	\$0.39
Total Value (\$/cow)	\$15.17	\$12.68	\$15.34	\$12.88
Herd Value (\$/day)	\$3,033.90	\$2,536.20	\$3,068.50	\$2,576.69

¹Prices taken from FMMO 33 for February, 2018 (NMPF, 2018; USDA, 2018).

Table 3. Contribution of microbial N to metabolizable protein amino acids (AA) in a typical dairy cow.

Amino Acid	Microbial AA (g AA/100 g true protein) ¹	Microbial AA Flow (g/day) ²		
		Decreased 3%	Average	Increased 3%
Ala	7.4	120.1	123.9	127.6
Arg	5.3	86.0	88.7	91.4
Asp	13.4	217.6	224.3	231.0
Cys	2.2	35.7	36.8	37.9
Glu	15.0	243.5	251.1	258.6
Gly	6.2	100.7	103.8	106.9
His	2.1	34.1	35.1	36.2
Ile	7.0	113.6	117.2	120.7
Leu	9.2	149.4	154.0	158.6
Lys	9.4	152.6	157.3	162.1
Met	2.6	42.2	43.5	44.8
Phe	6.4	103.9	107.1	110.3
Pro	4.3	69.8	72.0	74.1
Ser	5.4	87.7	90.4	93.1
Thr	6.3	102.3	105.4	108.6
Trp	1.4	22.7	23.4	24.1
Tyr	6.1	99.0	102.1	105.2
Val	6.9	112.0	115.5	119.0

¹Composite microbial AA taken from Sok et al. (2017, Table 4).

²Average microbial AA flow based on Hristov (2007) at 325 g/day of microbial N.

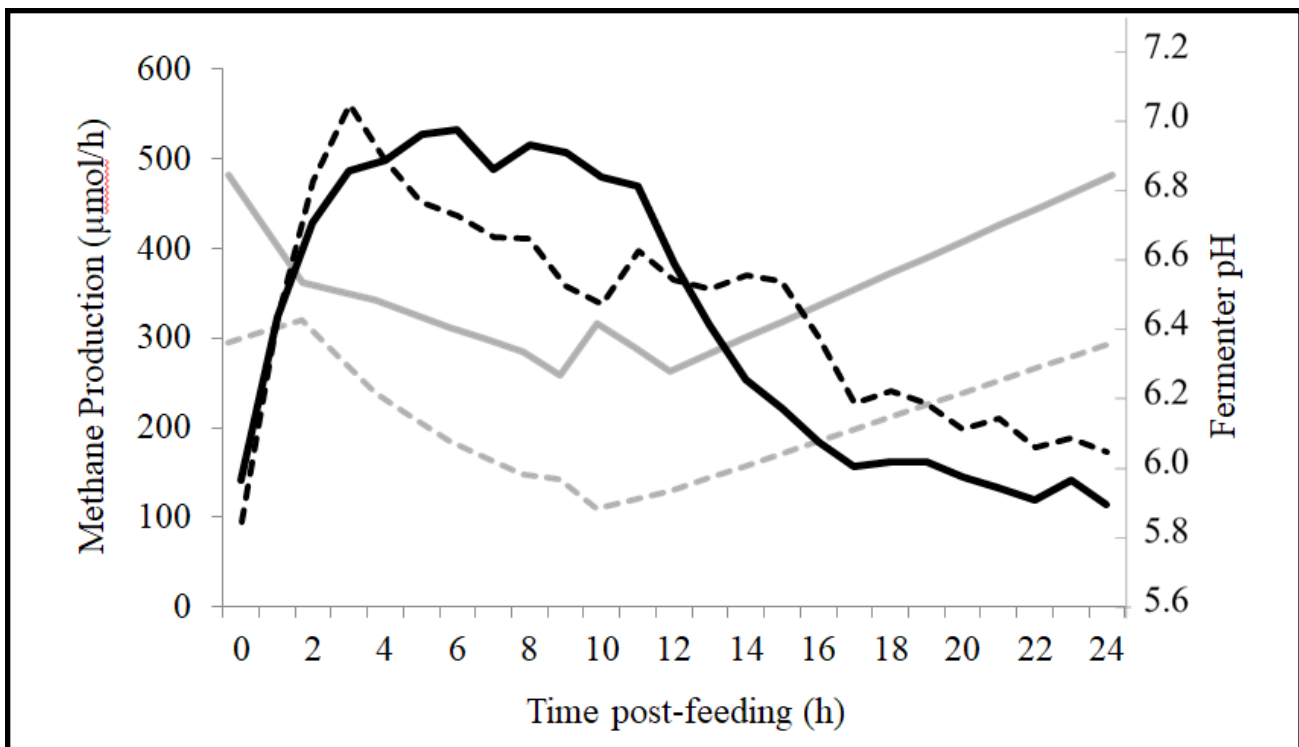


Figure 1. The effect of continuous culture fermenter pH on fermentation activity as quantified by methanogenesis on the same diet (adapted from Wenner et al., 2017; gray lines = pH, black lines = methane, dotted lines = low pH, and solid lines - control). The recovery of pH enables fermentation to compensate in later hours post-feeding.

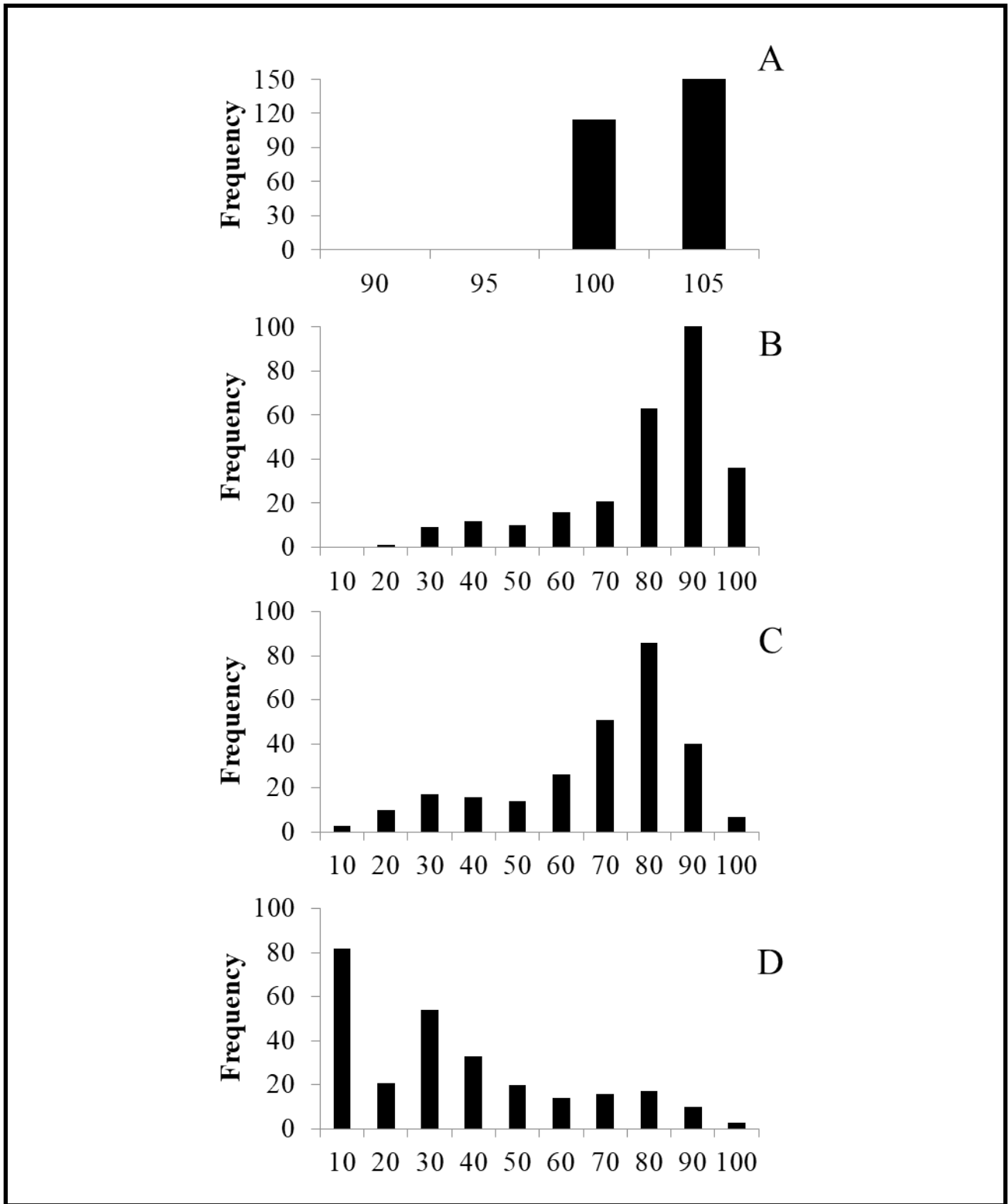


Figure 2. Relative distribution of a composited dataset of 2017 blood meal samples (n = 270). Panel A represents frequency of CP (%DM). Panel B represents RUP (%CP). Panel C represents digestible RUP (%DM). Panel D represents unavailable (%DM) (Ross, 2013). Courtesy of both Cumberland Valley Analytical Services and Dairyland Laboratories, Inc.

Relationships Among Changes in Body Condition Score and Reproductive Efficiency in Lactating Dairy Cows

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Abstract

This manuscript summarizes research, with emphasis on 3 recent publications, relating body condition score (BCS) or changes in BCS with reproductive performance in dairy cows. Cows with lower BCS have greater likelihood of anovulation with reduced reproductive performance due to decreased service rate in cows bred to estrus and reduced pregnancy per AI (P/AI) in cows bred to synchronized ovulation. Evaluation of BCS at first AI predicted P/AI in cows bred either to Ovsynch or Double Ovsynch with relative improvements of 30 to 50% in P/AI (BCS > 2.5 vs BCS < 2.5). Loss of BCS (calving to 21 days postpartum) was associated with a dramatic reduction in P/AI in cows bred to Double Ovsynch. One recent study found that cows with high BCS at 21 days before expected calving were more likely to lose BCS during transition period (21 days before calving to 21 days after calving) and cows that lost BCS had greater health problems and a large reduction in P/AI. Finally, one recent study showed that loss of BCS during the dry period (dry off to calving) was a critical predictor of health and reproductive performance. Excessive BCS at dry off (> 3.25) was the primary predictor of subsequent BCS loss. Thus, dairy herds need to implement evaluation of BCS at dry off, calving, 21 days after calving, and at first AI to diagnose nutritional and management factors associated with BCS change and thereby improve health and reproduction in these dairy herds.

Introduction

Efficient reproduction is key to profitability and sustainability of dairy operations. The reasons that efficient reproduction improves dairy farm profitability are manifold. First, the shape of the lactation curve, particularly in cows after their first lactation, shows that cows in early lactation are generally more profitable than later lactation cows. Thus, optimization of calving intervals can improve milk production from the herd and improve efficiency of milk production. In most models relating reproduction to profitability, the improvement in milk production is a major factor in improved profitability with improved reproduction.

There are also obvious genetic advantages in herds with efficient reproduction. High quality dams become pregnant to the best AI sires producing exceptional replacement heifers. This advantage will require about 2 years before it will begin to impact herd profitability but the impact can continue for many years. These longer-term advantages generally are of much greater economic impact than the short-term costs of the reproduction program.

Finally, one of the other major advantages of efficient reproduction is the improvement in overall quality of the dairy herd. A dramatic improvement in reproduction in a dairy herd can have a surprisingly rapid impact on the

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management and genetics of a herd, even before the quality replacement heifers produced by the program have entered the milking string. This fairly rapid impact of reproductive management is due to changes in culling practices in a herd. It seems obvious that herds with better reproductive efficiency would cull fewer cows due to poor reproduction; however, it is not as obvious that overall cull rate for the herd may not differ for a herd with good vs poor reproduction, the change is in the type of cow that is culled. If the 21-day pregnancy rate is 15%, then about 27% of cows will not be pregnant by 222 days in milk. Thus, reproductive culls could well represent almost all of the culling on this dairy. In contrast, herds with a 25% 21-day pregnancy rate (an exceptional rate) would have only 10% of cows that are not pregnant at 222 days in milk. Thus, the herd with good reproduction has much greater flexibility to cull "lower value" cows in their herds.

Thus, improvements in milk production, genetics, reproductive costs, and overall quality of the dairy herd can result from improving reproduction. Unfortunately, many dairy farms do not attain optimal reproduction due to many factors related to management, health, and physiology of high-producing dairy cows (Caraviello et al., 2006). The issues involved in reproduction of lactating dairy cows are complex, but increasingly, the interactions between nutrition, the hormonal systems, and altered reproduction in dairy cattle are being elucidated.

Figure 1 provides a generalized summary of 3 general areas in which there is a relationship of nutrition with reduced reproductive performance of lactating dairy cows. Inadequate consumption of nutrients can lead to anovulation and reduced reproductive performance. Inadequate consumption of energy leading to loss of BW and BCS is the primary

topic of this manuscript. The middle box shows the effect of high feed consumption on liver blood flow leading to altered reproductive physiology. Finally, excessive levels of carbohydrates, dietary protein, or other feed ingredients can also reduce reproduction (right box). These last 2 areas have been discussed in previous reviews (Sartori et al., 2002, Wiltbank et al., 2006, Santos et al., 2010, Bisinotto et al., 2012, Wiltbank et al., 2014). Thus, the remainder of this manuscript will focus on how BCS and changes in BCS are related to reproductive efficiency in dairy herds.

Measurement and Uses of BCS

Evaluation of BCS is a useful management tool to assess body fat stores of Holstein dairy cows (Wildman et al., 1982, Waltner et al., 1993). Body condition score has received considerable attention as a tool to aid in the management of nutritional programs in dairy herds (Waltner et al., 1993, Roche et al., 2009). The BCS of cows at calving, the nadir BCS, and the postpartum BCS loss are associated with differences in milk production, reproduction, and health (Pires et al., 2013). Overconditioned cows with a BCS greater than 4.0 at calving had greater circulating concentrations of non-esterified fatty acids (NEFA) in early lactation compared with cows with moderate or low BCS (Pires et al., 2007). Hyperlipidemia, in turn, caused insulin resistance in dairy cows (Hayirli, 2006), consistent with studies linking high BCS to reduced peripheral insulin sensitivity in the lipomobilization state (Ospina et al., 2010b).

The association of energy status during the transition period and reproductive efficiency in dairy cows has been demonstrated in multiple studies. For example, a retrospective analysis of 7 studies of prepartum nutrition found that feeding a high energy diet during the close-up period resulted in increased BCS loss post-partum and increased time to pregnancy

(Cardoso et al., 2013). In addition, 2 studies found that increases in NEFA concentrations during the transition period were predictive of reduced risk of pregnancy by 70 days after the voluntary waiting period (**VWP**) in evaluations of >2000 lactating dairy cows or reduced 21-day pregnancy rate in herd-level evaluations of 60 free-stall herds (Ospina et al., 2010a,b). Recently a study of 156 lactating dairy cows (Garverick et al., 2013) reported that the probability of pregnancy at first timed AI (**TAI**) was decreased as serum NEFA concentrations on day 3 post-partum increased. Other studies also indicate a negative relationship between post-partum NEFA or loss of BCS and fertility (López-Gatius et al., 2003, Chapinal et al., 2012a). In contrast, no effect of increased NEFA or β -hydroxybutyric acid (**BHBA**) concentrations during the transition period was found in a recent large ($n = 2,365$), multi-region study (Chapinal et al., 2012b). Unfortunately, none of these studies provided detailed information on reproductive management protocols, except for Garverick et al. (2013).

One critical issue is the relationship of lower BCS and increased anovulation in dairy cattle. Early studies demonstrated that the day of energy balance nadir was related to timing of first ovulation ($r^2 = 0.72$) with first ovulation occurring, on average, 10 days after the energy balance nadir (Butler and Smith, 1989). In a large collaborative study (> 5,000 cows evaluated) done by 3 different reproductive physiologists (Milo Wiltbank, Paul Fricke, and Jose Santos) and two geneticists (George Shook and Rebecca Bamber), it was found that anovulation had a fairly high heritability for a reproductive trait ($h^2 = 0.171$) in dairy cattle (Bamber et al., 2009). In addition to the genetic findings in this study, the study reported the percentage of cows with anovulation at ~60 days after calving, which as would be expected, was greater in cows with lower BCS (Figure 2). For example, cows

with very low BCS (< 2.00) had over 40% anovulation, whereas cows with 2.75 or greater BCS had 20.9% anovulation. However, less than 5% of cows had very low BCS (< 2.00) and only 25.8% had low BCS (< 2.50), demonstrating that in US dairy herds most cows have reasonable BCS by time of expected AI (~60 DIM) and most cows are cycling. Nevertheless, herds that have severe loss of BCS and consistently low BCS during the early post-partum period would be expected to have a high percentage of anovular cows. High percentage of anovular cows can dramatically reduce reproductive performance in lactating dairy cows (Gumen et al., 2003, Santos et al., 2016a, Santos et al., 2016b). One other complication is that when pregnancy is established in anovular cows, these cows are more likely to undergo pregnancy loss compared to cyclic cows (Santos et al., 2004, Sterry et al., 2006, Santos et al., 2009b). Thus, lower BCS (< 2.75) increases percentage of cows that are anovular and anovular cows reduce reproductive efficiency either by reducing percentage of cows that receive AI due to lack of expression of estrus and ovulation, decreased fertility after induction of ovulation and timed AI, and increased pregnancy loss in cows that become pregnant.

Effect of BCS at Time of AI on Fertility

A study summarizing early research (before 2003) from 11 studies with a total of 7,733 cows (López-Gatius et al., 2003) categorized cows by as low, intermediate, and high BCS (BCS < 2.5 ; between 2.5 and 3.5; and > 3.5 , respectively). Cows calving with a low BCS (BCS < 2.5) had a decreased relative risk of pregnancy at first AI (relative risk = 0.91) compared to cows calving with an intermediate BCS ($2.5 < \text{BCS} < 3.5$). The relative risk of pregnancy at first AI, however, did not differ between cows calving with an intermediate BCS and cows calving with a high BCS. Detection of estrus was the primary reproductive management

tool used in these early studies; therefore, cows that were not cycling would not receive AI. Current programs use GnRH and prostaglandin (PGF) to synchronize ovulation, allowing all anovular cows to be bred by timed AI, thereby increasing service rate in these cows, but fertility remains suboptimal.

An early study from our laboratory evaluated the effect of BCS near AI on fertility in cows that were bred to Ovsynch. The cows with low BCS (< 2.5) had lower P/AI compared to cows with normal (> 2.75) BCS (28.1%, 32/114 vs. 43.7%, 125/286; $P < 0.05$) at the pregnancy diagnosis near 60 days after AI (Souza et al., 2007). To calculate the relative improvement in fertility, differences between BCS classes are calculated ($43.7 - 28.1 = 15.6$), then the difference is divided by the percentage pregnant in the low BCS ($15.6/28.1 = 55.5\%$). So there are greater than 50% more pregnancies after an Ovsynch and timed AI program in cows with good BCS as compared to low BCS. This observed effect of BCS on P/AI is somewhat greater than calculated relative reductions in P/AI in other studies that compared fertility in cows with lower vs. higher BCS: 33.1% (Ribeiro et al., 2011), 38.6% (Ribeiro et al., 2012), 15.2% (Santos et al., 2009a), 25.3% (Escalante et al., 2012), and 45.7% (Moreira et al., 2000). Thus, lower BCS can dramatically reduce fertility, even when Ovsynch is used to induce ovulation and allow timed AI.

In a more recent study (Carvalho et al., 2014), we evaluated the effect of lower BCS at the time of AI in cows that are bred with the Double Ovsynch protocol. Compared to a Presynch-Ovsynch protocol, a Double-Ovsynch protocol dramatically decreases the proportion of cows initiating the Ovsynch protocol in a low P4 environment (Souza et al., 2008, Herlihy et al., 2012, Ayres et al., 2013). This is important for interpreting our research because it is well-

described that cows with low P4 at the beginning of the Ovsynch protocol have decreased P/AI compared with cows with high P4 concentrations (Silva et al., 2007, Denicol et al., 2012, Giordano et al., 2012a, Giordano et al., 2012b, Giordano et al., 2013). In our study using Double-Ovsynch for all breedings (Carvalho et al., 2014), there was a decrease of 8.8% in P/AI in cows with low BCS compared to high BCS (40.4% vs. 49.2%; $P = 0.03$) which calculates to a relative increase in P/AI of 21.8% ($8.8/40.4$). We continue to update data from this research (Carvalho, P.D., unpublished). From a total of 30 to 40 lactating cows bred with Double Ovsynch at first AI, only 24.7% ($n = 752$) had lower BCS (< 2.50). These cows had lower P/AI (40%) compared to cows with better BCS (> 2.50; 52.6%, 1203/2288) or a relative increase of 31.5% in P/AI (Figure 3). Thus, timed AI programs allow all cows to be bred, but cows with BCS less than 2.75 have much lower fertility than cows with better BCS, even when a program like Double Ovsynch is utilized that is expected to induce ovulation in anovular cows.

Effect of Change in BCS After Calving on Fertility

A frequently-discussed hypothesis that was first introduced by Britt (1992), postulated that energy status during the early post-partum period could alter follicular/oocyte quality resulting in negative effects on subsequent fertility in lactating dairy cows. This early study compared cows that lost BCS ($n = 30$) to cows with little BCS change ($n = 46$) during the early postpartum period (Britt, 1992). The P/AI was lower in cows with high BCS loss than in cows with little BCS loss either at first AI (62% vs 25%) or at all AIs (61% vs 42%). Interestingly, the cows that had the high BCS loss had much greater BCS at calving than cows with little BCS loss (3.15 vs 2.78). Thus, this early study introduced the concept that loss in BCS score after calving reduced fertility.

The summary of early research mentioned above (López-Gatius et al., 2003) also categorized change in BCS from calving until AI (7,733 cows from 11 studies) as: increased (gain in score), slightly decreased (0 to 0.5 point loss), moderately decreased (0.6 to 1 point loss), or severely decreased (>1 point loss). The effect of BCS change decreased risk of pregnancy (relative risk = 0.9) but only for cows categorized as suffering a severe BCS loss (> 1 BCS point) between parturition and first AI compared with cows categorized as undergoing increased or slight BCS loss. The relative risk of pregnancy did not differ between cows with a slight vs. moderate loss in BCS between parturition and first AI. Cows with severe loss in BCS between parturition and first AI also remained open for 10 days longer compared with cows undergoing a slight BCS loss. There was no difference in days open for cows with moderate or slight BCS loss. A more recent study (Santos et al., 2009a) also reported that cows losing more BCS between calving and first AI had lower P/AI and were more likely to undergo pregnancy loss than cows with little BCS loss. Moreover, cows that lost more BCS were also more likely to be anovular by 65 days in milk (**DIM**; Santos et al., 2009a). By contrast, Ruegg and Milton (1995) reported no association between BCS or BCS change from parturition to first AI and days to first estrus, days to first postpartum insemination, or number of inseminations required for cows to become pregnant.

We recently published a study in which we evaluated BCS of lactating dairy cows ($n = 1,887$) at time of calving and 21 days after calving. Cows were categorized by BCS change and then received timed AI after a Double Ovsynch protocol. Overall, only 7.3% of cows lost 0.5 or more BCS points (139/1,887). There was no difference between cows that lost 0.5 or more BCS points compared to those that lost 0.25 BCS points in P/AI at 40 days (27.3% vs. 24.6%;

$P > 0.15$) or at 70 days (24.6% vs. 22.3%, $P > 0.15$) after TAI, or in pregnancy loss between first and second pregnancy examination (7.9% vs. 9.4%, $P > 0.15$). Therefore, we combined these cows into a single group for all subsequent analyses (i.e., cows that lost BCS between calving and 21 DIM). Overall, the proportion of cows that lost, maintained, and gained BCS between calving and 21 DIM was 41.8%, 35.8% and 22.4%, respectively (Table 1). At the 40 days pregnancy examination (Table 1), P/AI differed ($P < 0.001$) dramatically among BCS change categories and was greater for cows that gained BCS (83.5%; 353/423), intermediate for cows that maintained BCS (38.2%; 258/675), and least for cows that lost BCS (25.1%; 198/789). Similarly, at the 70 days pregnancy diagnosis (Table 1), there was a dramatic effect of BCS change on P/AI ($P < 0.001$) but no effect on pregnancy loss ($P = 0.34$). There was an effect of parity (primiparous vs. multiparous) on BCS at parturition (2.82 vs. 2.98; $P < 0.001$) and at 21 DIM (2.76 vs. 2.90; $P < 0.001$) and on P/AI at 40 days (50.1% vs. 35.4%; $P < 0.001$) and 70 days (47.0% vs. 32.6%; $P < 0.001$) after TAI but no effect ($P = 0.41$) on pregnancy loss. However, both primiparous and multiparous cows had a similar effect of BCS on P/AI (Carvalho et al., 2014).

The median calving to pregnancy interval differed (Log-Rank test, $P < 0.001$) between BCS groups and was 84, 113, and 128 days for cows with gaining, maintaining, and losing BCS between calving and 21 days postpartum, respectively. Cows gaining BCS between calving and 21 days postpartum were 3.0, and 2.5 times more likely to be pregnant by 300 DIM compared with cows losing and maintaining BCS (HR = 3.0, $P < 0.001$; and HR = 2.5, $P < 0.001$, respectively). Cows maintaining BCS between calving and 21 days postpartum were 1.2 times more likely to conceive by 300 DIM compared with cows losing BCS (HR = 1.2, $P = 0.01$).

A commonly accepted idea regarding postpartum energy balance in dairy cows is that all or nearly all cows lose BCS or weight during the postpartum period and that cows only differ in the degree to which they lose BCS or weight. We were surprised at the relatively small degree of loss of BCS or BW observed in this experiment. Only 41.8% (789/1887) of cows lost BCS during the first 21 days postpartum and this was similar for the 2 farms that were utilized in this study. Further, the observation that only 7.3% of cows lost 0.5 or more BCS (139/1,887) during the first 21 days after calving seems somewhat at variance with previous reports of BCS losses of 1 or more units during the early postpartum period (López-Gatius et al., 2003, Gumen et al., 2005, Santos et al., 2009b). Even more surprising was the observation that 33.5% (358/1070) of cows on Farm 2 gained BCS during the first 21 days after calving and that almost 60% of cows on either farm maintained or gained BCS during this early post-partum period.

Recently (Carvalho et al., 2014), we have done weekly BW evaluation from calving until AI in lactating dairy cows ($n = 72$). As shown in Figure 4, Quartile 1 cows gained about 2.5% of BW from the first to third week after calving, Quartile 2 cows maintained BCS, Quartile 3 cows lost ~4% of BW by 6 weeks post-partum (~0.25 BCS), and only Quartile 4 lost ~7.5% of BW in the early post-partum period, equivalent to about 0.5 BCS unit. Thus, the BW changes in this study were consistent with our large study indicating that there are many cows that do not lose BCS or BW during the early post-partum period and that losses in BCS under current management conditions may be less than previously reported.

Table 2 shows the effect of quartile of BW loss on embryo characteristics. First, there was no effect of parity on any of the embryo

characteristics so all parities were combined for the analysis. Superovulatory response did not differ ($P > 0.15$) among quartiles (Table 2). Similarly, total unfertilized structures, total structures recovered, and recovery rate did not differ (not shown) and total fertilized structures and percentage fertilization also did not differ ($P > 0.15$) among quartiles. Total degenerated embryos were greatest for Q4 cows, lowest for Q1 and Q2, and intermediate for Q3. Percentage of degenerated embryos was similar among Q1, Q2, and Q3 cows and was greatest for Q4 cows (Table 2). Similarly, the percentage of fertilized structures that were classified as degenerate embryos was greatest for Q4 and least for Q1, Q2, and Q3 ($P = 0.04$). Conversely, percentage of fertilized structures classified as quality 1 and 2 ($P = 0.05$) or quality 1, 2, and 3 ($P = 0.04$) was lower for Q4 than for other quartiles (Table 2).

Thus, the most fascinating results in our study were the dramatic differences in P/AI that were observed in cows due to BCS change during the early post-partum period. In our study, cows with an increase in BCS had increased P/AI (at 70 days pregnancy diagnosis) by an astonishing 42.3% (78.3% - 36.0%) compared to cows maintaining BCS and 55.5% (78.3% - 22.8%) compared to cows losing BCS during the first 3 weeks post-partum. This difference could also be observed in the dramatic improvement in time to pregnancy in the cows that gained BCS during the early post-partum period. The BCS at parturition was slightly greater for cows that subsequently lost BCS (2.93) compared to cows that maintained (2.89) or gained (2.85) BCS; however, this minor difference seems unlikely to explain the extraordinary fertility differences. In addition, the parity differences between BCS categories seem unlikely to explain the results since primiparous and multiparous had similar differences in fertility based on change in BCS. Overall, our results agree with the Britt (1992) hypothesis, which postulates that negative

energy balance during the early postpartum period is associated with decreased P/AI at first AI. In addition, it seems clear that loss of BW during the early postpartum period was associated with reduced quality of embryos after superovulation. This suggests that effects of postpartum BCS/BW loss directly impact the early embryo, perhaps by direct effects on the oocyte during this period.

Effect of Changes in BCS During Dry Period on Fertility

In subsequent research, it has become clear that the loss of BCS during the early postpartum period is reflective of changes that are already occurring in BCS during the dry period. A recent study from our group (Barletta et al., 2017), categorized cows by whether they gained, maintained, or lost BW during the entire transition period (-21 days before calving to +21 days after calving). The percentages of cows that gained, maintained, or lost BCS from -21 to 21 DIM were 28, 22, and 50%, respectively. At Day -21, the cows in the group that lost BCS had the greatest BCS (2.97), following by Maintained (2.70), and the Gained group (2.57) had the lowest BCS ($P < 0.01$; Table 3). The Lost group had greater percentage of cows with BCS > 3 on Day -21 ($P < 0.01$) than the other groups. However, all cows had similar BCS on Days -7 (2.71; $P = 0.99$) and Day 7 (2.71; $P = 0.91$). At Day 21 postpartum, BCS was greater for cows that gained (2.90), intermediate for cows that maintained (2.70) and lower for cows that lost (2.54) BCS ($P < 0.01$; Table 3). Almost all cows that were over 3.0 BCS at Day -21, lost BCS during the transition period (Barletta et al., 2017). Thus, BCS at the start of the transition period is the primary driver of BCS loss during the transition period.

There are dramatic changes in health and reproduction in cows that lose BCS during

the transition period (Barletta et al., 2017). As shown in Table 4, days to first ovulation was much longer in cows that lost BCS (47.1 days), shorter in cows that maintained BCS (37.9 days), and cows that gained BCS had the earliest days to first ovulation (33.9 days). The P/AI either at the first pregnancy diagnosis (32 days) or second pregnancy diagnosis (70 days) varied substantially by BCS loss group (Table 4). For example, cows that gained BCS had almost 3-fold greater P/AI (45.5% P/AI at 70 days) compared to cows that lost BCS (15.7%). Cows that lost BCS were also more likely to have 2 or more health problems during the early postpartum period (62.9%) than cows that gained BCS (39.4%), which may partially underlie the observed reproduction problems. There were no differences in milk yield among the groups. It appears that many of the health and reproduction problems have already been determined before the transition period due to elevated BCS in some cows.

A recent study is also consistent with this idea (Chebel et al., 2018). This study evaluated records from 2 dairy farms in California ($n = 16,104$ lactations in 9,950 cows) and classified cows by BCS at dry off and parturition as having excessive BCS loss ($- 0.75$ or more; 9.9% of lactations), moderate loss (-0.5 to -0.25 ; 39.9%), no change in BCS (0; 29.9%), or gained BCS during dry period (> 0.25 ; 20.2%). The factor that explained the greatest percentage of the variation in the statistical model for BCS loss during the dry period was BCS at dry-off (94.7%) with only ~5% of variation explained by all other variables in the model (temperature-humidity index, calf sex, parity, days dry, number of calves, etc.). In this study, cows were bred by detection of estrus during a Presynch program (2 prostaglandin treatments 14 days apart) followed by timed AI using various Ovsynch modifications in cows that were not detected in estrus. Evaluation of P/AI

at first AI shows that BCS loss during the dry period had a substantial effect ($P < 0.001$) on reproductive performance. For example, cows that gained BCS had greater P/AI (41.9% P/AI at 67 days pregnancy diagnosis) compared to cows with excessive BCS loss (20.8%; Odds ratio = 0.36), moderate BCS loss (28.3%; OR = 0.55), or no change in BCS (33.1%; OR = 0.68). The authors concluded that “loss of BCS during the dry period was a predisposing factor associated with health disorders and reduced productive and reproductive performance in Holstein cows.”

Conclusions

It seems clear that evaluation of BCS could be a critical method for evaluating current management strategies on a dairy and as a predictor of future health and reproductive problems. For the research reviewed in this manuscript, BCS should be evaluated at dry off, near calving, at 21 days after calving, and at the time of first AI. The actual BCS at first AI is a clear predictor of expected outcome from the AI, even when excellent programs are utilized to induce cyclicity in cows, such as Double Ovsynch. It is also impressive that cows that are gaining BCS have dramatically better P/AI compared to cows losing BCS, based on measurements of BCS change: 1) during the period from calving to 21 days, 2) during entire transition period (-21 to + 21 days), or 3) only during the dry period (at dry off to calving). The key determinant of BCS loss seems to be that cows with excessive BCS have the least likelihood of gaining BCS and have the greatest BCS loss, on average. Excessive BCS at dry off seems to be any cows that is > 3.25 (Chebel et al., 2018), whereas at calving, it seems to be any > 3.0 (Carvalho et al., 2014; Barletta et al., 2017). Evaluation of BCS at these key times will allow clear analysis of current management practices by producers, veterinarians, and nutritional professionals and

rational development of strategies to correct problems, such as excessive BCS at dry off, resulting in dramatic improvements in health and reproduction in their dairy herds.

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Table 1. Effect of BCS change from calving to 21 days in milk (DIM) on pregnancies /AI (P/AI) for cows classified as losing, maintaining, or gaining BCS.

Measure	Lost BCS	Maintained BCS	Gained BCS	<i>P</i> Value BCS Change
% of cows	41.8 (789/1887)	35.8 (675/1887)	22.4 (423/1887)	
P/AI (40 days)	25.1 (198/789) ^c	38.2 (258/675) ^b	83.5 (353/423) ^a	<0.001
P/AI (70 days)	22.8 (180/789) ^c	36.0 (243/675) ^b	78.3 (331/423) ^a	<0.001
Pregnancy loss	9.1 (18/198)	5.8 (15/258)	6.2 (22/353)	0.34
BCS at calving	2.93 ± 0.01 ^a	2.89 ± 0.02 ^b	2.85 ± 0.02 ^b	0.005
BCS at 21 DIM	2.64 ± 0.01 ^c	2.89 ± 0.02 ^b	3.10 ± 0.02 ^a	<0.001

^{abc}Values within a row with different superscript letters differ at $P < 0.05$.

Table 2. Changes in embryo quality based on quartile of body weight loss (Carvalho et al., 2014; Figure 4).

	Quartile (Q)				<i>P</i> -value
	Fourth Q Lost +	Third Q Lost	Second Q Maintain	First Q Gain	
Corpus luteum (#)	18.4 ± 2.6	18.4 ± 1.7	19.0 ± 1.7	16.0 ± 2.0	0.67
Fertilized (%)	76.9 ± 7.1	77.0 ± 6.6	77.6 ± 7.6	78.4 ± 7.1	0.99
Quality 1 & 2 (%)	38.0 ± 8.7	61.3 ± 8.2	60.6 ± 9.4	63.4 ± 8.6	0.14
Degenerate (%)	35.2 ± 8.5 ^a	12.6 ± 4.6 ^b	14.5 ± 6.3 ^b	9.6 ± 3.7 ^b	0.02
Quality 1 & 2 of Fertilized (%)	48.4 ± 9.5 ^a	78.3 ± 6.6 ^b	72.6 ± 9.5 ^b	77.7 ± 7.4 ^b	0.05
Degenerate of Fertilized (%)	46.9 ± 9.6 ^a	17.4 ± 6.4 ^b	24.8 ± 9.3 ^{ab}	16.2 ± 7.0 ^b	0.04

^{ab}Values within a row with different superscript letters differ at $P < 0.05$.

Table 3. Body condition score (BCS; least squares means \pm SEM) on days -21, -7, 7, and 21, in relation to calving, for cows that lost, maintained, or gained BCS during the transition period (Barletta et al., 2017).

Item	Change in BCS ¹			P-value
	Gained	Maintained	Lost	
n	69	54	122	-
BCS at -21 DIM	2.57 \pm 0.03 ^c	2.70 \pm 0.04 ^b	2.97 \pm 0.03 ^a	< 0.01
BCS at -7 DIM	2.72 \pm 0.04	2.71 \pm 0.06	2.71 \pm 0.04	0.99
BCS at 7 DIM	2.72 \pm 0.04	2.71 \pm 0.06	2.69 \pm 0.04	0.91
BCS at 21 DIM	2.90 \pm 0.04 ^a	2.70 \pm 0.04 ^b	2.54 \pm 0.03 ^c	< 0.01

^{abc}Values within a row with different superscript letters differ at $P < 0.05$.

¹Cows had their BCS evaluated during the transition period (-21 to 21 DIM) using a 5-point scale with 0.25 increments.

Table 4. Effect of changes in body condition score (BCS) during the transition period (-21 to 21) on diameter of the ovulatory follicle, pregnancies per AI (P/AI), pregnancy loss, days postpartum at first ovulation, and percentage of cyclic cows at 50 DIM for cows that lost, maintained, or gained BCS (Barletta et al., 2017).

Item	Change in BCS ¹			P-value
	Gained	Maintained	Lost	
Cows, % (no./no.)	28 (69/245)	22 (54/245)	50 (122/245)	
Cyclic cows at 50 DIM, %	100 ^a (69/69)	94.4 ^b (51/54)	81.1 ^c (99/122)	0.015
First ovulation, days	33.9 \pm 0.5 ^c	37.9 \pm 0.7 ^b	47.1 \pm 1.0 ^a	< 0.01
Ovulatory Follicle, mm	18.5 \pm 0.5	19.0 \pm 0.8	18.4 \pm 0.4	0.76
P/AI 32 days, % (no./no.)	53.0 (35/66) ^a	26.9 (14/52) ^b	18.3 (21/115) ^b	< 0.01
P/AI 70 days, % (no./no.)	45.5 (30/66) ^a	25.0 (13/52) ^b	15.7 (18/155) ^b	< 0.01
Pregnancy loss, % (no./no.)	14.3 (5/35)	7.1 (1/14)	14.3 (3/21)	0.79

^{abc}Values within a row with different superscript letters differ at $P < 0.05$.

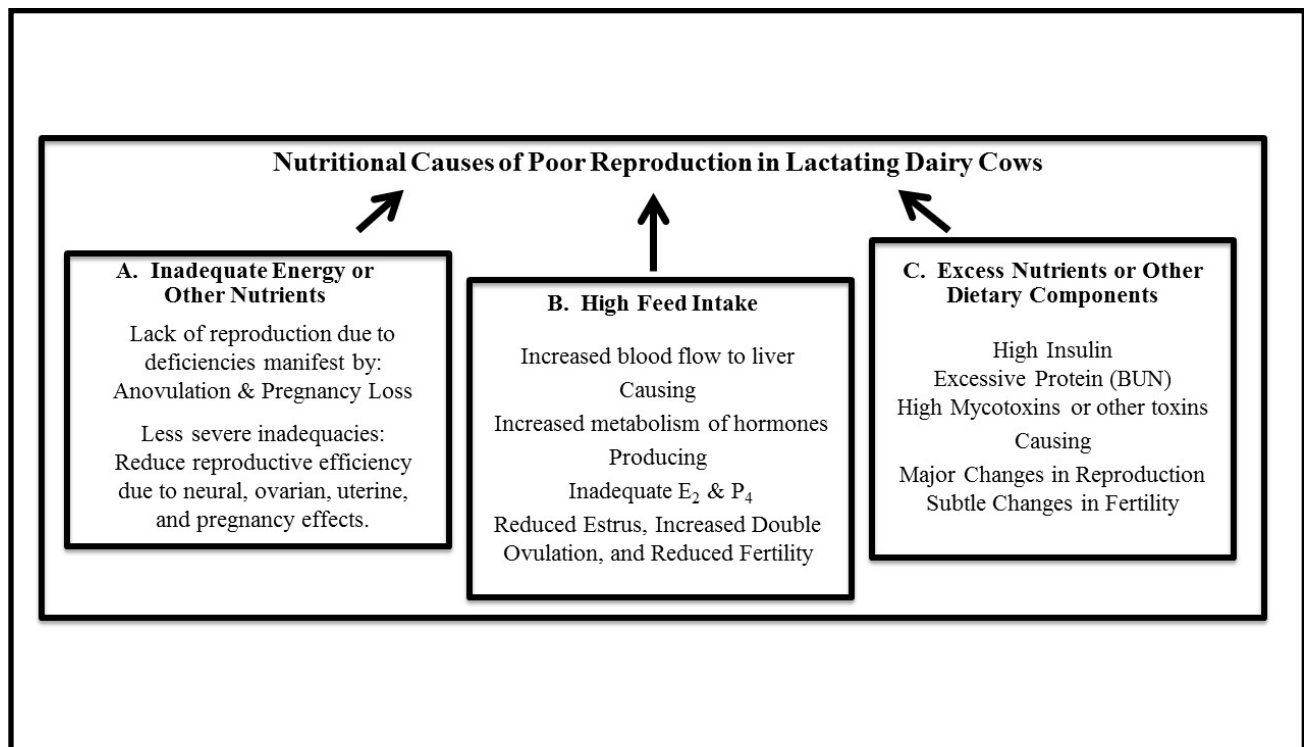


Figure 1. Summary of 3 different ways in which nutrition can produce reduced reproductive efficiency in lactating dairy cows. A. Inadequate intake of energy or other nutrients can lead to deficiencies which may be manifest as anovulation or pregnancy loss or as decreases in fertility and other measures of reproductive efficiency. B. High feed intake leads to high liver blood flow due to the high blood in the digestive tract that flows through the hepatic portal vein to the liver. This pathway leads to high metabolism of steroid hormones such as estradiol (E₂) and progesterone (P₄), causing reduced circulating E₂ and P₄ which causes large changes in reproductive physiology in lactating dairy cows. C. Excess consumption of certain nutrients, such as high carbohydrate diets leading to increased insulin or excessive protein leading to high blood urea nitrogen (BUN) can cause decreases in reproductive efficiency in certain situations. Consumption of feeds that contain certain toxins, such as high phytoestrogens, can produce dramatic changes in reproductive physiology, including reduced fertility.

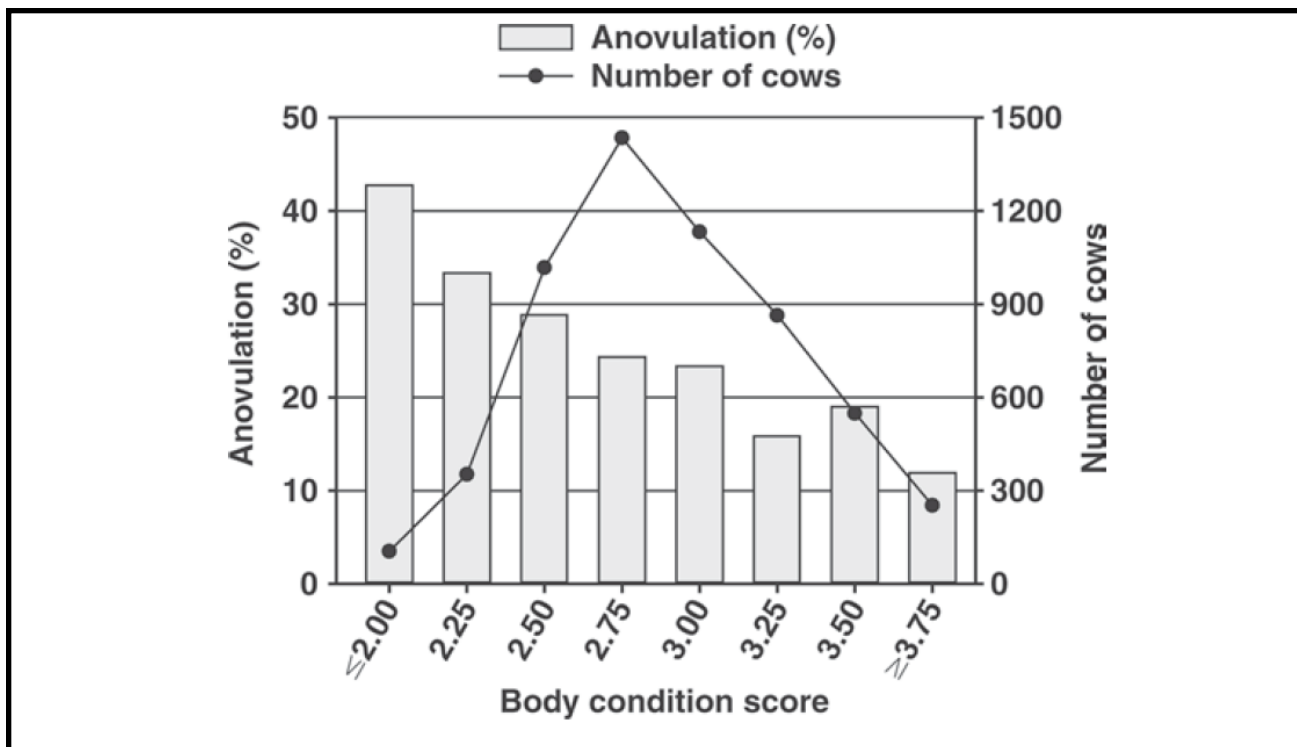


Figure 2. Relationship between BCS and percentage of cows that are anovular (Bamber et al., 2009). The low BCS cows (< 2.5) were a low percentage of the total cows (25.8%) but had a greater percentage of cows that were anovular (30.9%) compared to cows with greater BCS (74.2% of cows; 20.9% anovular).

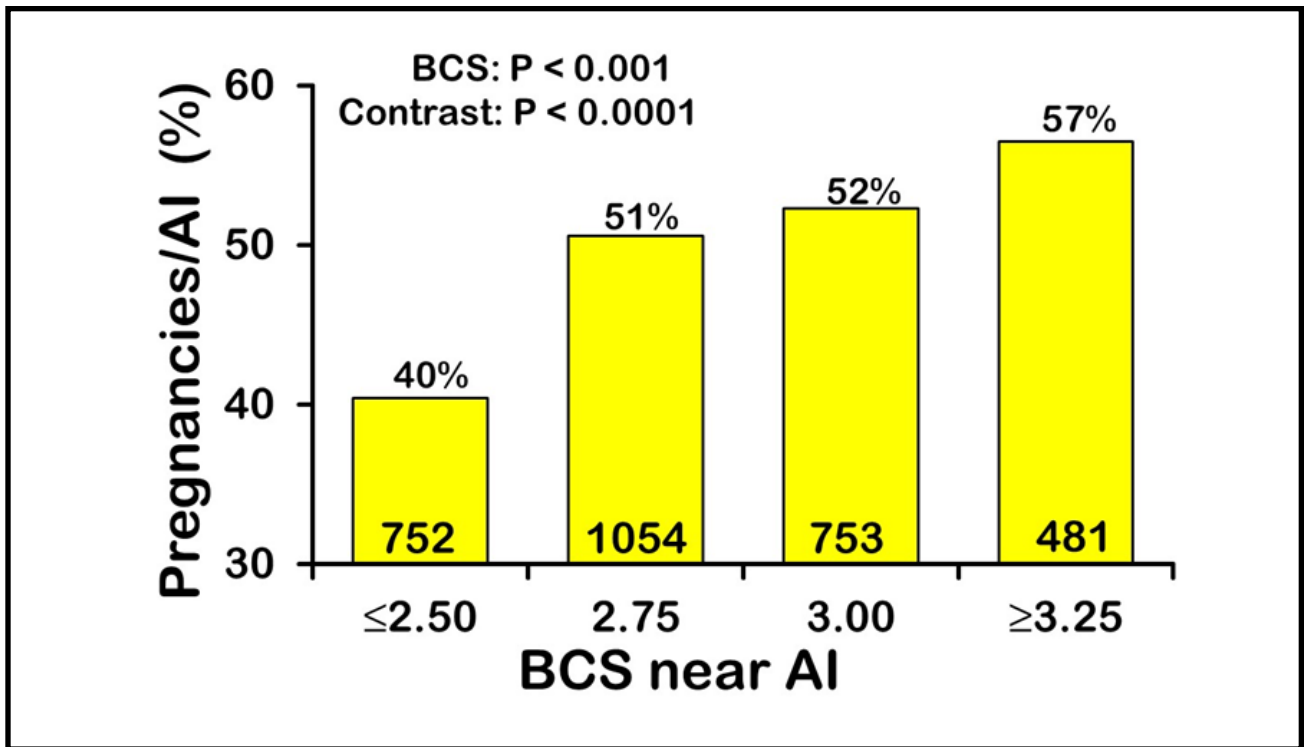


Figure 3. Effect of BCS at the time of AI on pregnancies per AI after Double Ovsynch and timed AI (Carvalho, P.D., unpublished). The contrast compared cows with low BCS (< 2.50) to cows with greater BCS (> 2.75).

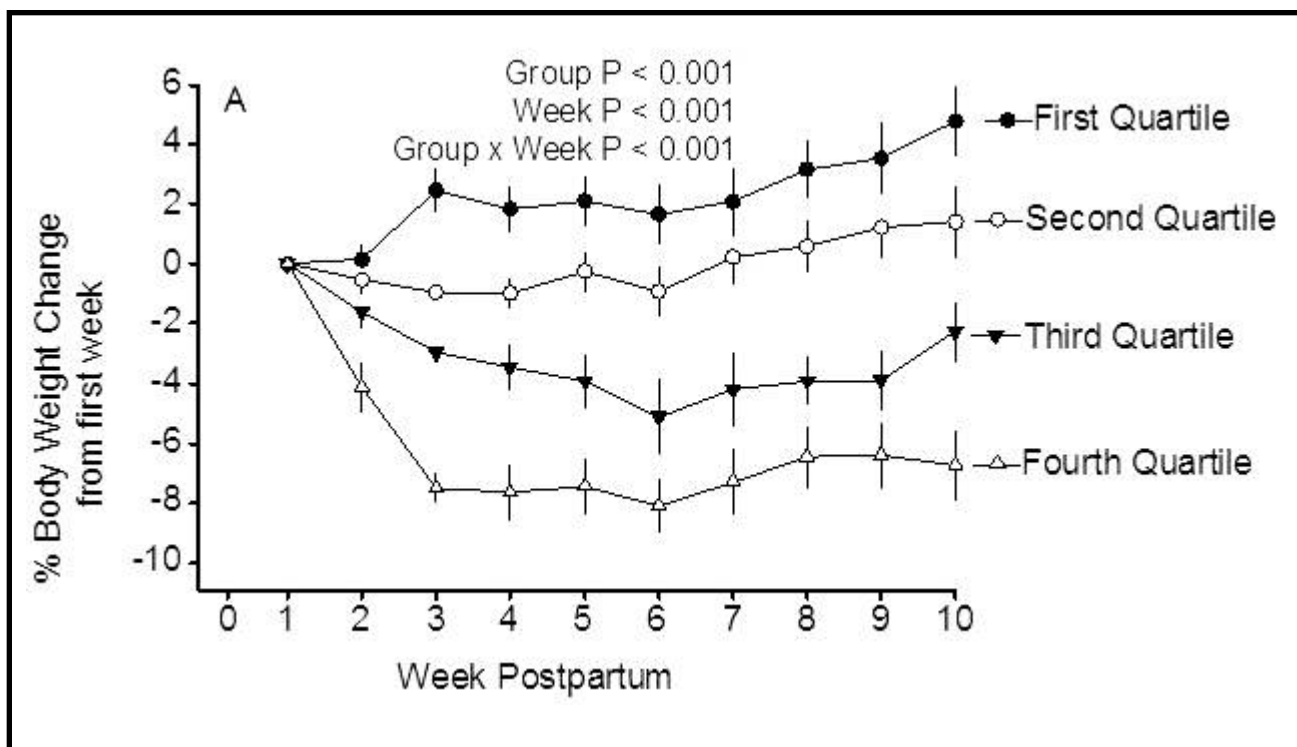


Figure 4. Percentage body weight change based on weight during first week postpartum (Carvalho et al., 2014). Cows were ranked according with % of body weight change from first to third week postpartum and divided into quartiles. Cows in the first quartile gained body weight, whereas cows in the second quartile maintained a relatively constant body weight. The third and fourth quartiles lost body weight with the third quartile losing ~4% of body weight and the fourth quartile losing ~8% of body weight. At the end of this time period cows in all four quartiles were superovulated and embryos (n = 560) were evaluated for fertilization and quality (Table 2).