IMPACT OF TRACE MINERAL VARIATION WITHIN FORAGES ON THE RATION FORMULATION PROCESS

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INTRODUCTION

While trace mineral (TM) concentrations of forages are often lower than the TM requirements of most domestic livestock, they are not negligible. Endogenous minerals in forages are highly available upon digestion, while minerals arising from soil contamination are poorly available. Frequently, dairy rations are balanced either assuming the TM are zero or using standard reference values, e.g. NRC (2001). If forage TM concentrations are not considered in the feed formulation process, it is not likely that the targeted dietary concentrations will be met. There is a greater risk of excess minerals in the ration than deficiency due to the skewed distributions in forage TM concentrations (Figure 1) or if the forage minerals are set to zero. This risk is of concern especially with Cu, where liver accumulation occurs when dairy cattle consume diets with 20 mg/kg Cu (Balemi et al., 2010) and may be detrimental to animal health and performance (Weiss & Faulkner, 2015). Using TM concentrations for forages and other basal feed ingredients will reduce the risk of mineral imbalances, will improve the efficacy of TM supplementation, and can reduce TM excretion into the environment via manure. The objectives of this research were to: 1) quantify the variation in trace mineral concentrations in forages, 2) evaluate the contribution of U.S. geographical location and harvest season to the TM variation, and 3) determine how variation in forage TM concentrations affects ration TM concentrations under different supplementation strategies.

GEOGRAPHICAL DIFFERENCES

Data from Cumberland Valley Analytical Services for forages from the 2009 to 2014 growing seasons with concentrations of major nutrients as well as mineral concentrations were used. Data were statistically filtered to remove misidentified feeds and outliers based on macronutrient concentrations using the procedures outlined in Yoder et al., 2014. After this step, the corn silage, legume hay, mixed mostly legume (MML) silage, and mixed mostly grass (MMG) silage data sets contained 20654, 8856, 8631, and 2914 observations, respectively.

As expected, TM concentrations for corn silage, legume hay, MML silage, and MMG silage displayed skewed distributions (Figure 1). TM values were log normalized before analysis of variance with location, season, and their interaction as independent effects and total ash concentration as a covariate (Proc Mixed, SAS/STAT 9.4). Geographical variation in copper and zinc concentrations were graphed using Proc Gmap (SAS/GRAPH 9.4) and a zip code data set (U.S. Census Bureau).

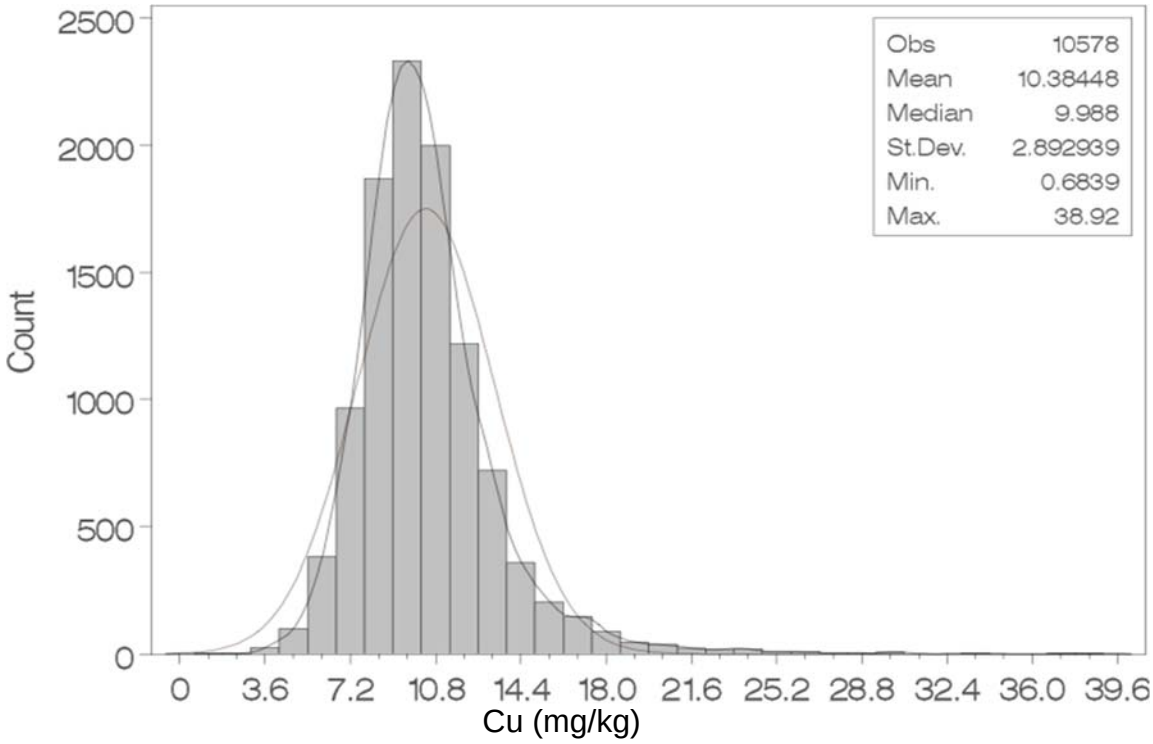

Figure 1. Representative histogram showing skewed distribution of trace mineral concentrations in forages before normalization.

Geographical location and total ash content were the largest sources of variation in forage TM concentrations ($p<0.0001$). Differences between median values for lowest and higest variation in TM concentrations are small, but the range between the 5th and 95th percentiles is illustrative of the large differences in TM consistency (Table 1). Ranges in TM concentrations of forages from the most variable regions of the U.S. were 4 to 10 times greater than the more consistent forages grown in other regions (Table 1). Growing season was often a non-significant ($p>0.20$) contribution to total TM variation, while the interaction between location and season was significant ($p<0.05$). The interaction implies that a portion of the variation attributed to geographical area is dependent on weather conditions during the growing season, e.g. dust carried by winds, or harvesting and storage practices that differ between regions.

Soil contamination can contribute to higher TM concentrations, especially Mn and Fe. Titanium (Ti) concentration in forages is considered by agronomists to be the gold standard in determining soil contamination of forages. However, Ti is not measured in routine nutrient analysis of feed ingredients. Soil contamination of forages reduces the concentration of organic nutrients, and soil Fe can decrease the absorption and utilization of dietary copper and perhaps other minerals (NRC, 2001; references within Hansen & Spears, 2009 and Spears, 2013). In the past, Fe from soil contamination has been assumed to be non-reactive and not interfere with absorption of other trace minerals (TM). However, *in vitro* studies have shown that soil Fe solubility and bioavailability can be increased during ensiling (Hansen & Spears, 2009).

Soil contamination in forages was estimated using a modification of the residual ash (RA) calculation from Cary et al. (1986):

$$RA\ (\%DM) = \text{total ash} - (CaO + K_2O + MgO + Na_2O + P_2O_5 + SO_4 + Cl)$$

Few corn silage samples showed more than 4% soil contamination, but 10 to 23% of legume hay, MML and MMG silages had levels greater than 4% (Table 2). These levels of soil contamination are associated with Fe concentrations greater than 800 mg/kg with the most extreme samples exceeding 2000 mg/kg. Both total ash and Fe concentration are highly correlated with the level of soil contamination estimated by residual ash (R^2 ranging from 0.47 to 0.75).

Table 1. Geographical areas with the highest variation in forage copper and zinc concentrations (mg/kg) have ranges that are 4 to 10 times greater than those in more consistent forages (lowest variation) although differences between median concentrations are small. p5 = 5th percentile, p95 = 95th percentile back calculated from log normalized data. Reference values from Table 15.3 (NRC, 2001) given in last column for comparison.

Cu	Lowest variation			Highest variation			
	median	**p5**	**p95**	**median**	**p5**	**p95**	**NRC 2001**
Corn silage	6.0	4.9	7.3	7.4	3.2	16.4	6
Legume hay	9.6	7.2	11.4	8.2	6.0	34.0	9
MML silage	7.8	5.7	9.6	10.9	5.7	30.1	9
MMG silage	8.8	6.1	9.4	7.8	4.7	37.9	9

Zn	Lowest variation			Highest variation			
	median	**p5**	**p95**	**median**	**p5**	**p95**	**NRC 2001**
Corn silage	23.2	21.4	28.5	26.6	15.3	60.7	24
Legume hay	24.0	21.1	29.5	27.8	20.9	49.2	24
MML silage	28.3	25.8	35.0	35.7	21.8	69.7	28
MMG silage	23.8	20.0	27.6	29.1	18.4	97.5	30

Table 2. Percentage of samples in arbitrarily defined levels of soil contamination based on Residual Ash and the associated Total Ash (% DM), and Fe concentrations (mg/kg) of commonly used dairy forages, given as mean ± SD. However, note that ash and Fe concentrations are not normally distributed within a forage type. MML= Mixed Mostly Legume, MMG = Mixed Mostly Grass.

Level of Soil Contamination		Corn Silage	Legume Hay	MML Silage	MMG Silage
<1%	Percentage	63.5	18.3	8.3	19.3
	Total Ash	3.28 ± 0.47	9.20 ± 1.24	9.21 ± 1.19	6.08 ± 1.56
	Fe	133 ± 83	212 ± 128	265 ± 151	219 ± 170
1 to 4%	Percentage	36.0	71.9	78.1	57.6
	Total Ash	5.39 ± 0.94	10.68 ± 1.25	10.55 ± 1.24	8.41 ± 1.77
	Fe	234 ± 139	353 ± 238	423 ± 261	355 ± 252
> 4%	Percentage	0.5	9.8	13.6	23.1
	Total Ash	8.02 ± 0.60	13.35 ± 1.35	13.51 ± 1.56	12.07 ± 2.03
	Fe	555 ± 313	872 ± 553	1155 ± 576	850 ± 605

RATION FORMULATION AND TM SUPPLEMENTATION

Nutritionists want to know how to best manage the variation in forages to reduce variation in the finished rations. They also want to know how to supplement under conditions of varying TM concentrations in ingredients. The first step is to know how much variation is occurring. This requires appropriate sampling and testing. Frequency of sampling will be determined by how much variation there is in forages and how often forage ingredients are changed in the ration (Figure 2), with more variation requiring more sampling and analysis. Amount of TM supplementation will depend on the median TM concentrations in the forages (Figure 2).

This data on forage variation when combined with data on TM concentrations in grains and protein meals allows us to predict TM concentrations in rations. Mixing feeds together always reduces nutrient variation compared to the variation in individual ingredients, and using more variable ingredients at lower inclusion rates can also reduce variation. Most dairy rations in the U.S. do require supplementation with Cu, Zn, and Mn to reduce the incidence of deficiencies (Table 3). Supplementing in the range of 11-14 for Cu, 30-50 for Zn, and 40-60 for Mn (mg/kg) should nearly eliminate the possibility of any individual rations being deficient (Table 3).

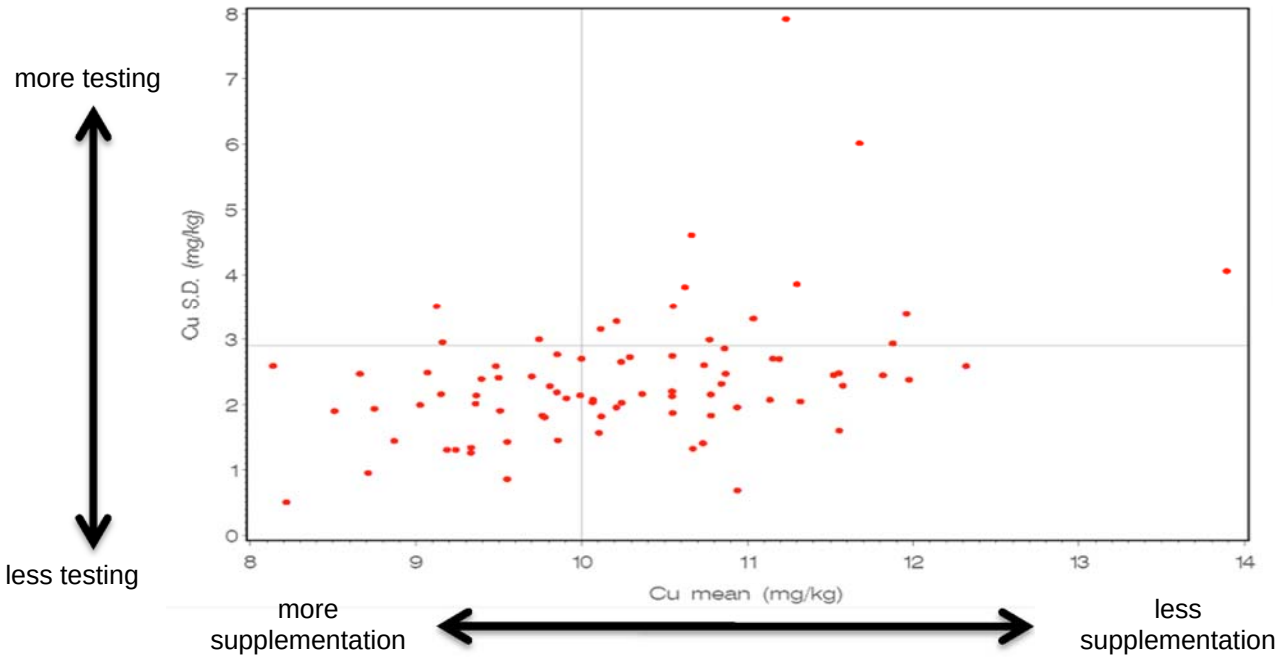

Figure 2. Recommendations for testing and supplementation will vary according to the observed variation in TM concentrations. Example given is in legume hay, median Cu = 10.0, S.D. = 2.9 mg/kg. Each dot represents an individual mailing center, an area defined by the first 3 digits of a zip code.

Table 3. Feed mixing reduces TM variation in finished rations. Predicted TM concentrations in total mixed rations with 0, 1x, or 1.5 supplementation of basal ingredients. Dietary levels (mg/kg) of 11, 52, 40, and 17 were set as minimums for Cu, Zn, Mn, and Fe, respectively (NRC, 2001).

Supplementation Level	Zero	1x	1.5 x	S.D.
Cu ave	6.0	17.0	23.0	1.9
Cu < 11 mg/kg % failures	99.58	0.05	0	
Cu > 30 mg/kg % failures	0	0	0.01	
Zn ave	33.2	85.2	111.2	8.5
Zn < 52 mg/kg % failures	98.75	0	0	
Mn ave	35.1	75.1	95.1	10.0
Mn < 40 mg/kg % failures	69.02	0.01	0	
Fe ave	204	221	230	86.2
Fe < 17 mg/kg % failures	0.06	0.01	0	

Basal ingredients: corn silage, legume hay, flaked corn, dried distillers' grains, corn gluten feed, soybean meal
1x supplementation (mg/kg): 11 Cu, 52 Zn, 40 Mn, 17 Fe added
1.5 x supplementation (mg/kg): 17 Cu, 78 Zn, 60 Mn, 25.5 Fe added

Knowing the basal TM concentrations in forages is key to accurate and precise supplementation! Higher levels of copper supplementation should be avoided to reduce long-term Cu accumulation in liver and potential chronic Cu toxicity (Weiss and Faulkner, 2015). Obviously, excess supplementation of other minerals should be avoided to reduce feed costs, and also to reduce excretion into the environment.

CONCLUSIONS

Geographical location and total ash content are significant sources of variation in TM concentrations in commonly used dairy forages. There are areas in the U.S. that have consistently low TM concentrations, while forages in other areas have high concentrations with high variation. Soil contamination may contribute to variation and can be attributed to weather patterns, soil types, and harvesting and storage practices. Variation in TM concentrations can be reduced with standard feed mixing protocols, but requires knowledge of concentrations for accurate and precise formulation of dietary TM levels. These results support sampling and analysis of forages and formulation of dairy rations for TM based on analytical results rather than reference values.

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GUT MICROBIOME AND NEUROCHEMICAL-BASED INTERACTIONS BETWEEN HOST, MICROBIOTA AND DIET: IMPLICATIONS FOR BEHAVIOR AND DISEASE

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The ability of the gut microbiome to influence various aspects of host health beyond more traditionally associated functions such as digestion of food is increasingly being recognized (Flint, 2012; Shreiner et al., 2015; Tuddenham and Sears, 2015). While interest over the past decade has grown dramatically, our understanding of the interface between the microbiome and host is still largely, but certainly not exclusively, based on *correlational* studies. Such correlational studies by definition do not demonstrate *causality*. Clearly, the need to identify the mechanisms by which the microbiome may influence the host remains paramount and an area for which the great bulk of research lies in the future.

This short review seeks to discuss one of those possible mechanisms by which the microbiota contained within the gastrointestinal system may impact host health, including behavior. It relies on the evolutionary relationship between the microbiota and host's neurophysiological system. This field of study has been termed microbial endocrinology. As will be discussed, the microbiota possesses the capacity to not only recognize neurochemicals produced by the host such as in response to stress, but also synthesize the same neurochemicals as produced by the host. The ability of the microbiome to produce and release neurochemicals that can influence the host, known as microbial endocrinology, provides for a mechanistic basis with which to examine the ability of stress to influence the health and behavior through the microbiome-gut-brain axis (Lyte, 2013a; Lyte and Cryan, 2014; Neuman et al., 2015).

MICROBIAL ENDOCRINOLOGY – CONCEPTUAL FRAMEWORK

Microbial endocrinology represents the intersection of two seemingly disparate fields, microbiology and neurobiology (Figure 1). The field of microbial endocrinology was founded in 1993 when the term was first coined (Lyte, 1993). Although the concept of microbial endocrinology was founded just over 2 decades ago, there has been published evidence by numerous investigators over the preceding six decades going back to 1930 that demonstrate the validity of uniting the fields of microbiology and neurobiology as a conceptual framework with which to understand interactions between the microbiota and the host, although at the time it was not conceived that a host-derived neurochemical could interact with a prokaryotic microorganism such as the infectious bacterium *Clostridium perfringens* (Lyte, 2010a).

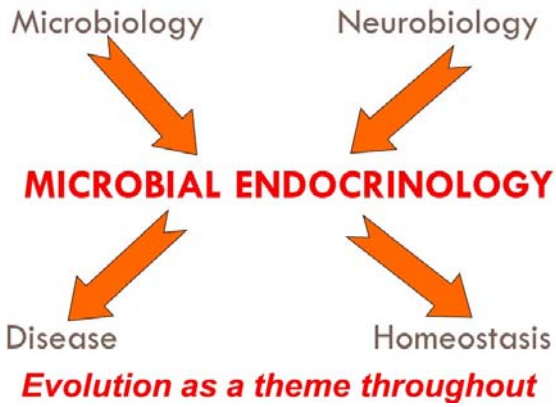

Figure 1. Conceptual basis of microbial endocrinology.

It is somewhat surprising to learn that what are often most thought of as exclusively mammalian in origin are in fact found widely disseminated throughout nature. This is expressly the case for a wide spectrum of neurochemicals extending from epinephrine to somatostatin (LeRoith et al., 1986; Lenard, 1992; Lyte, 2010a). A comprehensive analysis of the wide spectrum of neurochemicals and related cognate receptors that have been isolated form microorganisms highlights the presence in microorganisms of what are otherwise thought to be more commonly associated with mammalian systems (Roshchina, 2010). In general, the precise role of these neuroendocrine hormones in bacterial physiology is largely unknown. The diverse nature of these neurochemicals strongly suggests that from an evolutionary perspective the possession of what are normally considered to be specific to vertebrates implies that microorganisms have a means to recognize neurohormones within a vertebrate host and initiate changes in physiology that would prove advantageous to its survival.

ANATOMICAL ASSOCIATIONS THAT FOSTER MICROBIAL ENDOCRINOLOGY

The question must be asked if there is a spatial relationship between the gut microbiota and elements of the host nervous system that would enable interactions that are based on a shared neurochemistry. It is perhaps under-appreciated by most microbiologists that the gut is a highly innervated organ that possesses its own nervous system known as the enteric nervous system (ENS) that is in constant communication with the central nervous system (CNS) through nerves such as the vagus which directly connect portions of the gut to the brain (Figure 2).

The ENS is composed of over 500 million neurons. The extensive nature of this network is best shown in Figure 3 which demonstrates that the innervation extends not only to the tips of the villi themselves (Figure 3A) but also around the base of the crypts (Figure 3B) (Powley et al., 2011). It is through this ENS-vagus connection that information derived from elements of the ENS that innervate the gut is transmitted to the brain (Furness et al., 2014). Further contributing to the amount of information obtained in the gut are the luminal epithelial chemosensors, which can respond to and transmit information regarding bacterial metabolites such as neuroactive compounds that are contained within the luminal space (Breer et al., 2012). This gut-to-brain communication

has been the subject of intensive study for many years and is now recognized to play an important role in the ability of gut-related pathologies to also result in mental health–related issues such as depression (Foster and McVey Neufeld, 2013). The inclusion and recognition that microorganisms interact with elements of the ENS and thereby contribute to the information that is received by the brain concerning the physiological state of the gut has led to the relatively new field of study known as the microbiota-gut-brain axis (Lyte and Cryan, 2014).

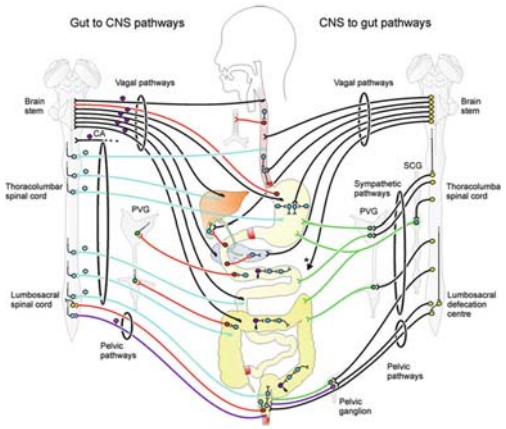

Figure 2. Innervation of gastrointestinal tract from Furness, 2014.

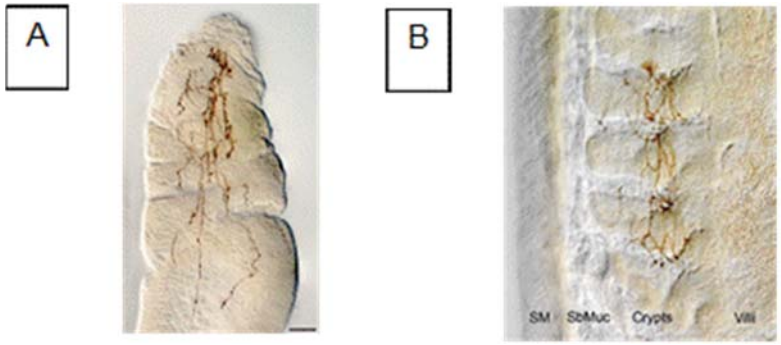

Figure 3. Presence of afferents in the intestinal villi (A) and crypts (B). From Powley, 2011.

Indeed, one of the most dramatic examples of how information that is gathered in the gut by components of the ENS can selectively influence the brain was shown following the interruption of the vagal nerve connection between the gut and brain by a procedure known as sub-diaphragmatic deafferentation (Klarer et al., 2014). Following this surgical procedure which involves transection of the vagus nerve, it was shown that specific behavioral responses of the animal, such as anxiety-like behavior or learned fear, could be selectively affected depending on whether the information from the vagal villus or the vagal crypt efferents were involved (Klarer et al., 2014). While this points out that “bottom-up” information collected by the components of the ENS have effects outside of the gut, left unanswered is the question of what in the lumen of the gut, namely the microbiota, may have on the information that is gathered by these ENS elements.

STRESS: THE PROTOYPICAL EXAMPLE OF MICROBIAL ENDOCRINOLOGY

To date, one of the most potent neurophysiological events that have been shown to influence host health, specifically susceptibility to infectious disease, and behavior is that of stress. Numerous studies have purported to show that stress can affect gut microbiota composition, influence microbiota-gut-brain communication, and result in behavioral alteration (Grenham et al., 2011; Cryan and Dinan, 2012; Collins et al., 2013). Both physical and psychosocial stress, as well as alteration of circadian rhythm, have been shown to alter microbiota community structure within the gut (Bailey et al., 2011; Bangsgaard Bendtsen et al., 2012; Thaiss et al., 2014).

There is a common evolutionary pathway in which stress-related neurochemicals first evolved in bacteria and, through lateral gene transfer, were acquired by mammals (Iyer et al., 2004). This means that a *mechanistic bi-directional* signaling pathway for these neurochemicals exists between gut microbiota and the host in response to stress as shown in Figure 4.

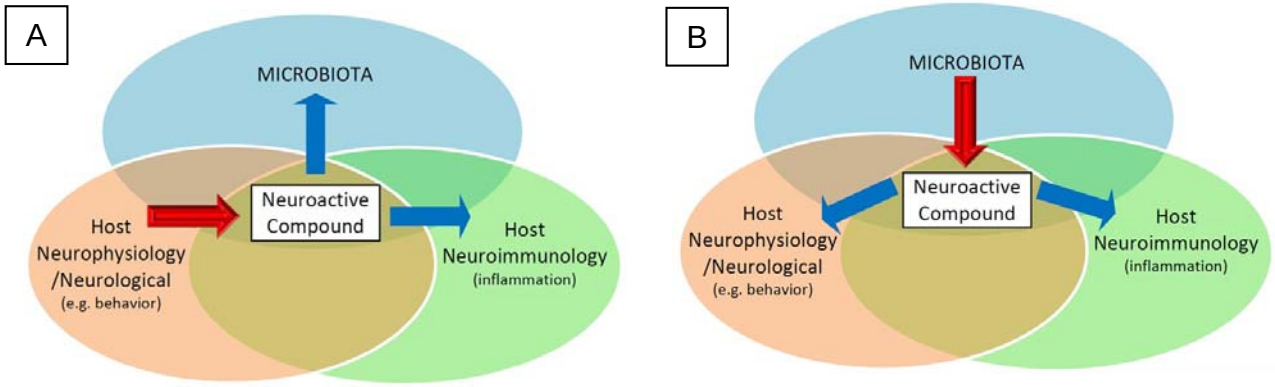

Figure 4. Bi-directional nature of microbial endocrinology in which neurochemicals produced by the host can influence the microbiota (A) and the very same neurochemicals produced by the microbiota can influence the host (B). The evolutionary-based neurochemical signaling pathway between microbiota and host means that a neurochemical(s) produced by the host can influence the microbiota (A) and at the same time a neurochemical(s) produced by the microbiota can, in turn, influence the host (B).

The microbiota community structure within the gut can rapidly change due to influx of host stress-related neurochemicals into the lumen. One of the principal classes of neurochemicals produced during periods of stress is the biogenic amines, notably the catecholamine family (dopamine, norepinephrine and epinephrine). Bacteria were first shown to be responsive to the catecholamines as reflected by changes in growth (Lyte and Ernst, 1992; Kinney et al., 1999; Roberts et al., 2002; Vlisidou et al., 2004), gene expression (Nguyen and Lyte, 1997; Anderson and Armstrong, 2006; Oneal et al., 2008) and transfer (Peterson et al., 2011). Release of catecholamines from neurotoxin-injured enteric neurons into the intestinal lumen result in the rapid alteration of microbiota

community from one dominated by Gram-positive taxa to one dominated by Gram-negative taxa (Lyte and Bailey, 1997). Further evidence of the association of neuronal activity to microbiota composition came from the observation that as injured nerves re-healed over a two week period, the microbiota community structure returned to normal (Lyte and Bailey, 1997). Remarkably, gut bacteria can also produce the very same neurochemicals produced by the host. For example, the *in vivo* production by gut bacteria of physiological levels of norepinephrine and dopamine capable of affecting host physiology has been observed (Asano et al., 2012). This further highlights the bi-directional nature of host-microbial interaction.

MICROBIAL ENDOCRINOLOGY AND INFECTIOUS DISEASE

The ability of infectious microorganisms to respond to neurochemicals and alter growth and virulence has now been reported by a number of groups (Lyte et al., 1997a; Kinney et al., 1999; Vlisidou et al., 2004; Nakano et al., 2007b; Bearson et al., 2008; Sandrini et al., 2010; Freestone et al., 2012; Sandrini et al., 2014). Although the mechanisms governing the ability of neurochemicals such as the biogenic amines to modulate the growth and production of virulence-related factors have not yet been completely elucidated, recent results have shown the ability of biogenic amines such as norepinephrine to induce transcriptional changes in mRNA transcript levels for a number of genes in a number of respiratory and intestinal pathogens as well as increase the rate of conjugative transfer between enteric bacteria (Nakano et al., 2007a; Oneal et al., 2008; Peterson et al., 2011).

From a clinical standpoint the ability of pharmacologically-relevant concentrations of neurochemicals, such as the catecholamines and related analogs (i.e. inotropes based on catecholamine structure such as dobutamine) have their greatest impact through the induction of biofilms. Early work demonstrated that dopamine and dobutamine, both used in the clinical intensive care setting for the support of cardiovascular and renal function, could induce biofilm formation from exceedingly low inocula of *Staphylococcus epidermidis* in physiologically-relevant plasma containing medium on materials used in the manufacture of indwelling medical devices (Lyte et al., 2003). Subsequent work has shown that catecholamines can induce the formation of biofilms by *Pseudomonas aeruginosa* which may provide a mechanistic explanation for its prevalence in ventilator-associated pneumonia (Freestone et al., 2012). Recent reviews have addressed the numerous and increasing number of studies which have examined the ability of neurochemicals to influence the pathogenesis of infectious disease through direct interactions with microorganisms, both prokaryotic and eukaryotic (Clemons et al., 2010; Lyte, 2015; Sandrini et al., 2015).

DIET AND BEHAVIOR – ROLE OF THE MICROBIOTA-GUT-BRAIN AXIS AND MICROBIAL ENDOCRINOLOGY AS A MEDIATING MECHANISM

The concept that bacteria in the gut can communicate with the brain thereby influencing behavior, and that the host nervous system can, in turn, influence the composition of the gut microbiota, has given rise to the concept of a microbiota-gut-brain

axis (Lyte and Cryan, 2014). An ever-growing number of studies have demonstrated the ability of bacteria to influence brain function for which a number of possible mechanistic routes have been proposed (Bravo et al., 2011; Lyte, 2011; Neufeld et al., 2011; Reid, 2011; Cryan and Dinan, 2012; Collins et al., 2013; Desbonnet et al., 2013; Lyte, 2013b; Wall et al., 2014). Due to shared neurochemicals between host and microbe, microbial endocrinology has been proposed as one of the mechanisms by which such reciprocal communication between brain (nervous system) and microorganisms in the gut can occur (Lyte, 2014b, a).

The ability of diet to alter the composition of the microbiome has been recognized for decades (for review see (Flint, 2012)). What is not known, however, is if diet-induced changes in the microbiome can directly and in a causal manner lead to changes in behavior via microbial endocrinology-based mechanisms. Such a proposal, that diet can influence bacteria to produce neurochemicals that interact with the ENS, or directly are absorbed into the portal circulation, would represent a new mechanism by which nutrition could impact the host and ultimately influence various aspects of behavior as well as food preferences and appetite. It should be noted that it has now been proposed that a positive feedback loop exists between the host's dietary preferences and the microbiome (Norris et al., 2013). The Norris et al. paper therefore represents one of the first proposals, along with that proposed earlier (Lyte, 2010b), that suggests that the nutritive state of the host and the microbiome influence one another through bi-directional microbial-based mechanisms that had not been previously envisioned as part of nutrition.

The presence of neurochemicals in plants and processed foods has long been recognized. For example, the source material used to demonstrate the biological role of the neurotransmitter acetylcholine in muscle contraction was obtained from the leaves of the common nettle before it was ever isolated from a vertebrate source (Roshchina, 2010). From a nutritional standpoint, these neurochemicals, which include the biogenic amines, have not been viewed as a significant dietary energy source. Their impact on health and well-being has in the past been primarily restricted to direct physiological or patho-physiological effects in the host such as following the consumption of foods containing vasoactive substances. The ability to demonstrate that the nutritional value of a particular food may extend beyond the more commonly accepted understanding of components such as carbon and nitrogen content (as well as protein content as typical examples) to that of providing a common signaling mechanism, namely neurochemicals, between the microbiome and host would add to our understanding how diet may affect the composition of the microbiota. That in turn would aid in deciphering the mechanisms by which the microbiota-gut-brain axis is capable of modulating behavior.

Figure 5 illustrates how the proposed neurochemical-based facets of diet and microbiome can interact to influence the microbiota-gut-brain axis and thereby influence cognitive processes that ultimately result in modulation of behavior. These involve microbial endocrinology-based pathways by which neurochemical compounds produced by both the host and the microbiota can serve as a mechanism by which the brain and behavior can be modulated within the microbiota-gut-brain axis (Lyte, 2013a).

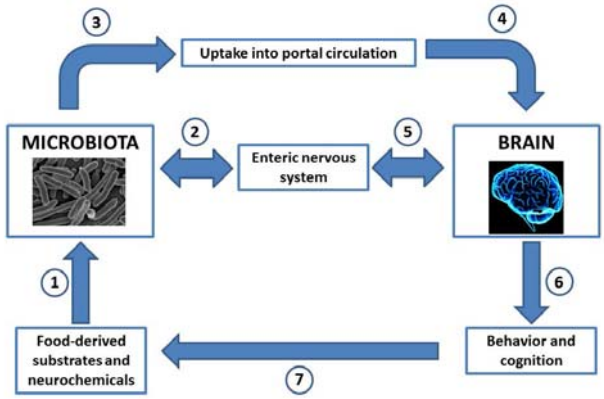

Figure 5. Microbial endocrinology-based pathways by which diet can influence the microbiota-gut brain axis. From Lyte, 2013a.

As shown in Figure 5, food ingested by the host contains both the substrates needed for neurochemical production by the host and the microbiota as well as fully functional neuroactive components (1). The microbiota in the gut is capable of either forming neurochemicals from the substrates present in the ingested food; or responding to the neuroactive food components themselves; or responding to neurochemicals secreted into the gut by components of the host enteric nervous system (2). Neurochemicals produced by the microbiota in the gut have two pathways by which to influence the host; they can either be taken up from the gut into the portal circulation (3) or they can directly interact with receptors found on components of the enteric nervous system which innervates the complete length of the gastrointestinal tract (2). Once in the portal circulation, microbiota-derived neurochemicals can influence components of the nervous system and ultimately the brain (4). Microbiota-derived neurochemicals can also influence components of the nervous system such as the brain through ENS-CNS communication (5). The result of either pathway (4) or (5) on the brain may result in an alteration of behavior or cognition (6) as well as food preferences and appetite (7) [82-85]. This should not be viewed as a one-way direction of only gut-to-brain since the brain may influence the composition of the microbiota through the specific release of neurochemicals into the gut lumen (2).

CONCLUDING STATEMENT

The ability of microorganisms to both produce and recognize the exact same neurochemicals that mammalian hosts (as well as plants and insects) produce offers a new mechanistic pathway by which to understand the ability of the microbiota to influence both behavior and disease.

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IMPACT OF MYCOTOXINS ON THE HEALTH AND PRODUCTIVITY OF DAIRY CATTLE

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INTRODUCTION

The term mycotoxin covers a broad group of secondary metabolites produced by fungi. By definition, these metabolites must be toxic in nature to the species of focus; in this case dairy cattle. That said, not all fungal metabolites are considered to be mycotoxins. Mycotoxins of interest include aflatoxins, ergot alkaloids, fumonisins, ochratoxins, patulin, trichothecenes, and zeralenone (Bennet and Klich, 2003). Past research has focused on cereal grains, byproducts, and dry-stored forage as primary sources of mycotoxins, however, ensiled feeds can also be prominent source for dairy cattle (Driehuis et al., 2008). This review will focus on mycotoxin degradation in the rumen and the effects of mycotoxins on gastrointestinal function. Production responses will also be highlighted.

Ruminal degradation of mycotoxins and the effect of mycotoxins on gastrointestinal function

There is no doubt that ruminants have greater tolerance to feed contaminated with mycotoxins than monogastrics. The improved resistance is largely related to the ability of the ruminal microflora to degrade mycotoxins converting them into less potent intermediates (Hussein and Brasel, 2001; Fink-Gremmels, 2008). For example, ochratoxin is degraded into ochratoxin α and phenylalanine by mixed microbes isolated from the reticulo-rumen and omasum; both of which have reduced toxicity (Hult et al., 1976). Kiessling et al. (1984) evaluated the degradation of aflatoxin, ochratoxin, zearalenone, T-2 toxin, diacetoxyscirpenol (**DAS**), and deoxynivalenol (**DON**) using in vitro incubation with mixed rumen microbes or with protozoa, or bacteria alone. That study led to several important findings. Firstly, ochratoxin degrading activity appears to respond to feeding cycles with lowest activity immediately after a meal and greatest activity prior to the meal. Secondly, the greatest ochratoxin, zearalenone, DAS, and T-2 toxin was found for the protozoa rich incubation (Hussein et al., 2001; Fink-Gremmels, 2008). In contrast, Keissling et al. (1984) found that aflatoxin and DON were not degraded in vitro. The lack of ruminal degradation of aflatoxin has been confirmed by subsequent studies (Westlake et al., 1989; Hussein et al., 2001) while degradation of DON appears to be variable (Westlake et al., 1989) with nearly complete degradation being predicted in vivo using cattle fitted with a duodenal cannula (Dänicke et al., 2005). Finally, the Keissling et al. (1984) study reported that when sheep were fed a high-grain diet, capability for toxin degradation was reduced by 20%. This may suggest that dietary scenarios that partially defaunate the rumen may decrease the ability of microbial

degradation of mycotoxins and thereby reduce tolerance of ruminants to mycotoxins in feed.

Microbial degradation may not be sufficient to reduce the potency of all mycotoxins. Despite some degradation of ergot alkaloids by ruminal microbes to yield lysergic acid (Duringer et al., 2007), lysergic acid may be transported across the gastrointestinal tract at a greater rate than most ergot alkaloids suggesting that the overall toxic dose may not be reduced (Guerre, 2015). Moreover, while there is degradation of ergot alkaloids, there was 35% recovery of ergovaline in feces from sheep and nearly 250% recovery of lysergic acid (DeLorme et al. 2007). This suggests that despite some capacity for degradation, exposure of gastrointestinal tissues and potentially systemic tissues to ergot alkaloids is possible. T-2 toxin is catabolized to HT-2 toxin and zeralenone is degraded to zeralenol (among others). Zeralenol has a greater affinity to estrogen receptors than zeralenone demonstrating that microbial degradation can, at times, worsen the challenge (Marczuk et al., 2012). In addition, the role of microbes to degrade mycotoxins may come at a cost as there is clear evidence demonstrating that mycotoxins may alter the ruminal microflora activity. High doses of aflatoxin B_1 and G_1 (1.0 µg/mL of rumen fluid) reduced DM digestion of hay by 50 and 20% respectively (Westlake et al., 1989). El-Ayouty and El-Saadany (1990) as well as others (Escoula, 1992; Puel et al., 2005) also reported reductions in DM and OM digestibility associated mycotoxin contaminated feed. Perhaps this is not surprising given that some mycotoxins are known to have antimicrobial properties (Fink-Gremmels, 2008; Strickland et al., 2011). Future studies should evaluate the impact of mycotoxins on the ruminal microbiome to evaluate species that increase and decrease in relative abundance when exposed to mycotoxins.

Corresponding to antimicrobial properties of some mycotoxins, May et al. (2000) evaluated the effect of DON and fusaric acid on *Ruminococcus albus* and *Methanobrevibacter ruminantium*. Fusaric acid inhibited the growth of *R. albus* and *M. ruminantium* with concentrations as low as 15 µg/mL while DON had no effect. Fumonsin B_1 was reported to have little influence on short-chain fatty acid production and microbial activity; however, it should be noted that only 12 to 18% of the fumonsin B_1 was degraded after 72 h of incubation (Caloni et al., 2000). Feeding mycotoxin contaminated grain has also been reported to increase ruminal ammonia concentration and reduce the microbial protein flow the small intestine (Dänicke et al., 2005).

Mycotoxins such as slaframine (Froetschel et al., 1986) and ergot alkaloids (Koontz, 2015) can directly impact rumen function. Froetschel et al. (1986) reported marked reductions in rumen motility and increases in rumen fluid volume and rumen liquid outflow (Froetschel et al., 1987). Ergot alkaloids can bind with G-protein coupled receptors, beta-adrenergic receptors, and biogenic amine receptors (Koontz, 2015). The ability of ergot alkaloids to act as ligands for a variety of receptors presents a challenge for a clear diagnosis in field conditions. However, reduced serum prolactin is a common response. Cattle fed feed contaminated with ergot alkaloids also have reduced blood flow caused by vasoconstriction and consequently reduced SCFA absorption from the rumen (Foote et al., 2012). The reduction in SCFA absorption appears to be largely in

response to the reduced blood flow and low feed intake (Foote et al., 2012) and independent of epithelial function as exposure to ergovaline ex vivo did not alter SCFA transport despite the ability to detect the movement of ergovaline across the isolated ruminal epithelia in under the same model (Foote et al., 2014). Interestingly, exposure of the rumen epithelium to 50 or 250 ng ergovaline/mL did not alter barrier function of the ruminal epithelium. It is not clear whether other mycotoxins, such as patulin (Mahfoud et al., 2002) or fumonsin B1, alter barrier function of the gastrointestinal tract in ruminants as reported for monogastrics.

In monogastrics, it is known that species differ for the potential to absorb mycotoxins across the gut with rapid rates for aflatoxins, poor intestinal absorption for fumonsins, and moderate for DON (Grenier and Applegate, 2013). However, entero-hepatic recycling via the bile may contribute to increased exposure of intestinal epithelial cells to mycotoxins and mycotoxin incorporation into micelles may increase intestinal absorption (D'Mello et al., 1999; Mahfoud et al., 2002; Grenier and Applegate, 2013). Indeed, DON has been shown to reduce the absorptive surface area in poultry (Awad et al., 2006a, Awad et al., 2006b, Awad et al., 2011). Supporting the reduction in absorptive surface area, Awad et al. (2007) also noted that DON inhibited the glucose-dependent increase in short-circuit current of the jejunum in Ussing chambers. This suggests that DON, and potentially other mycotoxins, may modulate sodium transport by intestinal epithelium. Given the potential for detoxifying effects in the rumen, it would be expected that the effective dose ingested to induce such effects in ruminants would be much greater than monogastrics, but it could be expected that similar responses would occur. Others have also shown a reduction in Na-dependent transport processes with ochratoxin (Maresca et al. 2001), and DON (Maresca et al., 2002). Thus, it appears that mycotoxins have the potential to alter Na-dependent nutrient absorption in monogastric species but to the authors' knowledge, there is no work evaluating similar processes in ruminants. As stated above, addition of ergovaline was not reported to alter SCFA transport ex vivo (Foote et al., 2014) suggesting that reduced blood flow was the primary mechanism decreasing SCFA absorption in vivo (Foote et al., 2013).

In addition to reduced nutrient transport capability, there is evidence in monogastric species demonstrating that exposure to ochratoxin, fumonsin B1, and DON can reduce trans-epithelial resistance thereby increasing the paracellular permeability of the intestinal epithelium (Grenier and Applegate, 2013). It appears that basolateral exposure to DON down-regulates tight-cell junction associate proteins (zona occludin-1 and occludin) suggesting that movement of DON across the epithelia may be required to induce negative effects on barrier function. As with nutrient absorption, data is limited on whether such a response occurs in ruminants.

Effect mycotoxins on the productivity of dairy cattle

The symptoms of mycotoxin exposure for dairy cattle are general and vague. Symptoms include low feed intake, reduced milk production, exacerbated negative energy balance, and hemorrhagic enteritis and diarrhea, reproductive failure, mastitis, and laminitis (Fink-Gremmels, 2008; Marczuk et al., 2012). Given the non-specific

symptoms, variation in mycotoxin concentration in feeds, and diagnostic challenges, confirming chronic mycotoxin exposure is difficult. (Fink-Gremmels, 2008) suggested that exposure to aflatoxins may reduce liver function and contribute to development of fatty liver disease, especially for cows in early lactation. However, a recent case-study report in Poland (Marczuk et al., 2012) demonstrated detectable plasma concentrations of DON and elevated levels of zeralenone. The exposure to DON and zeralenone was prolonged and 5 cows died during the study. Associated with the elevated plasma DON and zeralenone, was an increase in PCV, Ca, and leukocytosis.

Although mycotoxins are often implicated with reduced milk production, supporting evidence is difficult to find in published literature. Studies evaluating adsorbants as a strategy to reduce the impact of mycotoxins have reported that low doses of mycotoxins likely do not affect DMI or milk production (Xiong et al., 2015). For example, Queiroz et al. (2012) reported that a diet containing 75 µg/kg aflatoxin B1, did not affect DMI or milk yield for dairy cattle but decreased the concentration of milk CP supporting the inhibitory effect of aflatoxin on protein synthesis. Firmin et al. (2011) reported no effect of aflatoxin B1 or M1 on DMI, milk yield but reduced milk fat content for sheep. Even long-term exposure to DON did not affect hepatic function in dairy cattle (Kinoshita et al., 2015). While aflatoxin and trichothecenes seem to have little effect on DMI and milk yield but reduce milk CP, ergot alkaloids have a potent effect to reduce milk yield. Early work in rats demonstrated that administration of a variety of ergot alkaloids inhibited prolactin secretion thereby inhibiting lactation (Shaar and Clemens, 1971). Work in pre-partum heifers (Bernard et al., 1993) and cows supported these findings demonstrating that even pre-partum exposure to endophyte infected fescue decreased prolactin concentration prior to parturition; however, prolactin concentrations recovered and milk yield was only numerically lower for cows exposed to alkaloids relative to their counterparts. Part of the discrepancy between anecdotal field evidence and controlled research studies may be related to the additive effect of multiple mycotoxins present in feeds at once compared to the single or dual mycotoxin evaluation treatments that have been investigated in research studies and perhaps the duration of the exposure.

CONCLUSIONS

Ruminants have a greater tolerance to mycotoxins than monogastrics due to ruminal metabolism. However, potential effects of mycotoxins on intestinal epithelial cells may still be present although there is limited data to support the outcomes in dairy cattle. Although mycotoxins have been suggested to affect DMI and milk yield, there is limited data to support such a conclusion. Future work is needed to evaluate combinations of mycotoxins on production parameters.

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OPPORTUNITIES TO IMPROVE GUT HEALTH IN RUMINANT PRODUCTION SYSTEMS

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INTRODUCTION

In the past decade there have been a large number of publications describing how the gastrointestinal tract (GIT) plays a pivotal role in health and disease (Spor et al., 2011). These findings coincide with the advancement of molecular based sequencing techniques that have allowed researchers to investigate the expression of genes in gut tissues and the microbial communities with great efficiency and specificity. This highly advanced branch of molecular research has recently been applied in livestock studies (Frank et al., 2011), leading to the term “gut-health” becoming a buzz-word in the animal nutrition industry. This is an interesting turn of events given that the industry has traditionally been rooted in highly quantitative research. In spite of the evolving interest in this field, the term “gut-health” remains loosely defined, even scientifically, thus careful consideration of what the gut-health promoting action of a particular nutrient or feeding strategy requires close consideration. In this review, the main principles of gut health will be defined, and a description of the key target areas for future advancement in ruminant production will be provided. Furthermore, we will examine what has been done thus far in the ruminant sector with respect to the development of nutritional additives that positively impact gut health.

DEFINING GUT HEALTH

The GIT is the largest organ in the body involved in digestion and nutrient absorption, and invests great effort into maintaining a fine balance between its highly dense resident gut microbiota and the gut-associated immune system. The absence of gut microbiota results in an underdeveloped gut-associated immune system and peripheral organs (e.g. the spleen; Guarner, 2006). Conversely, an altered gut microbiome is associated with chronic metabolic disorders (obesity), inflammatory bowel disease (IBD), allergies, and autoimmune conditions, including type1 diabetes (Sekirov et al., 2010). In addition to the gut microbiota, the GIT epithelial barrier plays a vital role in maintaining the health of the gut and the host. The epithelial barrier that physically separates microbiota in the gut lumen and the mucosal immune system contains nearly 70% of total leukocytes and 80% of total secreting cells of IgA (mucosal antibody) in the body (Vighi et al., 2008) and interacts with the gut microbiome to maintain intestinal homeostasis and gut health. Inasmuch, defining gut health should take all the involved components and their complex interactions into account.

MICROBIOTA AND GUT HEALTH

The mammalian GIT is considered to be sterile *in utero* and undergoes rapid colonization with an array of microbiota during and after birth. This process of colonization is influenced by maternal microbiota, and delivery mode during birthing (Fanaro et al., 2003), while diet, lifestyle and antibiotic treatments may also largely influence the microbial composition after birth (Fouhy et al., 2012; Rodriguez et al., 2015). The neonatal gut colonization is a crucial period for the developing gut and the naïve immune system (Hansen et al., 2012) and may have long-term health effects on the animal (Conroy et al., 2009). The “hygiene hypothesis” suggests that increased hygienic conditions in western countries has reduced infant exposure to microbes, resulting in higher incidences of atopic diseases (atopic eczema, allergic rhinitis and asthma) (Kalliomaki and Isolauri, 2002). The administration of probiotics or cultures of healthy gut microflora though has been shown to reduce the development of atopic eczema significantly (Kalliomaki and Isolauri, 2002). Therefore, both gut colonization and the composition of early microbiota are important factors for long-term gut health.

A recent study revealed that the establishment of host-specific gut microbiota is required for the development of the mucosal immune system (Chung et al., 2012). The development of mucosal T-lymphocytes in human-microbiota colonized mice was similar to that of germfree mice and the cell numbers were less than that of mouse-microbiota colonized mice (Chung et al., 2012). Further, the susceptibility to *Salmonella* infections was higher in human-microbiota colonized, compared to that of mouse-microbiota colonized mice (Chung et al., 2012). The same phenomenon has also been suggested in different livestock animals, such as swine (Mulder et al., 2011) and cattle (Oikonomou et al., 2013). For example, restricted exposure to microbiota during early life in piglets interferes with the development of gut epithelium, while promoting a greater immune activation (Mulder et al., 2011). Similarly, higher bacterial diversity and prevalence of *Faecalibacterium prausnitzii* during the first week of life have been shown to increase body weight gain and decrease diarrhea incidence in older calves (Oikonomou et al., 2013). These results highlight the importance of gut microbiota establishment and gut health in early life.

The increased beneficial bacteria in the gut may influence gut health via different mechanisms, such as the prevention of enteric pathogens colonization, increasing digestive capacity, lowering of pH, and improving mucosal immunity (Uyeno et al., 2015). For example, *Bifidobacterium* protects the host against enteropathogenic infections by competing for nutrients and space, and by producing acetate (Hsieh et al., 2015). Additionally, *Bifidobacterium* has also been shown to closely regulate the intestinal epithelial barrier via the modulation of intercellular tight junction proteins (TJs) (Ulluwishewa et al., 2011). Given the intricate nature of these interactions and outcomes, it seems incredibly important to understand the role of microbiota in gut health with respect to individual animal species if manipulations of gut microbiota are to be used to improve health and production of livestock.

BARRIER FUNCTION AND GUT HEALTH

An important factor for gut health is maintaining proper epithelial barrier function of the GIT, which is highly orchestrated by the presence of nutrients and microbes within the gut (Shen et al., 2011). The barrier function of the GIT in the ruminal and intestinal epithelium are managed by a combination of cell junctions, including anchoring junctions (desmosomes, hemidesmosomes and adherence junctions), gap junctions and tight junctions (Turner, 2009). Cell junctions are the most common range of transmembrane proteins that interact with actin cytoskeleton of cells to maintain cell-to-cell adhesion in the intestinal epithelium (Ulluwishewa et al., 2011).

Proper regulation of cell junctions is crucial for the maintenance of intestinal homeostasis (Ulluwishewa et al., 2011). The increased permeability of the epithelial barrier is a common indication of different gastrointestinal diseases, such as IBD (Edelblum and Turner, 2009). Crohn's disease and ulcerative colitis also display variations in the expression of TJs from the claudin family (Edelblum and Turner, 2009). Cell junction-mediated changes in the epithelial barrier permeability are regulated by the production of cytokines (Edelblum and Turner, 2009). Other than the mucosal cytokines, dysbiosis in gut microbiota has also been shown to alter TJs leading to an increase in intestinal permeability during IBD (Hold et al., 2014).

In ruminants, the feeding of calf starter has been suggested to decrease alter the expression of TJs at the mRNA level during weaning transition (Malmuthuge et al., 2013). Moreover, increasing the diet in rapidly fermentable carbohydrates has been shown to decrease the expression of TJs in the rumen epithelium (Steele et al., 2011) and increase the expression of inflammatory genes in the hindgut epithelium (Tao et al., 2014). However, how these observed changes in cell junctions may impact intestinal permeability and gut health is still unclear. Also, while the role of cell junctions in gut health has been studied extensively in humans, the corollary knowledge in livestock species remains quite limited.

OPPORTUNITIES FOR GUT HEALTH IN RUMINANT PRODUCTION

There are several key phases and challenges in dairy production that can impact both GIT function and economic profitability, including pre-weaning, weaning, and the transition to highly fermentable diets.

Pre-weaning

The pre-weaned calf is the most at risk of all cattle on the farm, with digestive disorders and diseases resulting in morbidity and mortality rates above 50% and 10%, respectively, while the majority of which come from scours (USDA, 2007). An increase in intestinal permeability and fecal scores have been observed in younger calves (2-week-old) compared to their 4-week-old counterparts (Marquez, 2014), suggesting higher prevalence of disruptions in gut barrier function in younger animals. Therefore, it is important to understand the role of the intestinal microbiota and epithelial barrier

function in the prevention of calf diarrhea to improve gut health and to decrease calf deaths.

Weaning

The time of weaning for calves can be classified as one of the most dramatic GIT transformations in nature. Dairy and beef calves can suffer from weaning stress associated with gastrointestinal ailments, such as parakeratosis (Bull et al., 1965) as well as sudden and dramatically increased gut permeability (Wood et al., 2005). Opportunities to improve rumen development and lower gut adaptations would be immensely beneficial in decreasing the stress associated with weaning in ruminants.

Transition to Rapidly Fermentable Diets

Dramatic shifts in rapidly fermentable carbohydrates are commonly associated with GIT ailments in ruminants. The most notable being ruminal acidosis, a disorder characterized by a depression of ruminal pH, which alters GIT microbiota and barrier function. Ruminal acidosis is estimated to affect a large proportion of lactating dairy and beef feedlot cattle, and as such, is of great interest to researchers seeking to develop feed additives to alleviate the detrimental impact of this digestive disorder.

FEEDING THE GUT

The history of animal nutrition has been largely based in quantitative analysis and assessment of ingredients and balancing for energy and protein. In addition to supplying a consistent and balanced source of nutrients, producers are now investing in ingredients that are fed for the sole purpose of improving health, a correlation that is not easily quantified in the form of milk or meat production. Within the livestock industry, the number of ingredients commonly used for gut health applications is far greater in monogastrics than in ruminants. Several bioactive ingredients exist with gut health applications, such as probiotics, prebiotics, metabolites, essential oils, and bioactive proteins and fats; however, the number of studies examining their effects and impact on ruminant health remains quite limited. To maintain the scope of this review, the most utilized bioactive ingredients in ruminant nutrition, such as probiotics, prebiotics and metabolites will be discussed. Other classes of ingredients, such as essential oils, bioactive fats and proteins have received limited attention in ruminants and thus will not be included in this review.

PROBIOTICS AND PREBIOTICS

A probiotic is defined as a live microorganism which when administered in adequate amounts confers a health benefit on the host. The expectation of a probiotic is to (1) promote the development of a healthy microbiota predominated by beneficial bacteria, (2) prevent enteric pathogen colonization, (3) enhance gut tissue maturation and integrity, and (4) improve mucosal immunity (de Lange et al., 2010). The manipulation of microbiota to improve gut health using direct fed microbials and

probiotics has been widely studied in human medicine and nutrition, as well as livestock nutrition. The commonly used probiotics in ruminant rations are live yeast (*Saccharomyces cerevisiae*), lactic acid producing bacteria (e.g., *Lactobacillus* and *Enterococcus* spp.) and fungi (e. g. *Aspergillus oryzae*). In ruminant production, probiotics were initially used in young ruminants to aid in the establishment of microflora for feed digestion and health. Further advancements in the field led to more focused research on fibre digestion and optimizing ruminal fermentation and health (McAllister et al., 2011). Most ruminant probiotic research is focused on dry matter intake and milk production, with limited attention given to the underlying mechanisms and overall health effects.

A recent effort has been made to reduce ruminal acidosis using direct fed microbials (Krehbiel et al., 2003). Ruminal acidosis is a common digestive disorder in the cattle industry, caused by the transition to highly fermentable diets designed to improve production (Nagaraja and Titgemeyer, 2007). In addition to the accumulation of short-chain fatty acids (SCFA) or lactate, the composition of rumen microbiota is also altered in cattle with ruminal acidosis (Nagaraja and Titgemeyer, 2007). Live yeast, such as *Saccharomyces cerevisiae*, has been shown to attenuate ruminal acidosis in cattle by altering the microbiota of the rumen (Chaucheyras-Durand and Durand, 2009; AlZahal et al., 2014). *Megasphaera elsdenii* has been successfully used to increase ruminal pH and decrease the production of lactate and has been recommended as a direct fed microbial to prevent high-grain diet induced acidosis (Krehbiel et al., 2003). This technology however is not currently available in the market. More research characterizing how probiotics can impact host-microbial interactions will provide more insight into how they can be formulated into rations to improve ruminal health.

The use of probiotics in calves has focused primarily on maintaining intestinal health in the first weeks of life or aiding in the development of the rumen during weaning. It has been well established that in certain environmental conditions, feeding lactic-acid bacteria during the pre-weaning phase is associated with improved weight gain (Frizzo et al., 2011). However, only a small number of samples evaluate health related metrics. For example, the administration of *Lactobacillus* and *Bifidobacterium* to newborn calves during the first week of life has been shown to increase weight gain, feed conversion ratios and health (Abe et al., 1995). Similarly, Timmerman et al., (2004) showed reduced diarrhea and improved health when calves were supplemented *Lactobacillus* in the milk. Probiotics have also been supplemented in the dry feed offered to calves to improve performance during the weaning period (Lesmeister et al., 2004; Yohe et al., 2015). Still, it remains unclear whether these benefits come from improved rumen growth or function, and thus they warrant further investigation.

A prebiotic is a non-digestible feed ingredient that can be used to alter the composition or metabolism of the gut microbiota in a beneficial manner. In practice, prebiotics have been used almost exclusively to increase the proportion of *Bifidobacterium* and *Lactobacillus* in the gut (Gibson et al., 2004). Most of the work with prebiotics has been conducted in calves, leaving a paucity of information regarding mature cattle. For example, feeding fructooligosaccharides enhances the growth

performance of veal calves by decreasing feed conversion ratios and increasing carcass weight; however, the possible mechanisms behind these performance measures were not investigated (Grand et al., 2013). Recent galactooligosaccharide prebiotic supplementation research in newborn calves has shown increases in the abundance of *Lactobacillus* and *Bifidobacterium* (Marquez, 2014), underscoring the potential to improve gut health via increasing the establishment of beneficial bacteria. This same study also showed that intestinal permeability was not affected by prebiotics, which suggests that they may not influence gut health via modulating intestinal epithelial barrier, but only via promoting the colonization of beneficial microbiota. The effect of supplementing milk with the prebiotics inulin and lactulose on GIT immunology of pre-ruminant calves was recently evaluated and the mRNA expression of genes involved in inflammation were downregulated in the intestine (Masanetz et al., 2011). These studies showcase that both prebiotics and probiotics influence microbiota and the overall health of the host; however, the particular mechanisms and modes of action require more research in ruminants.

METABOLITES

The most studied metabolites with respect to ruminants are SCFA, which are the end-products of microbial fermentation in the rumen and hind gut. They are also commonly regarded as luminal growth factors and increasing their production alters GIT function in ruminant and non-ruminant models (Sakata and Yajima, 1978). Of the SCFA, butyrate has been reported to be the most potent stimulator of epithelial proliferation in colonic epithelial cells, and is the primary energy source of the ruminant GIT. The supplementation of butyrate is known to induce ruminal epithelial proliferation *in vivo* (Sakata and Tamate, 1978). Recent research in mature dairy cows showed that genes involved in differentiation and growth were activated in the rumen epithelium by ruminal butyrate infusions (Baldwin et al., 2012). Furthermore, butyrate supplementation in dairy cows during acidosis impacted the cytokine and host defense immune expression (Dionnisopoulous et al., 2013). Interestingly, young calves fed a milk replacer fortified with butyrate exhibited increased ruminal papillae length, width, and surface area (Gorka et al., 2011), suggesting cross-talk between the lower gut and rumen.

While ruminant studies have focused primarily on butyrate, research in monogastrics has identified a significantly larger catalogue of metabolites that can be used to improve GIT health. For example, precursors of butyrate have been studied as a mean of increasing butyrate supply (de Lange et al., 2010). In addition, osmoregulator metabolites, such as betaine have been shown to improve gut barrier functions (Eklund et al., 2005) and medium chain fatty acids have been shown to increase the proportion of beneficial bacteria within the GIT (de Lange et al., 2010). This body of knowledge in monogastric research provides a useful framework to explore and implement new nutritional technologies in ruminant production.

ADDITIONAL INGREDIENTS

Over the past decade an array of novel ingredients designed to improve gut health have entered the market. One class of ingredients that has received attention, as an alternative to antibiotics, is phytochemicals (also referred to as essential oils) derived from plant extracts. Phytochemicals are volatile plant components that are known for their antimicrobial activity and have been shown to have a positive impact on GIT microbial activity and community structure (de Lange et al., 2010). Recent research highlights how essential oils influence not only the microbiota but also neuroendocrine system function of the host (Furness et al., 2013). To date, the majority of studies have only evaluated the use of phytochemicals in relation to production parameters (growth and milk production) without evaluating the specific measurements of GIT function.

The monogastric industry has a history of feeding bioactive proteins and fats to improve gut health. For example, plasma proteins and feed enzymes have been extensively used in monogastrics; however, their efficacy cannot be translated into ruminant production due to feed ingredient regulations and safety (de Lange et al., 2010). In addition, the use of essential fatty acids for improving gut immunity and health has been well characterized in monogastrics, but scarcely evaluated in ruminants (Garcia et al., 2015). There remain great opportunities to translate this knowledge to ruminant research, especially calves, which function in a similar physiological manner to monogastrics.

CONCLUSION

Gut health has become a popular topic in livestock agriculture, but the ruminant livestock are the least developed in the sense of scientific research and commercial application and provide the most opportunity for growth in the industry application. The recent influx of nutrition research investigating how ingredients can influence gut health represents a shift in the approach towards ruminant nutrition, which has been historically rooted in quantitative findings. In order to adequately and aptly discuss gut health, two major principles must be considered: microbiota and the barrier integrity of the GIT. There are several opportunities at different stages of life and different points in the production cycle (e.g. pre-weaning, weaning and transition to rapidly fermentable diets) to improve gut health in ruminants. The breadth of nutritional technologies now common in monogastric livestock species, but untested in ruminants, also offers valuable insight into potential developments and applications for the ruminant sector.

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OPTIMAL USE OF SUGAR IN DIETS FOR DAIRY CATTLE

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There are many different sources of dietary sugar for use in rations for dairy cattle. Common ingredients used to increase the dietary sugar concentration in North America include molasses-based products (beet or cane molasses and beet pulp), citrus products, or byproducts from cheese processing such as whey permeate. While these ingredients contain different types of monosaccharides and disaccharides, current dietary recommendations only pertain to total dietary sugar and total non-structural carbohydrate (**NSC**) concentrations and common analysis do not differentiate sugar type. This paper will review the potential of dietary sugar to modulate dry matter intake, ruminal fermentation and production outcomes for lactating dairy cattle, and will investigate whether identification of disaccharides should be included rather than a single classification system for sugar.

Characterizing Sugar in Diets for Dairy Cattle

Characterization of feed components has greatly improved including the understanding of carbohydrate fractions. Under conventional analytical approaches carbohydrates can be classified as neutral detergent insoluble (neutral detergent fiber) and neutral detergent soluble fractions. The neutral detergent soluble fraction has been of interest as it contains organic acids, simple sugars and disaccharides, oligosaccharides, starch, pectins, and soluble fibre (Hall, 1999; Lanzas et al., 2007). Obviously, the neutral detergent soluble fraction is diverse and the resulting fermentation rates within the rumen for this fraction also differ markedly. For example, according to the Cornell Net Carbohydrate System starch from corn and barley are degraded at 12 and 30%/h, respectively. This compares to rates for hydrolysis for sucrose and lactose of 1311 and 331%/h, respectively and fermentation of glucose and fructose of 521 and 530%/h and that of galactose estimated at 439%/h (Weisbjerg et al. 1998). Thus, it could be expected that the potential for short-chain fatty acid (**SCFA**) production (an hence energy supply) and acidification of the rumen digesta would also differ not only among starch and sugar sources but also between different disaccharides.

Given the rapid rates of hydrolysis and fermentation of disaccharides and monosaccharides, respectively, there is no doubt that increasing the sugar content without a concomitant reduction in starch will result in reduced ruminal pH. In fact, simple sugars such as glucose (Krehbiel et al., 1995; Penner et al., 2009a; Oba et al., 2014) and oligosaccharides (Gressley et al., 2011) have been used to induce ruminal acidosis. That said, a common inclusion strategy for sugar inclusion in diets is through the replacement of dietary starch such that the total dietary NSC does not change. Under such a dietary scenario, sugar inclusion can result in numerous benefits.

Effects of dietary sugar on DMI

Sugar is a palatable component within diets for dairy cattle. Early work had suggested that inclusion of sugar may be one strategy to improve DMI and could represent an opportunity for cows in early lactation (Nombekela et al., 1994; Nombekela and Murphy, 1995). The suggestion for potential to improve DMI in early lactation was supported by Penner and Oba (2009) where cows fed a diet containing 8.7% ethanol soluble carbohydrates (using sucrose) consumed nearly 1 kg/d more during the first 4 wk of lactation than cows not provided with supplemental dietary sugar. It is important to note that sugar was included in the diet in that study as a partial replacement for dietary starch. Broderick and Radloff (2004) evaluated the inclusion rate of dried molasses and reported that as dietary sugar concentration increased from 2.6 to 7.2%, DMI increased linearly. In the same manuscript, the authors also evaluated liquid molasses inclusion and reported a cubic response for DMI where a cows fed a dietary concentration of 4.9% (DM basis) sugar had greatest DMI. DeFrain et al. (2004) reported a tendency (P = 0.09) for a linear increase in DMI (1.6 kg increase) as lactose inclusion increased from showed that DMI tended to increase linearly as lactose increased from 5 to 13% in the diet on a DM basis. In contrast, numerous studies have reported no effect of sugar on DMI (Nobekela and Murphy, 1995; Ordway et al., 2002; DeFrain et al., 2006; Penner et al., 2009; Chibisa et al., 2015) reported no effect of sucrose on DMI. It is not clear why sugar inclusion does not consistently improve DMI, but it should be noted that sugar inclusion not appear that sugar inclusion would reduce DMI as summarized in Figure 1.

Effect of sugar inclusion on ruminal fermentation

A common response observed with the inclusion of lactose into diets for ruminants is an increase in the concentration of butyrate in ruminal fluid (DeFrain et al., 2004; Chibisa et al., 2015); however, sucrose does not seem to elicit the same response (Broderick and Radloff, 2004; Vallimont et al., 2004; Penner et al., 2009) except under a challenge model (Oba et al., 2015). The differential response may suggest that pathways of fermentation also differ. Interestingly, when cows were provided with the lactose, sucrose, or corn starch with a dose that would balance the quantity of hexose provided, sucrose increased the short-chain fatty acid (**SCFA**) concentration in the rumen to a greater extent than lactose and corn starch. The differential response between sucrose and lactose suggests that perhaps dietary evaluation and predictive models should consider the type of sugar in addition to the total sugar concentration.

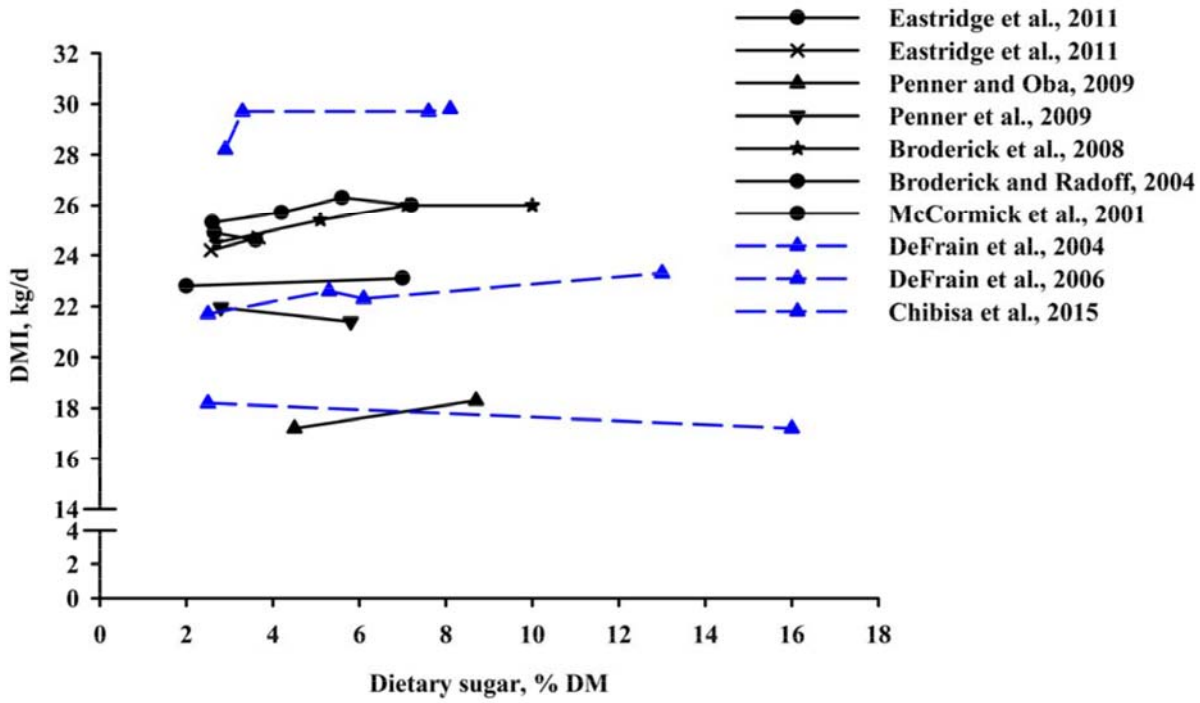

Figure 1. Relationship between dietary sugar concentration and DMI. Data compiled from treatment means from 9 separate studies. Solid lines indicate treatments with sucrose as the primary sugar source and dashed lines indicate treatments with lactose as the primary sugar source.

Although under challenge models it is clear that sugar can be used to reduce ruminal pH (Oba et al., 2015), use of sugar as a partial replacement for starch does not reduce ruminal pH (Chibisa et al., 2015). Past in vitro studies evaluating sugar inclusion in vitro have noted either no effect of sugar on pH of the incubation media (McCormick et al., 2001) or a tendency for increased pH for high sugar compared to low sugar incubations (Vallimont et al., 2004). Supporting the in vitro results, Broderick and Radloff, (2004), Penner et al. (2009), Penner and Oba (2009), and Chibisa et al. (2015) all reported that sugar inclusion did not decrease ruminal pH or tended to increase pH (Figure 2). The mechanisms for why sugar does not depress ruminal pH are not fully understood. However, the finding that pH is not affected in vitro (McCormick et al., 2001; Vallimont et al., 2004) and that pH is not reduced or may be improved in vivo suggests that the underlying mechanisms are likely related to microbial utilization of sugar.

Regarding microbial utilization, it has been shown that sugar inclusion increases the lag time and increased incorporation of C into microbial contents (Hall and Weimer, 2011). While it was suggested that this C incorporation was likely attributed to amino acid synthesis, it is now accepted that microbes, primarily protozoa, will accumulate reserve carbohydrates (Hackmann and Firkins, 2015). In particular, isotrichid protozoa are efficient at converting glucose to glycogen (Hall, 2011). Hall (2011) evaluated glycogen accumulation in response to sugar when incubations were performed under

faunated and defaunated conditions. That study demonstrated that total microbial glycogen accumulation increased with sugar and that protozoal glycogen accumulation represented 51% of the total glycogen recovered. Interestingly, glycogen did not accumulate in protozoa during the defauntated incubation supporting the model and total microbial glycogen accumulation was reduced by nearly 45% relative to the faunated incubations. The accumulation of carbon into microbial reserve carbohydrates could help explain why ruminal pH is not reduced when sugar replaces starch as the total amount of rapidly fermentable carbohydrate that is fermented would be reduced. The storage of carbohydrates by the rumen microbes rather than immediate fermentation may also explain why ruminal ammonia concentrations often increase or are at least not reduced with addition of sugar into diets (Penner et al., 2009; Oba, 2011; Oba et al., 2015). It could be expected that glycogen deposition by ruminal microbes may also diminish some of the potential productivity benefits arising with the inclusion of dietary sugar into diets for dairy cattle and may support microbial maintenance functions.

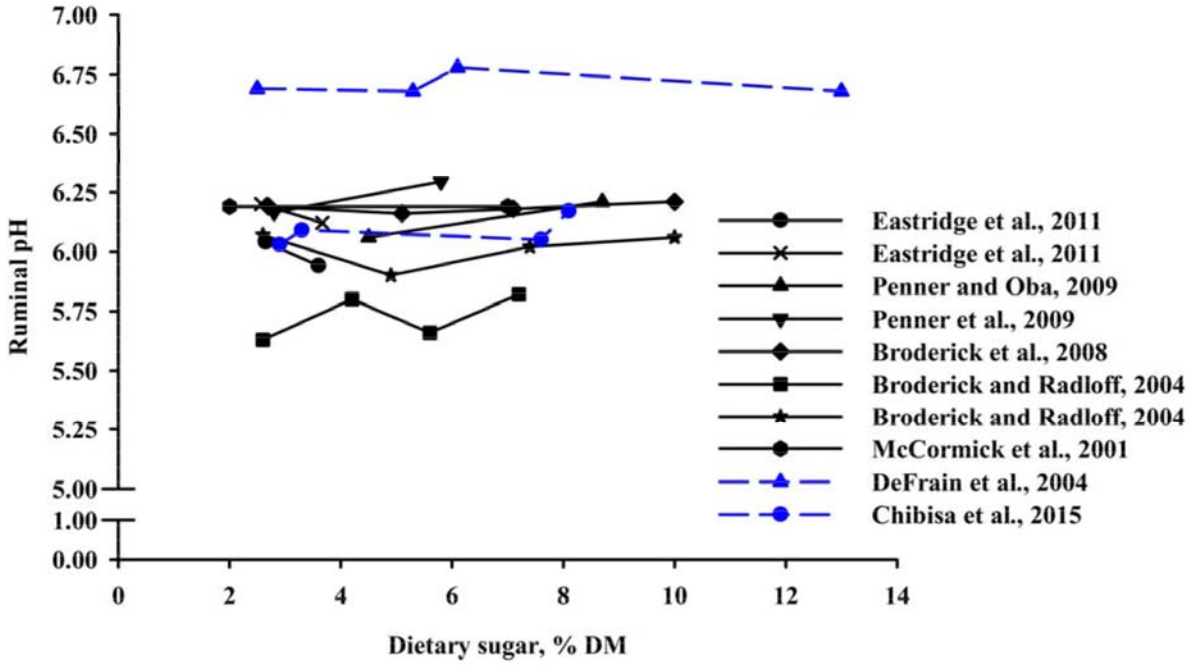

Figure 2. Relationship between dietary sugar concentration and ruminal pH. Data compiled from treatment means from 9 separate studies. Solid lines indicate treatments with sucrose as the primary sugar source and dashed lines indicate treatments with lactose as the primary sugar source.

While the ruminal pH response associated with dietary sugar inclusion is consistent between in vitro and in vivo studies, recent studies have indicated that presence of sugars in the diet may up regulate sugar transport (Moran et al., 2014). Another possible reason or the positive effect of sugar on rumen pH may be related to SCFA transport across the rumen epithelium. The mechanisms involved in SCFA

transport across the rumen epithelium have been described by Aschenbach et al. (2011) and primarily include passive diffusion, $SCFA^-$/ HCO_3^-exchange. Passive diffusion of SCFA may not result in complete proton removal from the rumen as a significant proportion of the protons can be recycled back into the rumen contents through the action of Na^+/H^+ exchangers. In contrast, absorption of SCFA via the $SCFA/HCO3^-$ exchange will result in the neutralization of a proton as $HCO3^-$ reacts with H^+ in the carbonic anhydrase reaction. In a study at the University of Saskatchewan, 4 cows were used in a Latin square design comparing low (2.6%) vs. high (8%) sugar diets (lactose as a sugar source) when the basal concentrate was corn or barley. While DMI did not differ averaging 29.5 kg/d, cows fed the high sugar diet had a greater reliance on $SCFA^-$/ $HCO3^-$ exchange than cows fed the low sugar diets. This suggests that sugar may not only affect the rumen microbial community and function but may also affect epithelial function.

A second study was conducted at the University of Saskatchewan to further evaluation the effect of sugar inclusion and sugar type on ruminal epithelial function (Penner et al., unpublished). A total of 18 lambs were fed a diet that contained no added sugar (2.6% sugar) or diets where either dried whey permeate or dried molasses were used to increase dietary sugar to 6%. As with previous work, sugar inclusion and sugar type did not affect ruminal pH and ruminal SCFA concentrations were not affected. Despite these findings, serum BHBA was greater for lambs fed lactose than sucrose and the total flux of acetate was reduced for lambs fed sugar compared to the control. However, total propionate flux tended to be greater for lambs fed lactose than those fed sucrose and the reliance on bicarbonate-dependent transport of SCFA was greater for lactose than sucrose for propionate (P = 0.043) and tended to be greater (P 0.10) for butyrate. While not commonly investigated, this study also showed that glucose uptake by the ruminal epithelium was twice as great for lambs fed diets with added sugar than the control and the SGLT-1 dependent portion of glucose uptake also tended to increase (P = 0.09). This supports work evaluating the inclusion of artificial sweeteners on glucose uptake by the intestinal epithelium (Moran et al., 2014). However, the quantitative importance of glucose uptake by the ruminal epithelium is not known.

Effect of dietary sugar on milk yield and composition

As with the effects of sugar on DMI, the results of dietary sugar inclusion on milk yield and milk composition are mixed (Figure 3). For example, Broderick and Radloff (2004) reported a cubic response for milk yield with increasing dry molasses inclusion resulting in dietary sugar values ranging between 2.6 and 7.2%, and with liquid molasses inclusion resulting in dietary sugar concentrations ranging between 2.6 to 10%. That work suggested that the optimal sugar concentration to induce both positive effects on DMI and milk yield was 5.9%. However, that study also noted that as dietary sugar concentration increased, there was a linear decrease in FCM. Most other studies have reported no effect of dietary sugar on milk yield or milk composition (DeFrain et al., 2006; Broderick et al., 2008; Penner and Oba, 2009; Penner et al., 2009; Chibisa et al., 2015). Collectively, it appears that sugar inclusion does not result in improved milk yield

or altered milk composition.

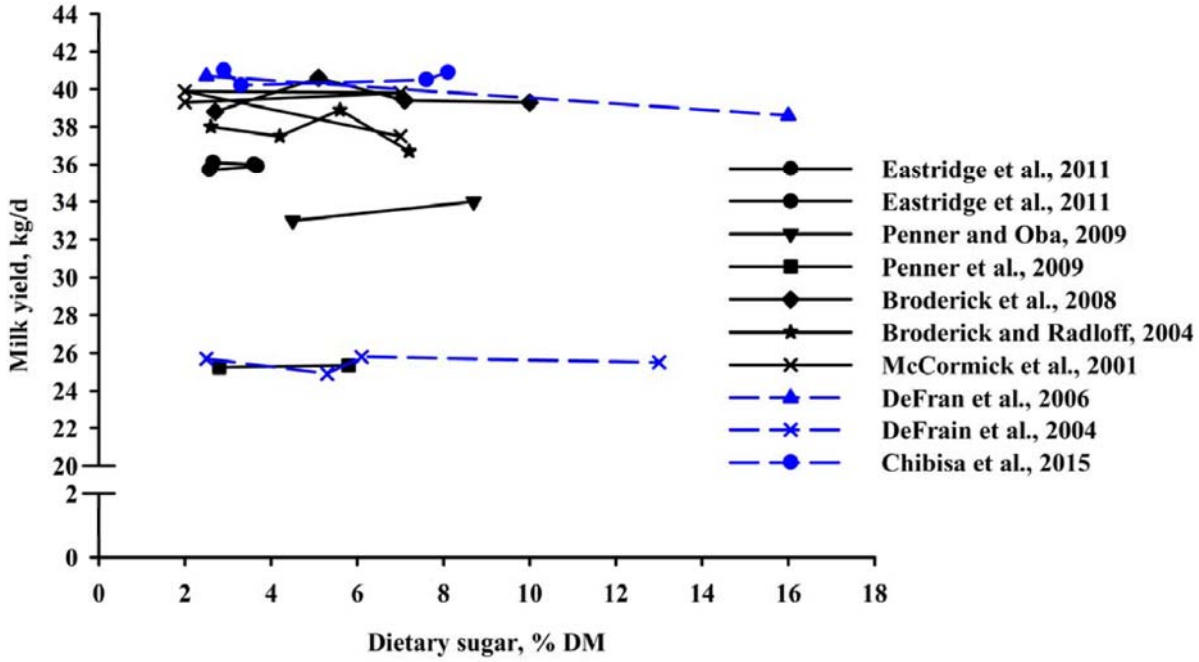

Figure 3. Relationship between dietary sugar concentration and milk yield. Data compiled from treatment means from 9 separate studies. Solid lines indicate treatments with sucrose as the primary sugar source and dashed lines indicate treatments with lactose as the primary sugar source.

CONCLUSION

Although there are studies that show a positive response of increasing dietary sugar, overall it does not appear that dietary sugar affects DMI, milk yield, or milk composition. Interestingly, inclusion of sugar as a partial replacement for starch does not negatively affect ruminal pH which is likely related to an increase lag time in the rapidly fermentable carbohydrate, increased glycogen accumulation by mixed microbes, and increased bicarbonate- dependent SCFA transport and potentially increased glucose uptake. Benefits of including sugar in diets for lactating cows may be limited to situations where sugar inclusion is cost-competitive on a hexose unit basis with starch.

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ROLE OF 16- AND 18-CARBON FATTY ACIDS IN DAIRY RATIONS

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INTRODUCTION

In most Federal Milk Market Orders milk fat and protein yield are the major contributors to the price that producers receive for milk. The addition of supplemental fatty acid (FA) sources to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production and solids yield. The ability to understand and model FA, the effects of individual FA, and different FA supplements on production parameters has direct impact on dairy industry recommendations and the usefulness of FA supplementation strategies. The emphasis of the current paper is on biological processes and quantitative changes during the metabolism of FA in the rumen and the effect this has on FA availability to the dairy cow, the digestibility of these FA, and their overall impact on performance and energy partitioning. We will focus on recent research supplementing palmitic acid (C16:0) and stearic acid (C18:0)-enriched supplements, on feed intake, milk production, milk composition, and energy partitioning.

Lipid Metabolism in The Rumen and Mammary Gland

As well as being derived from specific supplements, FA in the dairy cow's diet are also present in forages and concentrates. Each feed/fat source is composed of a different mix of individual FA. The majority of FA in dairy cow diets contain 16 and 18-carbons. Generally, most cereal grains and seeds contain a high concentration of linoleic acid (C18:2 n-6), whereas linolenic acid (C18:3 n-3) is typically the predominant FA in forage sources. For example, corn, cottonseed, safflower, sunflower, and soybean oils are high in C18:2 n-6, whereas linseed is high in C18:3 n-3. Unsaturated FA are toxic to many rumen bacteria, thus an extensive metabolism of dietary lipids occurs in the rumen that has a major impact on the profile of FA available for absorption and tissue utilization (Palmquist et al., 2005). The two major processes that occur are hydrolysis of ester linkages in lipids found in feedstuffs and the biohydrogenation of unsaturated FA. Biohydrogenation of unsaturated FA results in the conversion of unsaturated FA to saturated FA, mainly C18:0, through a series of biohydrogenation intermediates (conjugated C18:2 and *trans* C18:1 FA). The major substrates are 18:2 n-6 and 18:3 n-3 and the rate of rumen biohydrogenation is in the range of 70-95% and 85-100%, respectively (Jenkins et al., 2008); thus C18:0 is the predominant FA available for absorption by the dairy cow under typical feeding situations (Bauman and Lock, 2006). A series of recent in vitro studies concluded that biohydrogenation occurs to enable rumen bacteria to survive the bacteriostatic effects of unsaturated FA, and that the toxicity of unsaturated FA is probably mediated via metabolic effects rather than disruption of membrane integrity. Furthermore, it appears that the degree of toxicity of different unsaturated FA varies for individual ruminal bacteria species; all the main species that

comprise the ruminal cellulolytic bacteria appear vulnerable to inhibition by unsaturated FA (Maia et al., 2007, 2010).

FA supplements are often used as a means to increase the energy density of the diet and many of these are referred to as inert. In this case inertness simply means that the FA supplement has minimal affects on rumen fermentation. Although deemed inert at the level used, they can still be hydrolyzed, if a triglyceride, or biohydrogenated, if unsaturated. Often, Calcium-salts of palm FA or canola are referred to as 'protected'. However, these are not protected from rumen biohydrogenation, but rather are considered to be ruminally inert with regard to their effects on the microbial population (Palmquist, 2006).

Lipids in milk are primarily in the form of triglycerides (98%) with phospholipids and sterols accounting for 1.0 and 0.5 % of total lipids, respectively. Bovine milk is extremely complex and contains about 400 FA, a large proportion of which are derived from lipid metabolism in the rumen (Jensen, 2002). Milk FA are derived from 2 sources; <16 carbon FA from de novo synthesis in the mammary gland and >16 carbon FA originating from extraction from plasma. 16-carbon FA originate from either de novo or preformed sources. Substrates for de novo synthesis are derived from ruminal fiber digestion and dietary FA supply preformed FA for direct incorporation into milk fat (Palmquist, 2006). Microbial synthesis of branched and odd-chained number FA in the rumen and absorption of biohydrogenation intermediates also contribute to the diversity of FA secreted in milk fat. Under typical conditions, about half of the FA in milk are synthesized de novo, 40 to 45 % originate from FA in the diet, and less than 10% are derived from mobilization of adipose tissue (Palmquist and Jenkins, 1980). However, nutrition can substantially alter the balance between mammary de novo FA synthesis and uptake of preformed FA. C16:0, C18:0 and *cis*-9 C18:1 are the major FA in milk fat. The relatively high melting point of C16:0 and C18:0 requires the production of de novo synthesized FA or the conversion of C16:0 and C18:0 to *cis*-9 C16:1 and *cis*-9 C18:1, respectively, in the mammary gland in order to maintain fluidity.

Overall Impact of Fa Supplements

There is a wide range of FA supplements available for lactating dairy cattle. For example, Calcium-salts of free FA and prilled saturated free FA are two common types of supplements used in the dairy industry and they differ in FA content and FA profile. Calcium-salt supplements typically contain 80-85% FA and these typically provide approximately 50% saturated and 50% unsaturated FA. By comparison prilled saturated free FA contain approximately 99% FA which are approximately 90% saturated, 10% unsaturated. A summary of the FA profile of some commonly used supplements is provided in Table 1. Although in general FA supplementation has been shown to increase milk yield, milk fat yield, and the efficiency of milk production, great variation has been reported in production performance for different FA types, and indeed the same supplement across different diets and studies. This is evident in a meta-analysis examining the effect of FA supplementation to diets of dairy cows (Rabiee et al., 2012). In general milk production and milk fat % and yield increased, DMI and milk protein %

decreased, and milk protein yield was not affected by FA supplementation. There was a wide range of responses (~5 standard deviations) for all variables, indicating varied and marked biological effect of the different FA supplements (Rabiee et al., 2012).

Table 1. Fatty acid composition of common fat supplements (Data from our laboratory).

Fatty Acid, g/100 g	Tallow	Ca-salt PFAD	Saturated free FA	C16:0-enriched
C14:0	3.0	2.0	2.7	1.6
C16:0	24.4	51.0	36.9	89.7
C18:0	17.9	4.0	45.8	1.0
C18:1	41.6	36.0	4.2	5.9
C18:2	1.1	7.0	0.4	1.3

Utilizing a larger data set than Rabiee et al. (2012), we recently performed a meta-analysis of production responses to commercially available FA supplements (Boerman and Lock, 2014a). Available data were collected from 133 peer-reviewed publications of which 88 met our selection criteria, comprising 159 treatment comparisons. Calcium-salts of palm FA distillate (PFAD; n=73), saturated prilled FA (PRILLS; n=37), and tallow (n=49) supplemented at ≤ 3% diet DM were compared to non FA supplemented diets used as controls. Treatment comparisons were obtained from either randomized design (n=99) or crossover/Latin square design experiments (n=60). Preliminary results from the meta-analysis are shown in Figure 1.

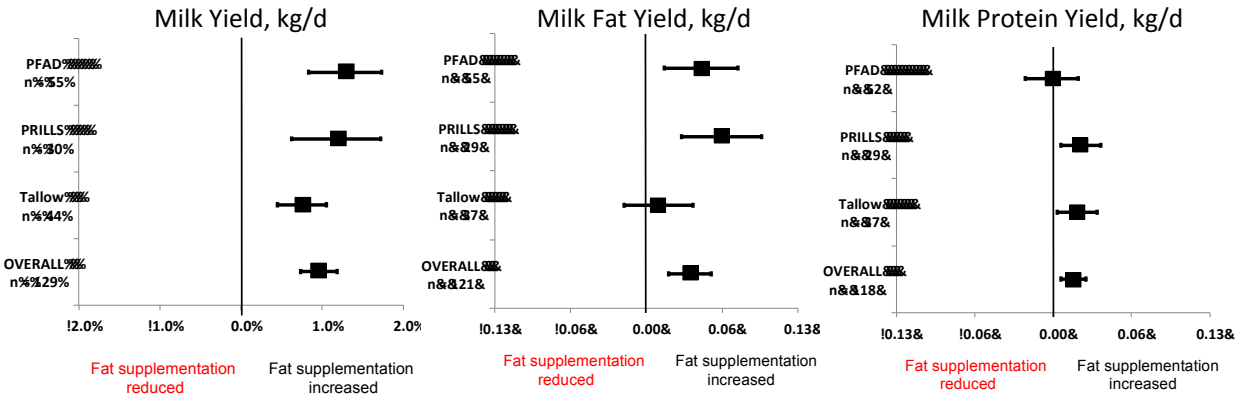

Figure 1.Effect of commercially available FA supplements on yield of milk, milk fat, and milk protein (Boerman and Lock, 2014a). All data reported in peer-reviewed journals in which FA supplements were included at ≤ 3% diet DM compared to control with no added FA supplement. All studies had to have measurements of variance reported. PFAD – calcium salts of palm FA distillate (~ 50% 16:0, ~ 50% unsaturated 18-carbon FA); PRILLS – saturated FA prills (> 80% saturated FA [16:0 and/or 18:0]); Tallow – animal fat labeled as tallow (~ 50% 16:0 and 18:0, ~ 45% 18:1). Data analyzed using *Comprehensive Meta-Analysis (CMA) version 2.0 (Biostat, Englewood, NJ), calculating difference between FA supplemented and control diets using a random effects model.*

Overall, FA supplementation increased yield of milk and milk components and reduced DMI. However type of supplement influenced response with PRILLS not reducing DMI, tallow having no effect on milk fat yield, and PFAD having no effect on milk protein yield. It is important to note that the majority of the studies reported in Figure 1 simply compared a single commercial FA supplement with a non FA supplemented control diet. This makes direct comparisons between different FA supplements difficult to interpret and importantly provide accurate answers to commonly asked questions (by farmers and nutritionists) as to which are the best FA supplements to use. There are limited reports in the published literature that have undertaken direct comparisons between different commercially available FA supplements. Results from the meta-analysis also suggest that responses to FA supplements interact with other dietary components, and this should be examined further.

Impact of Supplemental 16- And 18-Carbon Fa on Fa Digestibility

Under typical feeding situations, C18:0 is the predominant FA available for absorption by the dairy cow, regardless of the diet fed. As result, this FA has an important impact on total FA digestibility as recently observed in a recent meta-analysis and meta-regression examining the intestinal digestibility of long-chain fatty acids in lactating dairy cows (Boerman et al., 2015a). We observed a negative relationship between the total flow and digestibility of FA (Figure 2A). Furthermore, the decrease in total FA digestibility appears to be driven by the digestibility of C18:0 because a negative relationship between the duodenal flow and digestibility of C18:0 was also was detected (Figure 2B).

The exact mechanisms for the reduction in digestibility are not understood; however, potential causes include limits in lysolecithin or competition for absorption sites (Drackey, 2000). Lysolecithin also acts as an amphiphile (substance with both water and lipid-loving capacity) and further increases the solubility of saturated FA (Freeman, 1969). During FA digestion in the small intestine, bile secretions supply bile salts and lecithin, and pancreatic secretions provide enzymes to convert lecithin to lysolecithin and bicarbonate to raise the pH. Lysolecithin is an emulsifier compound and together with bile salts desorb FA from feed particles and bacteria, allowing the formation of micelles, which is critical for absorption (Lock et al., 2005). Once micelles are formed they facilitate transfer of water-insoluble FA across the unstirred water layer of intestinal epithelial cells, where the FA and lysolecithin are absorbed. Additional research to understand the observed reduction in C18:0 digestibility and how this may be overcome or improved is required.

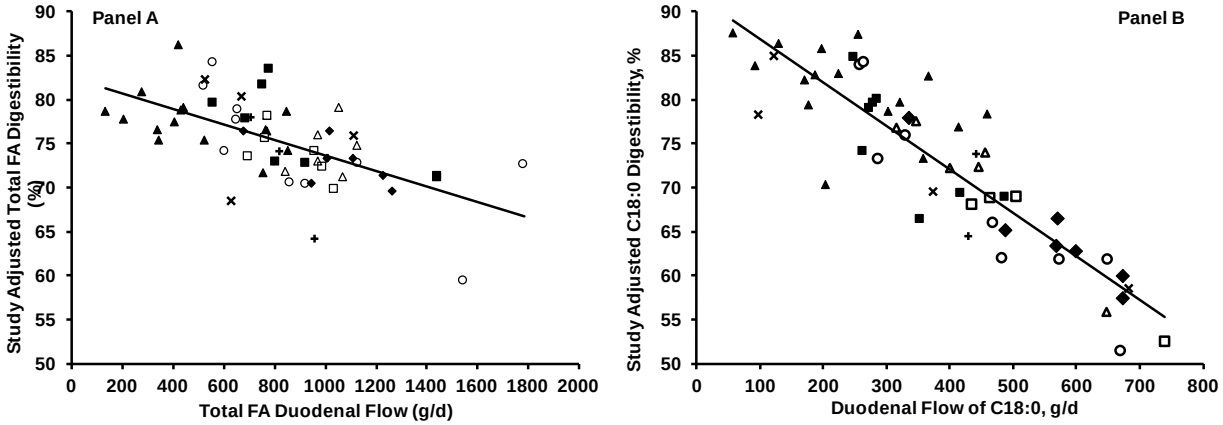

Figure 2. Relationship between study adjusted total FA intestinal digestibility and total FA duodenal flow (Panel A) and study adjusted C18:0 intestinal digestibility and duodenal flow of C18:0 (Panel B). Results from a meta-analysis using 15 published studies that measured duodenal flow and intestinal digestibility of fatty acids in dairy cows (Boerman et al., 2015a). Control treatments represented by black triangles; animal-vegetable fat treatments represented by black diamonds; calcium salt treatments represented by black squares; tallow treatments represented by open circles; vegetable oil treatments represented by open triangles; seed meal treatments represented by open squares; whole seed treatments represented by black addition sign; and other treatments represented by black multiplication sign.

Our recent FA digestibility research has utilized and focused on C16:0 and C18:0-enriched supplements. Of particular importance, Boerman et al. (2014b) fed increasing levels of a C18:0-enriched supplement (85% C18:0) to dairy cows and observed no positive effect on production responses, which was likely associated with the pronounced decrease in total FA digestibility as FA intake increased (Figure 3A). Similarly, de Souza et al. (2015) fed increasing levels of a C16:0-enriched supplement (87% C16:0) to dairy cows and even though a positive effect was observed on production response up to 1.5% diet dry matter, we observed a decrease in total FA digestibility as FA intake increased (Figure 3B). Considering the results presented in Figure 3, given that the range on FA intake is similar across both studies, the decrease in total FA digestibility is more pronounced when there is increased intake/rumen outflow of C18:0 rather than C16:0, similar to our observations in Figure 2.

To further understand what factors influence FA digestibility, we recently utilized a random regression model to analyze available individual cow data from 5 studies that fed a C16:0-enriched supplement to dairy cows (unpublished results). We observed that total FA digestibility was negatively impacted by total FA intake, but positively influenced by the intake of *cis*-9 C18:1. This suggests that a combination between 16-carbon and unsaturated 18-carbon FA may improve FA digestibility, but reason for this effect needs to be further determined.

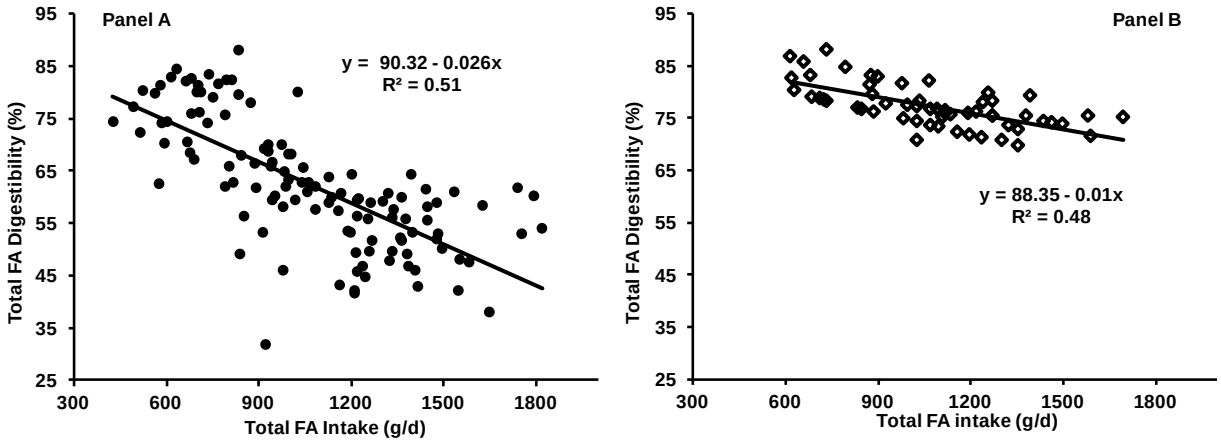

Figure 3. Relationship between total FA intake and total FA digestibility of dairy cows supplemented with either a C18:0-enriched supplement (Panel A) or a C16:0-enriched supplement (Panel B). Results in Panel A utilized 32 mid-lactation cows receiving diets with increasing levels (0 to 2.3% dry matter) of a C18:0-enriched supplement (85% C18:0) in a 4 X 4 Latin square design with 21-d periods (Boerman and Lock, 2014b). Results in Panel B utilized 16 mid-lactation cows receiving diets with increasing levels (0 to 2.25% dry matter) of a C16:0-enriched supplement (87% C16:0) in a 4 X 4 Latin square design with 14-d periods (De Souza et al., 2015).

Impact of Supplemental 16- And 18-Carbon Fa on Production Responses

In the 1960's Steele and co-workers performed a series of studies using relatively pure sources of C16:0 and C18:0 and and their findings suggested that C16:0 supplementation induces a higher milk fat response (concentration and yield) as compared to C18:0 supplementation. More recent work from Enjalbert et al (1998) suggests that the uptake efficiency of the mammary gland is higher for C16:0 than for C18:0 and *cis*-9 C18:1. We recently carried out a series of studies examining the effect of individual saturated FA on production and metabolic responses of lactating cows (Lock et al., 2013, Piantoni et al., 2013, Rico et al., 2014, Piantoni et al., 2015). These results indicate that C16:0 supplementation has the potential to increase yields of milk and milk fat as well as the conversion of feed to milk, independent of production level when it was included in the diet for soyhulls or C18:0 (Table 2).

Rico et al. (2013) fed increasing levels of a C16:0-enriched supplement (87% C16:0) to dairy cows and observed a quadratic response with a positive effect on milk fat yield, 3.5% fat-corrected milk and feed efficiency up to 1.5% diet DM (Table 3). Furthermore, we recently utilized a random regression model to analyze available individual cow data from 10 studies that fed a C16:0-enriched supplement to dairy cows (unpublished results). We observed that energy partitioning toward milk was increased linearly with C16:0 intake, as a result of a linear increase in milk fat yield and 3.5% fat-corrected milk with increasing intake of C16:0.

Table 2. Summary of DMI, milk production and composition, body weight, and BCS for cows supplemented with C16:0 and C18:0 supplements. The C16:0 supplement contained ~ 99% C16:0 and the C18:0 supplement contained ~ 98% C18:0.

	Piantoni et al. (2013)[1]			Piantoni et al. (2015)[2]			Rico et al. (2014)[3]		
Variable	**Control**	**C16:0**	**SEM**	**Control**	**C18:0**	**SEM**	**C16:0**	**C18:0**	**SEM**
DMI, kg/d	27.8	27.8	0.54	25.2^{n}	26.1^{m}	0.42	32.1	32.3	0.44
Milk yield, kg/d	44.9^{b}	46.0^{a}	1.7	38.5^{n}	40.2^{m}	0.71	46.6	45.8	2.02
Fat yield, kg/d	1.45^{b}	1.53^{a}	0.05	1.35^{n}	1.42^{m}	0.03	1.68^{y}	1.59^{z}	0.05
Milk fat, %	3.29^{b}	3.40^{a}	0.11	3.60	3.59	0.12	3.66^{y}	3.55^{z}	0.09
Protein yield, kg/d	1.38	1.41	0.04	1.14^{n}	1.19^{m}	0.02	1.50	1.49	0.05
Milk Protein %	3.11	3.09	0.05	3.00	2.99	0.05	3.24	3.29	0.05
3.5% FCM	42.9^{b}	44.6^{a}	1.35	38.6^{n}	40.5^{m}	0.76	47.5^{y}	45.6^{z}	1.64
3.5% FCM/DMI	1.54^{b}	1.60^{a}	0.03	1.53	1.55	0.04	1.48^{y}	1.40^{z}	0.05
Body weight, kg	722	723	14.7	727	730	12.8	720	723	13.6
BCS	2.99	2.93	0.15	2.67	2.67	0.11	2.93^{z}	2.99^{y}	0.11

[1]Treatments were either a control diet (with 2% of diet DM as added soyhulls) or a C16:0-supplemented diet (with 2% of diet DM as C16:0). Means within a row with different superscripts ($^{a, b}$) differ ($P < 0.05$).

[2]Treatments were either a control diet (with 2% of diet DM as added soyhulls) or a C18:0-supplemented diet (with 2% of diet DM as C18:0). Means within a row with different superscripts ($^{m, n}$) differ ($P < 0.05$).

[3]Treatments were either a C16:0-supplemented diet (with 2% of diet DM as C16:0) or a C18:0-supplemented diet (with 2% of diet DM as C18:0). Means within a row with different superscripts ($^{y, z}$) differ ($P < 0.05$).

Piantoni et al. (2015) reported that C18:0 increased DMI and yields of milk and milk components, with increases more evident in cows with higher milk yields, indicating that there was significant variation in response. Reasons why only higher yielding cows responded more positively to C18:0 supplementation than lower yielding cows remains to be determined. However, when we directly compared C16:0 and C18:0 supplementation the yield of milk fat and 3.5% FCM increased with C16:0 regardless of level of milk production (Table 2, Rico et al., 2014). In a recent dose response study with mid lactation cows feeding a C18:0-enriched supplement (85% C18:0) increased DMI but had no effect on the yields of milk or milk components when compared to non-FA supplemented control diet (Table 4), which is probably associated with the decrease in FA digestibility (Figure 3A, Boerman and Lock, 2014b).

There is mechanistic data to support the concept that individual FA can impact milk fat synthesis differently. Hansen and Knudsen (1987) utilized an in vitro system and reported that C16:0 stimulated de novo FA synthesis and incorporation into triglycerides whereas other FA were either neutral or inhibitory. In addition, there were only minor differences in the esterification efficiency into triglycerides of various FA, except for C16:0, which was a better substrate than the other FA tested. These results in association with the digestibility results suggest that C16:0-enriched supplement improve performance of dairy cows, while understanding factors that affect the digestibility of C18:0 with increasing intake/duodenal flow may allow the development of strategies to overcome this possible limitation.

Table 3. DMI, milk production and composition, body weight, and BCS for cows supplemented with increasing levels of a C16:0-enriched supplement (Rico et al., 2013). The C16:0 supplement contained 87% C16:0.

	C16:0 supplementation, % diet DM					
Variable	**0%**	**0.75%**	**1.50%**	**2.25%**	**SEM**	***P*-value**
DMI, kg/d	28.8	28.8	28.6	27.4	0.83	0.05
Milk yield, kg/d	43.7	43.5	44.5	42.5	1.73	0.06
Fat yield, kg/d	1.63	1.69	1.78	1.70	0.09	0.01
Milk Fat, %	3.78	3.88	4.01	4.03	0.17	0.01
Protein yield, kg/d	1.36	1.36	1.40	1.32	0.06	0.08
Milk Protein, %	3.17	3.15	3.18	3.16	0.07	0.32
3.5% FCM, kg/d	45.3	46.1	48.0	45.9	1.91	0.02
3.5% FCM/DMI	1.57	1.60	1.68	1.68	0.07	0.21
Body weight, kg	703	705	701	701	25.7	0.76
BCS	2.66	2.48	2.71	2.84	0.05	0.94

Table 4. DMI, milk production and composition, body weight, and BCS for cows supplemented with increasing levels of a C18:0-enriched supplement (Boerman and Lock, 2014b). The C18:0 supplement contained 85% C18:0.

	C18:0 supplementation, % diet DM				**SEM**	***P*-value**
Variable	**0%**	**0.80%**	**1.50%**	**2.30%**		
DMI, kg/d	28.5	29.1	29.6	30.0	0.61	0.13
Milk Yield, kg/d	38.3	38.6	38.2	37.8	1.65	0.51
Fat Yield, kg/d	1.43	1.40	1.40	1.42	0.04	0.61
Fat, %	3.79	3.72	3.74	3.82	0.08	0.29
Protein Yield, kg/d	1.33	1.33	1.32	1.30	0.05	0.49
Protein, %	3.49	3.50	3.48	3.49	0.05	0.91
3.5% FCM/DMI	39.8	39.4	39.3	39.3	1.40	0.77
FCM/DMI	1.43	1.39	1.35	1.33	0.04	0.03
Body weight, kg	738	739	735	737	12.0	0.58
BCS	3.44	3.40	3.39	3.42	0.08	0.37

Supplemental Fat Interactions with Other Dietary Components

The composition of the basal diet can also be an important element of production responses to FA supplementation. In high producing dairy cows an interaction was observed between forgage:concentrate ratio and response to supplemental FA (Weiss and Pinos-Rodriguez, 2009). In high-forage diets increased energy intake from supplemental saturated FA (mixture of C16:0 and C18:0) was directed mostly to body reserves, whereas in low-forage diets the increased energy intake from the saturated FA supplement was directed mostly to milk production. Using lower producing cows Grum et al. (1996) compared diets at 2 different forage:concentrate ratios either without or with added saturated FA (mixture of C16:0 and C18:0). At both forage:concentrate levels supplemental saturated FA increased milk fat concentration and yield, whereas saturated FA supplementation had opposing effects on DMI when supplemented in the low and high forage:concentrate diets. In early lactation cows, van Knegsel et al. (2007) fed either high

FA or high starch diets with the same concentrate to forage ratio (40:60). Additional FA in the high FA diet were provided by Ca-salts of palm FA and palm oil. Cows fed the high FA diet partitioned more energy to milk than cows fed the high starch diet and had a higher milk fat yield. No differences were found for energy retained as body protein, but energy mobilized from body fat tended to be higher in cows fed the lipogenic diet (van Knegsel et al., 2007).

In a recent study using high producing post-peak dairy cows we fed either a high fiber and FA diet (HFF) containing a 50:50 ratio of forage to concentrate containing a C16:0-enriched supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate (Boerman et al., 2015b). The two treatments resulted in similar apparent energy densities and intakes but the HS treatment partitioned more energy toward body gain whereas the HFF treatment partitioned more energy toward milk (Table 5). In established lactation, cows are usually in positive energy balance and the goals are to maximize milk and component yields and reduce excessive conditioning. We recently observed that reducing starch concentration (32 to 16% diet DM) reduced BW gain in late lactation cows and diminished the incidence of over conditioning, while supplementation with a C16:0-enriched supplement increased milk fat yield and fat-corrected milk (Garver et al., 2015). Further work is necessary, but higher fiber and FA diets (particularly diets supplemented with palmitic acid) may diminish the incidence of over conditioning in mid and late lactation cows.

CONCLUSION

The addition of supplemental FA to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production. Although in general FA supplementation has been shown to increase milk yield, milk fat yield, and the efficiency of milk production, great variation has been reported in production performance for different FA supplements, and indeed the same supplement across different diets and studies. Further work is required to characterize the sources of variation in response to FA supplementation. Just as we recognize that not all protein sources are the same it is important to remember that not all FA supplements are the same. The key is to know what FA are present in the supplement, particularly FA chain length and their degree of unsaturation. Once this information is known it is important to consider the possible effects of these FA on DMI, rumen metabolism, small intestine digestibility, milk component synthesis in the mammary gland, energy partitioning between the mammary gland and other tissues, and body condition. Interactions with other dietary components and the level of milk production are also important in determining the response to various FA supplements. The extent of these simultaneous changes along with the goal of the nutritional strategy employed will ultimately determine the overall effect of the supplemental FA, and the associated decision regarding their inclusion in diets for lactating dairy cows.

Table 5. Body weight, body condition score, and calculated energy values for cows fed a high fiber diet containing a palmitic acid-enriched supplement or a high starch diet containing a mixture of dry ground and high moisture corn (Boerman et al., 2015b).

	Treatments[1]			
Variable	**HFF**	**HS**	**SEM**	***P*-value[2]**
DMI, kg/d	26.9	27.4	0.38	0.02
3.5% FCM, kg/d	49.1	47.6	1.59	0.03
Change in BW, kg/d	0.33	0.78	0.10	0.003
Change in BCS, pt/28 d	- 0.01	0.24	0.03	0.001
Calculated energy values[3]				
Apparent NE_L of diet Mcal/kg	1.78	1.79	0.02	0.64
Milk, Mcal/d	32.8	32.6	1.05	0.05
Body Tissue Gain, Mcal/d	1.95	4.90	0.58	0.001
Maintenance, Mcal/d	10.6	10.7	0.17	0.02
Partitioning				
Milk, %	72.8	67.9	1.11	< 0.001
Body Tissue Gain, %	4.03	10.1	1.16	0.001
Maintenance, %	23.2	22.0	0.43	0.01

[1] Treatments were either a high fiber and FA diet (HFF) containing a 50:50 ratio of forage to concentrate containing a palmitic acid-enriched supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate containing a mixture of dry ground and high moisture corn.
[2] *P*-value associated with treatment differences (HFF vs. HS; Trt).
[3] From the sum of milk energy output, maintenance energy calculated from metabolic BW, and body energy gain divided by DMI for each cow on each diet throughout the 28-d period.

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DIETARY FACTORS INFLUENCING THE DEVELOPMENT OF THE RUMINANT GASTROINTESTINAL TRACT

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The ruminant gastrointestinal tract (GIT) faces the challenge of protecting the host from the contents of the lumen, while controlling the absorption and metabolism of nutrients (Gaebel et al., 2001). In ruminant production, the GIT undergoes rapid development in early life leaving the young ruminant susceptible to gut health challenges. It is generally accepted that in all ruminant production sectors the neonatal and post-natal calves have significantly higher mortality and morbidity compared to the rest of the herd. Based on the most comprehensive survey available in the North America dairy industry (USDA, 2007), average mortality rates in dairy calves in the pre-weaning phase were estimated to be as high as 10% and morbidity rates over 46%, with gastrointestinal ailments being the number one culprit. Similarly, a survey conducted on commercial dairy farms in Ontario and Minnesota (2,874 calves from 0-3 months) reported that gastrointestinal infections were the first ailment experienced in the calf lifespan, with 23% of these calves requiring antibiotic treatment for diarrhea (Windeyer et al., 2014). In addition to a high degree of infections, antibiotic treatments and stress during the pre-weaning phase, calves have been traditionally limit-fed milk in dairy production at approximately 10% of bodyweight (half of normal consumption) and weaned early (Jasper and Weary, 2002; Khan et al., 2011). Weaning represents one of the most dramatic gastrointestinal transformations in nature and is associated with weaning distress, depressed growth and impaired gut health (Khan et al., 2011). Birth to weaning is a time of extreme gastrointestinal challenges in the young ruminants and the short-term and long-term biological outcomes of altered GIT development are poorly understood.

Over the past decade, several studies regarding young ruminants have challenged traditional feeding programs and showcased the benefits of enhancing early life nutrition to improve health, growth rates, feed efficiency, animal welfare and lifetime production (Jasper and Weary, 2002; Khan et al., 2011; Soberon et al., 2012; 2013). It is still unknown how many common nutritional practices in ruminant production impact GIT development, which represents an opportunity to improve health, performance and welfare. This review will focus on recent studies investigating how prenatal nutrition, colostrum management, pre-weaning feeding plane and weaning can impact gut development and health.

PRENATAL NUTRITION

Development of the GIT in ruminants begins during the first trimester (cattle – 30 days of gestation), followed by accelerated growth in the last trimester (Guilloteau et al., 2009). For example, the growth of the small intestine in bovine fetus during 175 – 280

days is two-fold higher than that of the whole body (Guilloteau et al., 2009). During this period of fetal development, maternal nutrition plays a vital role in the healthy growth of the GIT (Duarte et al., 2013). Restriction of maternal nutrition during early to mid-gestation is associated with variations in fetal gut development (Meyer et al., 2010); however, this is not the case during mid to late gestational nutrition restrictions (Duarte et al., 2013). These changes are mainly studied in terms of intestinal tract weight, crypt and villus development, as well as vascularity (Trahair et al., 1997; Meyer et al., 2010; Duarte et al., 2013). Nonetheless, the effect of maternal nutrition restriction on fetal gut development of calves and lambs has been suggested to vary depending on the dam's parity (Meyer et al., 2010). Nutritional restriction during the first half of the pregnancy decreases small intestinal weights of lambs born to multiparous ewes (Trahair et al., 1997). Although there are no differences observed in the small intestinal villus height and mucosa thickness, crypt depth has been decreased due to restricted maternal nutrition (Trahair et al., 1997). Moreover, the differentiation of enterocytes is abnormal in fetuses of nutrition-restricted ewes, compared to well-fed ewes (Trahair et al., 1997). In contrast, Meyer and colleagues (2010) reported an increase in intestinal vascularity and jejunal proliferation in calves born under restricted maternal nutrition during early gestation. The lack of studies and variation in results related to how prenatal nutrition can impact fetal GIT development of ruminants represents a great need and valuable opportunity to uncover more consistent results through future research.

COLOSTRUM FEEDING

Ruminant GIT development mainly occurs prenatally; however, it undergoes marked structural changes postnatally to adapt the dietary changes and to digest and absorb the nutrients (Guilloteau et al., 2009). Colostrum, the first diet of neonatal ruminants that facilitates passive transfer of immunity, has been shown to influence the early development of the GIT (Baumrucker et al. 1994; Blattler et al., 2001; Yang et al., 2015). Although the passive transfer of maternal antibody is the main focus of colostrum management in neonatal calves, growth factors (insulin-like growth factor- IGF-1 and hormones) present in colostrum have been shown to influence gut development (Baumrucker et al. 1994; Blattler et al., 2001). Feeding colostrum within the first 3 days of life enhances the growth of small intestinal villus (circumference, height) and crypt (depth) of neonatal calves, compared to that of formula milk (Blattler et al., 2001). However, these effects are more prominent in the duodenum compared to that of jejunum and ileum (Blattler et al., 2001), suggesting that the effect of colostrum may be more influential in the growth of proximal intestine than that of the distal intestine. Similarly, the rate of epithelial cell proliferation has increased with colostrum feeding compared to formula milk feeding (Blattler et al., 2001).

The feeding of IGF-1 in milk has been shown to increase epithelial proliferation in neonatal calves (Baumrucker et al., 1994). IGF is involved in a variety of metabolic and physiological activities, such as transmembrane transportation and metabolism of glucose, amino acids, and nucleotides, synthesis of proteins, regulation of cell proliferation and differentiation, as well as inhibition of apoptosis (Georgiev, 2008). The concentration of IGF-1 is highest in the first colostrum and gradually decreases over

time (Baumrucker et al., 1994). This suggests that the presence of high amounts of IGF-1 in colostrum, when compared to formula milk that contains only traces of IGF-1 (Blattler et al., 2001), may enhance the intestinal development of calves fed with colostrum. However, feeding of growth factors alone has less impact on intestinal morphology than feeding actual colostrum (Roffler et al., 2003), indicating that there are other colostral components that may influence the development of the neonatal intestine.

Feeding colostrum has also been shown to impact intestinal epithelial barrier functions in piglets, via inhibiting the epithelial cell apoptosis and stimulating mucin secretion (Oste et al., 2010; Puiman et al., 2011). Additionally, feeding colostrum increases the number of goblet cells and intestinal protein synthesis, compared to formula milk (Puiman et al., 2011), which also enhances the intestinal barrier functions. These enhanced intestinal barrier functions later decrease the incidence of necrotizing enterocolitis in pigs (Jensen et al., 2013), indicating that colostrum-driven changes in the intestinal barrier play a crucial role in host susceptibility to enteric infections. Feeding bovine colostrum has also been shown to lower necrotizing enterocolitis induced by formula milk feeding in preterm pigs by improving intestinal functions, such as lowering pro-inflammatory cytokines production and increasing villus height and brush-border enzyme activities (Stoy et al., 2014). While it is evident that feeding of colostrum soon afterbirth decreases the calf's susceptibility to enteric infections (Godden et al., 2012), there remains a lack of knowledge regarding the impact of colostrum feeding on intestinal barrier development in calves, as the majority of studies focus primarily on absorption of maternal immunoglobulin. Thus, it is necessary to further explore this area in order to fully understand the effects of colostrum feeding on the development of intestinal barrier functions and calf susceptibility to enteric infections.

Over the last decade, our knowledge of how gastrointestinal microbiota can impact metabolic diseases has been transformed by a series of experiments in rodent models (Turnbaugh et al., 2004). Adding to these findings is the evidence that colonization in the first day of life can have longstanding consequences on the gut microbiota and health later in life (Koeinig et al., 2011). Given these results, colostrum management during the first day of life could have a longstanding influence on the microbial community structure, and thus, a substantial impact on GIT development and health. Colostrum contains specific bacteria and a large collection of oligosaccharides that can impact microbiota in the newborn after feeding, which is why colostrum is often referred to as the complete probiotic/prebiotic (Mills et al., 2012). With respect to potential probiotic and/or prebiotic properties within colostrum in ruminants, a study comparing pasteurized versus fresh colostrum feeding determined that pasteurized colostrum maintained higher levels of *Bifidobacterium* colonization and less *E. coli* in the calf ileum during the first 12 hours of life (Malmuthuge et al., 2015). The pasteurized colostrum did not contain viable *Bifidobacterium,* leading the authors to speculate that the oligosaccharides in colostrum underwent structural changes, which specifically supported *Bifidobacterium* growth as a result of heat treatment. This study is the first to

show how on-farm feeding practices of colostrum to ruminants in the first day of life may play a key role in microbial colonization and gut development.

FEEDING PLANE

Calves have been traditionally limit-fed milk or milk-replacer in dairy production at approximately 10% of bodyweight (half of normal consumption) (Jasper and Weary, 2002). Over the past decade, it has been well established that increasing intake during the pre-weaning phase increases the growth rate of calves and potentially future production (Soberon et al., 2012; 2013). Yet very little is known about how this feeding strategy will impact gut development. Although elevated planes are being fed during the pre-weaning period, many of these feeding schemes still restrict milk intake during the initial week(s) of life. This may be due to the train of thought purporting that higher levels of milk in the first weeks of life increase the incidence of diarrhea, a notion that is completely unfounded scientifically (Soberon et al., 2012). A recent study by de Passille et al. (2014) suggests that calves that consume more milk in the first five days of life have greater intake, growth and health during the entire pre-weaning phase. Besides the quantity of milk in the first week of life, there may be opportunities to improve the quality of milk as well. For example, colostrum feeding has been mainly focused on maximizing the passive transfer of immunoglobulins before gut closure. However, other bioactive components, such as growth hormones, prebiotics and immune system stimulants elevated within colostrum and transition milk (milk collected from the first three days after parturition) may aid in the development and health of the GIT, but are often overlooked. Although, abrupt transition from whole milk to milk replacer after the first meal of life is often practiced, calves with free-access to colostrum during the first week displayed greater growth rates and transiently enhanced insulin status and reduced cortisol status (Hammon et al., 2002). In a recent study, it was determined that calves fed transition milk have elevated health status, however the specific impact on the gut has not been properly studied - an important aspect of this feeding practice (Conneely et al., 2013). More work related to the impact of elevated quantity and quality of nutrition during the first week of life and its impact on gut development would be of great value to our industry.

After the first meals of life, dairy calves are transitioned to a milk feeding scheme that is typically offered in less than two meals per day (Johnsen et al., 2015). This transition marks a stark contrast to the calves' natural tendency to nurse from their dams *ad libitum*, facilitating feedings of up to 10 times a day in the first weeks of life (Jensen, 2003). Increasing feeding frequency, when calves are fed larger volumes of milk, improves digestion and efficiency of nutrient utilization (van den Borne et al., 2006). This may also provide benefits to digestive health when compared to feeding two times a day. Ahmed et al. (2002) determined that increasing the number of meals raises abomasal luminal pH and reduces ulceration. If large quantities of milk are fed in two meals per day, the abomasal capacity may be surpassed and milk may overflow to the reticulorumen. This is often referred to as "ruminal drinking," and if prolonged, can result in bacterial fermentation of the milk, which may lead to ruminal acidosis, impaired abomasal curd formation and infection. There is evidence that feeding elevated planes

of nutrition in only two meals per day disrupts metabolic and endocrine functions, leading to insulin resistance and disturbed glucose metabolism in veal and dairy calves (Bach et al., 2013). The long-term implications of these findings on gut development and metabolism are unknown and require more detailed investigations.

WEANING

The most dramatic changes in diet and gut microbiota in the ruminant lifespan occur during weaning. In nature, the weaning process occurs gradually over many weeks when calves are left to nurse from their dams, and is completed at approximately 10 months of age (Jensen, 2003). In contrast, commercial production systems, such as dairy, where feeding milk is considered more costly than feeding solid feed, practice abrupt transitioning of calves from milk to solid feed as early as possible. Early weaning methods (1-2 months of life) were accomplished by encouraging solid feed intake early, through the restriction of milk feeding to approximately 10% of birth weight, less than half of *ad libitum* consumption (Khan et al., 2011). Weaning is marked by the rumen capacity increasing from 30 to 70% of the entire forestomach (Baldwin et al., 2004). This process requires an extensive increase in the surface area for the absorption of short chain fatty acids (SCFA) produced through ruminal microbial fermentation to meet the demands for growth. This results in tremendous gut and metabolic ramifications to calf growth rate, as tissues must convert from reliance on glucose supplied from milk to the metabolism of SCFA as primary energy substrates for gluconeogenesis in the liver (Baldwin et al., 2004). With the recent adoption of feeding elevated levels of milk, weaning becomes even more critical as solid feed intake prior to weaning will be depressed.

To date, most of the studies have focused on rumen development during weaning transition. Factors that contribute to ruminal development include: the onset of weaning, the level of dry matter intake, dietary starch, and probiotics in dry feed (Khan et al., 2011; Eckert et al., 2015). Of all of the proposed mechanisms of ruminal growth and development, ruminal butyrate has been reported to be the most potent stimulator of epithelial proliferation and differentiation (Sakata and Tamate, 1978; Baldwin et al., 2004). In addition, hormones such as IGF-1 and epidermal growth factor have also been shown to stimulate the proliferation and differentiation of rumen epithelial cells in culture (Baldwin et al., 2004). The rapid state of cellular proliferation and differentiation during weaning is often associated with the condition of ruminal parakeratosis (Bull et al., 1965). This is defined as an accumulation of the outermost layer of the epithelium, termed the corneum, which can reduce SCFA absorption.

Recently, a series of microarray experiments were conducted using rumen tissue comparing different diets (hay, grain and milk) to uncover the mechanisms governing rumen epithelial proliferation and differentiation. It was determined that transforming growth factor β1 (Connor et al., 2014), peroxisome proliferator active receptors (Connor et al., 2013; Naeem et al., 2014) and microRNA (Liang et al., 2014) may be key target growth factors controlling cellular differentiation and inflammation. Understanding the control mechanisms for rumen growth and differentiation will offer insight into how to

perform dietary manipulations to smooth the transition from a pre-ruminant to a ruminant during weaning.

From a microbiological standpoint, rumen colonization starts immediately after birth. Within minutes, the rumen is colonized with microbes, and within two days of birth anaerobic bacteria colonize (Fonty et al., 1987). The appearance of adult-like cellulolytic and methanogenic bacteria as well as anaerobic fungi occurs in the first week of life (Fonty et al., 1987). A recent study using next generation DNA sequencing techniques confirms that the ruminal microbiota prior to weaning has a similar functional capacity as that of a mature ruminant (Li et al., 2012). The appearance of these populations is not dependent on nutrient digestion, as during this period the rumen has no functional activity, and may play a role in long-term imprinting of the microbial community (Jami et al., 2013). Although there has been great progress in the techniques used to study microbiology over the past decade, the microbial changes that occur during weaning remain poorly described.

While the rumen has received the bulk of the attention in the literature, it has recently been shown that the lower gut also undergoes transformation during weaning. It is hypothesized that the gastrointestinal tract barrier function is compromised during weaning. Associated with the disrupted barrier functions, feeding starter in combination with milk close to weaning tended to increase the mRNA expression of toll-like receptors that recognize bacteria (Malmuthuge et al., 2013). A recent study supports this notion of altered barrier function during weaning, showing increased permeability of the GIT during weaning (Wood et al. (2015), suggesting the importance of investigating the lower gut rather than the rumen in isolation. Infant and piglet research has clearly documented inflammation and morphological changes of the intestine during weaning (Pie et al., 2014). The same can be expected to occur in ruminants, as the levels of starch in the diet are equally high. An increase in inflammatory markers has been shown during the weaning of dairy calves (Kim et al., 2011). It may be that ruminants suffer temporarily from hindgut acidosis, for they have shown elevated fecal starch levels during weaning (Eckert et al., 2015). Greater investigation of the lower gut microbiological, structural, and functional changes and how these changes contribute to weaning stress is a logical next step in research.

CONCLUSION

It has become clear over the last decade that the nutrition of young ruminants in early-life can have longstanding impacts on growth, development and long-term productivity. The gut development, especially at weaning, is one of the most dramatic transformations in nature. In addition, the young ruminant is highly susceptible to gastrointestinal ailments prior to weaning that may have longstanding impacts on development. An understanding of how commonly practiced nutritional protocols can impact on gut development has not been achieved. In particular, few studies have looked directly at how gut development of the young ruminant is influenced by prenatal nutrition, colostrum feeding frequency and duration, pre-weaning level of feeding and weaning duration and age on gut health and development. Although it is necessary from

an agricultural production standpoint to measure growth during early-life nutrition program evaluation, there is also great value in measuring the dietary impact on gut development in order to achieve progress in this field.

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HOW TO GET MORE OUT OF DIETARY STARCH AND LOW STARCH DIETS

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INTRODUCTION

Starch is an energy dense nutrient made up of glucose units linked by an α-1,4 glycosidic bond. Typically, most dietary starch is fermented ruminally by dairy cows, likely ranging from 50 to 90% of starch intake. Starch fermented in the rumen generates propionate, used both as an energy source and to support lactose synthesis, a primary osmoregulator of milk yield. Furthermore, starch fermented in the rumen supports increased microbial protein synthesis, increased flow of metabolizable protein to the small intestine, and greater milk protein yield. Starch that is not ruminally fermented will be primarily digested in the small intestine, supplying glucose for use as an energy source. The remaining starch not digested in the rumen or small intestine will be available for fermentation in the large intestine. Increases in ruminal starch digestion will decrease starch digestion in the small intestine, but increase total tract starch digestion (**TTSD**). Several factors can influence TTSD of starch sources including grain particle size, moisture content, length of fermentation, vitreousness or prolamin content, and exogenous enzyme application. Dairy producers and nutritionists should monitor starch digestion to ensure starch sources are adequately processed.

The amount of dietary starch is quite variable in rations for lactating dairy cows but typically ranges from 20 to 30% of DM. There has been considerable interest in decreasing dietary starch content over the past 5 to 7 years. Much of this interest has been due, until recently, the relatively high cost of corn compared to historic prices. More recently, dairy producers and nutritionists have witnessed benefits in lowering dietary starch content beyond cost, particularly when starch content is 28% or greater. These benefits include improved NDF digestibility, increased bulk tank milk fat percent, and improved ruminal health. Partial replacement of high starch feeds with alternative feed ingredients can have profoundly different effects on lactational performance. Understanding those differences may lead to improved knowledge on ideal feeding strategies to replace dietary starch.

Monitoring Starch Digestion

Starch digestibility should be routinely monitored to determine if adequate digestion is occurring. Starch digestibility can be monitored as little as one or two times per year or as routinely as desired. Historically, starch digestion has been monitored by qualitatively assessing the amount of whole grain kernels or pieces in manure. Excessive grain in manure suggested poor starch digestion, requiring changes in the diets or improved grain processing. More recently, total tract starch digestion has been quantitatively measured by submitting fecal samples for starch analysis.

Several equations have been developed to estimate TTSD from fecal starch. In a review of the literature, Owens and Zinn (2005) reported that TTSD % = 98.2 – (0.93 × fecal starch %) for dairy cows. In 2006, Ferguson (*personal communication*) found that TTSD % = 98.7 – (1.76 × fecal starch %) in dairy cows.

More recently, Fredin et al. (2014) reported that TTSD % = 100.0 – (1.25 × fecal starch %). It should be expected that the intercept is 100.0 since the maximum amount of digestible starch is 100%. Fecal starch accounted for almost all of the variation in TTSD (R^2 = 0.94), strongly suggesting that measuring fecal starch alone is adequate to predict TTSD. Therefore, additional measurements, such as starch content of the diet or marker concentrations of the feces or diet, should not be needed. Several labs now offer near-infrared reflectance spectroscopy equations to predict fecal starch, allowing for more rapid and inexpensive monitoring of TTSD.

I recently updated the equation published in Fredin et al. (2014). I included individual cow data from the University of Wisconsin (Madison, WI) and Miner Institute (Chazy, NY) that I had direct access too (Dann et al., 2014; Farmer et al., 2014; Fredin et al., 2015a; Fredin et al., 2015b). These studies were added to the original equation (Fredin et al., 2014). The updated equation is presented in Figure 1. Due to the presence of heteroscedasticity in the updated regression model for TTSD and fecal starch, a default heteroscedasticity-consistent matrix estimator was included to properly estimate standard error around the intercept and slope. The updated equation is: TTSD % = 99.8 – 1.23 × fecal starch. The updated equation is remarkably similar to the original equation and serves to support the precision of the original equation. Due to the simplicity of the original equation (TTSD = 100.0 – 1.25 × fecal starch), I would advocate for its continued adoption to estimate TTSD.

Figure 1. Regression plot of fecal starch (% DM) and total tract starch digestibility (% starch intake). Total tract starch digestibility % = 99.8 (± 0.06) – 1.23 (± 0.02) × fecal starch %; RMSE = 0.90; R^2 = 0.93; P < 0.001; n = 726.

Average TTSD from the data set used in the updated equation was 95.7%. This dataset contained several treatments that were designed to depress starch digestibility, such as diets that included unprocessed corn silage or coarsely ground corn grain and suggests that excellent TTSD can occur in dairy cows. Total tract starch digestibility

greater than 98% are considered good and are consistently achievable. Starch digestibility greater than 95% is considered adequate. If TTSD is below 95%, consider replacing starch sources. Researchers at the University of Pennsylvania found that a decrease of 1%-unit in total tract starch digestibility is estimated to result in a 0.33 kg/d decrease in milk yield. By monitoring TTSD and adjusting starch sources when necessary, milk yield can be improved, especially when energy is limiting in the diet.

The most effective methods to improve starch digestibility of grains when TTSD is below 95% and grain type is unchanged is to grind grain more finely, add steam-flaked grain, or to include grain fermented at a higher moisture content. Total tract starch digestibility improved from 93 to 98% as the particle size of dry ground corn grain was decreased from 1270 to 552 μm (Fredin et al., 2015b). In a meta-analysis describing the effects of cereal grain type and processing methods on nutrient digestion by dairy cows, TTSD was 93% for corn ground to ≤ 1.5 mm, 90% for corn ground to ≤ 3.5 mm, and 78% for corn ground to > 3.5 mm (Ferraretto et al., 2013). In a review, Firkins et al. (2001) reported that TTSD for dry ground corn was 90.7%, steam-flaked corn was 94.2%, and high-moisture ground corn was 98.8%. Ferraretto et al. (2013) reported that TTSD was increased for ensiled (94.2%) and steam-flaked corn (93.9%) compared with dry ground or rolled corn (92%). Proper processing can have profound impacts on TTSD, leading to greater milk yield.

Effect of Feeding Reduced-Starch Diets

Fluctuations in grain costs have led to the partial replacement of grains such as corn and barley in lactating dairy cow diets with less expensive feeds. Common strategies for replacing grain in diets include the use of non-forage fiber sources (**NFFS**) such as citrus pulp, dried distillers grains plus solubles, soyhulls, or wheat middlings; forages such as corn silage or grass and legume hays and grass and legume silages; or sugars and sugar byproducts including molasses or glycerol. However, reduced-starch diets have resulted in reduced DMI (Ferraretto et al., 2013) due to increases in NDF content causing rumen fill. Furthermore, reduced-starch diets decrease the amount of rumen fermentable organic matter in the diet, potentially limiting microbial protein synthesis (NRC, 2001) and a reduction in the production of the glucogenic precursor, propionate (Allen et al., 1997) decreasing milk and milk protein yields. However, reduced-starch diets have the potential to improve rumen function by increasing rumen pH when excessive amounts of ruminally fermentable starch are fed (Allen, 1997), thereby increasing DMI and lactational performance. Often, TTSD increases when feeding a reduced-starch diet since less digestible grain sources are typically the first starch sources replaced.

Recently, I conducted a meta-analysis to determine the effect of reduced-starch diets on DMI and lactational performance, as well as to identify feedings strategies that can mitigate potential negative effects of feeding reduced-starch diets. The data set for the meta-analysis contained 223 treatment means from 53 peer-review papers and 4 scientific abstracts published from September 1993 through January 2014 in the *Journal*

of Dairy Science, *Animal*, or *Animal Feed Science and Technology*[1]. Studies included in the data set measured lactational performance of dairy cows fed TMR.

Studies that did not report dietary starch content were not included in the data set. Dietary starch content (% of DM) of the high-starch diet was included as a covariate effect because high dietary starch content can result in excessive amounts of ruminally-fermentable carbohydrate, increased risk for subacute or acute ruminal acidosis, and reduced DMI and lactational performance. The primary strategies to reduce dietary starch content included the partial replacement of grain or starch with NFFS, forage, or sugar or sugar byproducts.

The dependent variables evaluated were DMI and milk, fat, protein, and lactose yield, and MUN content. To determine the effects of reduced-starch diets on the dependent variables, the dependent variables were transformed as follows: Dependent variables = [Dependent variable mean on the high-starch treatment – dependent variable mean on the reduced-starch treatment]. Treatment included the decrease in dietary starch content (as a % of DM) and were calculated from the following equation: Decrease in dietary starch content = [Starch content (% of DM) on the high-starch diet – starch content (% of DM) on the reduced-starch diet.

Descriptive statistics of selected diet nutrient composition of experiments used in the meta-analysis are listed in Table 1. Dry matter intake averaged 24.2 kg/d across all diets and was 0.4 kg/d greater for reduced-starch diets than high-starch diets. Diet CP content averaged 17.8% across all diets and was similar for high-starch and reduced-starch diets.

Diet NDF content averaged 31.8% across all diets and mean NDF content was 3.1% greater for reduced-starch compared with high-starch diets. Dietary starch content averaged 24.6% across all diets and averaged 28.7% for high-starch and 21.9% for reduced-starch diets. Suggested levels of dietary starch for lactating cows are not well defined. Kaiser and Shaver (2006) reported that dietary starch content ranged from 25 to 30% for high producing herds and Staples (2006) suggested an optimal dietary starch content of 24 to 26% from a literature review. Dietary forage content averaged 48.0% for all diets and was 2.7% greater for the reduced-starch compared to the high-starch diets due to the partial replacement of grains with forages in 25 of the studies. Standard deviations and minimum and maximum values for the reported diet nutrient compositions suggest that a wide range of diets are represented in the meta-analysis.

Descriptive statistics for lactational performance data are provided in Table 2. Milk yield averaged 36.2 kg/d across all trials and was 0.7 kg/d greater for high-starch compared to reduced-starch diets. Milk fat and protein yield averaged 1.30 and 1.13 kg/d across all diets, respectively. On average, fat and protein yields were similar between high-starch and reduced-starch diets. Milk urea-N averaged 13.53 mg/dL across all diets and was decreased on the high-starch compared to the low starch diets. The large SD

[1] A complete list of published papers and abstracts are available upon request.

and minimum and maximum values suggest a wide range in lactational performance among experiments included in the meta-analysis

Table 1. Descriptive statistics and select diet nutrient composition of experiments used in the meta-analysis[1]

Item	Average	SD[2]	Minimum	Maximum
All diets				
DMI, kg/d	24.2	2.6	17.5	31.6
CP, % of DM	17.8	2.3	13.2	31.9
NDF, % of DM	31.8	5.5	19.5	48.4
Starch, % of DM	24.6	6.1	5.2	41.5
Forage, % of DM	48.0	11.6	10.3	79.6
High-starch diets				
DMI, kg/d	24.0	2.4	18.1	28.9
CP, % of DM	17.5	2.2	13.2	28.8
NDF, % of DM	30.0	4.8	19.5	42.1
Starch, % of DM	28.7	4.6	16.9	41.5
Forage, % of DM	46.5	10.1	10.3	67.0
Reduced-starch diets				
DMI, kg/d	24.4	2.8	17.5	31.6
CP, % of DM	17.9	2.3	13.2	31.9
NDF, % of DM	33.1	5.4	19.9	51.8
Starch, % of DM	21.9	5.1	5.2	34.3
Forage, % of DM	49.2	12.3	10.3	79.6

[1]Number of treatment means were 218, 83, and 135 for all, high-starch, and reduced-starch diets, respectively.
[2]Standard deviation.

The adjusted effect of reduced dietary starch on DMI (kg/d) is listed in Figure 2A. Dry matter intake was decreased 0.10 kg/d per %-unit decrease in dietary starch (P = 0.001; RMSE = 0.80). Dry matter intake tended to decrease 0.07 kg/d per %-unit decrease in dietary starch when starch was replaced by NFFS (P = 0.06) and decreased 0.12 kg/d per %-unit decrease in dietary starch when starch was replaced with forage (P < 0.01; Table 3). Ferraretto et al., (2013) reported that DMI was unaffected by dietary starch content which may be caused by the opposing effects of low DMI when excessive rumen fill occurs as dietary starch is replaced by forage NDF or increased meal size due to reduced ruminal propionate concentrations on reduced-starch diets (Allen et al., 2009). The more pronounced effect on DMI when forage replaces dietary starch is likely due to the greater amount of physically effective NDF in forages compared with NFFS (Mertens, 1997). The relatively large RMSE for the effect of reduced-starch diets on DMI indicates that the response on DMI is quite variable and dependent on the ingredients used to displace high starch feeds. Including low digestible forages in diets will reduce DMI due to effects on rumen fill, whereas highly digestible NFFS or forage such as BMR corn silage may increase DMI. Unexpectedly, the y-intercept for DMI is not zero. This is true for all other dependent variables. Theoretically, this would suggest that when dietary starch is unchanged from the high-starch diet, DMI would increase. Biologically, this cannot happen and in all cases, the y-intercept is not statistically different from zero (P > 0.10).

Table 2. Descriptive statistics of lactational performance of experiments used in the meta-analysis[1]

Item	Average	SD[2]	Minimum	Maximum
All diets				
Milk yield, kg/d	36.2	5.69	17.4	52.1
Fat yield, kg/d	1.30	0.20	0.76	1.74
Protein yield, kg/d	1.13	0.17	0.59	1.57
Lactose yield, kg/d	1.76	0.29	0.66	2.60
MUN, mg/dL	13.53	3.03	6.94	25.7
High-starch diets				
Milk yield, kg/d	36.6	5.98	17.9	52.1
Fat yield, kg/d	1.28	0.21	0.78	1.71
Protein yield, kg/d	1.15	0.17	0.60	1.57
Lactose yield, kg/d	1.80	0.30	0.72	2.60
MUN, mg/dL	13.07	2.54	6.94	20.60
Reduced-starch diets				
Milk yield, kg/d	35.9	5.51	17.4	50.9
Fat yield, kg/d	1.32	0.20	0.76	1.74
Protein yield, kg/d	1.11	0.16	0.59	1.52
Lactose yield, kg/d	1.74	0.28	0.66	2.51
MUN, mg/dL	13.80	3.27	8.01	25.70

[1]Number of treatment means were 218, 83, and 135 for all, high-starch, and reduced-starch diets, respectively.
[2]Standard deviation.

The effect of reduced starch diets on milk yield (kg/d) is listed in Figure 2B. Milk yield was decreased 0.19 kg/d per %-unit decrease in dietary starch ($P < 0.001$; RMSE = 0.63). The negative effect on milk yield may be due to the reduction in DMI. Ferraretto et al., (2013) reported a tendency for milk yield to decrease by 0.09 kg/d per %-unit decrease in dietary starch. A potential difference between the two estimates for the effect of dietary starch content on milk yield is the meta-analysis by Ferraretto et al., (2013) was more expansive (n = 320) as it was not restricted to only trials that evaluated dietary starch concentrations. Partially replacing starch by forage NDF will also decrease the rate and extent of rumen fermentable OM and decrease production of propionate (Allen et al., 1997), a primary source of blood glucose and milk lactose. Twenty four treatment means for milk yield were greater for reduced-starch compared to high-starch diets, suggesting that positive lactational performance can be achieved when feeding reduced-starch diets. Milk yield tended to decrease by 0.16 kg/d per %-unit decrease in dietary starch when NFFS replaced grain ($P = 0.06$) and 0.32 kg/d when forage replaced grain ($P < 0.01$; Table 3). The greater reduction in milk yield when dietary starch was replaced by forage is likely due to reduced DMI and decreased ruminal degradation of forage NDF compared to non-forage NDF (Allen, 1997).

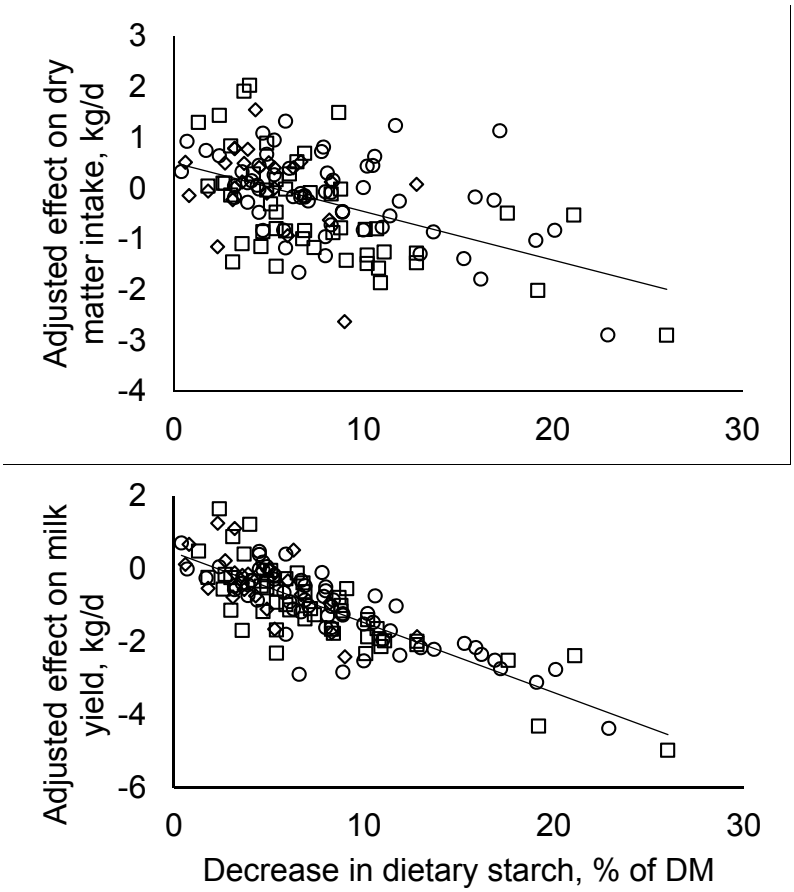

Figure 2. Effect of decreased dietary starch (% of DM; panel A) on DMI (kg/d; panel A) and milk yield (kg/d; panel B) adjusted for the random effect of trial. A) Effect on DMI = 0.52 + (–0.10 × decrease in % starch) + (–0.08 ± 0.79); RMSE = 0.80; *P* = 0.001; n = 135. B) Effect on milk yield = 0.44 + (–0.19 × decrease in % starch) + (0.00 ± 0.63); RMSE = 0.63; *P* < 0.001; n = 135. Strategies for decreasing dietary starch included partial replacement of grain with non-forage fiber source (○), forage (□), or sugar (◊).

The effect of reduced-starch diets on milk fat yield (g/d) is listed in Table 3. Milk fat yield decreased 6.9 g/d per %-unit decrease in dietary starch (*P* < 0.001). The decrease in milk fat yield is partly caused by the decrease in milk yield (Figure 2B). Milk fat yield decreased 5.4 g/d or 8.1 g/d when starch was replaced by NFFS (*P* = 0.05) or by forage (*P* = 0.01), respectively. Firkins et al. (2001) reported that increased amounts of grain intake reduced milk fat percent, whereas Ferraretto et al. (2013) reported a negative relationship between increased dietary starch content and milk fat percent, likely due to lower NDF intake, leading to milk fat depression. Both the composition and amount of unsaturated fatty acids influence the ruminal load of bioactive conjugated fatty acids that can cause milk fat depression and some NFFS such as barley distillers or corn distillers grains contain greater content of fat than grains, resulting in an increased ruminal unsaturated fatty acid load. Furthermore, barley or corn silages will have a similar fat content and fatty acid profiles to barley or corn grain, leading to similar ruminal

unsaturated fatty acid loads, negating potential increases in milk fat percent when replacing grain with forage.

Table 3. Effect of replacing dietary starch content (% of DM) on lactation performance[1]

Item	n[2]	Intercept	Slope	Cov[3]	*P*-value	RMSE[4]
All diets						
Fat yield, g/d	135	–181.1	–6.9	9.1	<0.001	72.7
Protein yield, g/d	135	16.7	–8.3	–0.1	0.001	28.5
Lactose yield, g/d	111	13.8	–11.5	-	<0.001	38.5
MUN, mg/dL	93	0.3	0.0	-	0.26	0.7
Non-forage fiber sources only						
DMI, kg/d	61	0.8	–0.07	-	0.06	0.8
Milk yield, kg/d	61	0.7	–0.16	-	0.01	0.6
Fat yield, g/d	61	–143.2	–5.4	7.6	0.05	65.3
Protein yield, g/d	61	31.6	–8.8	-	<0.01	23.9
Lactose yield, g/d	48	46.5	–14.7	-	<0.01	38.1
MUN, mg/dL	41	–2.8	0.0	0.1	0.68	0.9
Forage sources only						
DMI, kg/d	49	0.2	–0.12	-	0.01	1.6
Milk yield, kg/d	49	0.6	–0.32	-	0.01	0.8
Fat yield, g/d	49	–326.9	–8.1	13.7	0.01	123.3
Protein yield, g/d	49	–9.5	–11.1	0.7	<0.001	30.7
Lactose yield, g/d	46	5.3	–12.0	-	<0.01	90.0
MUN, mg/dL	34	–0.1	0.2	-	0.01	0.8

[1]Adjusted for the random effect of experiment.
[2]Number of treatment means.
[3]Cov = Covariate; Highest dietary starch content (% of DM) within study.
[4]Root mean square error.

Milk protein yield was reduced 8.3 g/d per %-unit decrease in dietary starch content ($P < 0.001$; RMSE = 28.65; Table 3). Milk protein yield was reduced 8.8 g/d per %-unit decrease in dietary starch content when starch was replaced by NFFS ($P < 0.01$) and 11.1 g/d when starch was replaced by forage ($P < 0.001$). There was actually a slight numerical increase in protein yield when starch was replaced with sugar (1.9 g/d). Nocek and Tamminga (1991) reported a positive correlation coefficient between starch intake (kg/d) and milk protein yield. Increased grain intake (Firkins et al., 2001) and dietary starch concentration (Ferraretto et al., 2013) also increased milk protein content. Furthermore, increased starch intake results in an increased amount (kg/d) of ruminal starch digestion (Nocek and Tamminga, 1991), leading to increased microbial protein synthesis and flow to the small intestine, increased amounts of metabolizable protein, and improved milk protein synthesis (NRC, 2001). Increased starch intake also increases the amount of starch flowing to the small intestine (Nocek and Tamminga, 1991). Greater starch flow and digestion (as kg/d) in the small intestine will result in greater utilization of glucose by small intestinal enterocytes as an energy source and reduced reliance on glucogenic AA. Sparing amino acids from metabolism by enterocytes will lead to increased uptake of amino acids into the portal vein and greater amounts of metabolizable protein for tissue

and milk protein synthesis (Nocek and Tamminga, 1991). Increased starch flow to the small intestine can also increases arterial concentrations of glucose and insulin, further resulting in improved milk protein content.

Milk lactose yield was decreased by 11.5 g/d per %-unit decrease in dietary starch content ($P < 0.001$; RMSE = 35.5). Milk lactose yield decreased 14.7 g/d per %-unit decrease in dietary starch when starch was replaced by NFFS ($P < 0.01$) and 12.0 g/d when starch was replaced by forage ($P < 0.01$). Lactose is a primary osmoregulator in mammary uptake of water and increased lactose synthesis increases milk yield. Increases in dietary starch increase the proportion of propionate in absorbed VFA (Allen et al., 2009). Up to 59% of absorbed propionate is converted to glucose in the liver of lactating dairy cows, 80% of glucose supply is utilized by the mammary gland, and 74% of the glucose extracted by the mammary gland is used for lactose synthesis (Hanigan et al., 2001). Greater supply of absorbed propionate from high-starch diets likely results in increased lactose synthesis. Increased amounts of dietary starch may also increase the amount of starch flow to the small intestine. Owens et al. (1986) reported that starch digested in the small intestine provides 42% more energy than starch digested in the rumen due to losses through methane, heat of fermentation, and futile energy cycling by microbes. The results from the meta-analysis suggest that increased dietary starch content increases both lactose yield and milk yield. This may be due to greater starch intake and potential increased flow of starch to the small intestine, leading to greater glucose absorption.

Unexpectedly, MUN was unaffected by dietary starch content ($P = 0.26$). Ferraretto et al., (2013) found that increased dietary starch content reduced MUN. Typically, highly fermentable, high starch diets will decrease MUN by increasing NH_3 utilization by rumen microbes to synthesize microbial protein.

CONCLUSIONS

Total tract starch digestibility can be effectively monitored by measuring fecal starch. Total tract starch digestibility greater than 98% is achievable and digestibility lower than 95% will result in the need to re-evaluate dietary ingredients or processing. Based on results of a meta-analysis, reduced starch diets result in decreased DMI and milk, fat, protein, and lactose yields. Milk urea-N content was unaffected by dietary starch content. More pronounced decreases in lactational performance were observed when starch was replaced with forage compared with NFFS. These data suggest that when lowering dietary starch content, consider replacing starch with NFFS. If forage is the only available ingredient, replace starch with a highly digestible forages to minimize production losses.

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BIOLOGICAL IMPORTANCE OF RUMINATION AND ITS USE ON-FARM

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INTRODUCTION

Rumination is controlled by dietary and management factors such as fiber amount and particle size, degree of overcrowding, grouping strategies, and other potential stressors in the management environment. Rumination reflects cow health and is highly sensitive to the state of well-being. However, direct observation of rumination is labor intensive and only a few cows may be monitored intensively at once. In recent years, commercial systems for monitoring rumination activity have become available, and published research indicates that there is reasonable correlation between visual and electronic monitoring systems (Schirmann et al., 2009). Current research and on-farm experiences are beginning to demonstrate the value of monitoring rumination to identify nutritional problems, find cows in estrus, detect health disorders earlier, streamline fresh-cow examinations, and adjust treatment protocols based on cow responsiveness. As research accumulates, we expect routine rumination monitoring to increase because rumination responds to stressors up to 24 h sooner than traditional measurements allowing for more effective cow management.

NUTRITIONAL ROLE OF RUMINATION

Rumination is defined as the regurgitation of fibrous digesta from the rumen to the mouth, remastication and reinsalivation, followed by swallowing and returning of the material to the rumen (Welch, 1982). This cyclical process is influenced by several primary factors including dietary and forage-fiber characteristics, health status, stress, and the cow's management environment (Grant and Albright, 2006; Calamari et al., 2014). Rumination is controlled both by the internal environment of the rumen and the external environment of the cow, i.e. the management environment. We have known for decades that receptors located within the reticulorumen are sensitive to friction or "scratch factor" from the fibrous components of the diet (Gordon, 1968). Rumination facilitates digestion, particle size reduction, and subsequent passage from the rumen thereby influencing dry matter intake. Rumination also stimulates salivary secretion and improves ruminal function via buffering (Beauchemin, 1991).

Rumination is positively related to feeding time and dry matter intake. Following periods of high feed intake, cows spend more time ruminating, usually after a 4-h lag. Restricting feed intake reduces rumination: a 1-kg decrease in dry matter intake has been associated with a 44 min/d reduction in rumination (Metz, 1975).

Cows ruminate 25-80 minutes per kilogram of roughage consumed (Sjaastad et al., 2003). Mertens (1997) reported that mean chewing time was 150 minutes per

kilogram of NDF for long grass hay. This relationship between NDF and chewing response forms the basis of fiber's physical effectiveness in the physically effective NDF (peNDF) feeding system. Physically effective NDF is based on the two fundamental properties of feeds that influence eating and ruminative chewing: fiber content and particle size. However, recent observations of Miner Institute's dairy herd suggest that more than simply the amount and quality of forage-fiber influence daily rumination time (Cotanch, 2015). It may be that cow and nutritional factors set a "normal" maximum amount of rumination activity, and as nutritionists and farm managers we essentially can reduce that maximal activity with non-ideal management.

Ruminant nutritionists have mostly focused on the component of rumination that is determined by fiber physical form and digestibility. However, we know that cows voluntarily control rumination and stop when disturbed. Under acute and chronic stress environments, rumination is depressed: rumination is highly sensitive to cow well-being. Increasingly, the management focus is shifting to these non-nutritional factors that greatly influence rumination.

RUMINATION AND MANAGEMENT

Figure 1 illustrates several key components of the management environment that may reduce the cow's expected rumination response to dietary peNDF, fiber digestibility, or fiber fragility. Rumination follows a 24-h rhythm and ordinarily mature cows will spend 480 to 540 min/d ruminating under ideal conditions (Van Soest, 1994). A wide range of management factors may depress rumination activity including overcrowding, mixed parity pens, excessive time spent in headlocks, and heat stress. If rumination is chronically depressed by 10 to 20% due to poor management, then we can reasonably predict compromised ruminal function and greater risk for associated problems such as sub-acute rumen acidosis, poorer digestive efficiency, lameness, and lower milk fat and protein output.

In particular, recent research shows that overcrowding influences rumination time, location, and cow posture during rumination (Hill et al., 2009). When cows are fed the same diet, as stall and headlock stocking density is varied from 100 to 142%, rumination time drops by 0.4 h/d, rumination while standing increases by 0.6 h/d, while recumbent rumination decreases 0.9 h/d.

Dominance hierarchy also affects rumination activity. Ungerfeld et al. (2014) compared the rumination activity of high and low ranked dairy cows and found that lower ranked cows ruminated 35% less than higher ranked cows. The lower ranked cows had shorter rumination bouts that reflected lower feed intake. The effect of social interactions within a group of cows on rumination needs to be considered when developing effective grouping strategies for a farm. This is especially important for mixed parity pens where we know that primiparous cows ruminate and lie down less when commingled with mature cows. In fact, we have measured up to a 40% reduction in rumination activity for primiparous cows when they are resting in stalls known to be preferred by dominant cows within a pen (Grant, 2012).

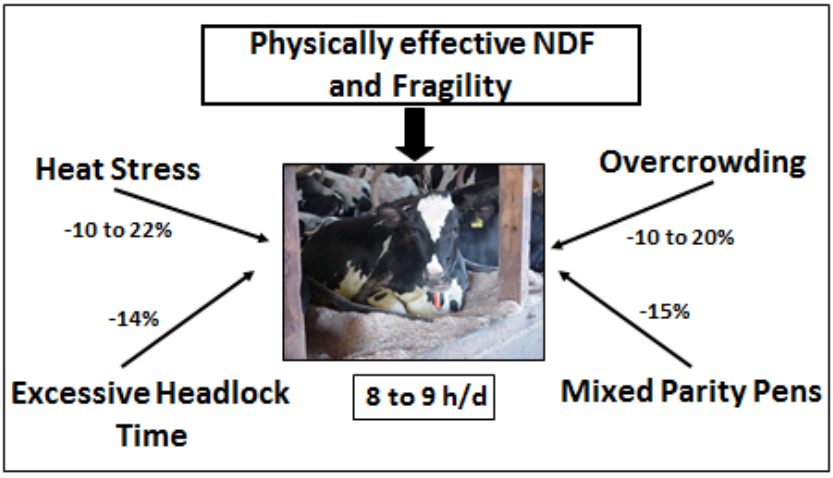

Figure 1. Physically effective NDF and fiber fragility drive rumination, but poor management substantially reduces rumination.

RUMINATION: MORE THAN SALIVATION

Rumination is an innate behavioral need of dairy cattle (Lindstrom and Redbo, 2000) and they exhibit stereotypies when it is inhibited. When ruminating, whether lying or standing, cows are quiet and relaxed, with heads down and eyelids lowered. Cows prefer to ruminate while lying down (Cooper et al., 2007; Schirmann et al., 2012) with rumination occurring in about 80% of resting bouts. Most rumination occurs at night and during the afternoon. Consequently, poor management that impairs lying time may also reduce rumination. The cow's favored resting posture is sternal recumbency with left-side laterality (55-60% left-side preference). This combination of left-side laterality and upright posture is thought to optimize positioning of the rumen within the body for most efficient rumination (Grant et al., 1990; Albright and Arave, 1997).

Total sleep time in cattle is short, and rumination provides the physiological rest and rejuvenation provided by sleep (Ruckebusch, 1972; Ewbank, 1978). Cattle experience about 3 h/d of non-REM sleep and 45 min/d of REM sleep (Ternman et al., 2012). The EEG patterns recorded during rumination are similar to sleep or somnolence (Bell, 1960). Rumination is closely associated with drowsiness and can even occur when the cow progresses into non-REM sleep. There may in fact be a behavioral continuum between rumination and sleep in ruminants. Sufficient sleep is critical for both metabolic and immune function and the relationships among rumination, resting, and sleep are critical for the health and well-being of dairy cows.

Rumination activity also increases with advancing age as do number of boli and time spent chewing each bolus. Total ruminative chewing increases linearly from 2 years of age forward (Gregorini et al., 2013). This trend toward greater rumination with advancing age may be compensation for reduced chewing efficiency.

USE OF RUMINATION ON-FARM AS A MANAGEMENT TOOL

Cows ruminate for approximately 450-550 minutes per day and a decrease in rumination time is typically a good sign that something is affecting ruminal function and cow well-being. Specifically, research and on-farm experience indicate that monitoring deviations in rumination from a baseline provides the most useful management information. Rumination often responds to a stressor 12 to 24 h sooner than traditionally observed measures such as elevated temperature or other clinical signs, depressed feed intake, or reduced milk yield (Bar and Solomon, 2010). Recently, on-farm systems have become available to monitor rumination as well as other behaviors such as activity.

Expected changes in rumination time for a variety of management routines and biological processes have been reported based on accumulated on-farm observations with a monitoring system that functions on sound created while chewing (SCR, 2013). Reported deviations in rumination include: calving, -255 min/d; estrus, -75 min/d; hoof trimming, -39 min/d; heat stress, -20 to -70 min/d; and mastitis, -63 min/d (SCR, 2013; Miner Institute data, 2014). A recommended target for making management decisions would be a deviation in rumination of greater than 30 to 50 min/d for either an individual cow or a group. Patterns in the variation in rumination should reflect the feed, feeding management, or the cow's physical and social environment. Key areas to assess would include standard operating procedure compliance, facility limitations, and management routines. Often, changes in rumination measured on-farm reflect changes in feed or feeding management, cow grouping or cow movement, and overall cow comfort.

Common challenges faced by dairy producers that would benefit from routine rumination monitoring include:

- Identifying nutritional problems,
- Finding cows in estrus,
- Detect health problems earlier such as metabolic disorders, mastitis, and lameness,
- Management issues such as grouping, stocking density, or heat stress abatement,
- Modifying traditional fresh-cow checks with less disturbance of cows and time in headlocks, less labor, and greater focus on high-risk cows, and
- Changing treatment and culling decisions because cows can be monitored after treatment to evaluate treatment efficacy.

Importantly, research to-date indicates that it is not necessarily the time spent ruminating each day that must be monitored, but the change in rumination time from day-to-day that is most important.

Rumination Monitoring and Transition Period

Several recent studies have demonstrated the usefulness of monitoring rumination activity during the periparturient period and in particular the first week of lactation as a means to identify in a timely manner those cows at elevated risk of developing a disease during early lactation (ex. Calamari et al., 2014).

Rumination normally decreases by about 70% at parturition and increases by approximately 50 min/d following calving (Soriani et al., 2012). However, severe inflammation around parturition is associated with a slower increase in rumination time following calving (Soriani et al., 2012). Additionally, more than 90% of cows that had low rumination during the first 3 to 6 days in milk experienced clinical disease in early lactation compared with only 42% for those cows that had greater rumination time. The average rumination time prior to calving was 479 min/d (from -20 to -2 d prepartum), and the value ranged from 264 to 599 min/d (Soriani et al., 2012). For the high-rumination cows during the first week postpartum, the increase in rumination time after calving was very rapid: by 3 days in milk rumination time had reached the average value observed for the entire first month of lactation. In contrast, the lower rumination cows did not reach a stable level of rumination similar to high-ruminators until 15 days in milk.

Earlier research has found that primi- and multiparous cows that have greater lying and ruminating activity for d -2 and -6 prepartum have greater dry matter intake and milk yield on d 1 to 14 postpartum (Daniels et al., 2003). Furthermore, cows with less rumination time prepartum tend to have less rumination time postpartum. Shorter rumination time is also associated with an elevated risk of several metabolic disorders (<420 min/d; Soriani et al., 2012). Figure 2 shows a screen shot from the SCR rumination monitoring system to illustrate how a fresh cow with low rumination activity may be tracked.

Most recently, Stangaferro et al. (2015a) compared prepartum rumination patterns of lactating dairy cows from -7 d to calving that developed health disorders to those cows that did not up to 30 days in milk. For all health disorders combined, rumination time was less for cows with health disorders (439 min/d) than for cows with no health disorders (456 min/d). Rumination time was lowest on the day of calving (391 min) than the 6 d preceding calving (range of 458 to 463 min) for all cows. These researchers concluded that, starting 7 d prepartum, rumination patterns are altered in cows that suffer health disorders within 30 days in milk. Specifically, rumination time is reduced in cows that suffer metabolic disease (such as abomasal displacement, ketosis, or indigestion) and metritis, but not in cows with retained placenta or mastitis.

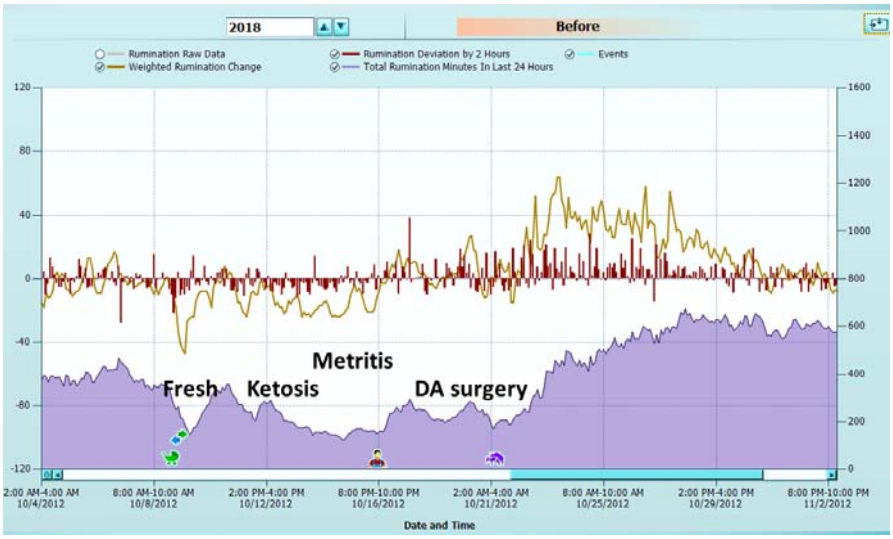

Figure 2. Example fresh cow with low rumination time and associated health problems.

Ability to relate rumination time to mastitis may be related to severity of systemic illness and type of mastitis-causing pathogen (Stangaferro et al., 2015a). Nonetheless, the rumination monitoring system identified cows with abomasal displacement, ketosis, metritis, and mastitis earlier than farm personnel (Stangaferro et al., 2015b). The mean days between clinical sign of disease to the day the disease was flagged by the rumination system was -3 d for abomasal displacement, -1.6 d for ketosis, -0.5 d for indigestion, -0.8 d for metritis, and -0.8 d for mastitis.

This research demonstrates that rumination technology may improve cow care and cow well-being by helping to identify health disorders more quickly. Research and on-farm observations have effectively related rumination activity and mastitis detection (Lacker and Bar, 2013), rumination and estrus (Pahl et al., 2015), rumination and grouping strategy (Grant and Albright, 2001), and rumination and calving pen management (Morrison et al., 2013). The relationship between rumination activity and lameness detection is less certain. Although Van Hertem et al. (2013) found that cows ruminated less at night (8:00 pm to 4:00 am) before being diagnosed as lame, Van Hertem et al. (2014) concluded that hoof trimming per se had relatively small effects on rumination and was dependent on several factors such as parity, stage of lactation, and effect of hoof trimming on subsequent distribution of locomotion scores.

Rumination and Reproduction

Pahl et al. (2015) found that rumination was reduced for about 30 hours around estrus but the primary drop occurred at 6:00 am on d -1 and noon on d 0. Their research indicates the potential to use changes in rumination as well as feeding times around estrus as a useful aid for early estrus detection. Rumination also shows great potential for monitoring of calving events (Pahl et al., 2014). In this study, cows stopped ruminating 123±58 min before calving and resumed ruminating 355±194 min following calving. Schirmann et al. (2013) found that daily rumination time decreased by about 63

and 133 min during the 24 h before and after calving, respectively. Similarly, feeding time was decreased by about 66 and 82 min per day before and after calving.

Rumination and Heat Stress

Heat stress negatively affects cow behavior, including rumination. Tapki and Sahin (2006) found that, as air temperature rose from 25 to 40°C, eating decreased 46%, standing increased 34%, locomotion decreased 19%, and rumination decreased by 22%. Higher producing cows (>32 kg/d) were more sensitive than lower producing cows, especially for lying and ruminating activities. More recently, Soriani et al. (2013) observed a negative relationship between daily maximum temperature-humidity index (THI) and rumination time with a reduction of 2.2 min of rumination time for every daily maximum THI unit over 76. Rumination time was negatively related to breathing rate and positively to milk yield. At Miner Institute, we have observed approximately 1 h difference in rumination time for cows that were exposed to minimal heat stress abatement (fans only over the stalls) versus fans and sprinklers over the feed bunk and the free stalls. This strong negative relationship between heat stress and rumination allows us to use rumination monitoring to gage the effectiveness of heat abatement strategies implemented by the producer.

Current Outlook for Using Rumination Monitoring

Despite the potential effectiveness of rumination monitoring, not all studies have found useful relationships. For example, Liboreiro et al. (2015) concluded that, although differences in daily rumination time and activity between cows that developed periparturient diseases and healthy cows were observed, further research is required to determine how rumination time and activity data can be used to diagnose cows that will develop disease earlier than using standard visual observations. They concluded, as have other research groups, that diagnosis of infectious and metabolic diseases works best when the focus is on change in rumination time from day-to-day.

The bottom line across nearly all of the published research and on-farm observations is that the results verify that rumination monitoring systems may provide predictive and actionable information that farmers can use to improve management of the individual cow, a group of cows, and the whole herd.

RUMINATION: THE BOTTOM LINE

Rumination is highly sensitive to changes in dietary peNDF and fiber digestibility, cow health and well-being. Its use as a routine on-farm monitoring tool is expected to grow since it will allow earlier identification of problems and more timely intervention.

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BALANCING DIETS WITH THE CNCPS v6.5 – WHAT'S CHANGED AND IMPLICATIONS FOR USE

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INTRODUCTION

With the release of CNCPS v6.5, there are changes to the predictions and requirements for energy, protein and amino acids and subsequently, some of the values that have been used to evaluate diets have changed or require review. Of particular interest are the amino acid requirements and supply with the updated library values and new post-absorptive efficiencies. Also, nutrient supply in dry cows, heifers and pasture fed or high forage fed cattle have to some degree been under-predicted and a new evaluation of passage rate equations for forages has been conducted and updated passage rate equations are being incorporated where needed.

Amino acids

With the update to the feed library (Higgs et al. 2015) one of the primary outcomes was the significant increase in the feed values for methionine (Met) content due to the updated chemistry that allowed for better recovery of the sulfur amino acids (AA) in feeds and the application of AA to the whole feed and not the insoluble residue. Other AA values changed with the update, but none as dramatic as Met. With the composition updates, the supply of Met in most cases doubled, thus efficiencies of use also were updated as one of the downstream offsets to the increased supply was to develop or adopt efficiencies that more closely reflect the post-absorptive metabolism of AA. The approach was to test and adopt the efficiencies described by Doepel et al. (2004) as re-evaluated by Lapierre et al. (2007). The efficiencies developed by Lapierre et al. (2007) were described as a function of MP supply, however, we have data that suggests the efficiencies should be described on an energy basis (Higgs, 2014). Therefore, to accommodate energy in the prediction, the efficiencies adopted were from the calculations where 100% of MP allowable AA were supplied. The assumption was that the AA supply at that point was at 100% of the MP allowable requirement which should be energy neutral, thus neither under or oversupplied and would represent the values consistent with routine formulation where energy should not be first limiting and protein and AA should not be under or overfed (Van Amburgh et al., 2015) (Table 1).

For comparison, the efficiencies developed by Higgs (2014) from the same dataset but using ME allowable energy as the basis for the AA efficiencies are also shown in Table 1. The efficiency of use of many of the AA developed for v7 are similar to those adopted from Lapierre et al. (2007) for use in v6.5 and provide some insight into the differences among models and also that within model structures, the efficiencies are model specific.

Table 1. The original efficiencies of amino acid utilization as published by O'Connor et al. (1993) and the combined efficiencies (%) of amino acid utilization for both maintenance and lactation adapted from Doepel et al. (2004) and Lapierre et al. (2007) and for comparison, the efficiencies from Higgs (2014) developed on an metabolizable energy allowable basis.

	CNCPS v6.0		CNCPS v6.5	CNCPS v7
Amino acids	Maintenance	Lactation	Combined Efficiency[1]	Efficiencies developed on an ME allowable basis[2]
Met	85	100	66	57
Lys	85	82	69	67
Arg	85	35	58	61
Thr	85	78	66	59
Leu	66	72	61	73
Ile	66	66	67	67
Val	66	62	66	68
His	85	96	76	77
Phe	85	98	57	58
Trp	85	85	65	65

[1]From Doepel et al., 2004 and Lapierre et al., 2007. [2]From Higgs 2014.

It is important to recognize that the efficiencies and breakpoints for AA supply, requirements and utilization are all going to be model specific and that applying the same ratios or grams for all versions of the CNCPS or the 2001 NRC are not appropriate and should not be expected to work effectively. All models are developed to be internally consistent and have particular offsets that allow them to be useful, and will provide different relationships that are not transferable among models.

Based on evaluations of AA supply and requirements, some relationships were developed that allow for formulation of the most limiting AA on an energy basis as a reference point. For example, the current formulation goal for Met (digestible Met, %MP) to optimize milk protein yield is 2.6% which is approximately 11% greater than the v6.0 and is difficult to achieve without adding rumen protected AA. Given the breakpoint analysis assuming energy is not first limiting, the grams of Met per Mcal ME was found to be between 1.12-1.15 g for lactating dairy cattle. For dairy cattle consuming 60 Mcals ME, that would equate to (60 Mcals * 1.12 g Met/Mcal) 67.2 g of metabolizable methionine to meet the requirements for milk protein yield. The requirements for milk protein concentration are greater by several grams, so the suggestion is to start with this approach and evaluate cattle responses before increasing the supply.

The breakpoint for lysine for milk protein yield is 7.0% digestible lysine %MP in v6.5, therefore, the updated ratio is (7/2.6 = 2.69). Thus, the lysine supply to optimize milk protein yield would be 2.69 * 67.2 g Met = 181 g and would increase with increasing

methionine and ME supply. Given the evaluations that have been conducted, we recommend that the user start with methionine calculations and then follow with the related lysine supply.

One additional modification to the amino acid supply is the inclusion of the tissue amino acid profile to the metabolizable protein that is generated when a cow is in negative energy balance. The CNCPS has inputs for body condition score change and when the change is a loss, the energy from mobilized tissue contributes to the ME allowable milk and the protein associated with the mobilization of that tissue contributes to the MP supply. The approach assumes that the profile of tissue mobilized is of similar composition to the tissue that was last deposited, so a cow mobilizing energy will mobilize tissue that is approximately 60-70% adipose tissue, 9-11% protein, some minerals and water. For example, a BCS loss of 0.5 over 30 days at 40 days in milk would result in approximately 8 Mcals of ME which is equal to about 21 lb of ME allowable milk, and about 320 g of MP which would provide for about 29 lb of MP allowable milk. The associated AA supply would be about 7 g of Met and 21 g of Lys based on the tissue composition of AA (Van Amburgh et al., 2015).

Rumen ammonia

With updates to the model, it is possible to formulate diets for high producing lactating cattle at or below 15% CP and when doing so, both MP supply and rumen ammonia balance are important to ensure adequate N for the rumen and protein and AA for the animal. Rumen ammonia is estimated from dietary soluble protein and rumen degradable protein that is converted to ammonia via degradation, and some urea recycling from the plasma urea pool. With the updates to the feed library and model, the rumen ammonia prediction is more accurate and sensitive to changes in carbohydrate fermentation and protein supply. The increase in sensitivity is partly due to the re-assignment of the soluble components of feeds to the liquid passage rates which increases the rumen escape of soluble proteins. This change is coupled with decreased rates of degradation of the soluble proteins, which when calculated together reduces rumen ammonia production, thus relying more on recycled nitrogen.

Generally, maintaining a rumen ammonia balance of 110 to 120% is adequate to ensure good ruminal NDF digestion. The robustness of rumen ammonia prediction assumes the user has described the cattle, feeds and DMI of the diets accurately and that feeding behavior follows normal time budgets and is not negatively influenced by overcrowding or excessive time spent away from the feed bunk (Gomez and Cook, 2010). Monitoring of MUN as a proxy for N status is acceptable if done on groups and not the bulk tank and the user is confident the laboratory conducting the MUN measurements have calibrated the system to changes in MUN below the standard ranges and basically close to zero to ensure linearity of the prediction. With the ability to formulate diets at lower crude protein levels, management factors around the cows can influence the meal pattern in such a way that the true dynamics of recycling is not completely captured since the model still integrates on a 24 hour basis. Most recommendations are for MUN to be between 8 and 12 mg/dl, however if the MUN values are less than 7 mg/dl, there is a

good possibility that the rumen N balance is negative during periods of the day and this could be exacerbated by time budgets of the cows and impacting microbial yields and forage digestibility. If diets are 15% -15.5% CP or less, monitor groups or individual cows within groups and if the feed intake and manure are not consistent, measure plasma urea nitrogen (PUN) to verify the MUN data.

aNDFom and uNDF

To account for ash contamination in NDF, aNDFom should be measured if at all possible to provide more accurate fiber levels for diet formulation. Depending on the forage type, and irrigation and harvest methods, laboratory data are demonstrating that in certain forages and in regions of the country there can be significant contamination of forages with soil. In regions where there are sandy soils and flood irrigation, the aNDFom content of total mixed rations has decreased up to 5 units compared to measurements of aNDF. The cows will be at greater risk of sub-acute ruminal acidosis and the solution is to increase the amount of forage fed to achieve the target intakes. The formulation objectives for aNDFom are the same as those for NDF and aNDF for total aNDFom, NDF as a percent of BW and all other goals related to fiber (Mertens, 2009).

With the implementation of uNDFom240 in place of lignin x 2.4 as the NDF unavailable to rumen digestion, the estimation of integrated of rates of aNDFom digestion are improved. The approach provides a more dynamic calculation of the rates of digestion and allows the user to account for the agronomic conditions the forages were grown in. Further, based on the data being generated from Miner Institute and University of Bologna, the ratio of rumen content to intake of uNDF is about 1.60 regardless of forage and intake. The range in uNDFom240 intake among studies has been observed between 0.30-0.48% if BW and the range in rumen contents is 0.48% to 0.62% of BW (Cotanch et al. 2014).

Feeding to a percentage is difficult, and the data further describe the amount of uNDF that is consumed by cattle among studies. The value has some variability, but is relatively consistent among the range in forage inclusion levels and digestibility of the studies from Miner Institute (Cotanch et al., 2014). Among all the treatments analyzed, the cattle consumed approximately 2.2 ± 0.24 kg of uNDF per day. This would represent total uNDF intake and the value can be used as benchmark to evaluate intake limitations due to the size of the pool. This value includes the uNDF from all feeds, so if the user is relying only on forages, the value will be lower and usually between 70% and 80% of the total diet. This is still an active area of research and the data are intriguing and provide the user with new values on which to estimate DMI potential of forages and diets.

Rate of passage for forages and NDF

The evaluations conducted and published on large lactating dairy cattle data sets indicated the model is doing a reasonable job of predicting ME and MP allowable milk, most limiting ME or MP and provides a good prediction of total MP supply (Van Amburgh et al., 2015). One of the problems with the evaluation was the dearth of information on

dry cows, heifers and pasture fed cattle. Feedback from users of the model over the last year has indicated that ME and MP supplies are being under-predicted in cattle fed high forage diets at more moderate intakes than a high producing lactating dairy cow. This is especially true for dry cows, heifers and pasture fed cattle. Consequently, another evaluation of the model is being conducted.

The forage passage rate in CNCPSv6.5 is predicted by an equation from Seo et al. (2006) and was built from the same database used to develop the 2001 Dairy NRC (NRC, 2001) equations. The predicted passage rate of forage from the evaluation was 0.04 h^{-1} with a range of 0.013 to 0.074 h^{-1} (Seo et al., 2006). Comparisons to omasal flow and rumen evacuation data in a large meta-analysis demonstrated that the predicted passage rate of forage from that equation is too fast and would underestimate the digestibility of fiber in the rumen (Krizsan et al., 2010). This discrepancy between measured flow using the omasal flow technique versus the prediction of Seo et al. (2006) was also identified during the development of CNCPS v7.0 and alternative equations were adopted (Higgs, 2014).

Several equations were evaluated during the development of v7.0 and the equation for forage and fiber passage rates was adopted from the NorFor modeling effort (NorFor, 2011). The equation is:

NDF(For) = 0.48+1.5106/(1+((ΣDMIi*NDFi)/(BW*7.484))^-3.198), where
ΣDMI is the total dry matter intake, NDFi is the NDF content of each feedstuff, and BW is the body weight of the cow.

As expected there was a significant difference in the predicted passage rate of forages between the two equations. Within one of the databases, the Seo equation mean kp prediction was 4.8% h^{-1} whereas the NorFor equation prediction was 1.7% h^{-1}. The predicted decrease in passage from the rumen with the NorFor equation allows for greater rumen residency time and greater ruminal NDF digestibility and subsequently increased ME and MP supplies. The increase in ME supply among lactating cattle diets evaluated was between 2 and 3 Mcal. A re-evaluation of the lactating cattle data set was conducted, but the data were incomplete due to a database issue, so no formal statistics are presented here, however, the evaluation suggested that ME was more positively influenced than MP and the change in MP was generally less than 100 g for the average diet.

As with all modifications to the model, there is an offset either downstream or upstream from these calculations that requires modification to allow for proper balance. In this case, the offset is in the intestinal digestibility of NDF. Since the inception of the model, the intestinal digestibility of NDF was set at 20% and this was done to account for potential hindgut fermentation. However, a review of the literature and remodeling the digestion of NDF through the entire gastrointestinal tract suggests that on average, post-ruminal NDF digestibility is approximately 5% (Higgs, 2014). Therefore, to allow for greater ruminal digestibility due to the adoption of the NorFor equation and offset such a high post-ruminal digestibility in v6.5, the post-ruminal digestibility was set to 5%.

The impact of the change in passage rate prediction was evaluated on dry cows with data from work being conducted in the Overton group. The impact on predictions of ME and MP supply for the dry cow diets demonstrated that with the NorFor equation, the increase in ME supply was more consistent with the observed energy balance of the cattle. Unpublished data from Sweeney and Overton were used to conduct the evaluation. In this evaluation, the data are from cattle on control diets from -21 days to calving. The diet characteristics are found in Table 2 and the body weight and body condition score change are in Table 3. Cattle were fed the diet starting between day -38 to day -31 of calving and measurements were taken between -21 and calving.

Table 2. Diet characteristics of dry cows -21 days prior to calving until parturition. (Sweeney and Overton, unpublished data).

Ingredient	pounds
BMR corn silage	14.4
Wheat straw	9.0
Amino Plus	2.6
Citrus pulp	1.1
Soybean hulls	0.7
Canola meal	0.7
Molasses	0.2
Calcium phosphate	0.1
Ground corn grain	0.1
Salt	0.1
Wheat midds	1.0
Calcium carbonate	0.9
Corn distillers (ethanol)	0.7
Magnesium oxide	0.2
Urea	0.1
Total DMI, lb/d	32.1

Over the period analyzed, the total body weight change was less than 2 lb, demonstrating that on average, energy balance was near zero. In this evaluation, the model predictions were improved with the implementation of the NorFor kp equations and there was a 1.5 Mcal increase in ME supply and a modest reduction in maintenance requirements which changed the ME balance from -3.1 to -1.1 Mcals (Table 4). These predictions were more consistent with the behavior of the cattle, especially the observed body weight and body condition score change, therefore we believe the adoption of the NorFor equation will reduce the amount of ME supplied to dry cows and minimize weight gain, adiposity and possible post-partum issues. We are continuing to build the database of dry cow data and will provide additional evaluations as data become available.

Some evaluations of growing heifers have been conducted and the updated passage rate equations appear to provide ME allowable gain predictions that are more consistent with the observed growth rates for heavier heifers, but not prior to puberty.

Before updating the equations for growing heifers, a more robust data set is required to fully evaluate the range in body weight and growth rates the model would be expected to predict for. At the time of this writing, there was a paucity of data, so a complete and satisfactory evaluation could not be accomplished.

Table 3. Body weight and body condition score and change over the 21 day treatment period. (Sweeney and Overton, unpublished data).

	Control
Body weight, lb	1,777
Body weight change, lb	-1.37
Body condition score (1-5)	3.37
Body condition score change	0.01

Table 4. CNCPS predictions with 6.5 (CNCPSv6.5) or 6.5 with 5% NDF intestinal digestibility and the NorFor equation for passage rate of forage NDF (CNCPSv6.5-_NorFor) (Sweeney and Overton, unpublished data).

	CNCPS6.5		CNCPS6.5_NorFor	
	ME (Mcals)	MP (g)	ME (Mcals)	MP (g)
Supply				
Diet	29.5	1305	31.0	1386
Body condition score	0.0	0.0	0.0	0.0
Total	29.5	1305	31.0	1386
Requirements				
Maintenance	21.0	707	20.6	671
Pregnancy	5.2	299	5.2	299
Growth	3.6	94	3.6	94
Desired reserves flux	2.7	5	2.7	5
Total	32.5	1105	32.1	1070
Balance	-3.1	199	-1.1	316

SUMMARY

The CNCPS is an evolving model and that is the primary reason it is useful. Like all models, there are offsetting errors and eventually, as components of the model are refined and improved, some of the offsets have to be fixed or replaced. The amino acid predictions and efficiencies of use are good examples of where a replacement was necessary. As long as the model predictions are improved and the predictions are consistent with the observed data, the process of model development works and creates a more useful and robust tool for evaluation and prediction.

We thank Brittany Sweeney and Tom Overton for dry cow data for evaluation.

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UNDERLYING FIBER CONCEPTS AND DEFINITIONS

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Fiber Concepts

- Historical development

NDF => NDR => aNDF => aNDFOM

- NDF – Sulfite, no Amylase
- NDR – Amylase, no Sulfite
- aNDF – Sulfite + Amylase
- aNDFOM – ash-free aNDF

Fiber Concepts

NDF => NDR => aNDF

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

CP contamination of forages is small especially for the aNDF (or NDF) method, which use sulfite

Fiber Concepts

NDF => NDR => aNDF

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

Can have huge differences in NDF and NDICP between the NDR and aNDF methods
CANNOT use NDICP measured by NDR to adjust aNDF for protein contamination

Fiber Concepts

aNDF => aNDFOM

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

For the aNDF method, the correction for ash contamination is typically greater than that for CP contamination and can be as high as 5-8%-units with soil contamination

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

Digestion Concepts

- Historical development
- Apparently digested => truly digested
  - Truly digested eliminates endogenous loss
- Terminology changes

  "digestible" => "digested"

  "undigestible" => "undigested"

  "undigested" => "indigestible"

Digestion Concepts

- "digested" nutrients differs from nutrient "digestibility"
  - To clearly define "digested" nutrients they should be identified by a lower case "d" prefix or "td" for true digestibility, which is expressed per unit or percent of DM, e.g., 26.1% daNDFOM (% of DM)
  - Digestion coefficients, or digestibility, should be identified with a capital "D" suffix, e.g., aNDFOMD, which is expressed as a fraction or percentage of the nutrient (% of NDF)

Digestion Concepts

- Traditionally we have referred to digestible or undigestible nutrients (e.g., digestible CP or DP)
  - The traditional term "digestible" is confusing because it literally means we measured what "can be digested"
  - Whether measured in vivo, in situ or in vitro, we measure what "was digested" and not what "can be digested"
  - Indigestible and undigestible mean the same thing, what "cannot be digested"

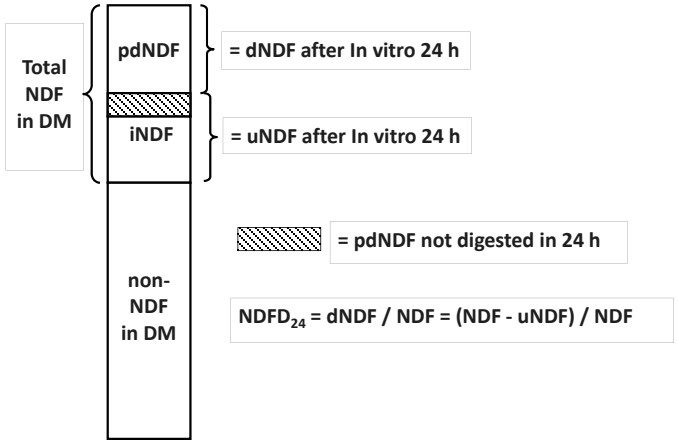
Digestion Concepts

- To clearly differentiate between “digested” and “digestible” nutrients, the latter is often called “potentially digestible” using the lower case “pd” prefix, e.g., pdNDF
- If dNDF refers to measured “digested” NDF then uNDFxx would refer to measured “undigested” NDF
- To distinguish it from uNDF, the model pool that cannot be digested is defined as indigestible NDF, i.e., iNDF (% of DM)

Digestion Concepts

- iNDF = the model pool that cannot be digested after infinite time of fermentation
  - Some models can estimate iNDF at infinite time!
- uNDF = the undigested NDF that is measured after fermentation under defined conditions (time)

Digestion Terminology



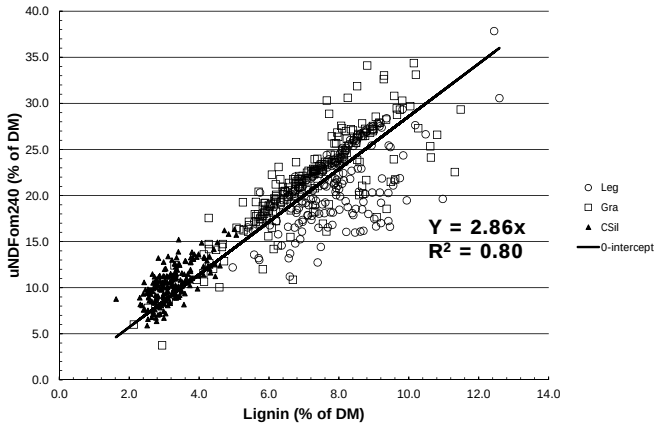
Kinetic Concepts

- Historical development

  NDFD (Lignin) => iNDF => $uNDF_{72}$ => $uNDF_{240}$ => I_2 & I_3
- Van Soest developed the acid detergent lignin system to measure lignin, which was thought to be the only indigestible fraction
- In 1972-73, we discovered that lignin complexed with other fibrous carbohydrates to make them indigestible

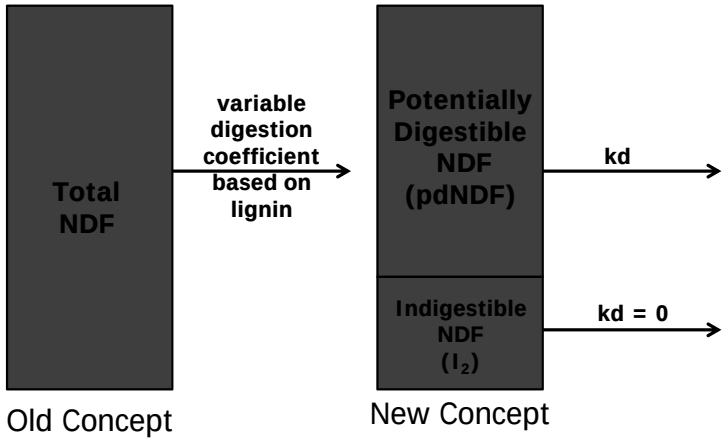
$uNDF_{240}$ is more than Lignin

(data courtesy of Dairyland Laboratories, Inc.)



Kinetic Concepts

NDFD => [pdNDF + I_2 ($uNDF_{72}$)]



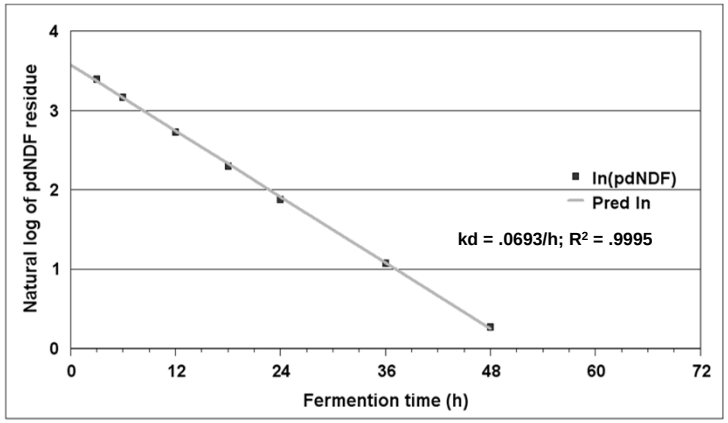
Kinetic Concepts

- Pools or compartments must have homogeneous kinetic properties
  - In the simplest kinetic model, total NDF was divided into two pools
    - Indigestible NDF (I_2)
    - Potentially digestible NDF (pdNDF)
  - Smith et al. (1972) used $uNDF_{72}$ to estimate I_2

Kinetic Concepts

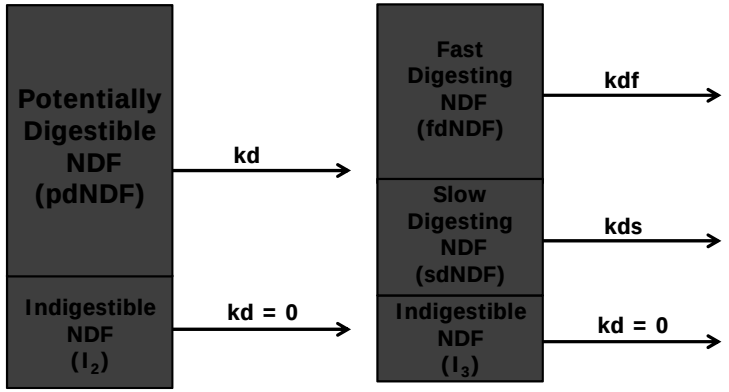
Time	Res(t)	pdNDF(t)	ln(pd)	Pred ln(pd)
0	50.00	50.00		
3	47.64	30.05	3.4029	3.3714
6	41.59	23.99	3.1778	3.1633
12	33.00	15.41	2.7350	2.7470
18	27.60	10.01	2.3034	2.3308
24	24.17	6.58	1.8840	1.9146
36	20.54	2.94	1.0788	1.0822
48	18.91	1.32	0.2771	0.2498
72	17.59			

Kinetic Concepts



Kinetic Concepts

[pdNDF + I_2(uNDF$_{72}$)] => [fdNDF + sdNDF + I_3(uNDF$_{240}$)]



Kinetic Concepts

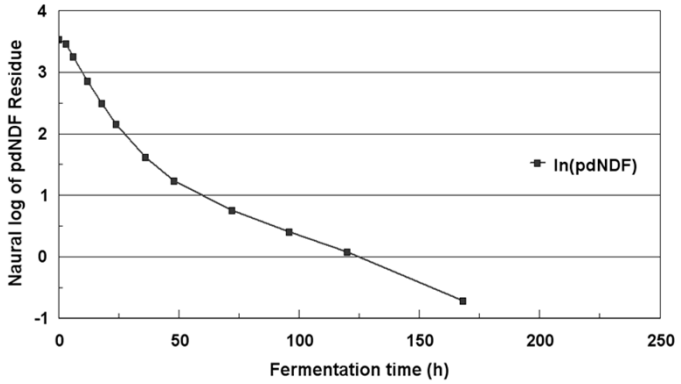
- 2-Pool Model
  $NDFres_{(t)} = pdNDF*e^{(-kd*t)} + I_2$
- 3-Pool Model
  $NDFres_{(t)} = fdNDF*e^{(-kdf*t)} + sdNDF*e^{(-kds*t)} + I_3$
- $I_2 > I_3$ can't use uNDF$_{240}$ for 2-Pool model

Kinetic Concepts

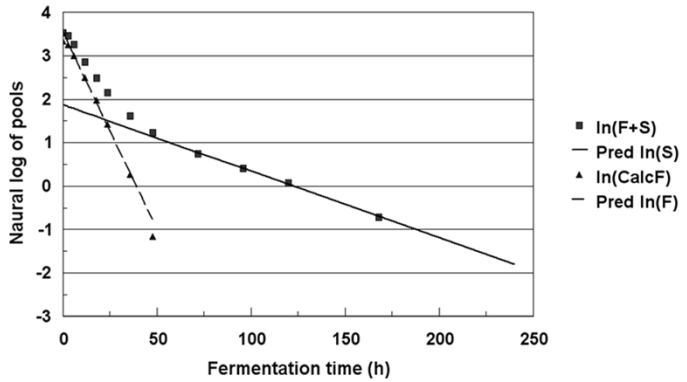
Time	Res(t)	pdNDF(t)	ln(pd)	
0	50.00	50.00		
3	47.64	32.18	3.4715	
6	41.59	26.13	3.2630	
12	33.00	17.54	2.8647	
18	27.60	12.14	2.4967	
24	24.17	8.71	2.1649	
36	20.54	5.08	1.6243	
48	18.91	3.45	1.2393	
72	17.59	2.13	0.7579	
96	16.97	1.51	0.4117	
120	16.54	1.08	0.0758	
168	15.95	0.49	-0.7118	
240	15.46			

Kinetic Concepts

What happens when using $uNDF_{240}$ instead of $uNDF_{72}$



Kinetic Concepts



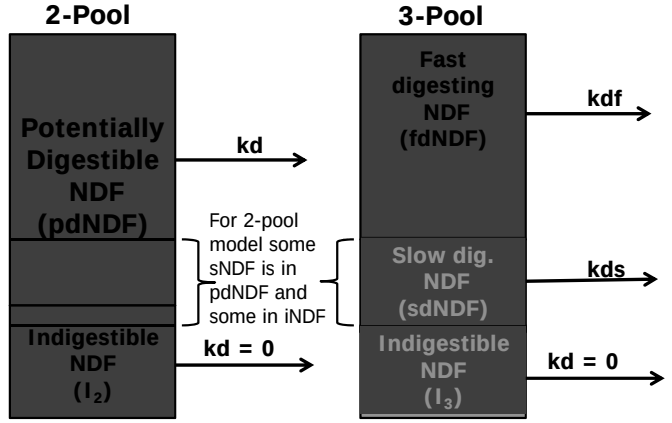
Kinetic Concepts

There is little Fast Pool remaining after 72 h

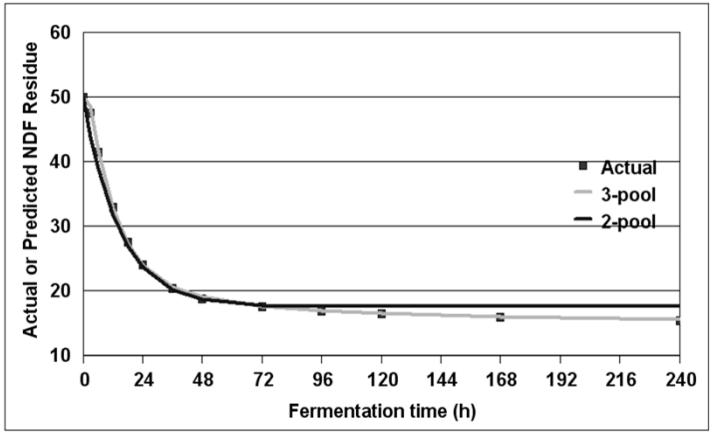
Time	Pool kd=.01/h	Pool kd-.08/h	pdRes(t)
0	5.00	35.00	34.54
3	4.95	27.69	32.18
6	4.80	21.78	26.13
12	4.52	13.48	17.54
18	4.26	8.34	12.14
24	4.01	5.16	8.71
36	3.56	1.98	5.08
48	3.16	0.76	3.45
72	2.48	0.11	2.13
96	1.95	0.02	1.51
120	1.54	0.00	1.08
168	0.95	0.00	0.49
240	0.46	0.00	

Comparison of Digestion Models

What happens when 3-pool data is fit to a 2-pool model?



Comparison of fitting 3-pool data to a 2-pool model



UNDERLYING FIBER CONCEPTS AND DEFINITIONS

QUESTIONS LATER

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CONSIDERING FORAGE NDF_{u30} AS A CONSTRAINT IN DAIRY RATIONS

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UNDIGESTED FIBER AND DMI

For dairy rations, it has long been known that dry matter intake (DMI) is related to dry matter digestibility (DMD) (Conrad, 1964). With the development of the NDF system, it was postulated by Goering and VanSoest that fiber digestibility was related to DMD.

DMD = NDF*NDFD + 0.98 NDS – 12.9

Mertens (2010) later mathematically rearranged this relationship to the following:

DMD = 87.1 – (0.98 – NDFD)*NDF

which was further simplified by Jones and Siciliano-Jones (2014):

$DMD = 87.1 – NDF_u$

This line of reasoning points to the conclusion that the size of the undigested fiber pool is related to dry matter digestibility which is in turn related to DMI. The convention of a subscript “u” is used to denote pool size whereas the capital “D” in Merten’s paper is used to denote digestibility as a percent of NDF. Empirically, across a similar range of NDF content, NDFD will be related to dry matter digestibility. For some time, the dairy industry became focused on NDFD (Oba and Allan, 1999). However, across forages, NDFD was a poor predictor of DMI since it did not account for pool size. In late 2013, Cumberland Valley Analytical Services began reporting NDF_u which is the percent of dry matter that is undigested fiber.

GUT FILL

Before discussing further the linkage between undigested fiber, DMD and DMI, it is important to review the notion of gut fill. Simply stated, gut fill is the retention and accumulation of particles in the rumen. Particle retention is controlled by digestion, reduction of physical size to allow passage, and overall passage rate. It is logical that as retention increases, gut fill increases. An important aspect of relating gut fill to particle retention is that gut fill will be diet and environment dependent. Consequently, the expectation that a single factor will describe gut fill for all herds is not realistic. However, under constant passage and physical reduction rates (for instance, with consistent forage types), it is reasonable that fiber retention would increase with an increasing undigested fiber pool.

For practical reasons, many measures of a dairy cow's performance are expressed on a daily basis. If the rumen of a dairy cow is at steady state (i.e., frequent meals), the time period is unimportant. Using a day as the unit of measure, it is realistic to assume that the daily consumption will be regulated to have no net accumulation of particles. Unfortunately, determining the steady state condition is very difficult, but changes from the steady state condition should be more predictable. In other words, it is hard to predict intake but it is easier to predict if a ration change will increase or decrease intake. If passage rate and particle reduction rate are not changed, an increase in the undigested NDF pool (e.g., decreased digestibility) will decrease DMI.

The main question remaining from the above discussion is "What measure of the undigested fiber pool size is most related to gut fill?". Relative to gut fill, digestion of particles is important only to the extent that the particle has not otherwise passed from the rumen. From a practical perspective, some feeds (e.g., soy hulls) contain NDF that is resistant to digestion in the rumen (NDF_{u30} = 7.5% of DM) but they naturally pass from the rumen very quickly due to their functional specific gravity and particle size. Consequently, this undigested fiber pool has limited impact on overall particle retention. Therefore, the undigested fiber pool should only contain particles large enough to readily resist passage. To easily implement this restriction, all finely ground commodities should be excluded from the undigested fiber pool for gut fill calculation.

NDF_{u30}

Laboratory estimates of fiber digestion are largely performed in an *in vitro* system. These systems estimate the amount of NDF that would be digested if it did not otherwise pass from the rumen. Ideally, the *in vitro* system would measure NDF digestion up to the time when the particles would normally pass from the rumen. Unfortunately, the mean particle retention time is different for different feeds (grasses vs corn silage, Lund et al., 2006; legumes vs grasses, Oba and Allan, 1999) and different environments (e.g., cold and heat stress and overcrowding; Kennedy et al., 1976).

Generally, for forage particles, the mean particle retention time is longer than 24 hours but shorter than 48 hours. Relative to gut fill, the mean particle retention time is very difficult to measure. For example, inert particles are not subject to digestion or particle reduction. Simply for convenience, a standard of 30 h *in vitro* incubation has become common for NDF digestibility. Across many different forage types, a 30 hour mean retention time is reasonably appropriate.

The thesis presented in this manuscript is that the undigested forage NDF pool after 30 hours of *in vitro* digestion (NDF_{u30}) is a contributor to gut fill. The underlying assumption is that forage particles generally remain in the rumen for 30 hours before passage during which time digestion is an influence on their overall passage retention time. Once the particle has passed out of the rumen, its digestibility is no longer relevant. However, NDF_{u30} is only one component of the factors influencing overall particle retention. Overall passage rate and physical reduction cannot be ignored.

PRACTIAL USE OF NDF_{u30}

It is not practical to predict DMI simply knowing the load of forage NDF_{u30} being consumed since particle passage rates are influenced by both intrinsic and extrinsic factors (Krämer, 2013). The current industry debate about the optimal forage NDF_{u30} load is not founded given different particle passage rates. We have seen diets where the forage NDF_{u30} varies from 4 pounds to over 6 pounds per cow per day. The herds consuming only 4 pounds have specialized high byproduct diets, while the herds containing 6 pounds tend to have finer chopped forages . However, changes in DMI when the load of forage NDF_{u30} is changed can be more predictable (Jones, 2014).

There are two main uses of forage NDF_{u30} for evaluating rations. First, if the herd has an acceptable DMI, ensure that the next ration change is constrained to the current forage NDF_{u30} load expressed as pounds/day. This becomes important when new forage is introduced or forage substitution needs to occur. For example, consider changing from a corn silage with a 15% NDF_{u30} to a new crop that is 18% NDF_{u30} where corn silage makes up 20 pounds of the ration dry matter. In the first instance, the corn silage contributed 3 pounds of NDF_{u30} while in the second instance; this corn silage provided 3.6 pounds of NDF_{u30}. Without adjusting for gut fill, the DMI will probably decrease.

The second use of forage NDF_{u30} is to evaluate herds that have poor DMI. Given a specific region and forages, it is feasible to select forage NDF_{u30} load that provides a reasonable gut fill. For example, if other herds feeding similar forages have a NDF_{u30} load of 5.5 pounds per day and the problem herd is at 6.5 pounds per day, the opportunity to minimize gut fill may exist. A different scenario arises when a herd has a low forage NDF_{u30} and low intake. In this case, subclinical acidosis may be inhibiting fiber digestion which would elevate the *in vivo* NDF_{u30} compared to the *in vitro* estimate. Low ruminal pH from highly fermentable feeds can decrease rate of fiber digestion and increase the filling effect of the diet (Allen and Mertens, 1988).

It is still unclear if the undigested fiber component of whole cotton seeds (WCS) contributes to gut fill. Generally, WCS contains 40% NDF_{u30} when measured *in vitro*. If we assume that WCS passes quickly from the rumen (< 30 hours), then the rate of digestion is not significant. However, if WCS has a higher residency time (> 30 h) and that 40% of it remains undigested, then the WCS will contribute to gut fill. If this is the case, a 3 pound inclusion of WCS will result in an additional 1.2 pounds of NDF_{u30} to the gut fill load.

It needs to be noted that in February 2015, the US Patent Office issued a patent which contains claims for the use of undigested NDF and starch digestibility for ration formulation (Weakley, 2015).

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USING 240 HOUR uNDF IN THE FIELD

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INTRODUCTION

The term uNDF (undigested NDF) is a relatively recent addition to the lexicon of ruminant nutrition (Mertens, 2013). It represents the undigested NDF residue after a given length of ruminal digestion time. Determination of uNDF is an old analysis, as it is how NDF digestibility is determined, by weighing what remains as a means of determining what disappears. What is new regarding uNDF is our use of it in determining the fast and slow fiber pools, calculation of rates of digestion, and how gut fill maxima and minima may be estimated. Cotanch et al. (2014) provide a thorough explanation of uNDF application in modeling and ration formulation.

Determination of uNDF requires specific methodology of individual sample digestions using the modified Tilley-Terry system as modified by Raffrenato and Van Amburgh (2010), with filter pore size of 1.5 µm in order to capture all of the small undigested fiber particles. Larger pore size filter systems result in an under-estimation of uNDF. Near infrared calibrations to the Tilley-Terry system are appropriate for analysis of uNDF across time points. Also, NDF residues must be ash-corrected and reported on an organic matter (om) basis.

Previous research conducted at Miner Institute (Cotanch et al., 2014) of high and low forage and high and low NDFd of corn silage-based rations, differing in dietary uNDF240om, resulted in a range of uNDF240om intakes, as % of BW, of 0.30 to 0.39. The high forage/low NDFd ration and low forage/high NDFd ration resulted in intakes of uNDF240om of 0.39% and 0.30% of BW. It was believed that these values could serve as initial reference points to mark gut fill limits of maximum and minimum fill. A possible rumen fill maximum of 0.40% BW and possible minimum of 0.30% was proposed to ensure adequate rumen fill of peNDF (Mertens, 1997). Summary of a second trial conducted at Miner Institute where diets ranged from 50-39% forage with substitution of hay crop silage with NFFS and straw, but similar dietary level of uNDF240om, showed similar uNDF240om intakes of 0.33-0.36% of BW. Of the total dietary treatments between the two studies, 7 of 8 diets resulted in ratio of rumen fill: intake of uNDF240om of 1.57-1.61. This led to the belief that uNDF and possibly uNDF240om could be used to better estimate DMI and gut fill max and mins.

A number of questions arose relative to the field application of this concept that warranted further investigation.

1. How does intake of uNDF240om vary across stage of lactation, or does it?
2. Is uNDF240om the best predictor of DMI and rumen fill or is some other time point, such as u30 more appropriate?

3. Sensitivity of the cows to uNDF?

HOW DOES INTAKE OF uNDF240om VARY ACROSS STAGE OF LACTATION?

To look at how intake of uNDF240om varies across DIM and stage of lactation, TMR samples and intakes were taken of the far dry, close-up dry (CUD), fresh, high and low lactating groups at Miner Institute. Analyses of NDFom, uNDF30om and uNDF240om were conducted using the Tilley-Terry system. Individual cow intakes were calculated from the pen average (Figure 1). Across pens, intake of uNDF240om ranged from 1.9 kg in CUD up to 2.6kg in the high group. Intake of NDFom and uNDF30om showed greater range across stage of lactation. Intake of NDF ranged from 7.2 to 9.6 kg/d from CUD to high and uNDF30om intakes ranged from 3.9 to 6.3 kg. Gut fill and DMI estimations appear more sensitive to NDF and uNDF30om compared to uNDF240om. Transitions of intakes across groups appear to be smooth and adequate, as production and herd health were good at this point in time.

The same data are expressed relative to the CUD group in Figure 2. This approach may be helpful in monitoring dietary transitions between groups. It also becomes clearer that uNDF30om may be more sensitive than NDFom or uNDF240om when monitoring intakes across stages of lactation.

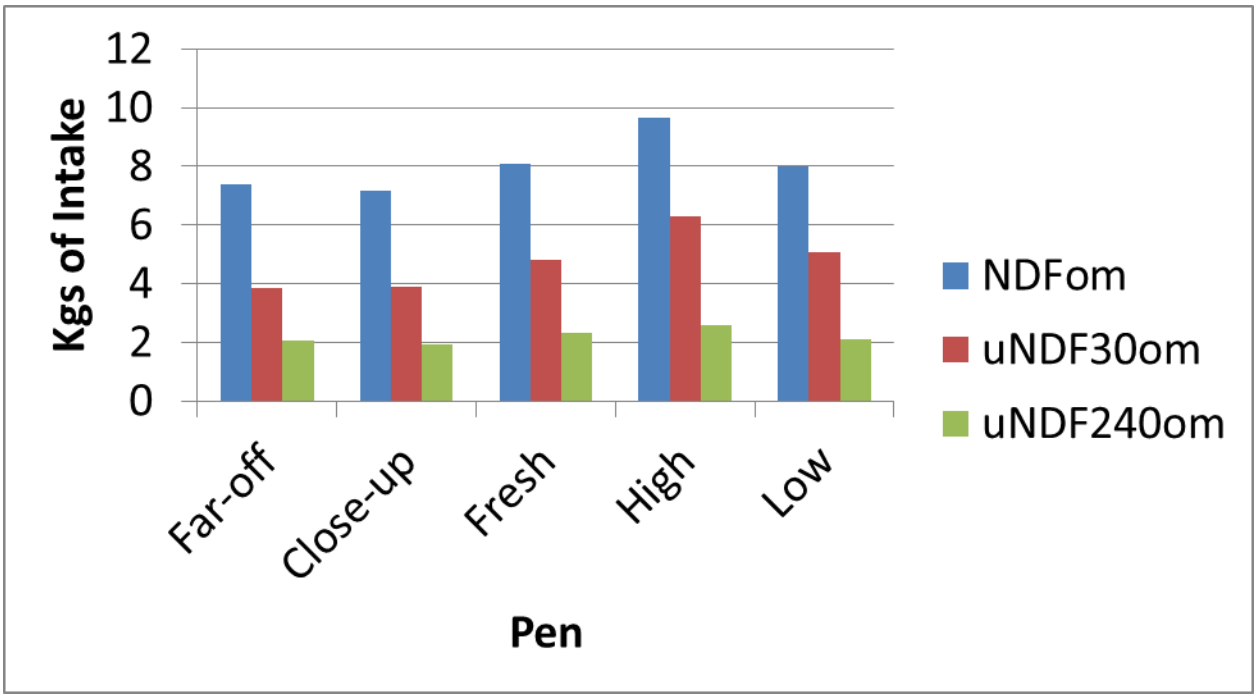

Figure 1. Intake of NDFom, uNDF30om, and uNDF240om across stage of lactation, (kg).

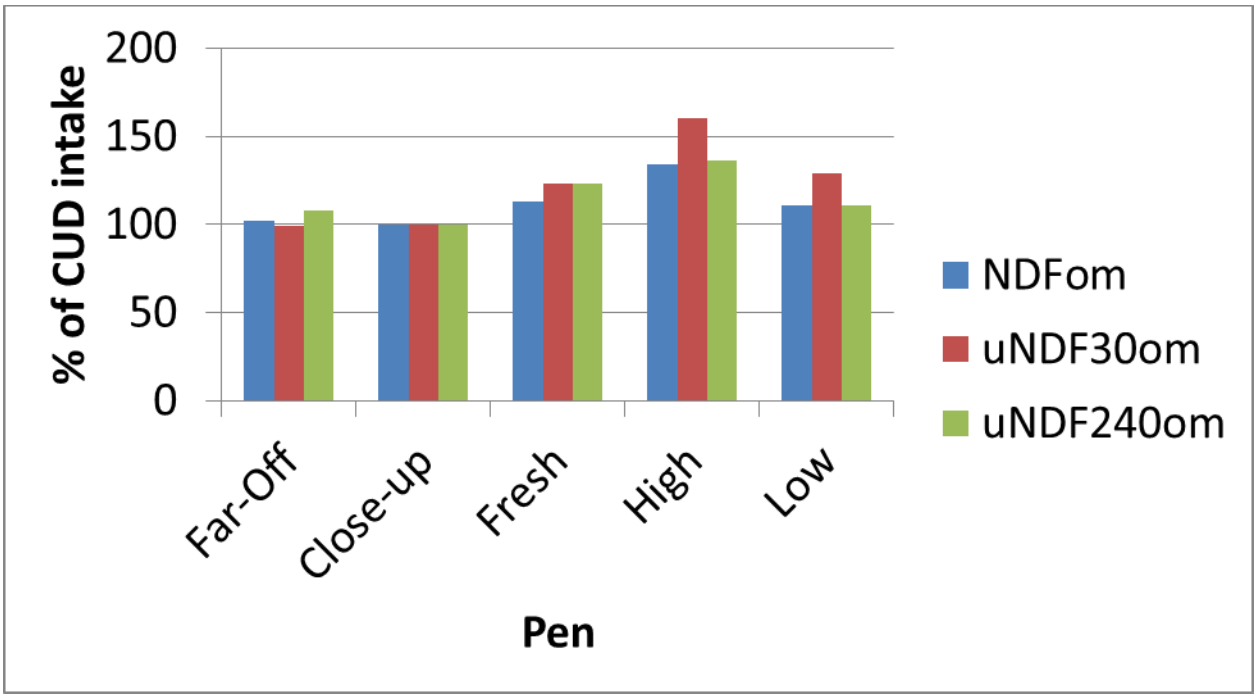

Figure 2. Intake of NDFom, uNDF30om, and uNDF240om relative to the CUD group across stage of lactation.

SENSITIVITY OF COWS TO uNDF

Samples and intakes for the data discussed above were collected in October of 2014, when intakes and milk production were high. A second round of samples were collected in February 2015 after a diet change where intakes and milk production were drastically reduced; 6.8 and 2.3 kg of milk in the high and low groups, respectively, and 2.3 kg of DMI in each group. Table 1 lists the uNDF240om for the high, low and far-dry groups with calculated estimates of per-cow intake of uNDF240om. Average cow body weight of 820 kg was used to calculate % of BW values. Of note is that dietary uNDF240om is nearly 4%-units greater in February 2015 when milk and DMI drastically dropped in both lactating pens. The far-dry cows experienced nearly a 1.8 kg drop in DMI as well. Intake of uNDF240om varied by stage of lactation. The high cows ate about 2.6 kg uNDF240om in October 2014 when consuming nearly 30.5 kg of DMI and averaging over 54.5 kg of milk. As a percentage of BW, this group was consuming about 0.32% of BW as uNDF240om. When forage quality dropped in February 2015, it appears gut fill of the high cows was limiting as uNDF240om intake was about 0.41% of BW, similar to the previously mentioned benchmark. However, for later lactation cows and dry cows, the benchmark values do not hold, as DMI decreases occurred when uNDF240om intake was only 0.32% of BW for both the late lactation and far dry cows.

Table 1. uNDF240om of diets and estimated intake kg (lb) and as percentage of BW based on pen intakes.

	Date	DMI, kg est.	uNDF240om, % of TMR	uNDF240om, kg DMI, est.	uNDF240om, % of BW est.
Pen 2 High	Oct 2014	30.5	8.5	2.6	0.32
	Feb 2015	28.2	12.0	3.4	0.41
Pen 5 Low	Oct 2014	24.1	8.7	2.1	0.26
	Feb 2015	21.8	12.1	2.6	0.32
Far Dry	Oct 2014	15.0	14.5	2.2	0.27
	Feb 2015	13.2	19.2	2.5	0.31

SUMMARY

From this quick summary of uNDF analyses of the Miner herd rations it appears that uNDF240om does play a role in limiting intake as well as providing sufficient gut fill. However, benchmarks of uNDF240om intake as kg or as % of BW seem to differ across stage of lactation and will likely vary from herd to herd. Tracking intake of uNDF240om across diet changes may benefit cows in order to provide sufficient gut fill while avoiding situations of unexpected gut fill limits.

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uNDFom: HOW DOES IT VARY ACROSS THE FORAGE POPULATION?

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With the introduction of the CNCPS 6.5 Biology came the need for new nutrient analyses. Previous versions predicted the indigestible NDF (iNDF) as (lignin x 2.4). The iNDF is used as the end point of fiber digestion and factors into the rate of digestion calculation (kd). Recent work (Raffrenato et al., 2010) has shown that the iNDF constant of 2.4 varies across feed types, thus a better measure is needed if we are to improve our ability to predict kd.

Research at Cornell has determined that measuring rumen in vitro NDF digestibility (NDFD) at 30, 120 and 240 hours will lead to the better prediction of kd. These measures along with NDF are performed on an organic matter (om) or “ash free” basis to reduce the artificial inflation of NDF in high ash samples. High ash can overwhelm the ability of the NDF solution to solubilize all of the minerals. The residual minerals contaminate the NDF residue leading to an overestimation of the fiber value. This may lead to rations that appear to be adequate in fiber, which in fact are deficient. The resultant over estimation of fiber may lead to problems often associated with low fiber diets such as reduced feed intake, rumen acidosis, foot problems, etc..

Along with the new measures comes new terminology to define and differentiate the new from existing values. Now NDF is labeled as aNDFom indicating that it has been treated with amylase and sodium sulfite and determined on an organic matter basis. The indigestible NDF (iNDF) or end point of fiber digestion is replaced by the undigestible fiber (uNDF) as measured after a 240 hour in vitro incubation in rumen fluid. To maintain consistency of terminology and reflect that the results are on an organic matter basis, the undigestible fiber and fiber digestibility are expressed as uNDFom and NDFDom, respectively, followed by the time point, e.g., uNDFom240 & NDFDom240. This applies to all time points. Undigestible was chosen to better define the NDF remaining at any specific time point.

The Dairy One Forage Lab began offering the new organic matter values in January 2015. The following tables and figures provide insight to the variation across the population of the primary forage types. The data were collected during the period of January to July 2015. Legume and grass categories were designated based on the customer supplied description. Legume and mixed mostly legume (MML) were grouped together as legumes. Likewise, grass and mixed mostly grass (MMG) were grouped together as grass. Unless otherwise noted, all nutrient composition values are expressed on a dry matter basis as a percentage of the dry matter. NDFD values are expressed as a percentage of the aNDFom.

Table 1. shows the base nutrients for the population of samples summarized. Corn silage and legume haylage were representative of and comparable to historical averages, while the grass data was better in quality and more representative of mixed mostly grass. All populations provided a good basis for evaluating the new component analyses.

Table 1. Base nutrient values (averages) of sample population

Forage	n	CP	ADF	aNDF	Lignin	ASH	FAT
Corn Silage	5,030	8.22	26.52	44.77	3.17	4.18	3.21
Legume Silage	2,280	21.01	34.89	46.99	7.09	10.81	3.89
Grass Silage	3,959	16.37	36.85	56.36	5.91	9.42	3.96

The aNDFom provides the base measure for subsequent fiber digestibility measures. Table 2. summarizes the typical differences between aNDF and aNDFom. Across feed types the difference ranged from 0 – 21.29 percentage points with an unweighted average of 2.01.

Table 2. aNDF vs aNDFom

	Corn Silage			Legume Haylage			Grass Haylage		
	aNDF	aNDFom	diff	aNDF	aNDFom	diff	aNDF	aNDFom	diff
n	5,030	5,030	5,030	2,280	2,280	2,280	3,959	3,959	3,959
mean	44.77	42.93	**1.84**	46.99	45.14	**1.85**	56.36	54.01	**2.35**
sd	5.36	5.57	1.51	5.48	5.57	1.18	6.90	7.16	1.56
min	28.65	19.72	0.00	31.61	27.04	0.00	35.64	31.44	0.00
median	44.32	42.49	1.30	46.57	44.84	1.50	56.00	53.72	1.62
max	79.74	78.84	16.59	69.94	67.85	11.88	85.05	80.85	21.29

Tables 3., 4. and 5. provide the base data for uNDFom measures. Rates of digestion are plotted in Figure 1. comparing the average values for a forage type to the least and most digestible samples as determined by uNDFom240. The spread between these lines demonstrates the potential range within the population.

Table 3. Corn silage om digestiblities

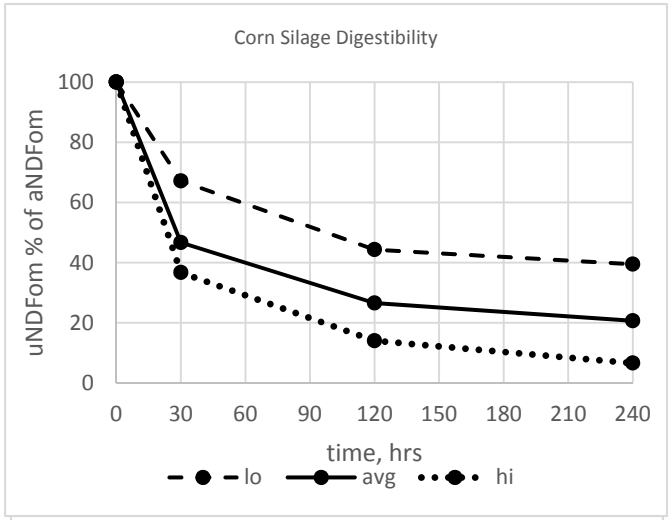
	aNDFom	uNDFom30	uNDFom120	uNDFom240	lignin x 2.4	constant	NDFDom30	NDFDom120	NDFDom240
n	5,030	5,030	5,030	5,030	5,030	5,030	5,030	5,030	5,030
mean	42.93	20.04	11.41	8.91	7.60	**2.83**	53.34	73.44	79.37
sd	5.57	3.86	2.73	2.31	1.51	0.59	6.37	5.00	3.91
min	19.72	6.27	3.34	2.06	1.18	1.04	15.22	48.70	60.36
median	42.49	19.74	11.23	8.77	7.49	2.83	53.33	73.53	79.25
max	78.84	52.62	34.73	30.95	22.90	11.03	81.58	92.29	94.85

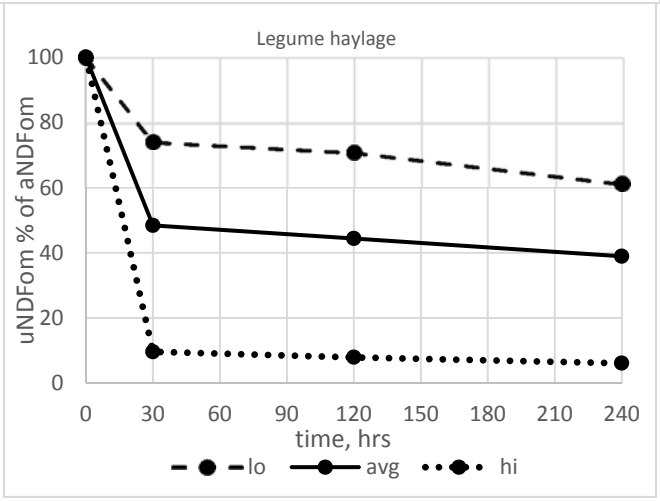
Table 4. Legume haylage om digestiblities

	aNDFom	uNDFom30	uNDFom120	uNDFom240	lignin x 2.4	constant	NDFDom30	NDFDom120	NDFDom240
n	2,280	2,280	2,280	2,280	2,280	2,280	2,280	2,280	2,280
mean	45.14	21.85	19.97	17.42	17.01	**2.46**	51.56	55.62	61.08
sd	5.57	4.93	4.56	4.31	3.40	0.40	9.14	8.94	9.44
min	27.04	3.59	2.94	2.29	1.34	1.05	10.02	27.62	37.28
median	44.84	21.59	19.75	17.40	17.11	2.45	51.46	54.75	59.89
max	67.85	47.71	42.76	36.94	31.99	6.55	90.38	92.12	93.86

Table 5. Grass haylage om digestiblities

	aNDFom	uNDFom30	uNDFom120	uNDFom240	lignin x 2.4	constant	NDFDom30	NDFDom120	NDFDom240
n	3,959	3,959	3,959	3,959	3,959	3,959	3,959	3,959	3,959
mean	54.01	23.88	19.79	14.82	14.20	**2.52**	56.43	63.70	72.67
sd	7.16	7.86	6.22	5.22	3.70	0.68	10.71	9.23	8.77
min	31.44	4.57	3.47	2.61	3.86	1.01	11.27	28.44	37.85
median	53.72	22.93	19.30	14.52	13.99	2.50	56.76	64.14	73.55
max	80.85	59.49	45.91	36.10	45.48	6.64	89.55	92.06	94.38





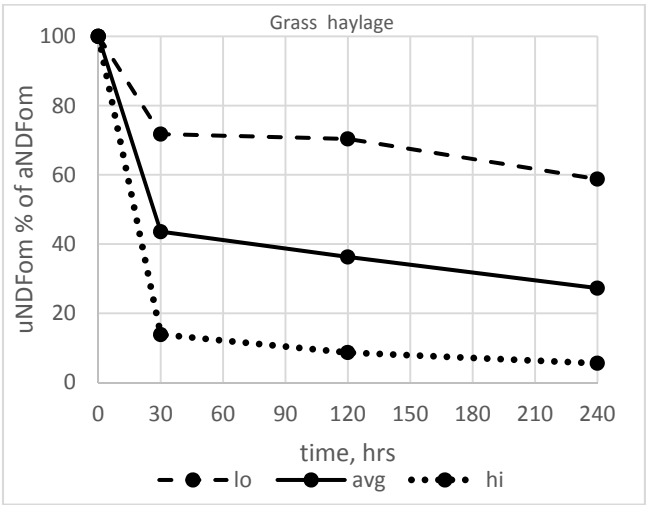


Figure 1. Rates of digestion with uNDFom expressed as a percentage of aNDFom

Tables 6. And 7. explore indigestibility and rate constants. At the heart of the new fiber measures was the desire to better define the end point of digestion and prediction of kd. Previous versions of the CNCPS model estimated this value as (lignin x 2.4). In CNCPS 6.5, the value is measured as uNDFom240. Figure 2. is from an earlier summarization of samples analyzed by our lab. These data were from a smaller population of samples (corn silage n = 1,171, legumes n = 419, grasses n = 1,083) than the current summarization and were used to bring more clarity to the graphics. There was a moderate positive relationship between iNDF and uNDFom240 across all forages, but the take home point is the wide degree of variation about the trend line illustrating the diversity of values in the population (Figure 2.). Likewise, the calculation of individual rate constants as [uNDFom240/lignin] yielded means of 2.46 and 2.52 across the haycrop populations with a combined range of 1.01 – 6.64 (Table 7.). The corn silage mean was similar at 2.83 (Table 7.), but ranged from 1.04 – 11.03 (the next highest value was 8.23).

Table 6. iNDF* vs uNDFom240

	Corn silage		Legume haylage		Grass Haylage	
	uNDFom240	iNDF	uNDFom240	iNDF	uNDFom240	iNDF
n	5,030	5,030	2,280	2,280	3,959	3,959
mean	8.91	7.60	17.42	17.01	14.82	14.20
sd	2.31	1.51	4.31	3.40	5.22	3.70
min	2.06	1.18	2.29	1.34	2.61	3.86
median	8.77	7.49	17.40	17.11	14.52	13.99
max	30.95	22.90	36.94	31.99	36.10	45.48

*iNDF = lignin x 2.4

Table 7. Indigestibility constant summary table (historic = 2.4)

Forage	n	mean	sd	min	max
Corn Silage	5,030	2.83	0.59	1.04	11.03
Legume Haylage	2,280	2.46	0.40	1.05	6.55
Grass Haylage	3,959	2.52	0.68	1.01	6.64

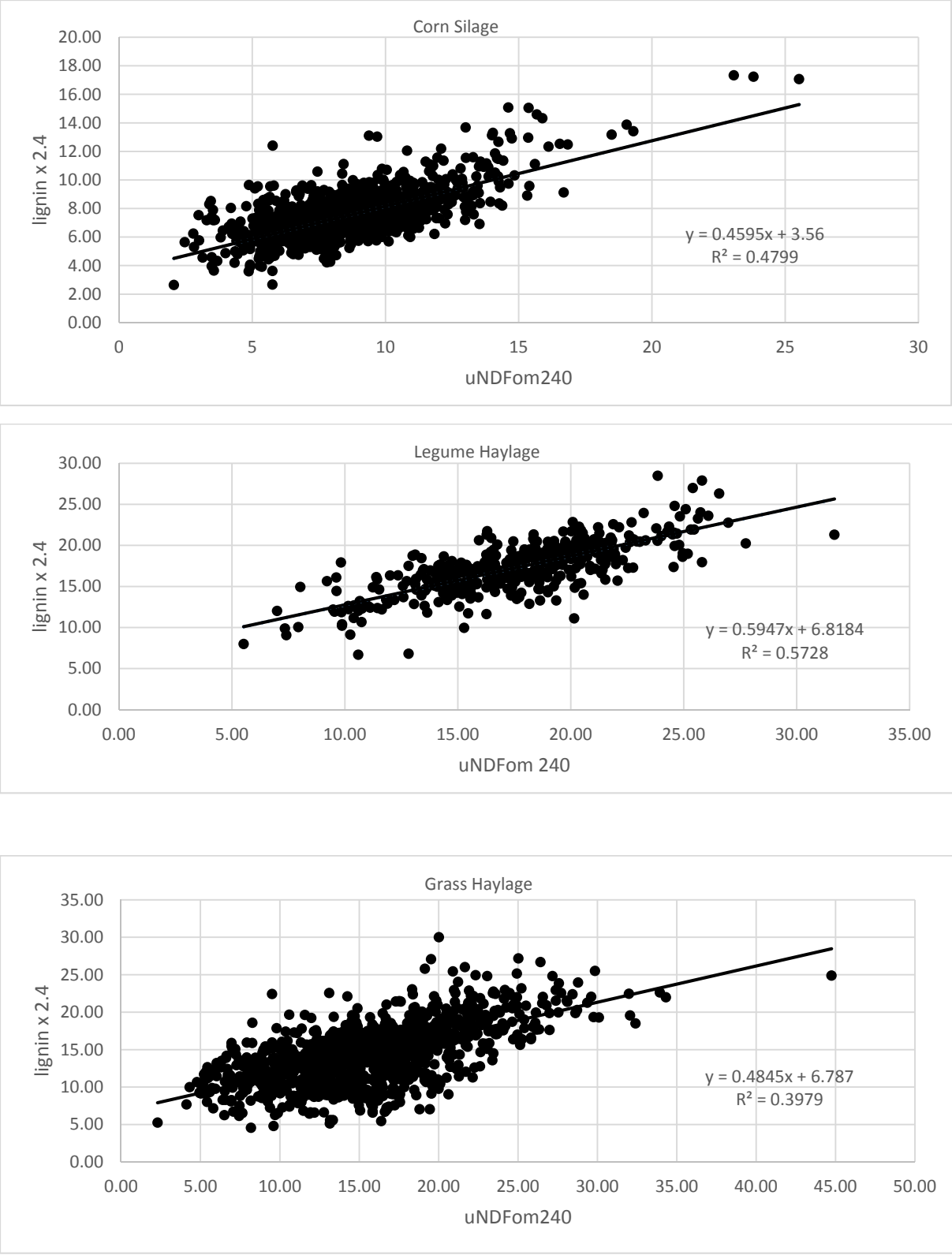


Figure 2. Lignin x 2.4 (iNDF) vs. uNDFom240

In conclusion, data collected from a large population of samples analyzed during the course of routine commercial forage analysis demonstrated sufficient variation in the determination of uNDFom240 to warrant routine analysis in favor of using fixed values in the course of rate predictions.

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CANOLA MEAL

True Value of Canola Meal

Essi Evans, Technical Advisory Services Inc.
Carson Callum, Canola Council of Canada

- Presented on Behalf of Afgritech, LLC
- Cornell Nutrition Conference, 2015

Perceptions are changing

- Older tables such as NRC suggest
  - Canola meal protein is degraded in the rumen
  - Canola is high in fiber
  - Fiber is not digested
  - Energy value is low
  - Takes up too much space in formulas
- Does not seem suitable for high producing cows

Observations of Users

- "I can replace soy protein with canola protein, and save money."
- "I replaced soy pound for pound, and have been cleaning up in the market."
- "I can't explain it - it just works."
- " Canola is my secret. Stop telling everyone about it!"

Canola Meal: Feeding the US Dairy Market

Meal	MT Dairy	Kg/cow/day	Lb./cow/day	Lb. CP/day
DDGS	10,125	3.60	7.90	2.13
SBM	3,519	1.25	2.75	1.24
CM	3,400	1.20	2.65	1.00

Something Seems Wrong

- Canola Council of Canada invested heavily in research to evaluate the feeding value of canola meal for the dairy industry and to obtain accurate tabular values
- Science Cluster program through AAFC
  - G. Broderick, USDA Forage Center, Emeritus
  - A. Faciola, University of Nevada
  - K. Kalscheur, USDA Forage Center
  - H. Lapierre, Agriculture Canada
  - T. Mutsvangwa, University of Saskatechewan
  - D. Ouellet, Agriculture Canada
  - P. Robinson, University of California

Values in Common Use are Outdated

- Huhtanen et al., 2011
  - Compared added ration protein from soybean meal to canola meal
  - Tested results from 122 studies
  - With the **same amount** of protein added, canola meal showed a 2.75 lb./cow/day greater milk yield
  - Concluded the protein value of soybean meal, relative to canola meal was over estimated
  - Protein entering the intestines was higher with canola meal than with soybean meal with the same amounts of protein

Values in Common Use are Outdated

Martineau, et al., 2013 Science Cluster

- Evaluated results from 49 trials
- When cows were fed the same amount of protein, researchers found that milk yield was increased by 3.1Lb/cow/day day with canola meal relative to all other vegetable proteins.
- Milk yield was increased by 1.7 lb. per day when canola meal was compared to soybean meal

- *Results do not support the values in common use in many matrices*

Less Protein is Destroyed in the Rumen Than Older Methods Assume*

	Soybean meal	Canola Meal
Protein, % of DM	49.4	42.5
Portion that escapes rumen breakdown, % of protein	52.5	60.4
Portion that escapes rumen breakdown, % of meal	25.9	25.7

*Based on actual results obtained by Afgritech LLC

Brito and Broderick, 2007

	Soybean meal	Canola meal
% of diet	12.1	16.5
Diet crude protein, %	16.5	16.5
Microbial protein, g/day	2,710	2,780
RUP, g/day	990	1,150
Total	3,700	3,930
Milk yield, lb	88.2	90.6
Protein yield, lb	2.71	2.80

More Fiber is Digested than Previously Thought

uNDF at 120h of fermentation

Feed	aNDFom (%DM)	uNDF (% aNDFom)	Lignin (% DM)	2.4 x Lignin (%aNDFom)
Beet Pulp	47	18	4.41	22.5
Canola Meal	29	32	7.37	61
Citrus Pulp	25	7	2.63	25.2

Canola meal is more digestible than previously indicated
- Supported by newer laboratory methods
- Supported by feeding trial results
- Fiber is about 30-35% undigested- not 60-70%!

Cotanch et al., 2014 Proc. CNC

Science Cluster Results

- Canola meal nutrient profiling
  - Glen Broderick, U.S. Forage Research Lab
  - Full in-depth analysis of 36 samples/year for 4 consecutive years
  - Include determination of rates of protein degradation and calculation of RUP using the inhibitor method
  - Preliminary results presented at ADSA
- Subset of samples sent to D. Ross for digestibility profiles
- Samples being analyzed in Nevada for uNDF

Canola Meal Quality Survey – data compiled

Component	Average
Moisture (%)	12.0
Crude protein (N x 6.25, %)	36.7
Rumen escape protein, NRC (%)	43.5
Rumen escape protein, CNCPS (%)	52.3
Fat (%)	3.3
Phosphorus (%)	0.99
Acid detergent fiber (%)	16.2
Neutral detergent fiber (%)	25.4
Sinapine (%)	1.0
Phytic acid (%)	2.3
Glucosinolates (µmol/g)	4.2

Fat: ranged from 2.8% to 4.8%

Science Cluster Results

Faciola et al., 2015 Univ. Nevada

	15% CP	15% CP	17% CP	17% CP
	Soybean meal	Canola meal	Soybean meal	Canola meal
DM intake, lb	54.6	55.6	55.4	56.1
Milk yield, lb	86.9	88.4	87.4	90.4
Protein yield, lb	2.62	2.66	2.66	2.73
Fat yield, lb	3.43	3.50	3.52	3.63

Noteworthy
MUN values lower with canola meal diets
Little change in DMI

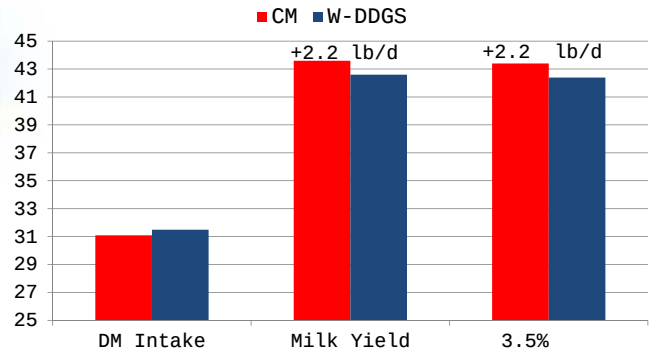
Science Cluster Results

Swanepoel et al, 2014 University of California

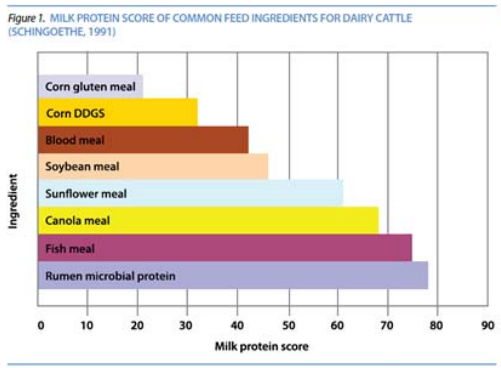
	100% Canola meal	67% Canola 33% C-DDGS	33% Canola 67% C-DDGS	100% High Pro Corn DDGS
DMI	53.6	54.6	53.7	53.0
Milk, Lb	104.4	105.5	104.5	99.0
Fat, Lb	3.50	3.58	3.62	3.44
Protein, Lb	3.04	3.08	3.05	2.87
Milk/Feed	1.95	1.93	1.95	1.87
Chg BCS	0.03	0.08	0.03	0.01

Science Cluster Results

Kiran and Mutsvangwa, 2014 , University of Saskatchewan



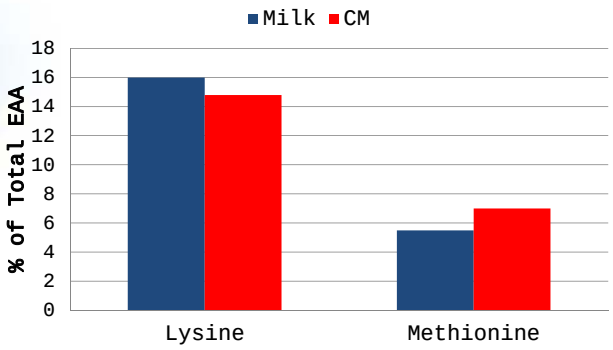
Amino Acid Profile



KEEP IT COMING

Science Cluster Results

Maxin et al., 2013 Agriculture and Agri-foods Canada



Amino Acid Supply, Corrected for Digestibility

		CM	SBM
Absorbable, G/kg	Met	4.03	3.15
	Lys	10.93	14.76
	Arg	11.70	17.67
	Thr	8.44	9.44
	Leu	13.42	18.39
	Ile	8.05	10.89
	Val	10.16	11.37
	His	4.98	6.29
	Phe	7.67	12.34
	Trp	2.34	3.15

Amino Acid Supply, Corrected for Digestibility

		CM	SBM
Absorbable/% of CP	Met	0.97	0.64
	Lys	2.63	3.01
	Arg	2.82	3.60
	Thr	2.03	1.92
	Leu	3.23	3.75
	Ile	1.94	2.22
	Val	2.45	2.32
	His	1.20	1.28
	Phe	1.85	2.51
	Trp	0.56	0.64

Soluble Protein

≠

Degraded Protein

Lignin * 2.4

≠

Indigestible NDF

canola council OF CANADA

Thank YOU

TRUE VALUE OF FEEDING CANOLA MEAL

E. Evans
Technical Advisory Services Inc.
Bowmanville, Ontario

Canola meal has been described in the past as a protein source that was readily degraded in the rumen, highly fibrous, poorly digested, low energy, and sometimes poorly palatable. Values in feed tables and feed formulation programs in general would not encourage use of this meal. In spite of such apparently poor quality, the use of canola meal by the dairy industry in the USA has been accelerating, with imports more than doubling in the last 10 years (Figure 1).

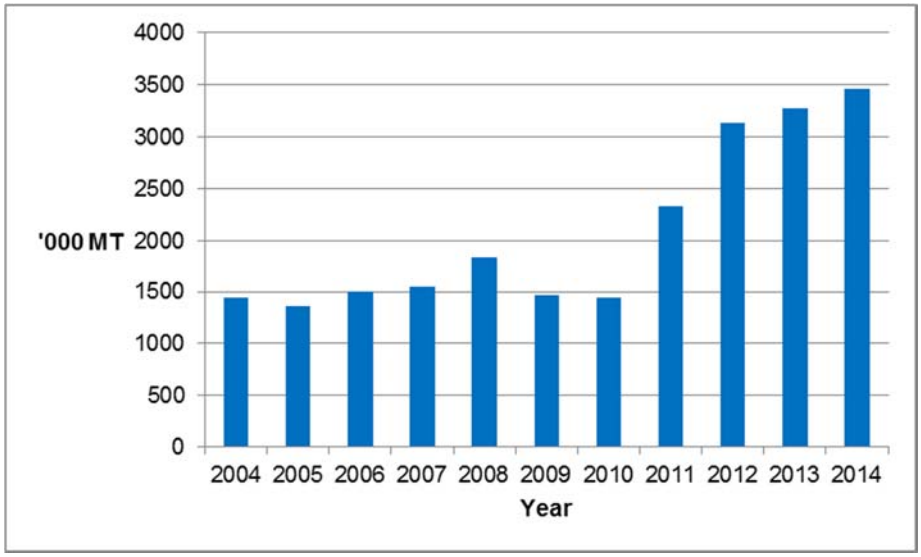


Figure 1. Export of Canadian canola meal to the USA by year

This increased rate of usage suggests that dairy nutritionists are using the meal, but possibly not the nutritive values at hand. A survey commissioned by the Canola Council of Canada in 2011 (Evans and Hodgins, 2012) confirmed that dairy nutritionists had a lot more respect for canola meal than tabular data suggested that they should have. Over 80% of the dairy nutritionists surveyed had used canola meal and 92.4% perceived the meal as being an excellent or good source of protein for dairy cows. The results revealed that 65.0% of canola meal users believed that the protein content should be higher. Surprisingly, only 10.3% stated that rumen solubility was a critical issue, and 46.1% stated that the rumen escape value was the most positive attribute of the meal. These responses along with other responses to the survey indicated, and in some cases stated, that users were largely satisfied with the meal, and were using nutrient values that differed from those published in NRC (2001) and feed formulation databases.

UNDERTAKING TO ADDRESS THE ISSUE

It was obvious to Canola Council that the needs of the industry were not being met with respect to understanding the true value of canola meal. In response to both the increasing demand for canola meal as well as the results of the survey, the Canola Council of Canada with financial assistance from Agriculture and Agrifoods Canada set out to research and assess the value of canola meal, and to describe the value in terms that can readily be used by the dairy feeding industry.

Research programs aimed at identifying various characteristics of canola meal for dairy cows were established at the University of California, University of Nevada, University of Saskatchewan, South Dakota State University, the University of Manitoba, the US Dairy Research Center in Wisconsin, and the Agriculture and Agrifoods Research Center, Quebec.

META-ANALYSES

One of the first undertakings commissioned was a review and meta-analysis of past research. Fortuitously, while this research was under way, Huhtanen et al (2011) published results from 122 studies in which dietary protein was elevated using either soybean meal (SBM) or canola meal (CM) and the higher protein concentrations were increased at the expense of grains. For every 1 kg increase in crude protein consumed, milk production increased by 3.4 kg with canola meal, and 2.1 kg with soybean meal, resulting in a net gain of 1.3 kg with canola meal. Much like the survey indicated, the authors concluded that “the current feed protein evaluation methods based on determination of RUP [rumen undegraded protein] by the in situ procedure fail to evaluate relative values of SBM vs. CM and untreated vs. heat-treated CM correctly”.

Members of the canola science cluster Martineau et al. (2013) compared the effects of replacing a protein source with the same amount of protein from CM. There were 49 trials in the data set, and CM intakes varied from 1 to 4 kg/cow/day. At the average level of inclusion (2.3 kg/day) of CM, milk yield increased by 1.4 kg when compared to all protein sources. The improvement was less when CM was compared to SBM (0.7kg). The researchers concluded “These data also indicate an underestimation of MP (metabolizable protein) supply associated with CM inclusion in dairy rations using the National Research Council (2001) model”.

In a follow up meta-analysis Martineau et al. (2014) compared response in plasma amino acids to changes in protein source in the diet. Essential amino acids were higher and urea nitrogen was lower when cows received CM than when they were given other vegetable proteins. The conclusion “…these results indicate that CM feeding increased the absorption of all EAA (essential amino acids)…”

Results from all three analyses underscored the need to supply the dairy feeding industry with more useful, and accurate feeding values for this ingredient.

CANOLA MEAL NUTRITIONAL VALUE SURVEY

The purpose of this study was to provide the feed industry with a complete and up to date set of nutrient values, using the most current methodology. To assess nutrient composition and quality, meal samples were collected from 12 crushing facilities across Canada. Three samples were collected from each plant for 4 consecutive years (2011-2014), and were analyzed for an extensive range of nutrients and anti-nutritional factors at the University of Manitoba and the US Dairy Forage Research Center. All samples were analyzed for rates of protein digestion and RUP using methods that would provide results that were consistent with NRC (2001). A subset of these samples was also provided to Cornell University for analysis by the method developed by Ross et al. (2013).

Results for an extensive list of nutrients have been published in the Canola Meal Feed Industry Guide (2015). This publication is available to download from the Canola Council of Canada website.

Table 1. Rumen undegraded protein (RUP) values as calculated by methods consistent with the NRC (2001) and the CNCPS 6.5 (2015) models

Reference	Canola Meal	Soybean meal
Broderick et al, 2015	35.5	25.7
Ross, 2015	52.3	45.2

As Table 1 shows, unlike results published in the past, newer methods of analyses show that the RUP value of solvent extracted CM protein is higher than untreated solvent extracted SBM. Clearly, this explains why, at equal amounts of protein, CM elevates plasma levels of all essential amino acids relative to SBM.

FIBER DIGESTIBILITY

Canola meal contains a considerable amount of lignin. Survey data revealed that CM contains 6.6% lignin and 10.1% total lignin plus polyphenols (DM basis). With 28.8% neutral detergent fiber (NDF), older models using a derivative of lignin to compute indigestible NDF indicated that the potential digestibility of NDF was extremely low. Early studies ((Mustafa, et al., 1996, 1997) indicated that approximately half of the NDF from CM was actually digested, which indicated that the potential digestibility is even greater. This was recently corroborated by Cotanch et al. (2014) who determined that the potentially digestible NDF in CM was 63%.

The difference between the determined digestibility values and the lignin method of estimation has an impact on the prediction of available energy. Recent feeding studies have not been able to demonstrate a noticeable lower energy value for diets where CM has been substituted for SBM or DDGS, as the data below will demonstrate. Studies are

currently underway to assess the potential digestibility of NDF using the survey samples available.

MILK PRODUCTION

When canola meal is substituted for other vegetable proteins, there appears to be a slight milk production advantage favoring diets with canola meal, and no difference in feed efficiency. In a recent study, Broderick et al. (2015a) measured a consistent increase in energy corrected milk production when CM was substituted for SBM and corn in diets with 15 and 17% protein. There were no differences in energy corrected milk to dry matter in this study. Milk urea nitrogen (MUN) concentrations were lower when canola meal was provided in the diet.

Table 2. Evaluation of diets containing soybean meal (SBM) or canola meal (CM) at two levels of crude protein (CP) on milk production in Holstein cows

	Treatment			
	15% CP		**17% CP**	
Item	**SBM**	**CM**	**SBM**	**CM**
Dry matter intake (DMI), Kg/day	24.8	25.3	25.2	25.5
Weight gain, Kg/day	0.23	0.55	0.50	0.41
Energy Cor. Milk (ECM), Kg/day	38.5	39.2	38.7	39.9
ECM/DMI	1.55	1.55	1.56	1.58
MUN	9.9	8.7	13.2	12.0

In an earlier evaluation (Table 3), Brito and Broderick (2007) compared urea, SBM, cottonseed meal (CSM) and CM in diets for lactating dairy cows. Concentrating on the vegetable protein diets and ignoring the urea treatment, cows receiving the diet with CM produced more fat corrected milk than cows given the diets with SBM or CSM. Efficiency values were similar for the vegetable protein treatments.

Wheat distillers' grains with solubles (W-DDGS) were compared to CM at two protein levels in a recently completed experiment (Mutsvangwa and Doranalli, 2014). Again, milk yield favored the use of CM, with milk to dry matter values in a similar range for all treatments (Table 4). Protein yields were higher with the CM diets at each level of protein.

Table 3. Evaluation of diets containing urea, soybean meal (SBM), cottonseed meal (CSM) or canola meal (CM) on milk production in Holstein cows

	Treatment			
Item	**Urea**	**SBM**	**CSM**	**CM**
Dry matter intake (DMI), Kg/day	22.1	24.2	24.7	24.9
Weight gain, Kg/day	0.58	1.23	1.00	1.25
Fat Cor. Milk (FCM), Kg/day	30.6	37.1	36.8	38.8
FCM/DMI	1.39	1.53	1.58	1.55
MUN	16.9	12.0	10.0	11.6

Table 4. Evaluation of diets containing wheat distillers' grains (W-DDGS) or canola meal (CM) at two levels of crude protein (CP) on milk production in Holstein cows

	Treatment			
	15% CP		17% CP	
Item	**W-DDGS**	**CM**	**W-DDGS**	**CM**
Dry matter intake (DMI), Kg/day	31.6	31.4	31.5	31.0
Milk , kg/day	42.2	43.2	43.2	44.2
Fat Yield, Kg/day	1.47	1.51	1.48	1.51
Protein yield, Kg/day	1.36	1.39	1.39	1.42
Milk/DMI	1.33	1.38	1.37	1.42

One probable reason for the higher milk and milk protein yields in these and other studies would be the amino acid balance provided by canola meal. Mutsvangwa and Doranalli (2014) measured the abomasal outflow of amino acids in the study described in Table 4. The CM diets showed a 20, 0, 28, 25 and 5 g/day advantage over W-DDGS for lysine, methionine, histidine, threonine and tryptophan, respectively. In a study comparing SBM, CM, W-DDGS and high protein corn distillers' grains (C-DDGS), Maxin et al (2013) found that the metabolizable protein provided when CM was fed to cows producing 35 kg of milk resulted in no amino acid deficiencies. In contrast, the supply of methionine was low with SBM, histidine was low with W-DDGS, and lysine was marginal with C-DDGS as the primary sources of supplemental protein. Brito et al (2007), in a continuation of the experiment described in Table 3, found that the abomasal outflow of lysine and methionine were highest when cows received the CM diet (Table 5).

Table 5. Evaluation of diets lysine and methionine outflow with diets containing urea, soybean meal (SBM), cottonseed meal (CSM) or canola meal (CM)

	Treatment			
Item, g/day	**Urea**	**SBM**	**CSM**	**CM**
Protein entering intestines	2880	3700	4060	3930
Lysine entering intestines	147	194	196	201
Methionine entering intestines	51	68	70	74

RUMEN PRODUCTION AND METABOLISM

With the higher RUP, and the apparent greater contribution of amino acids from canola meal beyond the abomasum, it would seem possible that the rumen nitrogen requirements might not be met. Recent studies suggest that this is not of major concern, and additional research is underway. De Paula et al (2015) compared SBM to two sources of CM in a duel flow continuous culture system. Rumen ammonia levels, total volatile fatty acid (VFA) concentration as well as molar percentages of acetate, propionate, butyrate, and isobutyrate were not affected by treatments. There were, however, differences in the branched chain VFA. Molar proportion of valerate was lower with the SBM diet, whereas molar proportions of isovalerate, and total branched chain VFA were lower for CM diets (Table 1). Microbial growth did not appear to be affected by these changes in that study.

Based on the meta-analysis of Martineau et al (2014), CM feeding results in lower plasma urea nitrogen than other vegetable protein meals. This could be due to either less urea being generated in the rumen, or less being produced post absorption due to the inefficient use of absorbed amino acids. Ouellet et al (2015) determined little difference in rumen urea production from SBM, CM, C-DDGS and W-DDGS, with lowest values obtained with W-DDGS. Urinary excretion of urea was significantly lower with the CM diet than with diets containing the remaining protein sources, indicating an efficiency advantage with the CM diet. In addition to this important finding, the researchers reported a considerable entry of urea back into the gut, which is then available to support microbial growth.

FEEDING LEVEL

There appears to be no practical restrictions to the amount of canola meal that can be included in diets for lactating dairy cows and several recent studies have illustrated this. Swanepoel, et al. (2014) provided dairy cows with diets that contained 20%CM, in replacement for 20% high C-DDGS. Milk production increased from 45.0 to 47. 5 kg/ cow/day with no difference in intake.. Brito, et al., (2007) replaced 12% soybean meal and 4.5% corn meal with 16.5% canola meal in diets for high-producing cows. Dry matter intake increased by 0.3 kg, while milk yield increased by 1.1 kg. Maxin et al (2013) compared a diet with 20.8% canola meal in place of SBM and corn grain. These researchers found no differences in dry matter intakes, milk yield, or milk component yield for cows producing 35 kg of milk/day.

Under practical feeding situations, canola meal can be included in formulations for dairy cows with no restrictions, and is a well-balanced source of RUP.

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IMPLEMENTING CNCPS 6.5

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AMTS LLC

INTRODUCTION

The current version of the Cornell Net Carbohydrate and Protein System (CNCPS6.5; VanAmburgh et al., 2015) introduces several new concepts. These updates pertain to moving from CNCPSv6.1 (Tylutki et al., 2008) to CNCPSv6.5. Taking full advantage of these new concepts requires adopting new feed analytical methods. As previously presented at this conference, these analytical methods include: aNDFom, multi-time point DNDF for carbohydrate C pool size and B3 pool size and rate calculations, amino acids, and nitrogen intestinal digestibility.

CNCPSv6.5 CHANGES

The commercial laboratories (namely CVAS, DairyOne, DairyLand, and Rock River) have been heavily focused on implementing the new assays. In the case of aNDFom, this required the laboratories to develop updated calibration equations. This is due to adding an additional step in the aNDF assay: ashing the residue post-aNDF. In conversations with the lab managers, the resulting calibration statistics are improved as would be expected. This is because an NIR relies upon carbon containing bonds for reflectance and soil contamination contains no carbon. In the case of wet chemistry, this extra step does increase turn-around time and cost as additional equipment and labor is required. However, in feeds such as hay crops and root crops (beets for example), soil contamination can easily account for 3-20 points of ‘NDF’.

The second area revolves around the new methods to determine carbohydrate pools B3 and C (CHOB3, CHOC) and the degradation rate for CHOB3 (kdCHOB3). For forages, the new method requires a 30, 120, and 240 hr DNDF while for non-forages, 12, 72, 120 hr DNDFs are required (Raffrenato et al. 2009). These results are then used with a non-linear, dynamic model, to calculate an integrated kd for CHOB3. This is opening many new areas for research as it appears that DMI is highly correlated with total CHOC in the rumen. Notice CHOC is being used here as the uNDF240 (and 120 for non-forages) is the CHOC pool inCNCPSv6.5. There is much confusion being introduced by groups discussing uNDF30 or uNDF240. The research to date has focused on the 240 hr relationship with rumen fill and dynamics. The laboratories have developed NIR predictions for the forage time points. Again, with very positive feedback from the labs regarding prediction statistics. However; for non-forages, the DNFD time points must be done via wet chemistry as there are insufficient sample numbers at this time to develop calibrations.

The third area focuses on amino acids (Van Amburgh, et al. 2015). CNCPS6.5 revamped the entire amino acid structure. These changes relate to several areas. The first is the composition of all feeds. Historically, amino acids were expressed as a proportion of the insoluble residue. In this method, a standard soluble protein assay was conducted and amino acids determined on the residue. This method had never been adopted by commercial labs. While it was available on special request, very few samples and products were analyzed resulting in a mixed feed library. Furthermore, the second issue related to analytical methods, specifically sulfur containing amino acids. The net result was that nearly every feeds MET values were under-reported nearly 50%. The third area was related to the efficiency of use for amino acids. Historically, CNCPS had different efficiency values for maintenance and lactation. LaPierre et al. (2007), at this conference, presented research results for combined values. CNCPS6.5 adopted these combined values along with a revamped feed library.

The fourth area relates to measuring nitrogen intestinal digestibility. Ross evaluated several different methods. Historically, CNCPS has relied upon the detergent system to estimate protein pools and digestibility. While adequate for forages, it has been shown to be poorly correlated with protein pools and digestibility's in other protein products. As an example, what do NDICP and ADICP in blood meal represent? The objective was to develop an assay that could measure intestinal digestibility accurately and able to be implemented by the commercial labs for all feeds. Other methods (e.g. Modified Minnesota Three-Step, Ceasectomized Rooster, Mobile Bag) require specially prepared animals. Additionally, Ross found that the enzymes utilized by some methods were inconsistent and, in some cases, did not match cattle intestinal enzyme profiles. The new Ross method includes a 16-hr *in vitro* to estimate RUP, an acid hydrolysis estimating abomasal action, and then enzyme exposure estimating small intestinal action. The value reported is estimated intestinal indigestible nitrogen (IUN). The assay was evaluated with a lactation study (Gutierrez-Botero et al., 2014). In this study, two blood meal sources were used representing two different intestinal digestibility. Diets were iso-nitrogoneous and formulated to be MP limiting. According to the assay, there was a 20 g difference in nitrogen digestibility. Trial results showed a 2 kg difference in milk production. Evaluations with CNCPS6.5 compared ADICP (predicted no difference) and the IUN. The IUN results predicted a 2.5 kg difference in milk production. This shows the sensitivity of this new assay.

IMPLEMENTATION

As a licensee, AMTS LLC implemented CNCPS6.5 biology with extreme care. The updates required repopulating all feed libraries and core biology changes. This required several interface changes and preparing multiple training materials prior to release. Throughout this time, AMTS evaluated diets from dairy farms and ingredients. One ingredient, AminoMax, was selected for further analysis incorporating all CNCPS6.5 updates. AFGRITec LLC, Watertown NY, manufactures AminoMax, a patented process to treat canola and soybean meal. The quality control program includes sampling all in- and out-bound loads. Given the manufacturing process, the company expressed a large

interest in evaluating the product with the assays. Samples were submitted from various production, and experimental, runs to a commercial laboratory and Cornell University.

Table 1 contains the NDF analytical values utilized in this evaluation. Nutritionists for years have reported cows respond differently to canola meal then standard analytical results and models would suggest. Given that AminoMax is a treated soy/canola blend, these results clearly show there is significantly more available NDF then previously reported. As Table 2 shows, the lignin x 2.4 relationship for determining CHO C pool size greatly over-estimates the undigestible pool compared with 120 hr DNDF. In this case, the relationship is 1.36 x lignin indicating significant lower lignin cross-linking within the NDF matrix. Shifting the potentially digestible NDF pool from 29.7 to 60.2% NDF has significant impacts on ME and MP flows. Assuming a 6% passage rate, this pool size shift, and new kd calculations, result in 256% greater potential NDF degraded. Utilizing AMTS.Cattle.Professional, when fed at 2 kg, this equates to 0.5 kg higher ME and MP allowable milk production, 13 g MP, 1 g LYS, 1 g MET, and 175 g lower CHO C when using the new NDF digestibility methods.

Table 1. AminoMax fiber components and digestibility.

	Analytical Values	Units
aNDFom	28.0	% OM
Lignin	8.2	% DM
12 hr DNDF	34.1	% NDF
72 hr DNDF	57.7	% NDF
120 hr DNDF	60.2	% NDF

Table 2. AminoMax Carbohydrate (CHO) C and B3 pool size and B3 degradation rate.

	CNCPS6.1 Based	CNCPS6.5 Based	Units
CHO C	70.3	39.8	% NDF
CHO B3	29.7	60.2	% NDF
CHO B3 kd	4.5	7.1	%/hr

Amino acid composition is shown in Table 3. As has been observed with all feeds, the change in methodology (%ISR vs %CP) changed values 1-20%. Some ingredients saw small changes, while others such as canola resulted in significant changes. Lysine in canola meal, for example, changed from 6.7% ISR to 5.7% CP. The CNCPS6.1 MET value (2.47% ISR) for AminoMax was a measured value. This highlights one of the issues Cornell identified with CNCPS6.1 amino acids. Namely, the improper hydrolysis and extraction prior to HPLC analysis accounting for approximately 50% of sulfur containing amino acids. Raw canola MET values changed from 1.4% ISR to 2.1% CP in the CNCPS6.1 to CNCPS6.5 transition. This highlights the issue of CNCPS6.1 library being confounded by improvements in amino acid analytical methods resulting in a ‘mixed’ library. Ingredients that were analyzed after the analytical error was determined, and corrected, all showed higher MET values. The analytical error was not well known or discussed, therefore, much confusion was observed when discussing MET with nutritionists.

Table 3. Amino acid composition of three ingredients comparing CNCPSv6.1 (%ISR) and CNCPS v6.5 (%CP).

	6.1	6.5	6.1	6.5	6.1	6.5
	Canola, expellers		Soybean Meal		AminoMax	
Methionine	1.4	2.1	1.3	1.3	2.5	2.0
Lysine	6.7	5.7	6.5	6.1	6.4	6.0
Arginine	6.8	6.1	7.7	7.3	6.8	6.7
Threonine	4.9	4.4	4.8	3.9	4.7	4.7
Leucine	8.0	7.0	8.7	7.6	8.2	8.2
Isoleucine	4.9	4.2	4.0	4.5	5.0	4.5
Valine	6.4	5.3	4.4	4.7	6.2	5.8
Histidine	4.0	2.6	2.7	2.6	3.8	2.9
Phenylalanine	4.7	4.0	5.2	5.1	4.9	4.8
Tryptophan	1.2	1.5	1.4	1.3	1.2	1.2

The Ross IUN results introduce a very interesting issue. Non-forage protein ingredients are critical for ruminant nutrition. It is well known that any process that involves heat decreases protein and amino acid intestinal digestibility. It is also well known that ADICP is inappropriate for estimating protein intestinal digestibility. As Cornell research has demonstrated, this assay is very sensitive allowing predicted performance to match observed milk production closely. This assay can be used as a component of manufacturing process control in the production of by-pass products. It is generally accepted that the IUN be implemented; however, given that this deals directly with commercial products, the first company to publish IUN results may be put at a competitive disadvantage as the values will be lower then any ADIN result.

Canola and soybean meal samples were submitted to Cornell for IUN analysis (Table 4). Five samples of unprocessed canola averaged 25.5% total N IUN (SD 2.6%). Processed canola (n=10) resulted in an average 19.9% total N IUN (SD 2.7%). At first glance, it would appear concerning that IUN of processed was lower then raw canola. However, this would suggest that the increase in RUP via processing results in a very highly digestible RUP fraction. Compared with the detergent methodology (ADICP), IUN is higher for all samples (unprocessed and processed, canola and soybean meal). These results should come as no surprise as the detergent system was never designed to evaluate protein digestibility.

Nutritionists have debated the value of different feeds and analysis for many years. This has resulted in over-feeding nutrients, thus potentially increasing cost and excretion. Canola is an excellent example of this conundrum. It is also important to understand that just because the Protein C fraction of canola increases 3x does not mean the canola is any worse. The new assay is akin to changing currency in that canola intestinal digestibility has always been lower but the detergent system was unable to describe this. Implementing the IUN assay will allow nutritionists to make more informed decisions and formulate more efficient diets. These statements are supported by data from Miner

Institute research where AminoMax was fed in a direct replacement (pound for pound dry matter) for another commercial by-pass protein. There was no statistical difference in any measured parameter with the exception of MUN with AminoMax fed cows lower (9.6 vs 11.4 mg/dL for AminoMax and competing product; respectively) (Tucker et al., 2015).

Table 4. Intestinally unavailable nitrogen (IUN %N) of unprocessed and processed canola and soybean meal.

	Canola Meal			Soybean Meal		
	Avg.	SD	n	Avg.	SD	n
Unprocessed						
ADICP (%CP)[a]	8.4	n/a	n/a	1.8	n/a	n/a
IUN (%N)	25.5	2.6	5	10.6	n/a	1
Processed						
ADICP (%CP)	9.4	0.7	6	1.2	0.3	5
IUN (%N)	19.9	2.7	10	10.8	1.6	3

[a]Values from CNCPSv6.5 feed library for Canola Meal, Expeller

FIELD IMPLEMENTATION

The new methods and updated amino acid composition/efficiency values implemented in CNCPSv6.5 allow the nutritionist to explain more production and formulation variance. Implementing CNCPSv6.5 should be done step-wise by nutritionists. AMTS.Cattle.Professional ver. 4 fully implemented CNCPSv6.5. During the upgrade process, user files were converted to implement the new amino acid composition values. While this is an important step, and allows nutritionists to evaluate amino acids with the latest information and improved confidence, it is only the first step. The CNCPSv6.5, and AMTS, feed libraries have fields for the new DNDF time points, uNDF, and IUN results. These fields are not populated with data however. And, if data is not present, the programs utilize CNCPS6.1 calculations. Data limitations, and normal variance between farm/source, make it nearly impossible to populate these fields. As commercial products are analyzed, a feed library could be developed; however, this takes time and resources. It is estimated that it would require approximately $2 million USD in feed analysis to fully populate the existing library for these new methods.

The major commercial laboratories, along with their affiliates, introduced aNDFom early 2015 via NIR and wet chemistry. These laboratories have very good NIR calibrations for aNDFom on forages. Non-forages that could potentially be high in soil contaimination should be analyzed wet chemistry for aNDFom. These would include ingredients such as beets, cottonseed, cotton burrs, almond hulls, and other ingredients that would be prone to soil contact.

Moving towards uNDF is also recommended. Again, the aforementioned commercial laboratories and their affiliates began offering 30, 120, and 240hr DNDF via NIR in early/mid 2015. These results need to be reported, and inputted, as %NDFom. Diets high in non-forage NDF feeds should be analyzed for 12, 72, 120 hr DNDF as well.

Unfortunately, this must be done via wet chemistry. Given that greater then 70% of total CHO C comes from forages in typical diets, adopting the 30, 120, and 240hr DNDF is the most sensitive component. Feeds high in NDF, and less processed, should be next. Examples of these would be cottonseed, wheat middlings, canola meal, etc.

Implementing the Ross assay would be the final step with a focus on high protein feeds. The IUN assay is also the most difficult to implement. Attempting to implement this with only one or two feeds could greatly alter the perceived value of these feeds while optimizing or evaluating purchasing options. Implementing with only one or two feeds (e.g. an animal protein and a by-pass vegetable product) can be a powerful tool to evaluate product consistency and relative differences between products within class (e.g. two different animal protein sources) if the IUN is measured from both suppliers. It is recommended that IUN be implemented in two phases. The first phase would be high RUP products or those with known or suspected product variance (e.g. commodity blood meal, distillers grains, etc.). Within CNCPSv6.5 and AMTS, inputting IUN initiates several changes in the code. A user can input the IUN, evaluate the diet, and then input zero IUN and compare. During this time, a user feed library populated with IUN results can be developed. As additional feeds are added, formulation can become more IUN based. The second phase would be lower RUP feeds (e.g. soybean meal). Individual consultants are at a disadvantage here given their access to limited sample numbers. Consultant groups and feed companies should develop internal projects to develop a IUN based feed library. Regardless, nutritionists should request IUN results for commercial RUP products. Given the commercialization of the assay, it is now possible to include this as a standard quality control assay.

CONCLUSION

Modeling is an evolutionary process. The CNCPS has been able to capture research results and improve accuracy and formulation. Many times, these improvements introduce new inputs and outputs while forcing nutritionists to re-evaluate current thinking. The move from CNCPSv6.1 to CNCPSv6.5 biology is one of these re-evaluation points. Modern formulation packages and the commercial laboratories have worked closely together to ensure the new assays and biological modeling is implemented for nutritionists to take advantage of. Future model enhancements will allow nutritionists to evaluate dry matter intake differently and further fine-tune formulations. The CNCPSv6.5 is a step towards a fully dynamic supply model (CNCPSv7) and many CNCPSv7 concepts are introduced in CNCPSv6.5. AMTS user feedback supports implementing CNCPSv6.5 biology rapidly due to improved accuracy and the ability to improve animal performance.

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FEEDING THE FRESH COW

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INTRODUCTION

Nutritional management of the transition cow has been a topic of intense research focus for more than 20 years (Grummer, 1995), and during this time many nutritional innovations for the transition cow have been developed and deployed within the dairy industry. Among others, these include decreasing the dietary cation-anion difference of the prepartum diet for management of hypocalcemia, the introduction of "controlled energy" dietary strategies for dry cows to improve the dynamics of energy metabolism and dry matter intake (DMI) during the peripartal period, increased focus on metabolizable protein and amino acid supply to the prepartum cow with some evidence of improved postcalving performance, and the targeted supply of nutrients (e.g., rumen-protected choline) to improve aspects of metabolic health and productivity of transition cows. Furthermore, the importance of management of nonnutritional factors (e.g. stocking density, commingling of multiparous and primiparous cows pre- and postpartum, streamlining grouping changes for transition cows, and mitigating heat stress) is now recognized as a pivotal part of optimizing transition cow health and performance (Cook and Nordlund, 2004; Tao and Dahl, 2013). Collectively, these improvements in both nutritional management of transition cows and management of nonnutritional factors have led to greatly improved health and performance on many dairy farms.

Ironically, the vast majority of transition cow nutritional management research conducted over the past 20+ years has focused almost exclusively on the dry cow. In most studies focused on transition cow nutrition, dietary treatments were imposed during the prepartum period only and cows were fed a common diet during the postcalving period. Fresh cow rations are common in the dairy industry, although often they are modest variations of the high cow ration, perhaps with slightly higher fiber content and/or the inclusion of modest amounts (1.5 lb or less) of straw or hay, lower starch content, additional rumen undegradable protein, increased amounts of supplemental fat, or targeted inclusion of other nutrients or additives (e.g., rumen-protected choline, additional yeast or yeast culture, additional monensin). Success of these strategies was gauged largely at the farm level, because until recently very few controlled research studies examined these factors in the ration fed during the immediate postcalving period. During the past several years, there has been a surge of research interest in the postpartum diet, fueled in part by discussions related to carbohydrate formulation of the postpartum diet and potential interactions with DMI (Allen et al., 2009). Our objective in this paper is to review recent research focused on starch and fiber content in ration formulation for the fresh cow and to speculate about potential interactions of dietary factors in rations that may lead to varying outcomes at the farm level.

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TO STARCH, OR NOT TO STARCH?

Optimizing DMI during the postpartum period is particularly important to provide sufficient energy to support milk production as well as maintain health and support the return of reproductive capacity. Because of increased demand for glucose to synthesize lactose, liver glucose production nearly doubles within 11 days after calving compared to prepartum glucose output (Reynolds et al., 2003). Propionate that is produced via fermentation of starch in the rumen is the main precursor for liver glucose production (Drackley et al., 2001). Although there is a large increase in the liver's utilization of lactate, glycerol, and the glucogenic amino acids postpartum, propionate remains quantitatively the greatest contributor to liver gluconeogenesis at about 60% of precursor supply (Reynolds et al., 2003). Because of this increased demand for glucose postpartum, the liver should have the capacity to direct any additional propionate supply towards glucose synthesis during this early postpartum period (Drackley et al., 2001).

Allen et al. (2009) and Allen and Piantoni (2013) proposed that liver energy status is a major regulator of DMI in dairy cows. Their premise is that when oxidative fuel metabolism (propionate and nonesterified fatty acids (NEFA)) by the liver exceeds the liver's energy requirements, the brain is signaled to reduce DMI. This hepatic oxidation theory would suggest that feeding diets that would increase propionate supply (e.g. greater amounts or fermentability of starch, addition of monensin) during early lactation would decrease DMI via this liver signaling mechanism. If the hepatic oxidation theory applies in this manner to the early lactation period, then reducing the dietary starch content during this period would likely increase DMI by reducing propionate production in the rumen and decreasing the hypophagic effect from propionate oxidation (Allen et al., 2009).

Collectively, our research groups have completed three experiments evaluating starch content of the postpartum diet (Dann and Nelson, 2011; Williams et al., 2015) and starch content of the postpartum diet and monensin supplementation throughout the periparturient period (McCarthy et al., 2015a, 2015b). Dann and Nelson (2011) fed 72 multiparous Holstein cows a controlled energy diet during a shortened (40-d) dry period and then one of three dietary starch regimes during early lactation – a low starch (21.0% starch) diet for the first 91 d postpartum (L), a medium starch (23.2% starch) diet for the first 21 d postpartum followed by a high starch (25.5% starch) diet through 91 d postpartum (MH), and a high starch diet (25.5% starch) for the first 91 d postpartum (H). Sources of starch among diets were primarily conventional, kernel-processed corn silage and dry corn meal; the differences in starch content across diets were achieved by replacing corn meal with soybean hulls and wheat middlings.

In this study, cows fed H throughout the trial period tended to have lower DMI for the first 13 wk postpartum than cows fed L (23.7 vs. 25.2 kg/d); cows fed MH had intermediate DMI (24.9 kg/d). Cows fed MH had higher milk yield than cows fed H (49.9 vs. 44.2 kg/d); cows fed L averaged 47.9 kg/d of milk. Overall, performance of cows in this study was best when fed MH rather than either L or H beginning at calving.

McCarthy et al. (2015a, 2015b) fed primiparous (n = 21) and multiparous (n = 49) Holstein cows diets containing either 26.2% or 21.5% starch from calving through d 21 postpartum; beginning on d 22 postpartum all cows were fed the diet containing 26.2% starch through d 63 postpartum. Starch sources were predominantly brown midrib corn silage and dry corn meal; the overall starch content was varied by replacing corn meal with citrus pulp and soybean hulls. Cows were also fed either 0 or 400 mg/d of monensin beginning 21 d before expected calving and either 0 or 450 mg/d of monensin beginning at calving and continuing through d 63 postpartum. Although there were no overall effects of starch level in the diet on milk yield, a treatment by week interaction existed such that cows fed the higher starch diet had a faster rate of increase in milk yield postcalving. A similar interaction of starch level and week for DMI suggested that cows fed the higher starch diet increased DMI faster during the postpartum period. Cows fed monensin had higher postpartum DMI and milk yield, regardless of starch level in the postpartum diet. Furthermore, cows fed higher starch had lower NEFA and BHBA concentrations postpartum; monensin did not affect plasma NEFA but decreased plasma BHBA concentrations.

The Miner and Cornell studies suggest apparently opposite responses to feeding low- and high-starch diets during the fresh period. A comparison of the low- and high-starch fresh diets between the two studies (Table 1) suggests that the overall starch levels and CNCPS (v. 6.1) predicted levels of fermentable starch (% of DM and % of total fermentable carbohydrate), and total fermentable carbohydrate (% of DM) were quite similar between the low and high starch diets in the two studies. The major apparent difference between the two studies relates to the diet fed during the prepartum period. In the Miner study, cows were fed a typical low starch (13.5% of DM), controlled energy diet for the entire 40-d dry period whereas in the Cornell study, cows were fed a moderate starch close up diet (17.4% of DM). We speculate that the large differences in starch levels and fermentability between the prepartum diet and the high starch postpartum diet in the Miner study compromised the transition of cows onto the high starch postpartum diet in that study. Likewise, we speculate that feeding the higher starch prepartum diet to cows in the Cornell study facilitated their transition onto the higher starch postpartum diet. However, we also noted that intake (kg/d and % of body weight) of starch and NDF was lower in the Cornell study than the Miner study during the first 3 and 9 weeks of lactation. Given the lower NDF digestibility (NDFD) of the low and high starch diets used in the Cornell study (~56% NDFD at 30-h) compared with the Miner study (58 and 54% NDFD at 24-h for the low and high starch diets, respectively), it is possible that the cows fed both the low and high starch diets at Cornell containing 11.5% of DM as straw were limited by gut fill during the first 3 wk of lactation with NDF intake of <1.1% of body weight. This reinforces the need to use highly digestible fiber sources when lower starch diets are fed whether it is high cows or fresh cows. Both studies used a “21-d fresh period”. However, the optimal duration of feeding a fresh diet is unknown and it most likely varies among cows given differences in rate of increase in DMI and milk production.

Studies in the literature that offer the opportunity to examine interactions of prepartum and postpartum diets are limited. Rabelo et al. (2003; 2005) fed cows and first calf heifers either low or high energy diets prepartum followed by either low or high energy

diets postpartum until d 20 postcalving, then all cows were fed the high energy diet through d 70 postcalving. The prepartum diets were based upon alfalfa silage, corn silage, a comparatively small proportion (10 to 15% of DM) of chopped straw, and grain mixes consisting predominantly of corn meal. Prepartum, the "low" energy diet contained 39.7% NDF and 38.2% NFC and the "high" energy diet contained 32.2% NDF and 44.6% NFC. The postcalving diets were based upon alfalfa silage and corn silage, and grain mixes consisting predominantly of corn meal – the "low" energy diet contained 29.9% NDF and 41.4% NFC; the "high" energy diet contained 24.9% NDF and 47.2% NFC. Cows fed the high energy diet prepartum had higher prepartum DMI and no difference in postpartum DMI, and cows fed the high energy diet postpartum tended to have higher DMI and had higher energy intake from d 1 to 30 postpartum; overall effects of treatment from d 1 to 70 postcalving were not significant. Rates of increase of milk production were greater for cows fed high energy diets postcalving, and plasma concentrations of BHBA were substantially lower for cows fed the high energy postcalving diet when measured on d 7 and 21 postcalving. Interactions of prepartum and postpartum diets were largely not significant for response variables in this experiment; however, we note that both prepartum diets were comparatively high in NFC by current standards and the high energy postpartum diet was higher in NFC than current diets – starch values for diets were not reported in this study.

Table 1. Comparison of prepartum and postpartum rations from Miner and Cornell studies focused on varying starch levels in the fresh diet (Dann, personal communication).

Study & Group	Starch, %DM	Ferm.[1] starch, %DM	Ferm. starch, % Total ferm. CHO[2]	Total ferm. CHO, %DM
Miner (Dann and Nelson, 2011)				
Dry	13.5	11.5	29.7	39.4
Low fresh	21.0	16.8	40.1	42.4
High fresh	25.5	20.2	50.3	44.1
Cornell (McCarthy et al., 2015a,b)				
Close-up	17.4	15.3	36.3	42.2
Low fresh	21.5	16.8	42.1	39.9
High fresh	26.2	21.5	53.2	40.4

[1] Ferm = fermentable
[2] CHO = carbohydrate

Most of the early discussion surrounding the importance of prepartum diet on ruminal adaptation to postpartum diet focused on the potential importance of diet on ruminal papillae development (Dirksen et al., 1985). However, subsequent research (Andersen et al., 1999; Reynolds et al., 2004) did not support the idea that changes in ruminal papillae and mucosa were important factors in ruminal adaptations during the transition period under typical dietary strategies. Of course, the adaptation of the ruminal

microbiota to lactation would be a potentially important adaptation as well as overall adaptation of rumen fermentation to postpartum diets. Research conducted by Penner et al. (2007) suggested that cows have a dramatic increase in the amount of time with a ruminal pH between 5.5 and 5.8 (categorized as mild acidosis in their study) between d 2 and 5 postcalving; in their experiment ruminal acidosis was much lower during the dry period as well as at d 17, 37, and 58 postcalving. Interestingly, varying the forage to concentrate ratio of the prepartum diet did not affect postpartum incidence or severity of ruminal acidosis.

Recently, Williams et al. (2015) examined the effects of starch content of the postpartum ration on subacute ruminal acidosis and the acute phase response in 16 multparous Holstein cows. Cows were fed a close-up diet based upon conventional corn silage, haycrop silage, straw, and a grain mix (TMR contained 43.6% NDF and 15.5% starch). The postpartum treatment diets both contained the same proportions of conventional corn silage (28.3% of DM), haycrop silage (21.7% of DM) and straw (2.0% of DM); the high starch (27.2% of DM) and low starch (21.3% of DM) diets had different proportions of corn meal and nonforage fiber sources (soybean hulls and wheat middlings). Cows fed the high starch diet had lower rumen pH and increased time with ruminal pH < 5.8 during the first 3 weeks after calving. Also, cows fed the high starch diet hadincreased concentrations of haptoglobin and serum amyloid A in serum with the greatest differences occurring in the first 2 weeks. These data suggest that higher postpartum starch levels contribute to lower rumen pH and increased inflammation during the fresh period.

WHAT ABOUT FIBER?

Although there has been substantial discussion within academia and the industry over the past several years regarding starch levels in the postcalving diet, there has been very little discussion regarding fiber levels in the postcalving diet. In particular, we are interested in the fiber fraction that contributes toward rumen mat formation and proper rumen function that typically has been referred to as physically effective NDF(peNDF), although the more recent focus on undigestible NDF (uNDF) intrigues us as a more objective measure of fiber that may contribute to rumen structure and proper rumen function.

Our interest in fiber levels in postpartum diets stems specifically from our experience in the early stages of the experiment described above (McCarthy et al., 2015a) that caused us to essentially restart the experiment. The first cows calving onto the experiment developed a number of health issues, including quite strong clinical ketosis beginning 3 to 7 d postcalving and a high proportion of DA, particularly on the high starch treatment (See treatments HSLF and LSLF in Table 2). We identified that the NDF levels of both the BMR corn silage and legume silage used in the postpartum diets were lower than those used for formulation. We decided to increase the inclusion rate of straw in the diet, replacing an equal amount of BMR corn silage. We kept the inclusion rates of the legume silage and the grain mix inclusion rates and formulation the same. Ingredient and chemical composition of the prepartum diet and the high starch and low starch diets

before the change in straw and BMR corn silage (HSLF and LSLF) and after the change in straw and BMR corn silage (HSHF and HSLF) are presented in Table 3. These changes resulted in substantially increased NDF and $uNDF_{240}$ content of the postpartum diets following this change.

The effects of this change on postpartum health outcomes were immediate and lasted for the duration of the re-started experiment. Prior to the change, 5 out of 17 cows had clinical ketosis and 6 out of 17 cows had DAs. After the change, there were no DAs and 10 cases of clinical ketosis out of 77 cows calved (Table 2).

Prepartum DMI and intakes of $uNDF_{240}$ (both lb/d and expressed as a % of BW) are presented in Table 4. Although means are reported by treatment and statistical analysis conducted, we remind the reader that all cows were fed the same prepartum ration throughout the study and no changes were made at the time the changes were made to the postpartum ration. Thus, the general lack of treatment differences is logical. Multiparous cows did consume less $uNDF_{240}$ lb/d (~4.5 lb/d vs. ~ 5.1 lb/d; $P < 0.02$) after the ration change. We suspect that this related to either changes in the conventional corn silage or seasonal/heat effects on overall DMI as the experiment was started in early April 2012, the change was made in early May, and cows calved throughout the summer. Interestingly, multiparous cows consumed about 0.30% of their BW as $uNDF_{240}$ and primiparous cows consumed 0.20 to 0.22% of their BW as $uNDF_{240}$ during the prepartum period.

Postpartum DMI, intakes of $uNDF_{240}$, and milk yield are presented in Table 5. Following the partial replacement of BMR corn silage with wheat straw, overall DMI and intakes of $uNDF_{240}$ (both lb/d and as a % of BW) were increased. Before the ration change, multiparous cows consumed about 0.30% of their BW as $uNDF_{240}$ and primiparous cows consumed about 0.24% of their BW as $uNDF_{240}$. After the ration change, cows consumed about 0.38% of their BW as $uNDF_{240}$ and primiparous cows consumed about 0.31% of their BW as $uNDF_{240}$.

Although the ration change increased DMI and $uNDF_{240}$ intakes despite greater straw inclusion in the ration, of interest are the interactions between starch level and fiber level on postpartum DMI and milk yield. Cows fed the high starch ration concurrent with high fiber (after ration change) had the highest DMI; DMI of cows fed the low starch diet with either high or low fiber were intermediate, and DMI for cows fed the high starch diet with low fiber (before diet change) was the lowest of the four groups. This interaction is particularly evident in Figure 1 and is consistent with the higher overall incidence of clinical ketosis and DA for cows fed the high starch diet prior to the diet change.

We acknowledge that we need to interpret the direct comparisons of performance before and after the ration change with caution, given confounding with other factors (e.g., season, environment, other forage or feed changes); however, we interpret the results of this case study as evidence that starch level of the postpartum diet may depend in part on the fiber formulation in the ration. In situations where forage NDF is low to marginal, likely the best strategy is a lower (or less fermentable) starch ration. However, in

situations where forage NDF content in the ration is adequate, and perhaps at levels higher than most nutritionists typically consider, higher starch rations may yield better overall results. Currently, we are conducting an experiment (Williams et al., personal communication) designed to evaluate this hypothesis and determine the effects of varying dietary $uNDF_{240}$ level in the postpartum ration on postpartum performance, metabolism, and acute phase response in cows.

Table 2. Health events for cows fed either high or low starch diets for the first 3 wk postpartum before and after postpartum ration changes.

	Postpartum ration[1]				Parity		*P*-values[2]		
Item[3]	HSLF	LSLF	HSHF	LSHF	Primi	Multi	S	F	P
Multiparous, n	3	8	27	28					
Primiparous, n	4	2	11	11					
Clinical ketosis[3]	4	1	4	6	6	9	0.23	0.05	0.14
DA[4]	4	2	0	0	4	2	0.22	<0.001	0.06
RP[5]	1	2	2	1	3	3	0.32	0.05	0.20
Total disorders	9	5	6	7					

[1] HSLF = high starch, low fiber (pre-change); LSLF = low starch, low fiber (post-change); HSHF = high starch, high fiber (post change); LSHF = low starch, high fiber (post-change).
[2] S = effect of starch; F = effect of fiber; P = effect of parity.
[3] Clinical ketosis defined as rapidly decreased milk production and DMI and blood BHBA ≥ 2.6 mmol/L using Precision Xtra, displaced abomasum by auscultation
[4] Displaced abomasium diagnosed by auscultation.
[5] Placenta retained for ≥ 24 h postcalving.

Table 3. Ingredient and chemical composition of diets (± SD[1]) before and after postpartum ration changes (DM basis)

		Postpartum[2]			
Item	Prepartum	HSLF	LSLF	HSHF	LSHF
Ingredient (% of DM)					
Corn silage, conv.	42.1	---	---	---	---
BMR corn silage	---	46.1	46.1	38.5	38.5
Wheat straw	21.2	3.84	3.84	11.5	11.5
Legume silage	---	9.62	9.62	9.62	9.62
Corn meal, fine	4.28	21.0	10.3	21.0	10.3
Citrus pulp	7.23	1.01	7.15	1.01	7.15
Corn germ meal	---	2.52	5.56	2.52	5.56
Soybean hulls	7.08	---	3.58	---	3.58
Soybean meal	5.27	5.87	3.86	5.87	3.86
Canola meal	4.63	2.73	2.08	2.73	2.08
Blood meal	1.05	1.94	1.93	1.94	1.93
Expeller soy	1.78	1.70	2.34	1.70	2.34
Bypass fat	---	0.77	0.96	0.77	0.96
Anionic suppl.	1.33	---	---	---	---
Sodium bicarbonate	---	0.86	0.85	0.86	0.85
Minerals/vitamins	3.35	1.99	1.72	1.99	1.72
Chemical					
CP, %	13.0 ± 0.8	16.5	15.3	15.5 ± 1.2	15.4 ± 0.8
ADF, %	28.2 ± 1.2	17.7	22.3	22.7 ± 1.2	25.2 ± 1.2
NDF, %	42.9 ± 2.0	26.4	31.5	34.3 ± 1.5	36.9 ± 1.5
Sugar, %	4.9 ± 0.8	3.1	3.9	3.5 ± 0.6	4.5 ± 0.4
Starch, %	17.4 ± 1.2	28.3	22.0	26.2 ± 1.2	21.5 ± 1.0
Fat, %	2.6 ± 0.2	3.2	3.1	4.0 ± 0.2	2.2 ± 0.6
$uNDF_{240}$,[3] % of DM	14.9	7.7	8.9	10.5	10.9

[1] Chemcial composition was analyzed on 4-wk composite samples (n = 1 for HSLF, n = 1 for LSLF, n = 7 for HSHF, and n = 6 for LSHF).

[2] HSLF = high starch, low fiber (pre-change); LSLF = low starch, low fiber (post-change); HSHF = high starch, high fiber (post change); LSHF = low starch, high fiber (post-change).

[3] Determined using wet chemistry methods on a single composite sample from each diet (Cumberland Valley Analytical Services, Hagerstown, MD)

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Table 4. Prepartum DMI and $uNDF_{240}$ intakes for primiparous and multiparous animals fed high starch (HS) and low starch (LS) diets before (LF) and after (HF) partial replacement of BMR corn silage with straw in postpartum diets.

	Postpartum diet					P values		
Item	HSLF	LSLF	HSHF	LSHF	SEM	Starch	Fiber	Starch x Fiber
Prepartum DMI, lb/d								
Overall	28.0	28.7	29.5	28.0	1.7	0.74	0.71	0.38
Multiparous	35.0	34.5	33.0	31.4	2.8	0.54	0.12	0.75
Primiparous	19.7	19.9	21.9	20.0	1.3	0.76	0.60	0.64
$uNDF_{240}$ intake, lb/d								
Overall	4.01	4.01	4.17	3.97	0.20	0.56	0.73	0.56
Multiparous	5.11	5.05	4.63	4.41	0.37	0.54	0.02	0.74
Primiparous	2.84	2.76	3.00	2.84	0.26	0.55	0.55	0.84
$uNDF_{240}$ intake, % of BW								
Overall	0.26	0.27	0.27	0.26	0.01	0.92	0.73	0.36
Multiparous	0.32	0.31	0.30	0.29	0.01	0.59	0.08	0.81
Primiparous	0.20	0.20	0.22	0.21	0.02	0.65	0.36	0.87

Table 5. Postpartum DMI, $uNDF_{240}$ intakes, and milk yield for primiparous and multiparous animals fed high starch (HS) and low starch (LS) diets before (LF) and after (HF) partial replacement of BMR corn silage with straw in postpartum diets.

	Postpartum diet					P values		
Item	HSLF	LSLF	HSHF	LSHF	SEM	Starch	Fiber	Starch x Fiber
Postpartum DMI, lb/d								
Overall	39.2	43.8	47.3	44.4	2.4	0.63	0.01	0.03
Multiparous	43.9	53.0	51.3	48.3	3.1	0.11	0.48	0.002
Primiparous	32.5	30.2	36.4	34.5	1.3	0.12	0.005	0.89
$uNDF_{240}$ intake, lb/d								
Overall	3.35	3.68	4.98	4.74	0.24	0.81	<0.001	0.08
Multiparous	3.79	4.70	5.38	5.11	0.20	0.11	<0.001	0.004
Primiparous	2.82	2.73	3.81	3.66	0.13	0.32	<0.001	0.82
uNDF240 intake, % of BW								
Overall	0.26	0.28	0.37	0.36	0.01	0.95	<0.001	0.10
Multiparous	0.29	0.31	0.39	0.38	0.02	0.80	<0.001	0.13
Primiparous	0.24	0.24	0.32	0.3	0.01	0.58	<0.001	0.36
Milk yield, lb/d								
Overall	76.2	90.0	86.4	83.5	6.8	0.26	0.70	0.09
Multiparous	97.0	98.1	94.4	91.3	8.0	0.84	0.34	0.66
Primiparous	66.1	61.0	65.5	67.0	5.7	0.66	0.50	0.41

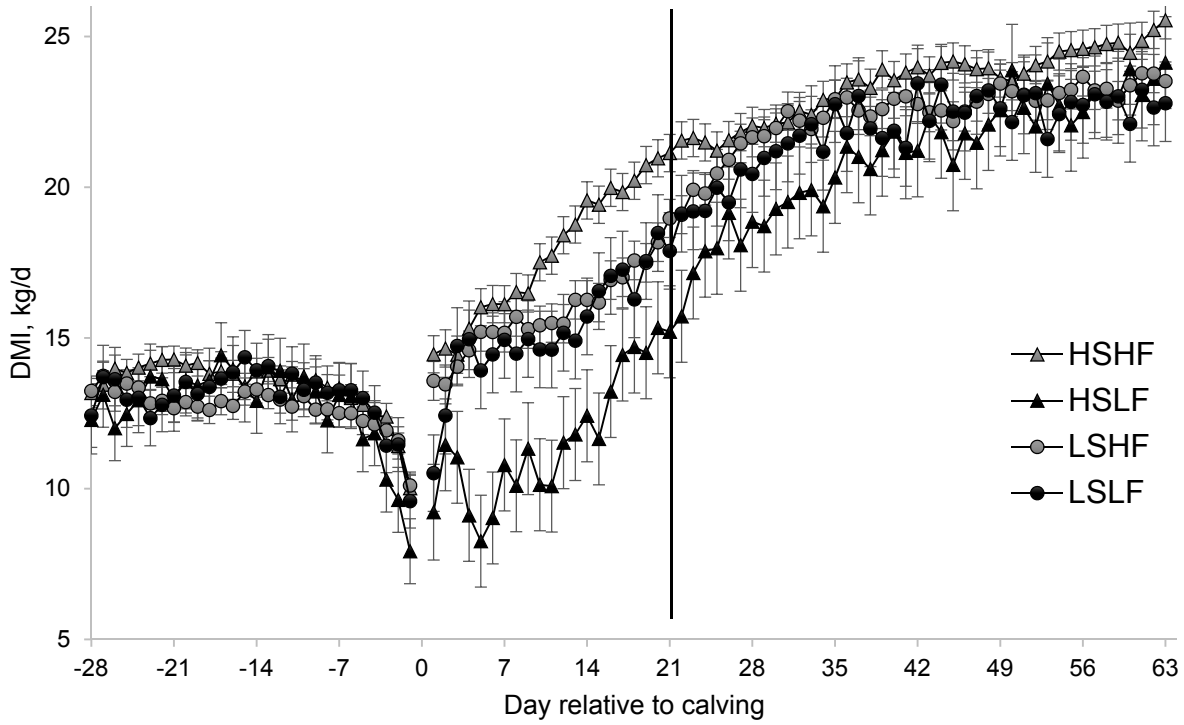

Figure 1. Dry matter intakes (DMI) of cows fed high starch (HS) or low starch (LS) before (LF) and after (HF) a ration change to partially replace BMR corn silage with straw and subsequently increase the NDF and $uNDF_{240}$ content of both rations. Treatment rations were fed from 0 - 21 DIM; at 22 DIM all cows were switched to the HS diet.

CONCLUSIONS

Studies conducted during the past several years are yielding important information regarding nutritional approaches for the cow during the immediate postpartum period, although we still have much to learn in the area of fresh cow nutrition. Although some studies suggest that feeding higher starch rations in the immediate postpartum period may improve metabolism and performance during early lactation, other studies suggest that feeding lower starch rations during the immediate postpartum period lead to better performance. Comparison of some of these studies suggests that feeding higher starch rations before calving may lead to better ability of cows to tolerate a higher starch ration after calving. Furthermore, case study work as part of an experiment in our group suggests that peNDF or $uNDF_{240}$ content of the postpartum is important to consider in ration formulation, and that nutritionists should consider feeding higher levels of straw or other feeds with high $uNDF_{240}$ content than they have typically feed. Furthermore, the optimum starch level for formulation may depend upon the peNDF or $uNDF_{240}$ content of the postpartum diet.

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UPDATE ON THE NEW U.S. DIETARY STANDARDS

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The Dietary Guidelines for Americans (DGA), first published by the U.S. federal government in 1980 and most recently updated in 2010, provide nutrition and diet guidance and information to help people ages two years and older promote lifelong health and to prevent chronic disease. The DGA is the cornerstone policy for all Federal food and nutrition programs, including those implemented by the Department of Health and Human Services (HHS) and the Department of Agriculture USDA). These two federal departments work together to update and publish the DGA every five years, as mandated by the U.S. Congress via the National Nutrition Monitoring and Related Research Act of 1990 (P.L. 101-445)**.** The 2015 (8^{th}) edition of the DGA, slated for release in late fall 2015, will be based on diverse input from government agencies, public and industry comments, and a scientific report from the 2015 Dietary Guidelines Advisory Committee (DGAC).

The 2015 DGAC was appointed by the HHS and USDA Secretaries and governed by the Federal Advisory Committee Act and was comprised on 14 nutrition science, public health, and medical experts. The committee members worked from spring 2013 until February 2015 with stellar support from HHS and USDA staff to provide the federal government with a scientific report on a vast array of nutrition topics. Ultimately, the report was organized into the following seven topics: 1) food and nutrient intakes and health: current status and trends; 2) dietary patterns, foods and nutrients, and health outcomes; 3) individual diet and physical activity behavior change; 4) food environment and settings; 5) food sustainability and safety; 6) cross-cutting topics of public health importance: added sugars, sodium, and saturated fat; and 7) physical activity.

Several aspects of the DGAC work are important to emphasize. First, the DGAC accomplished its work with unparalleled transparency. All full committee meetings were held in public forums; all working group and sub-committee meetings were conducted with federal staff oversight; all procedural and scientific activities are in the public record, and all resources used to develop the DGAC report are documented and publically available worldwide. Second, the scope and focus of the committees' work were developed in close communication and approval of HHS and USDA officials, which means that all of the topics scientifically assessed and evaluated were within the DGAC mandate. This is important to emphasize because after the DGAC report was published some people and interest groups have expressed concern that the 2015 DGAC addressed topics not covered by previous DGACs. Third, and perhaps most importantly, the DGAC members had absolutely no role in drafting or implementing DGA policy.

The themes, findings and conclusions presented in the DGAC report include the following:

- Most Americans do not consume a diet consistent with DGA recommendations, which likely contributes to suboptimal dietary and nutrient intakes and reduced health and higher chronic disease rates.
- Many people consume diets low in vegetables, fruits, whole grains, and dairy, and high in sodium, saturated fat, refined grains, added sugars, and energy (calories).
- Mixed dishes, which include burgers and sandwiches, pizza, and various meat/rice/pasta/grain dishes, and snacks and desserts are major sources of energy and large contributors of sodium, saturated fat, and added sugars to the diet.
- Research supports the benefits of consuming a variety of foods and beverages as part of healthy dietary patterns. “Common characteristics of dietary patterns associated with positive health outcomes include higher intake of vegetables, fruits, whole grains, low- or non-fat dairy, seafood, legumes, and nuts; moderate intake of alcohol (among adults); lower consumption of red and processed meat, and low intake of sugar-sweetened foods and drinks, and refined grains.”
- While there are many ways to achieve a healthy dietary pattern, the DGAC report emphasizes three USDA food patterns (Healthy U.S.-Style, Healthy Vegetarian, and Healthy Mediterranean-style).
- Successful and sustained improvements in a person’s diet and health are greatly influenced by their personal, social, economic, and cultural environments. Effective multi-component approaches and policies (more so than individual ones) may work together with a person’s own efforts to improve their diet and health.
- Compared to the current U.S. diet, a diet with more plant-based foods and less animal-based foods and energy promotes both improved human health and food security (including food sustainability associated with reduced environmental impact).

As noted above, low- or non-fat dairy is included in healthy dietary patterns. Indeed, “The USDA Food Patterns include 3 cup equivalents of dairy products per day in patterns that are targeted to preteens, teens, and adults. The amounts included for younger children are 2 cups for 2 and 3 year olds, and 2 ½ cups for 4 to 8 years olds.” The DGAC report identifies the Dairy Group for its relatively high nutrient density of numerous essential macro- and micro-nutrients, but also cautions that the Dairy Group also contributes relatively high amounts of sodium to the diet, especially from cheese. “Increasing the proportion of fat-free milk consumed to meet Dairy Group recommendations [by proportionately reducing cheese intake] would increase levels of magnesium, potassium, vitamin A, vitamin D, and choline in the patterns, and decrease amounts of sodium, cholesterol and saturated fatty acids. It especially boosts levels of potassium and vitamin D, nutrients that are below intake goals in all patterns.” Importantly, dietary modeling research shows that “None of the alternatives to milk and milk products provide a similar enough nutrient profile in terms of these impacted nutrients to be considered for inclusion in the Dairy Group. However, alternative calcium choices could be selected in combinations that together provide the range of nutrients needed.”

Collectively, this information underscores that dairy foods and beverages are important components of healthy dietary patterns.

In summary, the DGAC report contains reliable, scientifically rigorous information and conclusions to promote healthy eating for improved health in America. The committee urges the federal government to use this information as a foundation to make population health a national priority and to emphasize the importance of healthy diets to prevent chronic disease and to promote and sustain both human and environmental vitality.

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All information and quotes were obtained from the Scientific Report of the 2015 Dietary Guidelines Advisory committee, U.S. Departments of Health and Human Services and Agriculture, http://health.gov/dietaryguidelines/2015-scientific-report/ (accessed on September 1, 2015)

PREPARTUM NUTRITIONAL STRATEGIES TO MANAGE POSTPARTUM HYPOCALCEMIA

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INTRODUCTION

Dairy cows experience a negative calcium balance in the days immediately following calving as a result of the rapidly increased demand for calcium as the cow transitions from supporting growth of the fetus to supporting the needs of lactation. In order to maintain calcium homeostasis and support high levels of milk production, a coordinated hormonal response must increase the secretion of parathyroid hormone from the parathyroid gland, initiating increased activation of 1,25-dihydroxyvitamin-D_3. Together these hormones increase absorption of calcium in the intestine, increase osteoclastic activity at the bone to release stored calcium, and decrease the excretion of calcium at the kidney (Degaris and Lean, 2007). When this system is inadequate, hypocalcemia puts the animal at increased risk for several transition cow disorders including metritis, ketosis, displaced abomasum, and mastitis (Curtis et al., 1983, Chapinal et al, 2012, Martinez et al., 2012). Ultimately, cows with compromised blood calcium, despite having no clinical signs of paresis, have decreased reproductive performance and milk production (Chapinal et al., 2012). According to the 2002 Dairy NAHMS study, 47% of multiparous cows have subclinically low levels of blood calcium in the day after calving (Reinhardt et al., 2011).

Even cows that experience severe clinical hypocalcemia will respond with high PTH secretion; however, in late gestation cows, the ability of tissues to response to PTH may be diminished (Goff et al., 1989). Goff et al. (2014) showed that feeding a diet with a negative dietary cation-anion difference [DCAD; (Na + K) – (Cl + S)] resulted in increased sensitivity of tissues to PTH and a more efficient response to decreased blood calcium. Feeding a prepartum ration with a low or negative DCAD is an accepted strategy for improving the ability of cows to recover from the initial drop in blood calcium postpartum (Charbonneau et al., 2006). With the implementation of these strategies, the rate of clinical hypocalcemia has been reduced to less than 5% in the U.S. (Reinhardt et al., 2011); however, the use of negative DCAD feeding strategies to minimize subclinical hypocalcemia incidence is still being investigated.

Low potassium rations are commonly used with or without the addition of anion supplements to reduce dietary DCAD and to control the incidence of hypocalcemia in transition cows. Improvements in blood calcium status and performance are evident when the DCAD of the ration is reduced to -10 to -15

mEq/100 g of diet dry matter (Moore et al., 2000, DeGroot et al., 2010). However, many nutritionists and producers will implement a partial supplementation strategy, either due to starting cation concentrations in the ration as a result of available forages, to control costs, or because of ease of implementation given variability of mineral content of forages. The relative efficacy of a partial versus full anion supplementation strategy, in comparison to a low potassium ration without anion supplementation, has not been fully evaluated. Previous work compared varying levels of anion supplementation by feeding diets containing decreasing levels of DCAD (15 mEq, 0 mEq and -15 mEq/100 g of diet DM) in the prepartum diet; however, dietary calcium concentrations were increased as DCAD decreased. Multiparous cows fed the lowest DCAD had the highest plasma ionized calcium concentrations at calving, and tended to have the lowest incidence of hypocalcemia compared to cows fed the +15 mEq and 0 mEq/100 g DM rations (Moore et al., 2000). Dietary calcium in this study ranged from 0.44 to 1.50% of diet dry matter and therefore the impact of increasing calcium and decreasing DCAD likely both contributed to the differences in calcium status.

The objective of this study was to determine the effect of increasing anion supplementation, while maintaining a high dietary calcium, on plasma mineral status, intake and performance of multiparous Holstein cows. We hypothesized that feeding decreasing DCAD would result in improved plasma mineral status and increased intake and milk production.

EXPERIMENTAL APPROACH

All animal procedures were approved prior to the commencement of the trial by the Cornell University Institutional Animal Care and Use Committee. Multiparous Holstein cows (n=89) were fed a control diet (Table 1) beginning at 31 to 38 d prior to expected calving. At 24 d prior to expected calving cows were assigned randomly to receive the low potassium control ration (CON; n=30), a low potassium ration with partial anion supplementation (MedDCAD; n=30) or a low potassium ration with full anion supplementation (LowDCAD; n=29). Randomization was restricted to balance for previous mature equivalent 305 d milk yield, parity, and body conditions score (BCS). After calving, all cows were milked three times daily and fed a common postpartum ration through 63 DIM.

Diets were formulated using CNCPS version 6.1. The prepartum diets were based on brown mid-rib (BMR) corn silage, wheat straw, and a common grain mix (Table 1). Anion supplementation was administered with a small inclusion rate grain mix containing varying levels of anion supplement and distillers grains. Adjustments in inclusion rates of these mixes were made throughout the trial to maintain urine pH of the LowDCAD treatment between 5.5 and 6.0. Equal adjustments were made to all treatments to maintain equal nutrient composition (Table 1).

Table 1. Ingredient composition and analyzed nutrient composition of three prepartum diets with varying DCAD levels and the common postpartum diet.

	CON	MedDCAD	LowDCAD	Lactating
Ingredients (% of DM)				
BMR corn silage	44.8	44.8	44.8	37.3
Wheat straw	28.1	28.1	28.1	5.9
Alfalfa silage	-	-	-	9.8
Amino Plus[1]	8.1	8.1	8.1	7.1
Citrus pulp	3.3	3.3	3.3	3.9
Soybean hulls	2.3	2.3	2.3	-
Canola meal	2.2	2.2	2.2	5.9
Corn distillers grains	2.2	1.3	0.4	2.0
Corn gluten feed	-	-	-	3.9
Wheat midds	3.2	2.6	1.9	-
Ground corn grain	0.42	0.42	0.42	19.6
Molasses	0.67	0.67	0.67	-
LysAAMet[2]	-	-	-	0.78
Megamine L[3]	-	-	-	0.39
Alimet[4]	-	-	-	0.06
Megalac R[3]	-	-	-	0.39
Calcium diphosphate	0.46	0.46	0.46	-
Calcium carbonate	2.9	2.8	2.7	-
Magnesium oxide	0.56	0.42	0.25	-
Min-Ad[5]	-	-	-	1.6
Animate[6]	-	2.0	4.0	-
Urea	0.42	0.21	-	-
Salt	0.25	0.25	0.25	0.39
Sodium bicarbonate	-	-	-	0.78
Vitamin/mineral mix	0.14	0.14	0.14	0.22
Rumensin[7]	0.01	0.01	0.01	0.06
Chemical Analysis (Mean ± SD)				
DM (%)	46.3 ± 1.6	46.5 ± 1.3	46.4 ± 1.1	45.7 ± 1.8
CP (% DM)	13.0 ± 0.3	13.2 ± 0.4	13.2 ± 0.5	15.7 ± 0.2
ADF (% DM)	30.2 ± 0.7	30.5 ± 1.3	30.1 ± 1.3	20.6 ± 0.8
NDF (% DM)	44.3 ± 1.2	44.0 ± 2.1	43.2 ± 1.8	31.1 ± 1.0
Starch (% DM)	17.0 ± 0.5	16.0 ± 0.8	16.3 ± 0.9	26.0 ± 0.7
NFC (% DM)	33.6 ± 0.9	34.3 ± 2.5	35.0 ± 1.9	45.8 ± 1.2
Fat (% DM)	1.1 ± 0.1	1.3 ± 0.2	1.1 ± 0.3	2.3 ± 0.2
Ca (% DM)	1.54 ± 0.12	1.57 ± 0.14	1.57 ± 0.07	0.95 ± 0.03
P (% DM)	0.44 ± 0.01	0.43 ± 0.01	0.41 ± 0.01	0.41 ± 0.02
Mg (% DM)	0.47 ± 0.01	0.48 ± 0.03	0.50 ± 0.03	0.44 ± 0.02
K (% DM)	1.28 ± 0.07	1.26 ± 0.06	1.24 ± 0.07	1.37 ± 0.05
S (% DM)	0.20 ± 0.01	0.30 ± 0.02	0.41 ± 0.02	0.29 ± 0.01
Na (% DM)	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.44 ± 0.02
Cl (% DM)	0.27 ± 0.03	0.47 ± 0.05	0.69 ± 0.04	0.40 ± 0.02
DCAD (mEq/100g DM)	18.3 ± 0.8	5.9 ± 3.4	-7.4 ± 3.6	25.0 ± 1.5
Predicted MP (g/kg DM)	93.8	93.23	92.26	116.56

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[7]Elanco Animal Health, Greenfield, IN.

All cows were fed for ad libitum intake once daily at approximately 0800 h and feed delivered and refused was recorded daily. Samples of rations and ingredients were collected weekly and a subsample was used for dry matter determination. Four week composites of all rations and ingredients were analyzed for nutrient composition by wet chemistry methods (Cumberland Valley Analytical Services, Hagerstown, MD). Milk weights were recorded daily and milk samples were collected at three consecutive milkings once per week and analyzed for fat, true protein, lactose, total solids, urea nitrogen, and somatic cell count (Dairy One Laboratories, Ithaca, NY). Body weights were measured weekly. Body condition scores were assigned by two scorers weekly using a 5-point scale (Wildman et al., 1982) and the average of the two scorers was used for analysis. Data were collected through 63 DIM.

To determine degree of compensated metabolic acidosis, clean midstream urine samples were collected on one day during the week before assignment to treatments and three times weekly during the prepartum period at 9 h post feeding and urine pH was measured by a calibrated, glass electrode pH meter (model UP-5 pH meter, Denver Instruments, Denver, CO). Samples of blood were obtained from each cow via coccygeal venipuncture into lithium heparin vacutainers at 0700 h on one day during the week prior to assignment to treatment, twice weekly from 24 d prior to expected calving until calving, twice during the first 24 h postcalving, daily through 5 DIM and three times per week thereafter through 56 DIM. Plasma was harvested, snap frozen in liquid nitrogen and frozen at -20°C until analysis. Plasma minerals (Ca, P, Mg, K, Na, Cl, bicarbonate, and anion gap) were determined on one sample per a week prepartum and on all samples collected through 14 DIM. Samples were sent to the Cornell Animal Health and Diagnostic Center (Ithaca, NY) for mineral panel analysis.

Repeated measures data were analyzed using the REPEATED statement in the MIXED procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC). Prepartum and postpartum data were analyzed separately. Postpartum intake, milk production and milk composition were analyzed as wk 1 to 3 and wk 1 to 9 to determine effects that were manifested primarily in the early lactation period. The effects of treatment, time, parity (2^{nd} vs. 3^{rd} and greater), and all two way interactions were included in the model, when $P>0.10$ two way interactions with parity were removed from the model. Covariate measurements were included for plasma variables, urine pH, intake, and milk production. For repeated measures variables, four covariance structures were tested (autoregressive, heterogeneous autoregressive, compound symmetry and heterogeneous compound symmetry) and the covariance structure with the Akaike's information criterion closest to zero was used.

The Kenward-Roger option was used in the model statement to estimate degrees of freedom. F-tests for differences between treatment groups at individual timepoints were determined using the slice option in the LSMEANS statement. Linear and quadratic effects of decreasing DCAD were tested using orthogonal

contrasts. Fisher's exact test was used to determine differences in frequency of hypocalcemia for parity groups by day after calving. Least squares means are reported throughout. Significance was declared at P≤0.05 and trends are discussed at 0.05<P<0.10.

RESULTS

Feeding decreasing DCAD in the prepartum period resulted in a quadratic effect on urine pH (P<0.01) as depicted in Figure 1. Mean urine pH during the treatment period was 8.20, 7.84 and 5.98, for CON, MedDCAD and LowDCAD, respectively. These results suggest that the dietary strategies employed were effective in modulating cow physiology consistent with the principles of dietary DCAD manipulation.

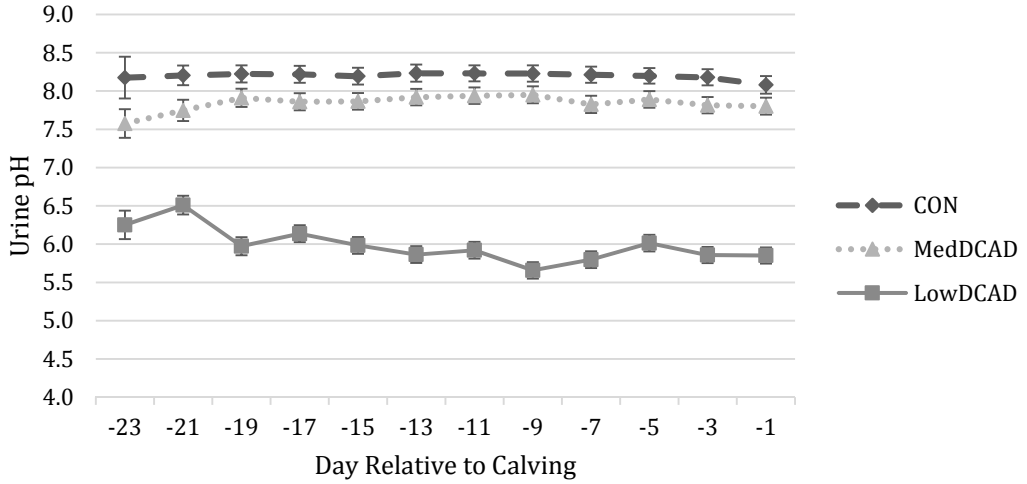

Figure 1. Urine pH least squares means and standard errors during the treatment period for cows fed one of three levels of DCAD prepartum.

Prepartum plasma mineral concentrations were not affected by DCAD treatment with the exception of a trend for a treatment by parity interaction for prepartum plasma magnesium. Older cows (3rd lactation and greater) fed the LowDCAD diet prepartum had lower plasma magnesium prepartum compared to older cows fed the CON and MedDCAD diets (1.89, 1.85 and 1.76 mg/dL for CON, MedDCAD and LowDCAD, respectively; P=0.07). There was a linear effect on mean postpartum plasma calcium over the first 14 DIM such that decreasing prepartum DCAD resulted in increased postpartum plasma calcium concentrations (8.84, 8.89 and 9.19 for CON, MedDCAD and LowDCAD, respectively; P<0.01) There was also a tendency for decreased postpartum plasma phosphorous with decreasing prepartum DCAD (4.74, 4.67 and 4.49 for CON, MedDCAD and LowDCAD, respectively; P=0.08). A trend for a treatment by day interaction was observed for postpartum plasma calcium (P=0.08) and a significant treatment by day interaction was observed for postpartum plasma magnesium (P<0.01). Plasma calcium was higher, or tended to be higher, for 5 d postpartum for cows

fed a lower DCAD and plasma magnesium was lower for cows fed a lower DCAD for 2 d postpartum (Figure 2).

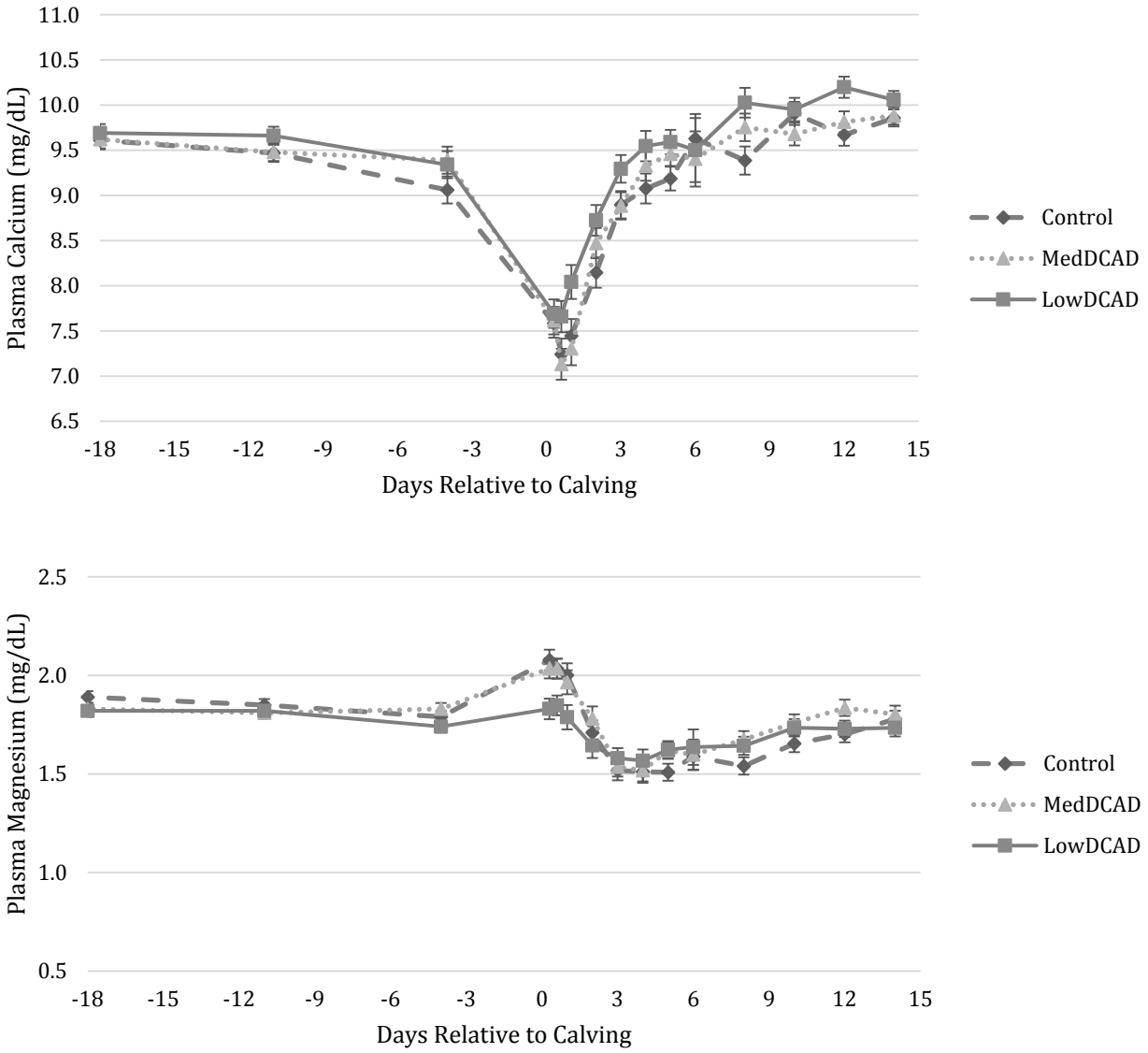

Figure 2. Least squares means and standard errors of plasma calcium (panel A) and plasma magnesium (panel B) in the period around calving for cows fed one of three levels of DCAD in the prepartum period.

A tendency for an interaction between treatment and parity was seen for postpartum plasma calcium such that older cows (3rd lactation and greater) had greater responses in postpartum plasma calcium concentration when fed the LowDCAD diet prepartum compared to the second lactation cows (Figure 3; P=0.06).

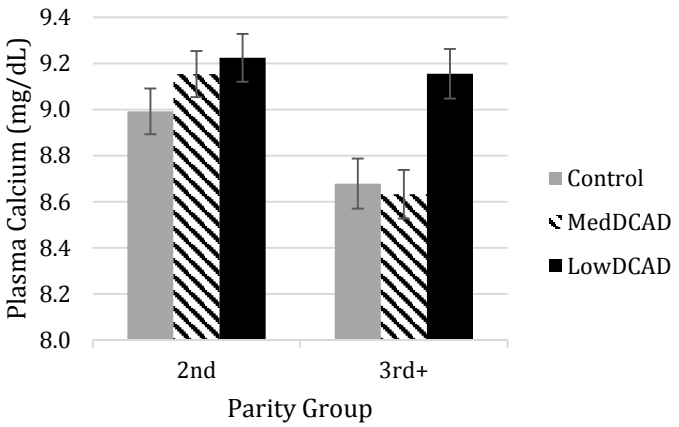

Figure 3. The interaction between parity group and prepartum DCAD level for mean plasma calcium over the first 14 DIM.

The effect of prepartum DCAD level on postpartum hypocalcemia incidence by day was also determined by testing the difference in the proportion of cows classified as hypocalcemic (plasma calcium <8.5 mg/dL) between treatments at each sampling point through 5 d postpartum. For second lactation cows, no difference in hypocalcemia incidence was observed between treatment groups at any of the sampling points in the 5 d postpartum. Incidence of hypocalcemia for older cows in each treatment group by day is shown in Figure 4. For older cows, there tended to be a difference in hypocalcemia incidence at the second sampling on d 0 (approximately 17.5 h postpartum), with significant differences in hypocalcemia incidence at d 1 (85%, 100% and 57% for CON, MedDCAD, and LowDCAD, respectively; P=0.01) and d 2 (69%, 57% and 14% for CON, MedDCAD and LowDCAD, respectively; P<0.01).

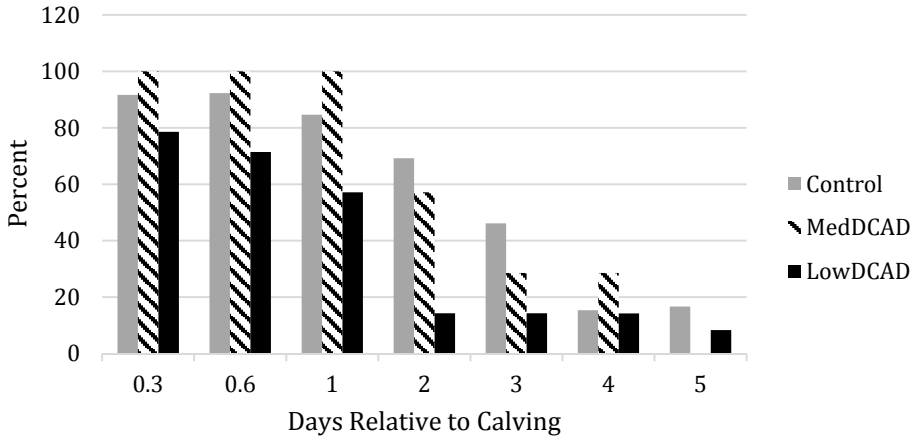

Figure 4. Proportion of cows in each treatment group entering their third lactation or greater with plasma calcium <8.5 mg/dL for each sampling point in the 5 days postpartum.

The effect of decreasing prepartum DCAD on DMI in the prepartum and postpartum periods is described in Table 2. A quadratic effect was seen such that cows fed MedDCAD in the prepartum period had the highest prepartum DMI (P<0.01); however, DMI were similar and within desired ranges for all groups during the prepartum period (14.6, 15.1, and 14.1 kg/d for CON, MedDCAD and LowDCAD, respectively). Individual F-tests indicated that intakes differed only at wk -4 and -3 relative to calving, with no difference in DMI detected between treatment groups at wk -2 and -1. A linear effect on postpartum DMI as a percent of body weight was seen such that cows fed decreasing DCAD prepartum had increasing postpartum intake as a percent of body weight in wk 1 to 3 (P=0.03), this effect tended to carry out through wk 9 of lactation (P=0.07). No effect of prepartum DCAD level on calculated energy balance was seen in the prepartum or postpartum periods.

Table 2. Least squares means of dry matter intake in the prepartum and postpartum periods for cows fed one of three prepartum DCAD levels.

	Prepartum Diet				P-values		
Variable	CON	MedDCAD	LowDCAD	SEM	Linear Contrast	Quadratic Contrast	Trt×Wk
Prepartum							
DMI, kg/d	14.6	15.1	14.1	0.2	0.15	0.01	0.45
DMI, % of BW	1.87	1.89	1.80	0.03	0.16	0.16	0.34
Energy Balance, Mcal/d	5.90	6.41	5.68	0.35	0.66	0.14	0.57
Postpartum (wk 1 to 3)							
DMI, kg/d	21.0	21.7	22.3	0.5	0.07	0.88	0.24
DMI, % of BW	2.94	3.04	3.15	0.07	0.03	0.99	0.37
Energy Balance, Mcal/d	-8.44	-8.51	-8.54	0.92	0.94	0.98	0.60
Postpartum (wk 1 to 9)							
DMI, kg/d	24.7	25.7	25.3	0.4	0.28	0.14	0.49
DMI, % of BW	3.48	3.61	3.61	0.05	0.07	0.23	0.48
Energy Balance, Mcal/d	-3.97	-3.39	-3.96	0.53	0.99	0.36	0.58

There was a linear effect of feeding a decreasing DCAD in the prepartum period on milk yield in wk 1 to 3 of lactation (P=0.03) such that cows fed decreasing prepartum DCAD had increasing postpartum milk yield (40.5, 42.1, and 43.8 kg/d for CON, MedDCAD and LowDCAD, respectively). A trend for a linear increase in fat-corrected milk yield (P=0.07) was seen for cows fed decreasing DCAD. Lactose yield (P=0.02) and total solids percent (P=0.01) linearly increased with decreasing DCAD, resulting in a trend for a linear increase in total solids yield (P=0.06) and energy corrected milk yield (P=0.08). A linear decrease in protein percent was observed with decreasing DCAD in wk 1 to 3 postpartum (P<0.01); however, there was no difference in protein yield. Milk urea nitrogen linearly decreased with decreasing DCAD (P=0.04). Results for milk production and composition in wk 1 to 3 are shown in Table 3. For data collected in wk 1 to 9, a linear effect of lower protein percent for cows fed a lower DCAD

remained (2.99, 2.98, and 2.84% for cows fed CON, MedDCAD, and LowDCAD, respectively; P=0.02) as well as a linear decrease in total solids percent (12.51, 12.51, and 12.28% for cows fed CON, MedDCAD, and LowDCAD, respectively; P=0.02). In wk 1 to 9, numerical differences in milk yield (47.1, 48.5, and 48.7 kg/d for cows fed CON, MedDCAD, and LowDCAD, respectively) and fat-corrected milk yield (48.6, 49.8, and 50.1 kg/d for cows fed CON, MedDCAD, and LowDCAD, respectively) were observed but there were no significant differences.

Table 3. Least square means for milk yield and milk composition during wk 1 to 3 for cows fed one of three levels of DCAD prepartum.

	Prepartum Diet				P-values		
Variable	CON	MedDCAD	LowDCAD	SEM	Linear Contrast	Quadratic Contrast	Trt×Wk
Milk yield, kg/d	40.5	42.1	43.8	1.1	0.03	0.97	0.35
Fat, %	4.38	4.36	4.24	0.08	0.21	0.63	0.10
Fat, kg	1.74	1.81	1.87	0.06	0.13	0.99	0.58
3.5 % FCM, kg/d	45.6	47.5	49.3	1.4	0.07	0.98	0.52
True protein, %	3.54	3.49	3.27	0.07	0.01	0.33	0.36
True protein, kg	1.36	1.42	1.42	0.34	0.21	0.57	0.09
Lactose, %	4.64	4.67	4.69	0.03	0.25	0.94	0.38
Lactose, kg	1.89	1.98	2.09	0.06	0.02	0.84	0.02
Total Solids, %	13.63	13.61	13.27	0.10	0.01	0.20	0.10
Total Solids, kg	5.42	5.65	5.86	0.17	0.06	0.96	0.13
ECM, kg/d	46.1	48.0	49.5	1.4	0.08	0.89	0.39
ECM/DMI	2.22	2.25	2.28	0.07	0.55	0.99	0.71
MUN, mg/dL	10.32	9.72	9.44	0.30	0.04	0.67	0.17
SCS	2.62	3.26	2.73	0.25	0.75	0.06	0.27

CONCLUSIONS AND IMPLICATIONS

Full anion supplementation to a low potassium ration in the prepartum period, with targeted urine pH values of 5.5 to 6.0, resulted in improved postpartum calcium status when compared to a low potassium ration with either zero or partial anion supplementation. Cows entering their third lactation or greater benefited the most from full anion supplementation and had significantly decreased incidence of hypocalcemia. Dry matter intake and milk production both linearly increased postpartum as prepartum DCAD decreased, particularly in the first 3 weeks of lactation. While some benefits are seen with partial anion supplementation, this study would suggest that full anion supplementation is necessary for improved calcium status as well as improved performance.

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CAN FEEDING DEFATTED MICROALGAE PRODUCE HEALTHIER ANIMAL FOODS?

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INTRODUCTION

The increasing demand of good quality animal products and limited supply of traditional feed lead to exploration of alternative and sustainable food and feed resources. Microalgae are microscopic or single cell algae, usually found in freshwater and marine systems (Thurman, 1997). Recently, they have gained popularity as a feedstock for biofuel production (Chisti, 2007). After oil extraction, the defatted microalgal biomass still contains good amounts of polyunsaturated fatty acids (PUFA), protein, carbohydrates, minerals, and vitamins (Brune et al., 2009; Shields and Lupatsch, 2012). PUFA, particularly n-3 fatty acids, in defatted biomass may bolster its appeal as a source for developing n-3 fatty acids-enriched animal products (Lum et al., 2013). Likewise, the desirable profile of protein, mineral and vitamin makes microalgae a potential replacement for soybean or corn meal in animal feed (Austic et al., 2013). In this paper, potential utilization of defatted microalgae in producing health-value improved animal foods will be discussed.

ENRICHMENT OF N-3 FATTY ACIDS IN CHICKEN EGGS AND MEAT

Dietary n-3 fatty acids can impact the n-3 fatty acid pattern in animal products. For example, the eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) concentrations in the yolk are highly dependent on dietary EPA and DHA concentrations (R^2 = 0.89) (Koppenol et al., 2014). EPA supplied from diet can be converted into DHA (Huang et al., 1990; Lemahieu et al., 2015). Concentration of tissue EPA is usually below 1% and reaches saturation in eggs at about 22 mg/egg (Coorey et al., 2015). Dietary α-linolenic acid (ALA) can contribute to ALA in tissues and can also be converted to EPA and DHA through desaturation and elongation. In humans, the conversion rate from ALA to EPA and DHA is less than 5% (Gerster, 1998; Brenna, 2002). However, chicks have shown a relatively higher conversion efficiency due to high activity of elongases 2 and 5 (Gregory and Geier, 2013). In this case, dietary supplementation of ALA has potential of enriching EPA and DHA in poultry products.

As a rich source of n-3 fatty acids, particularly EPA and(or) DHA, microalgae has been added to diets of dairy cattle, pigs and layers to enhance DHA contents in their products (Sardi et al., 2006; Stamey et al., 2012; Trentacoste, 2013; Park et al., 2015) The defatted microalgae (*Nannochloropsis oceanica*) used in our lab contained a high concentration of EPA (16.4%), but a low amount of ALA (0.12%). In a broiler chick study, we observed an increase of n-3 fatty acids in the liver, breast, and thigh with increasing doses of microalgae supplemented to the diet. Similar effects were observed in laying

hens. When hens were fed with 0-23% defatted *N. oceanica*, the level of n-3 fatty acids and n-3 to n-6 ratios were increased in their liver, breast, thigh, and eggs with the acclimation of microalgae supplementation. In eggs, for example, 23% microalgae supplementation resulted in an approximately 3-fold increase in DHA over the control. EPA concentration was also significantly increased by the microalgae feeding while its level was undetectable in control eggs. In another study, 3 or 5% flaxseed oil (FO) was supplemented alone or together with full-fat *Staurosira sp.*, defatted *N. oceanica,* and *Desmodesmus sp*. Surprisingly, FO alone efficiently increased ALA, EPA and DHA levels in eggs. Addition of full-fat *Staurosira sp.* did not have an obvious enhancement in egg n-3 fatty acids, but prevented 5% FO-induced negative effects on weight gain. While defatted *N. oceanica* (16.4% EPA) or defatted *Desmodesmus sp.* (22.1% ALA) was expected to induce more desirable changes in egg PUFA than full-fat *Staurosira sp.* (no ALA, only 2.3% PUFA), it turned out that together with 3% FO, all algae-supplemented groups accumulated similar amounts of ALA, EPA and DHA. Diet with 3% FO might provide sufficient ALA to saturate the tissue capacity of synthesizing EPA and DHA and thereby limiting any observable benefits of defatted *N. oceanica* and *Desmodesmus sp*. In a later study, we tried to reduce the FO to 1.5%. At the low level of FO, addition of 5% defatted *N. oceanica* exerted some positive effects on EPA and DHA in the liver, plasma, and eggs.

PROTEIN, MINERALS, VITAMINS, AND SO ON

There has been increased interest in microalgae as a viable protein source in animal feeds, particularly the defatted biomass from the biofuels industry (Lum et al., 2013). Microalgae can be high in essential amino acids comparable to soybean meal (Becker, 2007; Tibbetts et al., 2014). Previous work in our lab has shown that pigs and chicks were able to tolerate a moderate incorporation of microalgae into their diets as a protein source without any detrimental effects on their growth performance or egg production (Ekmay et al., 2014; Gatrell et al., 2014; Ekmay et al., 2015).

In addition to protein, microalgae are a promising source for minerals and vitamins. Our group have demonstrated the incorporation of defatted microalgal biomass into diets to be an adequate source of iron in weanling pigs (Kim and Lei, 2014). Volkman et al. summarized literature on microalgal strains that showed microalgae to be a bountiful source of ascorbic acid, β-carotene, niacin, α-tocopherol, and many other vitamins (Volkman et al., 2006). Apart from meeting nutritional requirements, the antioxidant activity of some of the vitamins such as β-carotene and α-tocopherol are potentially able to assist in the stabilization of eggs enriched with n-3 fatty acids (Sies and Stahl, 1995), similar to the role of vitamin E and organic Selenium (Ren and Perez, 2013).

SUMMARY

Defatted microalgal biomass supplementation can be an excellent source of protein, vitamins, minerals, and PUFA for animal nutrition (Spolaore and Joannis-Cassan, 2006). More importantly, the inclusion of the biomass in animal diets has potential to produce health value-added animal products such as EPA/DHA-enriched

eggs and meat. Future research will be required to find out the optimal species and doses of the biofuel-producing microalgae that can be used to improve nutritional quality, health value, and economic efficiency of animal products.

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SHREDLAGE IN DAIRY CATTLE RATIONS

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Dairy producers are growing more acres of corn silage and feeding higher levels of corn silage in dairy rations. This is the result of better agronomic information on corn silage management and improved nutritive value of corn silage. Factors including better hybrid genetics, selection of hybrids for fiber and/or starch digestibility, kernel processing (KP) and more attention to harvest dry matter and silo management are responsible for the improvement in nutritive value of corn silage. A new processing process called shredlage was introduced about 3 years ago. This process uses a special corn silage head that replaces the KP head on a Claas forage harvester. This process rips or tears the corn stalk into longer pieces. The rolls in the harvester are also tightened to better breakup the corn kernel. The TLC (theoretical length of cut) is recommended to be set at 26 mm for corn silage with a moisture content of 65 to 70%. The guideline is to have the rolls set at 1.5 to 1.75 mm. As corn silage gets drier (36 – 40% DM), the TLC is reduced to 21 to 23 mm and the rolls are set at 1.25 to 1.5 mm. Harvesting corn silage as shredlage is a slightly slower process, requires more power and uses more fuel. Custom harvesters are charging an extra $1-2 per ton to account for these differences.

Particle Size

Table 1 contains data on the impact of TLC on corn silage particle size distribution using the Penn State forage particle separator. The percent coarse material (top screen) decreases as TLC decreases. However, the total on the top 2 screens changes very little as TLC changes for the 3 shredlage samples. There was a lower total on the top 2 screens for the KP corn silage. The higher percent on the top screen for the 30 mm TLC shredlage may increase the risk of sorting.

Table 1. Corn Silage Particle Size Distribution[a]

Shredlage	% on Top Screen	% on Screen 2	% on Screen 3	% in the Pan
30 mm TLC	35	45	19	1
22 mm TLC	18	58	22	2
17 mm TLC	9	71	18	2
Conventional KP	8	60	30	3

[a] Source: Michelle Woodman, Landmark Services Cooperative, 2014

Previous Research

There have been 3 research trials conducted using shredlage in dairy cattle rations. Two of these were done at the University of Wisconsin by Dr. Randy Shaver and his research group. The first trial at the University of Wisconsin compared rations containing 50% of the total ration dry matter (DM) as either shredlage or KP corn silage

(Ferraretto and Shaver, 2012). These rations also contained 10% of the ration DM as alfalfa silage and 40% grain. This was a pen study with 14 pens of 8 cows in each pen. The rations were fed for an 8 week period. The key results from this trial are in Table 2. Both dry matter intake and energy corrected milk tended to be higher for cows fed the shredlage ration. Total tract starch digestibility was significantly higher for cows fed the shredlage ration. Feed sorting was not different between the 2 treatments.

Table 2. University of Wisconsin – Trial 1

	Shredlage Ration	**KP Ration**
Corn silage particle size[a]		
% on top screen	31.5	5.6
% on screen 2	41.5	75.6
% on screen 3	26.2	18.4
CSPS,% [b]	75	60.3
TMR particle size		
% on top screen	15.6	3.5
% on screen 2	38.2	52.9
% on screen 3	38.9	35.8
TMR Nutrient Composition		
CP, % of DM	17.2	17.3
NDF, % of DM	28.1	28.3
Starch, % of DM	25.4	25.5
Dry matter intake, lbs./day	55.9	54.3
Milk, lbs./day	95.9	94.2
Energy corrected milk, lbs./day	99.2	97.2
Milk fat, %	3.74	3.7
Milk true protein, %	3.18	3.21
Lbs. ECM/lb. DMI	1.76	1.77
30 hour in vitro NDF digestibility, % of NDF	50.9	50.8
7 hour in vitro starch digestibility, % of starch	78.5	75.4
Total tract starch digestibility, % of starch, week 8	99.4	97.5

[a] Using the Penn State forage particle separator
[b] Corn silage processing score

A second trial was conducted by the Wisconsin workers to examine the impact of shredlage when BMR corn silage was used (Vanderwerff et. al., 2015). A treatment was also added that replaced some of the corn silage with dry hay. There were 15 pens of 8 cows each in this trial and the rations were fed for 14 weeks. Table 3 contains the information from this trial. Milk production tended to be higher for cows fed the shredlage

ration compared with the KP ration. Milk production was significantly lower for cows fed the ration containing chopped hay but these cows had a higher milk fat content. Energy corrected milk was similar for the 3 rations. There were no differences in rumination time between the 3 rations.

Table 3. University of Wisconsin – Trial 2

Item	Shredlage ration	KP ration	KP+Hay ration
Corn silage, % of DM	45	45	35
Alfalfa silage, % of DM	10	10	10
Chopped hay, % of DM	-	-	10
Ration CP, % of DM	16.3	16.2	16.7
Ration NDF, % of DM	31.6	31.9	32.5
Ration Starch, % of DM	28.7	29.3	27.1
Dry matter intake, lbs./day	59.2	58.7	58.7
Milk, lbs./day	112.9	110.2	104.3
Energy corrected milk, lbs./day	108.9	106.9	106.5
Lbs. ECM/lb. DMI	1.84	1.82	1.82
Milk fat, %	3.29	3.31	3.67
Milk true protein, %	3.1	3.13	3.14
Rumination, minutes/day	504	503	499
Apparent total tract NDF digestibility, % of intake	38.9	40.8	41.7
Apparent total tract starch digestibility, % of intake	99.1	98.6	98.9

Cornell Trial

A trial was conducted at Cornell comparing shredlage versus KP corn silage. Conventional corn silage hybrids were used and 2 forage harvesters ran side by side during harvest. One had a shredlage head and the other a KP head. The corn silage from each harvester went into a separate bunker silo. This was done to minimize any

differences between the processing methods due to hybrid, maturity and dry matter. Samples of the incoming corn silages were evaluated using the Penn State forage particle separator and the 32 ounce Pioneer cup and the results used to adjust harvester settings during harvest. The bunker silos were opened after about 8 months of fermentation. The rations fed contained 50% corn silage, 14% alfalfa silage and 36% grain on a dry matter basis. This was a pen study using 4 pens of 32 cows per pen. Rations were rotated between pens each 4 weeks so that all pens were fed both shredlage and KP rations during the trial. Table 4 contains key information from this trial. There were no differences in dry matter intake, milk production or milk components in our trial. Total tract starch digestibility was similar between the 2 rations.

Survey Results

A survey of dairy producers' corn silage harvest and processing methods was recently reported (Salvati et. al., 2014). There were 69 dairy farms included in this survey. Some of the results from this survey are:

- 61% of the herds used a Class harvester with a shredlage processor.
- 95% of the herds stored corn silage in bunker silos.
- Theoretical length of cut:
    - > 26 mm = 14%
    - 26 mm = 47%
    - 22 mm = 32%
    - <19 mm = 7%
- Roll gap setting:
    - > 2.5 mm = 3%
    - 2.5 mm =16%
    - 2 mm = 48%
    - 1.5 mm = 18%
    - <1 mm = 15%
- Mean forage particle size (only shredlage herds):
    - 19.6% on the top screen of the Penn State box (range = 7.2 to 39.9%).
    - 75.7% on the top 2 screens of the Penn State box (range = 65.1 to 85.9%).
- Percent forage in the ration:
    - < 50% = 58.1%
    - 50 – 60 = 33.9%
    - >60 = 8.1%
- Percent corn silage in the diet when using shredlage:
    - Increased amount = 46.9%
    - No change = 50%
    - Reduced amount = 3.1%

- 40% of the herds reduced the amount of hay or straw fed when shredlage was used.
- 45.5% of the herds indicated an increase in milk production when shredlage was used while 39.4% indicated no change in milk production.

- 40% of the herds felt that silage pack density increased when shredlage was used while 43.1% indicated no change in silo pack density.

Table 4. Cornell Trial Results

Item	Shredlage Ration	KP Ration
Corn silage particle size[a]		
% on top screen	34.1	18.3
% on screen 2	40.2	57.2
% in pan	25.7	24.4
CSPS[b]	59.5	50.7
Corn silage composition		
DM, %	32.1	30.7
CP, %	7.34	7.9
NDF, %	41.7	44.1
30 hour in vitro NDFD, % of NDF	57.1	56.6
Starch, %	33.5	30.7
7 hour in vitro starch digestibility, % of starch	81.7	76.8
Packing density, lbs./cubic foot, wet basis	49.8	51.9
Packing density, lbs./cubic foot, DM basis	16.3	16.5
Ration CP, % of DM	17.9	18
Ration NDF, % of DM	33.7	33.2
Ration starch, %	23.1	22.9
Pen DMI, lbs./day	55.9	56.3
Milk, lbs./day	90.4	90.9
Milk fat, %	3.7	3.71
Milk true protein, %	3.01	3.0
Apparent total tract starch digestibility, % of starch	97.2	97.1
Apparent total tract NDF digestibility, % of NDF	45.6	46.6

[a] Penn State forage particle separator

[b] Corn silage processing score

SUMMARY

Shredlage provides another option for processing of corn silage at harvest. The primary advantage appears to be a higher ruminal and total tract starch digestibility due to more extensive kernel processing. In some herds, this improved starch utilization may support removing 1 – 2 pounds of corn grain from the ration. There seems to be limited effect of shredlage on NDF digestibility. In 2 of the trials reported, there has been a tendency for small increases in milk production when KP corn silage is replaced with shredlage. A key question is how much benefit there would be to shredlage if the corn silage processing scores were the same for KP processed corn silage and shredlage. One benefit of shredlage has been to raise the awareness of the importance of kernel processing and the relationship with total tract starch utilization. One measure of this has been the increase in corn silage processing scores reported by forage labs in the last few years.

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