STARCH AVAILABILITY, MEASUREMENT AND IMPLICATIONS FOR RATION FORMULATION

M. S. Allen
Department of Animal Science
Michigan State University

SUMMARY

Concentration and ruminal digestibility of starch in rations of lactating cows has important effects on productivity. Starch is more digestible and less filling than forage fiber and provides more glucose precursors than fiber from any source. Ruminal fermentability of starch is affected by grain and endosperm type, processing and conservation method, and diet and animal factors, and affects production of fermentation acids and microbial protein in the rumen. Excessive ruminal fermentability can decrease fiber digestibility, efficiency of microbial protein production, and alter ruminal biohydrogenation, decreasing synthesis of milk fat and increasing energy partitioned to body condition at the expense of milk.

The concentration and ruminal fermentability of starch affects feed intake, and energy partitioning of cows differently as they progress through lactation. High-producing cows in early to mid-lactation thrive on high-starch rations with highly fermentable starch sources while starch concentration and fermentability should decrease as lactation progresses to maintain yield of milk fat and prevent excessive body condition. Highly fermentable starch sources should be limited in rations for the first two weeks following parturition to avoid further depression in feed intake, and decrease risk of ruminal acidosis and displaced abomasum. Grouping cows by physiological state (fresh, early to mid, maintenance) is required to formulate diets for starch to optimize health and production.

INTRODUCTION

Starch is a highly digestible and energy dense feed component that typically ranges from less than 20% to greater than 28% in rations fed to lactating dairy cows. Forages are supplemented with cereal grains to increase energy density, provide glucose precursors, and decrease the filling effects of rations. Starch is composed of polymers of glucose (amylose and amylopectin) with bonds that are readily cleaved by mammalian enzymes. However, starch is packaged in granules that are embedded in a protein matrix in the seed endosperm, which varies in solubility and resistance to digestion (Kotarski *et al.*, 1992). These differences in endosperm type have great effects on ruminal fermentability of starch, which ranges widely; ruminal fermentability of starch from various cereal grains ranges from less than 30% to more than 90% (Nocek and Tamminga, 1991; Firkins *et al.*, 2001). Altering the concentration and ruminal fermentability of starch in rations affects digestibility of starch (Ngonyamo-Majee *et al.*, 2008), ruminal pH and fiber digestibility (Firkins *et al.*, 2001), and the type, amount, and temporal absorption of fuels (e.g. acetate, propionate, lactate, glucose) available to the

cow (Allen, 2000). This has great effects on lactational performance by affecting energy intake and partitioning as well as absorbed protein (Allen *et al.*, 2009). In addition, effects on animal performance depend upon physiological state of cows, which varies greatly through lactation (Allen *et al.*, 2005). Therefore the optimum concentration and ruminal fermentability of starch in rations of lactating cows vary through lactation. The objective of this paper is to discuss what determines site of digestion and total tract digestibility of starch, effects of concentration and ruminal fermentability of starch on animal performance, and considerations related to starch for formulating diets for lactating dairy cows.

STARCH FERMENTABILITY

Ruminal fermentability of starch is highly variable and affected by grain type, vitreousness, processing (e.g. rolling, grinding, steam flaking), conservation method (dry or ensiled), ration composition, and animal characteristics. Starch in wheat, barley and oats is generally more readily fermented than starch in corn, and starch in sorghum is most resistant to fermentation in the rumen and digestion by the animal (Huntington, 1997). These differences are largely because of differences in endosperm type rather than differences in starch composition (amylose vs. amylopectin) per se. Floury endosperm contains proteins that are readily solubilized, allowing greater access of enzymes to starch granules while vitreous endosperm contains prolamin proteins that are insoluble and resistant to digestion, decreasing access of enzymes to starch granules (Hoffman and Shaver, 2010). Starch sources vary in amount and proportion of the two types of endosperm and there is large variation in vitreousness of the endosperm (percent of the total endosperm that is vitreous) among varieties within certain grain types. Endosperm vitreousness in corn harvested dry ranges from 0% to greater than 75% and corn with more vitreous endosperm is more resistant to both particle size reduction by grinding and digestion (Hoffman *et al.*, 2010) than corn with more-floury endosperm. Vitreousness increases with increasing maturity at harvest (Phillipeau and Michalet-Doreau, 1997), so differences among corn hybrids are greatest when field dried. Because corn silage is harvested earlier than high moisture corn, the grain will have less vitreous endosperm and more moisture when harvested from the same field as whole plant silage compared with high-moisture corn. However, there can be large differences in vitreousness within corn silage harvested between 30% and 40% dry matter and within high moisture corn harvested between 60% and 75% dry matter (40 and 25% moisture) from the same field.

When grains are ensiled, ruminal fermentability of starch can be greatly affected by both grain moisture concentration and storage time. This is because ensiling solubilizes endosperm proteins over time, increasing starch fermentability. The increase in protein solubility and starch fermentability over time is greatest for grains with higher moisture concentration (Figure 1; Allen *et al.*, 2003). Therefore, the change is greatest for wetter corn silage and least for drier, high-moisture corn. This change is greatest over the first few months of ensiling and must be anticipated and accounted for when formulating rations. Because of this, it is recommended to wait several months after ensiling before feeding corn silage (Allen, 1998). However, the change continues

for months at a slower rate and corn silage and high moisture corn stored for long periods (one or two years or more) can be difficult to feed in high concentrations because it is so readily fermented.

Processing increases rate of starch digestion and the effects are greater for grains with more vitreous endosperm such as sorghum and corn (Huntington, 1997). Access of enzymes to starch granules is increased by steam flaking, which causes swelling and disruption of kernel structure, and reducing particle size by rolling or grinding whole grains, or processing silage to crush kernels, which greatly increases surface area. Dry grains can be finely ground, greatly decreasing effects of endosperm vitreousness on ruminal fermentability. Processing (rolling) corn silage is not as effective at increasing surface area as fine grinding; processing can reduce, but not eliminate, differences in digestibility of sources varying in vitreousness.

MEASURING STARCH CONCENTRATION AND FERMENTABILITY

Starch concentration is relatively consistent within cereal grain types but varies greatly within forages containing starch such as corn silage and small grain silages. Therefore, book values for starch concentration may be acceptable for cereal grains but starch concentration must be measured for forages from grain crops. For instance, the starch concentration of corn silage varies from less than 20 to over 50% of DM depending upon grain concentration, which, in turn is dependent upon genetics, environment and maturity at harvest. The starch concentration of corn silage is inversely related to concentration of NDF; fibrous stover fraction of the plant is enriched if kernels don't fill.

The non-fiber carbohydrate (NFC) concentration of diets should not be relied upon as a measure of starch concentration. The NFC fraction is calculated by subtracting measured components (NDF, CP, ether extract, ash) from total DM. It contains other carbohydrates such as sugars and pectin and can be underestimated to the extent that non-protein nitrogen is present. While starch, sugars and pectin are generally highly digestible, their effects on rumen microbial populations and fuels available to the animal differ greatly. Starch that is ruminally-fermented increases propionate production in the rumen (Sutton *et al.,* 2003) and starch that escapes ruminal fermentation provides glucose that is absorbed or metabolized to lactate in the small intestine (Reynolds *et al.*, 2003). Sugars are nearly completely fermented in the rumen and generally increase butyrate production (Oba, 2011). Most strains of pectin-degrading rumen bacteria produce acetic and formic acids and relatively little propionic acid (Dehority, 1969). Propionic and lactic acids are glucose precursors while formic, acetic, and butyric acids are not. In addition, propionate can decrease feed intake under some conditions (Allen, 2000) and starch, sugars, and pectin have different effects on microbial populations in the rumen that can affect fiber digestion and ruminal biohydrogenation of fatty acids. Therefore, NFC is not a useful proxy for starch when formulating rations for lactating cows.

Table 1. Effects of dietary treatment on passage rate (kp) of starch from the rumen[1].

Experiment	Treatment	kp, %/h	*P* value
Oba and Allen, 2000b	bm3 corn silage	12.9	0.02
	control corn silage	10.6	
	29% diet NDF	14.5	<0.0001
	38% diet NDF	9.0	
Oba and Allen, 2003a	high-moisture corn	15.4	0.07
	dry ground corn	19.7	
Voelker and Allen, 2003b	high-moisture corn	15.9	0.01
	24% beet pulp	23.5	
Ying and Allen, 2005	high-moisture corn	7.1	<0.0001
	dry ground corn	16.3	
	vitreous endosperm	16.0	<0.001
	floury endosperm	7.5	
Taylor and Allen, 2005	vitreous endosperm	21.2	0.10
	floury endosperm	16.2	
Allen *et al.*, 2008	vitreous endosperm	25.7	<0.001
	floury endosperm	16.0	

[1]Determined by dividing duodenal flux (g/h) by rumen pool size (g) and multiplying by 100.

Relative differences in rate of starch digestion can be determined by *in vitro* starch digestion (IVSD) with ruminal microbes. This can be done by incubating samples over time in rumen fluid with buffered media and evaluating the rate of starch disappearance or, less costly and equally informative, by evaluating starch disappearance over a period of time (e.g. 7 hours). We began using a 7-h incubation time over 20 years ago when our objective was to predict *in vivo* ruminal digestibility of starch because we thought it was a reasonable mean residence time of starch in rumens of lactating cows. However, we subsequently realized that was naïve because ruminal digestibility of starch *in vivo* is highly affected by the enzyme activity of the rumen fluid and particle size of the starch source, and that residence time of starch in the rumen is extremely variable, not only across cows, but also across sources of starch (Table 1). We continue to use IVSD with a 7 h retention time because we think it provides useful information about relative rates of fermentation among starch sources. However, it is very important to know that 7-h IVSD is a relative measure of rate of starch digestion among sources only. Samples must be ground before analysis, which removes important variation for many comparisons (e.g. processed vs. unprocessed corn silage). Comparisons must be done in the same *in vitro* run (at the same time) because IVSD of the same sources is highly variable across runs. This is because enzyme activities (amylases and proteases) of rumen fluid are highly variable from cow to cow, time relative to feeding, and diet consumed. In our laboratory, the coefficient of variation for 7-h IVSD across runs can be as high as 25% even after attempting to minimize variation by taking rumen fluid from several cows fed a specific diet at the same time of day relative to feeding. This is much higher than our coefficient of variation for 30-h *in vitro* NDF digestibility of less than 3%.

Because starch digestion is inhibited by insoluble proteins in the endosperm, the solubility of protein has been measured as an indicator of relative differences in starch digestibility. Like IVSD, determination of protein solubility requires grinding samples, removing variation among sources. Because it is a chemical rather than biological measure, it is less variable across runs than IVSD. Accuracy of ruminal starch digestibility prediction from protein solubility is limited by the relationship between protein solubility and rate of starch digestion as well as limited knowledge of passage rate of starch from the rumen. Therefore, like IVSD, measures of protein solubility provide some information related to ruminal starch digestion but cannot be used to measure ruminal starch digestibility accurately.

Prediction by Models

Although measurement of digestion rate of feed fractions *in vitro* and *in situ* can provide relevant information regarding relative differences among feeds, absolute, not relative, values are required by models to predict ruminal digestibility. Therefore, despite their promise, ration formulation models that include rumen sub-models such as CNCPS do not predict ruminal starch digestibility accurately even if *in vitro* rates of starch digestion are used as inputs (Allen, 2011). Accuracy and precision of prediction of ruminal starch digestibility was poor for several models including CPM and AMTS in a recent evaluation; AMTS and CPM over-predicted ruminal starch digestibility for corn grain by over 25 percentage units (~80% vs. 55%), leading the authors to conclude that the model estimates were not useful (Patton *et al.*, 2012). The primary factors limiting accurate determinations of digestion rate *in vitro* or *in situ* are 1) the inability to mimic the increase in surface area and breakdown of particle size by rumination, 2) variation in enzyme activity and ratio of enzyme to substrate in the rumen over time, and 3) lack of understanding and data on passage rates of starch.

Rates of starch digestion determined *in vitro* are much different than actual rates of digestion because feed particles containing starch that are consumed by cows are larger than what is required for *in vitro* analysis and because enzyme activity in the rumen is extremely variable depending upon diet, time since eating, and the cow. Grinding feeds is necessary to obtain uniform samples for analysis in the laboratory but grinding increases surface area accessible to microbes, increasing rate of digestion compared to intact feeds *in vivo*. On the other hand, not grinding at all will underestimate rate of digestion because feeds are crushed and ground by chewing over time, before they pass from the rumen. This is an unsolvable problem because simulation of the effects of chewing over time of incubation *in vitro* or *in situ* is infeasible.

The high variation in IVSD across runs prompted us to evaluate the effect of rumen fluid sampled before and after feeding on IVSD-7h which was 33% greater after feeding compared to before feeding (41.2 vs. 30.9%, $P < 0.01$; Fickett and Allen, 2002). Enzyme activity related to starch fermentation is also increased with higher starch diets; we reported that the fractional rate of starch digestion determined *in vivo* with the pool and flux method was greater for diets with higher starch concentration and lower NDF from forage (Oba and Allen, 2003a) or beet pulp (Voelker and Allen, 2003b). Therefore,

at least for starch, digestion is a second-order process dependent upon both substrate and enzyme activity. This is a problem for utilization of current data with most existing models in which digestion is modeled as a first-order process dependent on feed characteristics only.

Passage rate of starch was greatly affected by particle size, conservation method, and endosperm type for corn (Table 1; Ying and Allen, 2005; Allen *et al.*, 2008). However, little data exists for passage rates of starch and how it is affected by diet and level of intake. Because passage rate is as important as digestion rate for determining ruminal starch digestibility, accurate predictions by models that use digestion kinetics to predict starch digestibility are not currently possible. In addition, models such as CNCPS that use digestion rates for carbohydrate fractions but passage rates for entire feeds result in even greater inaccuracies for determination of ruminal starch digestion.

PRODUCTION RESPONSE

The filling effects and fermentability of rations are affected by the concentration and ruminal fermentability of starch and can affect DMI, nutrient partitioning, microbial protein production, and total-tract digestibility. Increasing the starch concentration of the ration offered to lactating cows from ~23 to ~34% (~24 to 16% forage NDF, respectively) resulted in variable effects on DMI and FCM yield depending upon the milk yield of cows (range in FCM: ~50 to ~130 lb/d); DMI response to the high-starch, low forage NDF ration increased linearly with increasing milk yield of cows throughout the range while FCM response increased only for cows above ~90 lb/d of FCM (Voelker and Allen, 2003a; Figure 2). Response for DMI was likely because the higher starch diet was less filling (16% forage NDF) and rumen fill is a greater limitation to feed intake as milk yield increases (Allen, 1996), while response for FCM likely depended upon effects of the ration on digestibility and energy partitioning among cows.

The physiological state of animals determines the effects of starch fermentability on DMI (Bradford and Allen, 2007) and production (Bradford and Allen, 2004) responses. High moisture corn compared with dry ground corn had opposite effects on milk yield for cows depending on initial milk yield, with no change for the group overall; high moisture corn increased concentration of milk fat and yield of FCM for cows producing over ~90 lb/day but decreased both for cows producing less than that amount (Bradford and Allen, 2004). Effect of treatment on DMI was not related to milk yield but was affected by physiological state of cows; depression in DMI by the high moisture corn compared with the dry corn treatment was related to plasma insulin concentration and insulin response to a glucose challenge (Bradford and Allen, 2007). Feed intake of cows with greater insulin concentration, and lower insulin response to a glucose challenge, was depressed to a greater extent by high moisture corn compared with dry ground corn. As lactation proceeds and milk yield declines, feed intake is increasingly dominated by metabolic signals. Highly fermentable diets often decrease feed intake in mid to late lactation, likely from stimulation of hepatic oxidation by propionate (Allen *et al.*, 2009). Reducing ruminal fermentability of starch by substituting dry corn for high

moisture corn in rations often increases energy intake and partitioning to milk for these cows.

Several experiments have fed diets differing in starch content in the postpartum period (Andersen et al., 2003; Rabelo et al., 2005; Dann and Nelson, 2011). Increasing diet starch content increased DMI and milk yield in experiments reported by Andersen et al. (2003) and Rabelo et al. (2005) but in those experiments grains were substituted for forage, increasing the forage NDF content of the diet. Forage NDF is very filling (Allen, 2000) and large increases in the forage NDF content of diets in these studies likely contributed to satiety by increasing ruminal distention, especially as lactation progressed and the lipolytic state diminished. Dann and Nelson (2011) substituted corn meal for non-forage fiber sources to increase diet starch content from 21% to 25.5% and the higher starch diet decreased DMI 1.5 kg/d. Non-forage fiber sources are much less filling than forage NDF (Allen, 2000) so the filling effects of the treatment diets were likely much more similar in that experiment than when grains are substituted for forage. To our knowledge, only two previous experiments have evaluated the effects of ruminal fermentability of starch in diets fed to cows in the postpartum period (Dann et al., 1999; Sadri et al., 2009). Increasing ruminal starch fermentability by substituting steam-flaked corn for cracked corn tended to decrease DMI by more than 1 kg/d over the first 63 d postpartum although interactions with time were not reported and greater ruminal fermentability would be expected to have a greater effect in the first few weeks of lactation (Dann et al., 1999). Sadri et al. (2009) compared grains varying in ruminal starch fermentability through the transition period and the more fermentable barley treatment decreased DMI compared with corn during both the prepartum and postpartum periods. These results are consistent with our expectations according to the hepatic oxidation theory of the control of feed intake (Allen et al., 2009).

Energy partitioning between milk production and body condition varies depending upon fuels available and as physiological state changes throughout lactation. Substitution of fiber for starch greatly alters fuels available for intermediary processes and often results in greater partitioning of energy to milk rather than body condition. Substitution of soyhulls for dry ground corn up to 40% of diet DM increased milk fat percent (linearly from 3.60 to 3.91%) and decreased body weight gain (linearly from 1.02 to -0.14 kg/d) with no effect on milk yield (~29 kg/d) and a slight decrease in DMI (tendency, linearly from 23.8 to 22.7 kg/g, Ipharraguerre *et al.*, 2002). We showed that beet pulp decreased BCS without decreasing yields of milk or milk fat when substituted for high-moisture corn up to 12% of diet DM (Voelker and Allen, 2003a). Furthermore, we showed that a 69% forage diet (0% corn grain) containing brown midrib corn silage increased energy partitioned to milk, decreasing body weight gain while numerically increasing FCM yield compared with a 40% forage diet (29 % corn grain) containing control corn silage (Oba and Allen, 2000a). In contrast, DMI and milk yield was reduced when the control corn silage, which had ~20% lower *in vitro* NDF digestibility (46.5% vs. 55.9) than the brown midrib corn silage, was fed in the higher forage diets.

As lactation proceeds, insulin concentration and sensitivity of tissues increase and energy is increasingly partitioned to body condition. Intravenous glucose infusion of

up to 30% of net energy requirement linearly increased plasma insulin, energy balance, body weight and back fat thickness, without affecting DMI or milk yield of mid-lactation cows (Al-Trad *et al.*, 2009). An experiment conducted with cows in the last 2 months of lactation showed that substitution of beet pulp for barley grain linearly decreased body condition score and back fat thickness, maintained milk yield and linearly increased milk fat yield and milk energy output (Mahjoubi *et al.*, 2009). Decreased body condition score and increased milk fat yield might have been because of a linear decrease in plasma insulin concentration which linearly increased plasma NEFA concentration.

High starch diets might result in greater insulin concentration, partitioning energy to adipose at the expense of milk, but they also often result in lower ruminal pH resulting in milk fat depression from altered biohydrogenation of polyunsaturated fatty acids in the rumen reducing milk energy output. While increased energy retention as body condition might be because of increased insulin as observed by Ipharraguerre *et al.* (2002) and Mahjoubi *et al.*, (2009), it might also be a result of altered gene expression in adipose tissue. Harvatine *et al.* (2009) reported that CLA-induced milk fat depression increased gene expression for enzymes and regulators of fat synthesis in adipose tissue. The energy spared from the reduction in milk fat synthesis was likely partitioned toward adipose tissue fat stores. Reducing ration starch concentration by increasing fiber from forages or non-forage fiber sources can maintain milk yield while decreasing gain in body condition.

Increasing ruminal degradability of starch generally increases microbial nitrogen flow to the duodenum but excessive ruminal starch digestion might decrease ruminal fiber digestibility, offsetting its effects (Firkins *et al.*, 2001). In addition, starch sources with faster rates of fermentation might decrease efficiency of microbial protein production; microbial growth can be uncoupled from OM fermentation under some conditions (Russell and Cook, 1995). Greater concentration of starch in rations (32 vs. 21% of DM) increased flow of microbial nitrogen from the rumen with no effect on efficiency of microbial nitrogen production in a study from our laboratory with lactating cows (Oba and Allen, 2003b). However, although ruminal starch digestibility was increased by high moisture corn compared with dry ground in that experiment, high moisture corn decreased efficiency of microbial nitrogen production compared with dry corn and did not affect flow of microbial nitrogen from the rumen. While flow of microbial nitrogen was positively related to true ruminal OM digestibility in that experiment, it was negatively related to rate of starch digestion across all cow period means. Microbial growth might be limited when rate of starch digestion is very fast (Oba and Allen, 2003b). Therefore, increasing ruminal starch degradation by increasing starch concentration of diets might improve flow of microbial nitrogen to the duodenum to a greater extent than increasing ruminal fermentability of starch.

FORMULATING RATIONS FOR STARCH

We know a great deal about what factors affect ruminal digestibility of starch that can be routinely used for ration formulation even if we cannot accurately measure rates of digestion and passage of starch. Starch concentration and ruminal digestibility is so

variable across feeds that we can measure starch concentration and use literature values for ruminal digestibility for initial formulation which can be adjusted using qualitative knowledge of factors that affect ruminal starch digestibility discussed above. Although we should strive to increase accuracy of prediction over time, we are not able to accurately predict animal responses to starch concentration and fermentability because of the many interactions that ultimately affect response such a stocking density, effective fiber concentration, milk yield, physiological state, etc. However, ration formulation should be an iterative process that includes cows in the loop; evaluation of cow response will provide feedback to optimize diets. Cow responses include DMI; yields of milk, fat, and protein; milk urea nitrogen; body condition; manure consistency; ketones; etc. Grains that differ in ruminal starch fermentability, but have high whole tract digestibility (e.g. high moisture corn and ground dry corn), allow evaluation of optimal ruminal starch digestibility without other confounding effects (e.g. effects of changing forage NDF concentration on feed intake) and diet starch concentration can be reduced by substitution of a non-forage fiber source, such as beet pulp, soyhulls, or corn gluten feed, for grains.

Group feeding complicates interpretation of responses for DMI and milk yield. Mean milk yield for the group masks effects of diets because large changes in milk yield of individual cows within the group might occur with no change in milk yield for the group overall. This is most evident when all lactating cows (with great differences in physiological state) are offered the same diet. Individual milk meters provide timely feedback regarding response of individuals within the group and are an important tool for diet formulation and grouping. The same is true for individual DMI response, but this is not feasible economically for group-housed cows. While that limits the usefulness of DMI determination for the group, it is still a very useful measurement, particularly in combination with milk yield to provide important clues for the effects of the diet change. Evaluation of cow response requires more attention by nutritionists and coordination with the management teams on farms. The extent to which nutritionists and the management team interact will vary from farm-to-farm, but this is an important determinant of the success of the nutrition program. The following recommendations for ration starch concentration and ruminal fermentability for cows as they progress through lactation should be adjusted as indicated by cow response.

Fresh Cow Ration (parturition to ~10-14 days postpartum)

Fresh cows are in a lipolytic state, are at increased risk for metabolic disorders, and feed intake is likely controlled by oxidation of fuels in the liver (Allen *et al.*, 2009). These cows require glucose precursors and rations should contain higher starch concentrations to the extent possible. However, they also have lower rumen digesta mass, which increases risk for ruminal acidosis and displaced abomasum. Highly fermentable starch sources increase fermentation acid production including propionate, which can stimulate oxidation of fuels in the liver, suppressing feed intake (Allen *et al.*, 2009). Therefore, highly fermentable starch sources should be limited during this period which lasts up to two weeks for most cows but even longer for cows with excessive body condition at parturition. Highly fermentable starch sources such as wheat, barley,

low-density steam-flaked corn, and aged (greater than 1 year old) high moisture corn and corn silage, should be limited **to allow greater starch concentrations (and glucose precursors)** with less risk of acidosis or displaced abomasum. Supplementing corn silage based diets with dry ground corn works well for this ration with a total starch concentration of up to 28% (DM basis) depending upon the fermentability of starch in the corn silage. Because feed intake is less limited by ruminal distention during this period, and greater rumen digesta mass is desirable, forage NDF concentration should be greater than 23% and use of non-forage fiber sources should be limited to diluting starch concentration, if necessary. Starch concentrations must be decreased when feeding highly fermentable starch sources.

Early to Mid-Lactation Ration

Cows in early to mid-lactation have high glucose requirement for milk production and partition relatively little energy to body reserves. They respond well to rations with lower forage NDF concentration (low fill) and highly fermentable starch. Starch concentration of rations should be in the range of 25 to 30% (DM basis) although the optimum concentration is dependent upon competition for bunk space, forage/effective NDF concentration, and starch fermentability. Higher starch, lower fill rations generally increase peak milk yield and decrease loss of body condition in early lactation. However, once cows replenish body condition lost in early lactation, they should be switched to a maintenance diet with lower starch concentration and ruminal fermentability.

Maintenance Ration (> 150 DIM and BCS of 3)

The maintenance ration is the key component of a ration formulation/ grouping system to increase health and production of cows. The goal of the maintenance ration is to maintain milk yield and body condition through the rest of lactation. Cows should be offered the maintenance ration when they are regaining BCS and reach a BCS of 3. If they continue receiving a high starch diet, BCS will continue to increase and they will be at increased risk for metabolic disease following parturition. Evidence presented above suggests that they are gaining condition because they are being fed rations with greater starch concentrations needed for their current requirement for milk production, increasing plasma glucose and insulin concentrations. Lowering ration starch concentration should limit body condition gain while maintaining and possibly improving feed intake and yields of milk and milk fat. The optimal concentration of starch is dependent upon the milk yield of the herd and physical groups possible but will likely be in the range of 18 to 22% (DM basis). Starch sources that are high fermentable (high-moisture corn, bakery waste, aged corn silage, etc.) should be avoided. Dried ground corn is an excellent starch source because it has lower ruminal digestibility (~60%) but high total tract digestibility (< 90%). The starch concentration of the maintenance ration should contain adequate, but not excessive forage NDF concentration to maintain DMI, and non-forage fiber sources (beet pulp, corn gluten feed, soyhulls, etc.) can be used to dilute starch to the target concentration. Monitoring BCS at dry-off is essential to adjust the starch concentration of the maintenance diet over time.

CONCLUSIONS

Concentration and ruminal fermentability of starch are highly variable among rations fed to lactating cows and have great effects on feed intake, energy partitioning, milk production, and health. The optimal starch concentration and starch source in rations varies by physiological state of cows, which changes through lactation. Cows should be fed different rations through lactation to maximize use of existing knowledge regarding starch nutrition.

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NDF - MAKING SOMETHING OLD, NEW AGAIN

M.E. Van Amburgh[1], R.J. Grant[2], K.W. Cotanch[2], A. Zontini[1], D.A. Ross[1] and A. Foskolos[1]

[1]Cornell University, Ithaca, NY
[2]Miner Institute, Chazy, NY

INTRODUCTION

Fiber digestibility and indigestibility are critical factors when assessing forage quality and formulating diets. Digestion characteristics of NDF influence feeding and rumination behavior, rate of particle breakdown, ruminal turnover and fill, dry matter intake, and overall efficiency of milk component output. Traditionally, nutritionists have focused on measures of NDF digestibility at specific timepoints and assumed that NDF was a relatively homogenous fraction. However, recently the focus has included indigestible fiber as well because of the recognition of its importance establishing the digestible portion or pool of NDF which leads to the extent of digestion and influences the rate(s) of fiber fermentation in the rumen. For purposes of nutritional modeling, indigestible NDF is required as the end point for fermentation to allow accurate estimation of the potentially digestible NDF fraction and its rate(s) of digestion. Measuring true NDF indigestibility would require infinite time, especially in aerobic systems, so in the actual rumen of a dairy cow or in an artificial rumen system, true indigestibility is never achieved. The standard nomenclature throughout the literature is “indigestible NDF (iNDF)” (Mertens, 1993; Huhtanen et al., 2006); however, to improve the accuracy of the standard terminology used to describe fiber fermentation dynamics, Mertens (2013) coined the term “undigested NDF (uNDF)” as the laboratory measure (typically in vitro or in situ) of indigestible NDF at a specified fermentation time. You will see both terms used, and for the most part, they are interchangeable as long as you know the method and time point used to determine the NDF digestion endpoint. However, moving forward, we will standardize our terminology to uNDF. To achieve iNDF requires estimations out to infinite time and that estimated residue might not be consistent with the interactive behavior of the forage and feed with rumen function.

WHY SHOULD WE USE uNDF?

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

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

- it can be used to estimate potentially digestible NDF(pdNDF) (NDF - uNDF),

- the uNDF fraction together with earlier time points of fermentation can be used to estimate the fast and slow pools of NDF digestion and their digestion rates (Raffrenato and Van Amburgh, 2010),
- measures of NDF pools and rates of digestion based on uNDF can help explain feeding and ruminating behavior, especially when chemical composition (i.e. ADL, NDF, ADF) are similar,
- chewing response to peNDF is likely influenced by forage uNDF,
- estimates of the slow pool of NDF and its rate of digestion plus the uNDF are related to dry matter intake and passage from the rumen,
- uNDF plays a critical role in maintaining the ruminal digesta load, and
- uNDF predicts forage quality because of the relationship between uNDF and OM digestibility (Nousiainen et al., 2003).

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

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

UPDATING THE ANALYSIS OF NDF TO aNDFom

One other related aspect of uNDF and NDF in general is the use of organic matter correction. Biogenic ash (ash integral to plant development) is soluble in NDF solution, so that is properly accounted for during the assay, however, soil ash is not soluble in NDF solution and if not removed or accounted for will falsely inflate the NDF values and the same is true for the uNDF. Moving forward, both the NDF and the uNDF should be ash corrected to remove any potential confounding by soil contamination. Management approaches that take advantage of practices like "hay in a hurry" along with large, high horsepower choppers will impact the amount of soil that is found in the forages. In addition, based on region of the country that forage is produced or sourced

will also affect the level of contamination. More sandy soils and irrigation practices such as flood irrigation can cause soil to be adhered to the plant. The easiest way to account for the contamination is to ash the residue after both the NDF and uNDF to correct the value. This also reduces bias in the estimation of rates of digestion since organic matter correction provides a more correct value for the true available NDF content. Thus, aNDFom analyses (NDF with sodium sulfite, amylase and ash correction) will provide nutritionists with more accurate information and in some cases significantly lower values.

There are no changes in the targets for aNDFom intake and in many cases, under reformulation, the amount of forage fed will increase 2-3% once the ash content of the NDF is accounted for. Under conditions where there was significant ash contamination, the amount of forage required to meet the typical dietary levels (e.g. 32%) can be increased by over 10% to maintain adequate aNDFom levels for normal rumen health. It is possible in certain situations, that inconsistent intakes, changes in rumination and rumen pH along with manure scores that are inconsistent can be an outcome of underfeeding forage and fiber because the NDF content of the diet was underestimated due to ash contamination. This most likely happens in the regions of the country where flood irrigation and sandy soils are more prevalent but it is still a possibility in the Northeast due to larger equipment, wide-swathing and variable field conditions.

HOW DO WE MEASURE uNDF?

The approach for estimating iNDF within the structure of the Cornell Net Carbohydrate and Protein System (CNCPS; Tylutki et al., 2008) has been through the use of acid detergent lignin (ADL) and a fixed factor of 2.4 calculated as ADL*2.4/NDF (Chandler et al., 1980). For other applications the approach most often used is that of Conrad et al. (1984) where a surface area relationship is described by a power function ((1- $lignin^{0.67}/NDF^{0.67}$) was used to describe the relationship between lignin and NDF to characterize the unavailable NDF. This approach is used in many of the net energy equations by commercial laboratories and the 2001 NRC (NRC, 2001).

More recently, iNDF has been estimated through long-time in vitro or in situ fermentations. The method recommended by the Cornell group requires 240 hours of in vitro fermentation using a Tilley-Terry system with modifications described by Raffrenato and Van Amburgh (2010). The fermentation end point *per se* is not important – it will vary with fermentation system. For example, the in situ approach published by Huhtanen et al. (2007) uses 288 hours to reach a similar fermentation endpoint to measure iNDF. The goal is to reach a point where the residue weight does not change significantly with additional hours of fermentation – this will be a measure of uNDF and the estimate of indigestible NDF for estimation of rates and extent of digestion. For commercial laboratory application and routine model inputs, we prefer the use of an in vitro approach which allows for sample submission from nutritionists and development of an adequate-sized database to develop NIR equations that will reduce the cost and increase the speed of sample analysis.

Examples of the chemistry related to NDF and NDF digestibility in four corn silages along with the calculated indigestibilities based on Chandler et al., and Conrad et al., are found in Table 1. The data in the table demonstrate the subtle differences that can be observed when analyzing for aNDFom compared with aNDF. The average difference among this very small sampling is 0.9 units of NDF, a very modest amount. However, we have analyzed or dealt with samples that were up to 10 units different after ashing, so again, it depends on where the sample is from and the agronomic an harvest conditions it is under. The uNDF as measured at 240 hr averages 24.8 %NDF whereas the lignin (%NDF)*2.4 value averages 41.9% and the power function of Conrad et al. (1984) averages 20.7%. The differences between the actual measurement and the calculations are significant and will result in biased estimations of total digestibility, rates of digestion and energy predictions. The Conrad et al. calculation average is biased because there is one sample that is very high compared to the rest, and that sample has the lowest measured uNDF of the four silages presented. Overall, this small example demonstrates that the values estimated by the previous methods using fixed factors as a function of the chemical measurement of lignin miss the potential interaction (cross-linking) between lignin and carbohydrate that actually impact the digestion capacity of the plant.

Table 1. Corn silage fiber chemistry, 240 in vitro indigestibilities (uNDF), and estimations of indigestible fiber by Chandler et al. (1980) (lignin (%NDF) x 2.4) and Conrad et al., 1984.

Corn silage	aNDF, %DM	aNDFom, %DM	Lignin, %NDF	uNDF, %NDF	Chandler et al. 1980	Conrad et al., 1984
1	38.1	37.5	6.61	23.6	42.3	16.4
2	39.5	38.9	6.46	25.6	39.2	16.89
3	41.5	40.9	7.47	27.3	43.4	17.7
4	43.7	41.9	7.51	22.8	42.8	31.8

Similar observations have been made for the non-forage fiber sources. Byproducts like beet pulp and citrus pulp that have good nutrient value and can be routine sources of energy for lactating dairy cattle have digestion behavior that is not dissimilar from forages. Data were generated to better understand when the uNDF is identified in non-forage fiber sources and that is in Table 2. For most non-forage feeds, the uNDF can be measured after 120 hr of in vitro digestion provided the samples are filtered on the appropriate filter paper (Whatman AH934 or equivalent). The only feed that had behavior more similar to forages was citrus pulp where the uNDF of the sample represented below was only identified at 240 h of fermentation.

Once the uNDF was identified and understood, it was important to evaluate the measured values from these non-forage fiber sources in a similar manner to the forages to better understand if the static calculations for uNDF and the measured uNDF were similar. The data in Table 3 demonstrate that the measured uNDF is both over- and under-predict for the feeds represented in this table and these inconsistencies will impact the estimation of digestible NDF and will also affect energy predictions from this group of feeds. Static values as a function of the lignin to NDF relationship do not

adequately account for the digestibility and uNDF of non-forage fiber feeds in a similar manner as forages, however it is expected that the variation in non-forage fiber feeds will not be as great as the forages due to the lack of agronomic conditions affecting their development.

Table 2. The aNDFom (%NDF) residues of feeds after 96, 120, and 240h of fermentation

	Time (h)				
	96	120	240	SEM	P-value
Beet pulp	22[a]	19[b]	17[b]	0.01	0.004
Canola meal	40	41	41	0.01	0.79
Citrus pulp	21[a]	20[a]	16[b]	0.01	0.002
Corn Gluten feed	16[a]	14[ab]	13[b]	0.01	0.028
Corn distiller	16	16	14	0.01	0.50
Corn germ	34	29	27	0.03	0.74
Flaked corn	14	14	12	0.02	0.73
Rice hulls	94	93	93	0.01	0.61
Soybean meal	11	9	9	0.01	0.95
Soy hulls	10[a]	9[ab]	8[b]	0.01	0.022
Wheat distiller	28	26	25	0.01	0.20
Wheat middling	36[a]	31[b]	30[b]	0.01	0.001

[a,b]Values with different letters are statistically different

Table 3. The neutral detergent fiber, acid detergent lignin and comparison of three methods of estimation of uNDF based on 120 hr fermentation, the Chandler equation or the Conrad equation, respectively.

Feed	aNDFom (%DM)	ADL (%DM)	uNDF (%aNDFom)	2.4 x ADL (%aNDFom)	$ADL^{2/3}/NDF^{2/3}$ (%aNDFom)
Beet pulp	47	5.4	19	28	24
Canola meal	29	8.8	41	73	45
Citrus pulp	25	1.94	20	19	53
Corn gluten feed	37	2.27	14	15	4
Corn distiller	41	4.4	16	26	23
Corn germ	63	5.9	29	23	21
Flaked corn	13	1.4	14	26	23
Rice hulls	71	0.8	93	20	5
Soybean meal	9	0.85	1	23	21
Soy hulls	72	1.3	9	10	7
Wheat distillers	38	3.8	26	29	22
Wheat middlings	45	4.9	31	17	23

IMPLICATIONS AND APPLICATIONS

Data being generated on lactating dairy cattle indicate the cow can "identify" with the values related to the uNDF measurements along with the rest of the pools (fast and slow digesting NDF pools) and these measurements are in some manner related to rumen fill, eating speed and ultimately, dry matter intake. Data generated in a forage digestibility study at Miner Institute with high and low forage inclusion levels demonstrated that the cow consumes approximately the same amount of uNDF as she excretes in her feces every day. The precision of the relationship was surprising as showing in Table 4. The relationship between uNDF intake and uNDF excretion was 1:1 and coupled with the relationship between the rumen contents of uNDF and the intake of uNDF suggests that if we understand the uNDF, we can directly estimate the rumen fill of total NDF and further, we should be able to predict intake among differences in TMR uNDF values.

Table 4. Intake of NDF and uNDF and rumen fill for Miner study

Item	LF-LD	HF-LD	LF-HD	HF-HD
NDF_{om} intake				
kg/d	8.87	8.95	8.48	9.88
% of BW	1.32	1.33	1.27	1.47
Rumen NDF_{om}				
kg	8.50	8.58	7.82	8.48
% of BW	1.27	1.28	1.17	1.27
$uNDF_{240om}$ intake				
kg/d	2.39	2.63	2.03	2.21
% of BW	0.36	0.39	0.30	0.33
Rumen $uNDF_{240om}$				
Kg	3.82	4.16	3.20	3.46
% of BW	0.57	0.62	0.48	0.52
Fecal uNDF, kg/d	2.41	2.64	2.04	2.24
Ratio rumen/intake uNDF	1.60	1.58	1.58	1.57
Ratio intake uNDF/fecal uNDF	1.0	1.0	1.0	0.99

SUMMARY

Studies are underway to evaluate the concept of aNDFom pools, chewing and rumination and feed intake. The data generated to date suggests that predictions for energy, rates of digestion, microbial yield and dry matter intake will be improved through the application of uNDF and the pool approach to defining NDF digestion. This is exciting and gives new life to an old topic, and might help explain differences in feeding behavior that nutritionists and others have observed but never been able to quantify.

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

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

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UPDATING THE ANALYSIS OF NDF TO aNDFom

One other related aspect of uNDF and NDF in general is the use of organic matter correction. Biogenic ash (ash integral to plant development) is soluble in NDF solution, so that is properly accounted for during the assay, however, soil ash is not soluble in NDF solution and if not removed or accounted for will falsely inflate the NDF values and the same is true for the uNDF. Moving forward, both the NDF and the uNDF should be ash corrected to remove any potential confounding by soil contamination. Management approaches that take advantage of practices like "hay in a hurry" along with large, high horsepower choppers will impact the amount of soil that is found in the forages. In addition, based on region of the country that forage is produced or sourced

will also affect the level of contamination. More sandy soils and irrigation practices such as flood irrigation can cause soil to be adhered to the plant. The easiest way to account for the contamination is to ash the residue after both the NDF and uNDF to correct the value. This also reduces bias in the estimation of rates of digestion since organic matter correction provides a more correct value for the true available NDF content. Thus, aNDFom analyses (NDF with sodium sulfite, amylase and ash correction) will provide nutritionists with more accurate information and in some cases significantly lower values.

There are no changes in the targets for aNDFom intake and in many cases, under reformulation, the amount of forage fed will increase 2-3% once the ash content of the NDF is accounted for. Under conditions where there was significant ash contamination, the amount of forage required to meet the typical dietary levels (e.g. 32%) can be increased by over 10% to maintain adequate aNDFom levels for normal rumen health. It is possible in certain situations, that inconsistent intakes, changes in rumination and rumen pH along with manure scores that are inconsistent can be an outcome of underfeeding forage and fiber because the NDF content of the diet was underestimated due to ash contamination. This most likely happens in the regions of the country where flood irrigation and sandy soils are more prevalent but it is still a possibility in the Northeast due to larger equipment, wide-swathing and variable field conditions.

HOW DO WE MEASURE uNDF?

The approach for estimating iNDF within the structure of the Cornell Net Carbohydrate and Protein System (CNCPS; Tylutki et al., 2008) has been through the use of acid detergent lignin (ADL) and a fixed factor of 2.4 calculated as ADL*2.4/NDF (Chandler et al., 1980). For other applications the approach most often used is that of Conrad et al. (1984) where a surface area relationship is described by a power function ((1- $lignin^{0.67}/NDF^{0.67}$) was used to describe the relationship between lignin and NDF to characterize the unavailable NDF. This approach is used in many of the net energy equations by commercial laboratories and the 2001 NRC (NRC, 2001).

More recently, iNDF has been estimated through long-time in vitro or in situ fermentations. The method recommended by the Cornell group requires 240 hours of in vitro fermentation using a Tilley-Terry system with modifications described by Raffrenato and Van Amburgh (2010). The fermentation end point *per se* is not important – it will vary with fermentation system. For example, the in situ approach published by Huhtanen et al. (2007) uses 288 hours to reach a similar fermentation endpoint to measure iNDF. The goal is to reach a point where the residue weight does not change significantly with additional hours of fermentation – this will be a measure of uNDF and the estimate of indigestible NDF for estimation of rates and extent of digestion. For commercial laboratory application and routine model inputs, we prefer the use of an in vitro approach which allows for sample submission from nutritionists and development of an adequate-sized database to develop NIR equations that will reduce the cost and increase the speed of sample analysis.

Examples of the chemistry related to NDF and NDF digestibility in four corn silages along with the calculated indigestibilities based on Chandler et al., and Conrad et al., are found in Table 1. The data in the table demonstrate the subtle differences that can be observed when analyzing for aNDFom compared with aNDF. The average difference among this very small sampling is 0.9 units of NDF, a very modest amount. However, we have analyzed or dealt with samples that were up to 10 units different after ashing, so again, it depends on where the sample is from and the agronomic an harvest conditions it is under. The uNDF as measured at 240 hr averages 24.8 %NDF whereas the lignin (%NDF)*2.4 value averages 41.9% and the power function of Conrad et al. (1984) averages 20.7%. The differences between the actual measurement and the calculations are significant and will result in biased estimations of total digestibility, rates of digestion and energy predictions. The Conrad et al. calculation average is biased because there is one sample that is very high compared to the rest, and that sample has the lowest measured uNDF of the four silages presented. Overall, this small example demonstrates that the values estimated by the previous methods using fixed factors as a function of the chemical measurement of lignin miss the potential interaction (cross-linking) between lignin and carbohydrate that actually impact the digestion capacity of the plant.

Table 1. Corn silage fiber chemistry, 240 in vitro indigestibilities (uNDF), and estimations of indigestible fiber by Chandler et al. (1980) (lignin (%NDF) x 2.4) and Conrad et al., 1984.

Corn silage	aNDF, %DM	aNDFom, %DM	Lignin, %NDF	uNDF, %NDF	Chandler et al. 1980	Conrad et al., 1984
1	38.1	37.5	6.61	23.6	42.3	16.4
2	39.5	38.9	6.46	25.6	39.2	16.89
3	41.5	40.9	7.47	27.3	43.4	17.7
4	43.7	41.9	7.51	22.8	42.8	31.8

Similar observations have been made for the non-forage fiber sources. Byproducts like beet pulp and citrus pulp that have good nutrient value and can be routine sources of energy for lactating dairy cattle have digestion behavior that is not dissimilar from forages. Data were generated to better understand when the uNDF is identified in non-forage fiber sources and that is in Table 2. For most non-forage feeds, the uNDF can be measured after 120 hr of in vitro digestion provided the samples are filtered on the appropriate filter paper (Whatman AH934 or equivalent). The only feed that had behavior more similar to forages was citrus pulp where the uNDF of the sample represented below was only identified at 240 h of fermentation.

Once the uNDF was identified and understood, it was important to evaluate the measured values from these non-forage fiber sources in a similar manner to the forages to better understand if the static calculations for uNDF and the measured uNDF were similar. The data in Table 3 demonstrate that the measured uNDF is both over- and under-predict for the feeds represented in this table and these inconsistencies will impact the estimation of digestible NDF and will also affect energy predictions from this group of feeds. Static values as a function of the lignin to NDF relationship do not

adequately account for the digestibility and uNDF of non-forage fiber feeds in a similar manner as forages, however it is expected that the variation in non-forage fiber feeds will not be as great as the forages due to the lack of agronomic conditions affecting their development.

Table 2. The aNDFom (%NDF) residues of feeds after 96, 120, and 240h of fermentation

	Time (h)				
	96	120	240	SEM	P-value
Beet pulp	22[a]	19[b]	17[b]	0.01	0.004
Canola meal	40	41	41	0.01	0.79
Citrus pulp	21[a]	20[a]	16[b]	0.01	0.002
Corn Gluten feed	16[a]	14[ab]	13[b]	0.01	0.028
Corn distiller	16	16	14	0.01	0.50
Corn germ	34	29	27	0.03	0.74
Flaked corn	14	14	12	0.02	0.73
Rice hulls	94	93	93	0.01	0.61
Soybean meal	11	9	9	0.01	0.95
Soy hulls	10[a]	9[ab]	8[b]	0.01	0.022
Wheat distiller	28	26	25	0.01	0.20
Wheat middling	36[a]	31[b]	30[b]	0.01	0.001

[a,b]Values with different letters are statistically different

Table 3. The neutral detergent fiber, acid detergent lignin and comparison of three methods of estimation of uNDF based on 120 hr fermentation, the Chandler equation or the Conrad equation, respectively.

Feed	aNDFom (%DM)	ADL (%DM)	uNDF (%aNDFom)	2.4 x ADL (%aNDFom)	$ADL^{2/3}/NDF^{2/3}$ (%aNDFom)
Beet pulp	47	5.4	19	28	24
Canola meal	29	8.8	41	73	45
Citrus pulp	25	1.94	20	19	53
Corn gluten feed	37	2.27	14	15	4
Corn distiller	41	4.4	16	26	23
Corn germ	63	5.9	29	23	21
Flaked corn	13	1.4	14	26	23
Rice hulls	71	0.8	93	20	5
Soybean meal	9	0.85	1	23	21
Soy hulls	72	1.3	9	10	7
Wheat distillers	38	3.8	26	29	22
Wheat middlings	45	4.9	31	17	23

IMPLICATIONS AND APPLICATIONS

Data being generated on lactating dairy cattle indicate the cow can "identify" with the values related to the uNDF measurements along with the rest of the pools (fast and slow digesting NDF pools) and these measurements are in some manner related to rumen fill, eating speed and ultimately, dry matter intake. Data generated in a forage digestibility study at Miner Institute with high and low forage inclusion levels demonstrated that the cow consumes approximately the same amount of uNDF as she excretes in her feces every day. The precision of the relationship was surprising as showing in Table 4. The relationship between uNDF intake and uNDF excretion was 1:1 and coupled with the relationship between the rumen contents of uNDF and the intake of uNDF suggests that if we understand the uNDF, we can directly estimate the rumen fill of total NDF and further, we should be able to predict intake among differences in TMR uNDF values.

Table 4. Intake of NDF and uNDF and rumen fill for Miner study

Item	LF-LD	HF-LD	LF-HD	HF-HD
NDF_{om} intake				
kg/d	8.87	8.95	8.48	9.88
% of BW	1.32	1.33	1.27	1.47
Rumen NDF_{om}				
kg	8.50	8.58	7.82	8.48
% of BW	1.27	1.28	1.17	1.27
$uNDF_{240om}$ intake				
kg/d	2.39	2.63	2.03	2.21
% of BW	0.36	0.39	0.30	0.33
Rumen $uNDF_{240om}$				
Kg	3.82	4.16	3.20	3.46
% of BW	0.57	0.62	0.48	0.52
Fecal uNDF, kg/d	2.41	2.64	2.04	2.24
Ratio rumen/intake uNDF	1.60	1.58	1.58	1.57
Ratio intake uNDF/fecal uNDF	1.0	1.0	1.0	0.99

SUMMARY

Studies are underway to evaluate the concept of aNDFom pools, chewing and rumination and feed intake. The data generated to date suggests that predictions for energy, rates of digestion, microbial yield and dry matter intake will be improved through the application of uNDF and the pool approach to defining NDF digestion. This is exciting and gives new life to an old topic, and might help explain differences in feeding behavior that nutritionists and others have observed but never been able to quantify.

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NDF Digestibility and uNDF: What does this mean and how can we apply it to make better decisions

Mike Van Amburgh, Rick Grant, Kurt Cotanch,
Alessandro Zontini, Debbie Ross and Andreas Foskolos

Dept. of Animal Science
Chazy, NY

Outline

- aNDFom – why and what it means
- aNDFom digestibility
- uNDF – definition
- uNDF and NDF pools
- Implications of using this approach
- Summary

NDF analyses

- Nutrition models/software have an input for NDF that is used primarily to calculate energy from available carbohydrates and effective fiber
- Mertens (2002) published the NDF method and gained AOAC approval – there are many approaches to measure NDF
- We want everyone to use of aNDFom – NDF with sulfite and ash correction – we are working to move labs in that direction
- Sniffen et al. 1992…

Why aNDFom?

- Hay in a hurry – wide swathing picks up dirt
- 600-800 hp choppers and big equipment that move fast make dust and dirt fly
- Flood irrigation moves soil
- Dirt/soil does not solubilize in NDF solution, thus if not corrected will inflate the NDF content
- Inflation of the NDF content means the diet as formulated is lower in actual NDF – intake and rumen health can be compromised

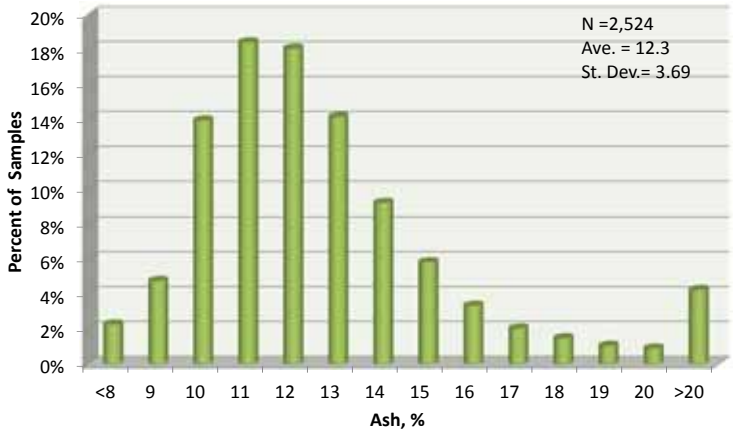
27 FIELD 316 SORGHUM X SUDAN

FIBER	% NDF	% DM
ADF	56.5	34.0
aNDF	→	60.2
aNDFom	→	55.4
NDR (NDF w/o sulfite)		
peNDF		~ 5 units
Crude Fiber		
Lignin	4.95	2.98
NDF Digestibility (12 hr)		
NDF Digestibility (24 hr)		
NDF Digestibility (30 hr)	60.2	36.3
NDF Digestibility (48 hr)		
NDF Digestibility (240 hr)	74.9	45.1
uNDF (30 hr)	39.8	24.0
uNDF (240 hr)	25.1	15.1

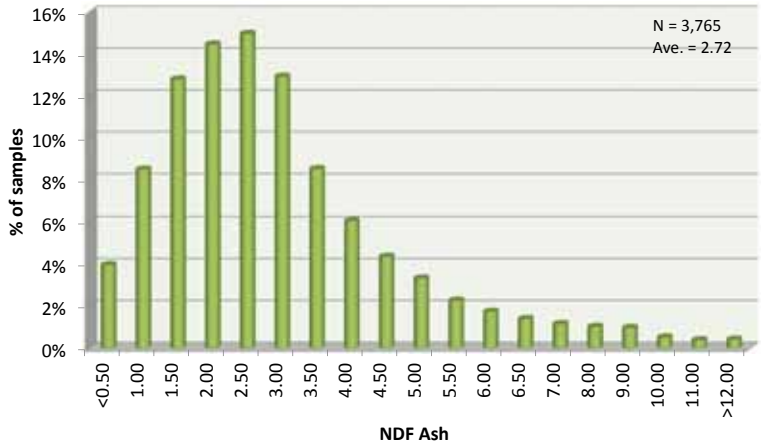
26 FIELD 308 TEST 2 SORGHUM X SUDAN

FIBER	% NDF	% DM
ADF	57.6	36.8
aNDF	→	63.9
aNDFom	→	53.7
NDR (NDF w/o sulfite)		
peNDF		10 units
Crude Fiber		
Lignin	4.86	3.11
NDF Digestibility (12 hr)		
NDF Digestibility (24 hr)		
NDF Digestibility (30 hr)	49.3	31.5
NDF Digestibility (48 hr)		
NDF Digestibility (240 hr)	77.0	49.2
uNDF (30 hr)	50.7	32.4
uNDF (240 hr)	23.0	14.7

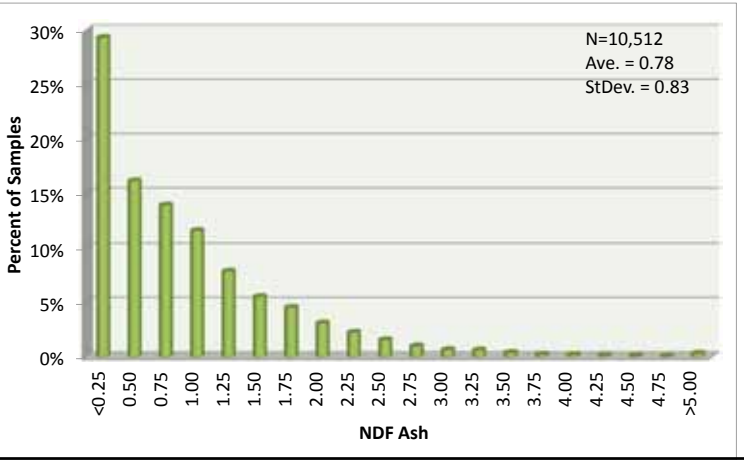
Distribution of Ash in Legume Silage
(CVAS 2010-2011, Chemistry)



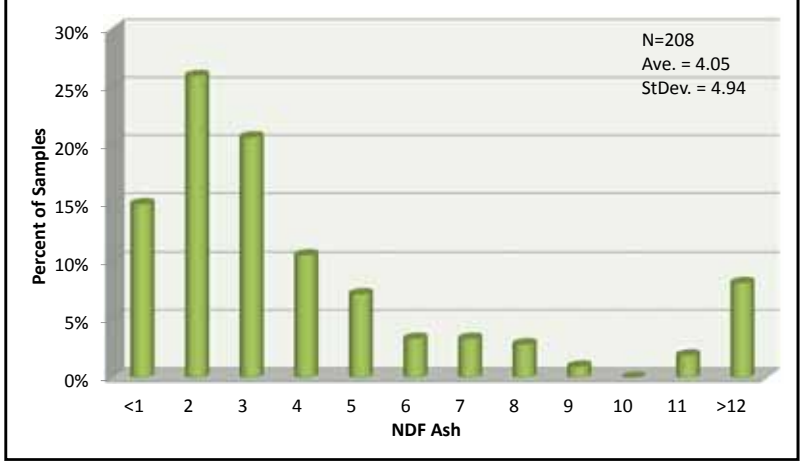
Distribution of NDF Ash in Haycrop Silage
(CVAS, 2013)



Distribution of NDF Ash in Corn Silage
(CVAS, 2012 crop)



Distribution of NDF Ash in Selected Soghum and Soghum/Sudan Samples
(CVAS, 2012 crop, chemistry)



Example of the Impact of Ash Contamination on NDF and NDF Digestibility Recovery

Sample	NDF	NDFom	NDFD30	NDFD30om
15081-068	54.6%		56.3%	

Ralph Ward

Example of the Impact of Ash Contamination on NDF and NDF Digestibility Recovery

Sample	NDF	NDFom	NDFD30	NDFD30om
15081-068	54.6%	**48.3%**	56.3%	**65.9%**

Ralph Ward

Example of the Impact of Ash Contamination on NDF and NDF Digestibility Recovery

Sample	NDF	NDFom	NDFD30	NDFD30om
15081-68	54.6%	**48.3%**	56.3%	**65.9%**
15085-56	60.1%		49.7%	

Ralph Ward

Example of the Impact of Ash Contamination on NDF and NDF Digestibility Recovery

Sample	NDF	NDFom	NDFD30	NDFD30om
15081-68	54.6%	**48.3%**	56.3%	**65.9%**
15085-56	60.1%	**50.9%**	49.7%	**61.9%**

Ralph Ward

Fiber degradation and iNDF

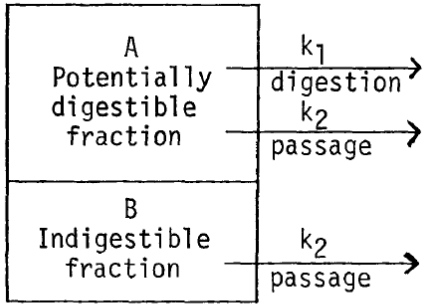


FIG. 1. Rumen cellulose split into two fractions—potentially digestible which disappear by digestion and passage and indigestible which disappears by passage.

Adapted from Waldo et al., 1972

How do we currently characterize NDF indigestibility? (iNDF)

Models like the CNCPS use (2.4 x lignin)/NDF

Dairy NRC (2001) and forage labs based on Weiss et al., 1992 use $(lignin/NDF)^{0.67}$

Van Soest and Lane Moore, 1963 USDA, Beltsville, MD right after Pete characterized NDF

Nomenclature slide - iNDF vs uNDF

Literature uses the term iNDF for indigestible NDF

We have an "Informal Fiber Working Group" that meets at least once per year around the Cornell Nutrition Conf. (Cornell, Miner Institute, Univ. of Bologna, Nutreco, ADM, Univ. of Parma, most commercial labs, Charlie Sniffen, Dave Mertens)

Mertens proposed a change in name from iNDF to uNDF –

> the NDF we call iNDF can digest, just not under anaerobic conditions, so to say indigestible is a misrepresentation – so we now use uNDF – undigested NDF

NDF Digestibility/Indigestibility

- Nousiainen et al. (2003; 2004) demonstrated in grasses that the relationship between lignin and digestibility was highly variable
- This was confirmed by Rinne et al. 2006 on legumes – methods used to determine this included 288 hr in situ (in a bag in the rumen) fermentations
- We were/are doing similar work at Cornell
  - Working to develop a procedure that could be used in a commercial lab
    Ph.D. work of Raffrenato (2011)

Corn Silage NDF Digestibility by NDF and Lignin Content

NDF, %DM	Lignin, %DM
42.3	3.01
42.6	3.32
42.6	3.24
42.6	3.24
42.3	3.18
42.3	3.00

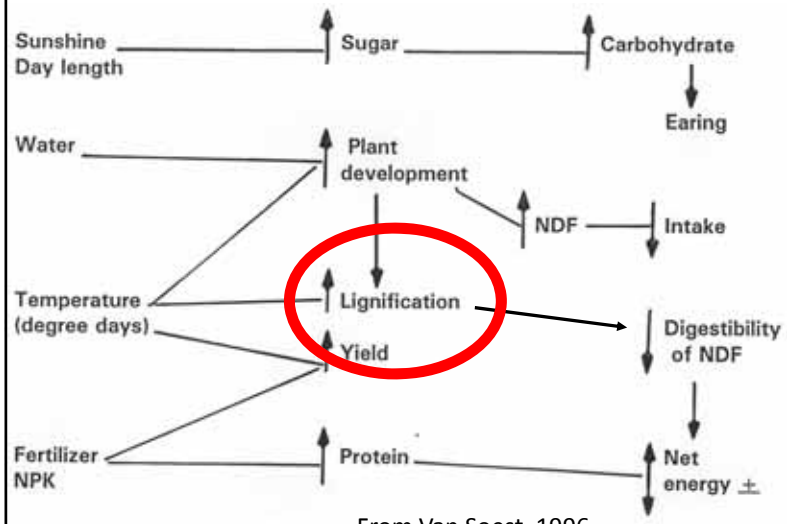
Corn Silage NDF Digestibility by NDF and Lignin Content

NDF, %DM	Lignin, %DM	NDFD% (30hr)	Est. NDF kd, %h
42.3	3.01	42.2	2.63
42.6	3.32	44.1	2.90
42.6	3.24	44.6	2.92
42.6	3.24	50.8	3.60
42.3	3.18	56.7	4.36
42.3	3.00	57.0	4.30

"Lignification" = cross linking between lignin and hemicellulose

- Light, heat and water interact at various stages of development
- For example, water stress causes greater cross-linking between lignin and hemicellulose
- Similar to the effect of building a very tall building

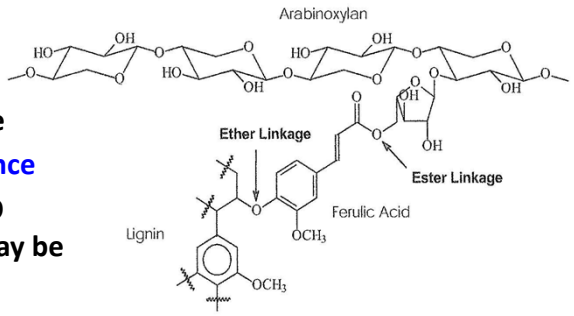
Factors Affecting Plant Development and Digestibility



From Van Soest, 1996

Lignin – Phenolic Acid – Hemicellulose Linkage

- **Ester & ether linkages to hemicellulose**
- **Steric hindrance**
- **Phenolic-CHO complexes may be toxic**



(Grabber, 2005)

Ratio of lignin to uNDF

Group	n	NDF	ADL	uNDF	Ratio (range)
		%DM	g/kg NDF		uNDF/ADL (%NDF)
Conventional C.S.	30	42.7	72.4	316.8	4.72 (1.73-7.59)
BMR C.S.	15	39.1	43.6	171.7	4.01 (3.14-5.45)
Grasses	15	47.2	62.1	222.8	3.63 (2.51-4.73)
Mature grasses	11	64.5	84.4	313.8	3.89 (2.60-5.64)
Immature grasses	13	44.1	59.3	232.2	4.16 (2.59-7.40)
Alfalfas	18	36.6	172.6	461.4	2.70 (2.43-2.95)

Raffrenato 2011

NDF Digestibility/Indigestibility

Weisbjerg et al. (2010) measured iNDF in legumes and grasses

- 288 h in situ,
- 12 µm porosity bags

Grasses range between 1.27-4.57 for ADL and iNDF

Legumes ranged between of 1.22-3.59 for ADL and iNDF respectively,

Corn silage example for uNDF 240 vs lignin*2.4 – 2013 corn silages

	CS 1	CS 2	CS 3	CS 4
NDF, %DM	45.4	44.5	40.3	50.2
aNDFom, %DM	44.4	43.8	38.8	49.3
Lignin, %DM	3.40	3.43	2.87	4.26
Lignin*2.4/NDF	18.4	18.7	17.9	20.7
uNDF, %NDF	11.8	10.7	10.9	14.2

Corn silage chemistry and uNDF by three methods, 240 hr uNDF, Chandler et al. (1980) and Conrad et al., 1984 equations

Corn silage	aNDF, %DM	aNDFom, %DM	uNDF, %NDF	Chandler et al. 1980	Conrad et al., 1984
1	38.1	37.5	23.6	42.3	16.4
2	39.5	38.9	25.6	39.2	16.9
3	41.5	40.9	27.3	43.4	17.7
4	43.7	41.9	22.8	42.8	31.8

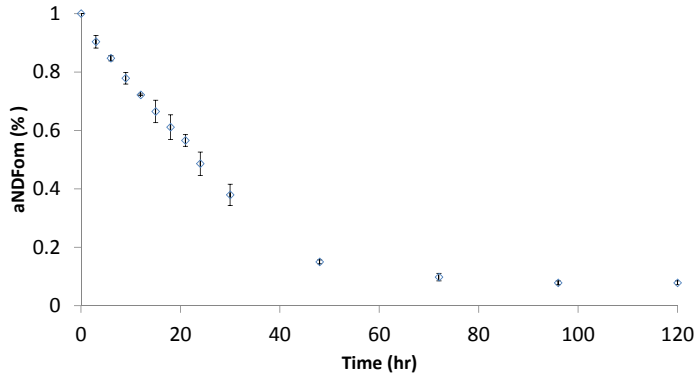
Opportunity with uNDF

- Improve predictions of energy from forages – more biologically appropriate measurement
- Eliminate the need for ADF and lignin measurements
  - Only do ADF to get to lignin
  - Only use lignin to calculate relationships to NDF (either CNCPS approach or Weiss et al 1992)
- Helps improve predictions of intake and rumen function – microbial production, etc

What about Non-forage Fiber Feeds?

- Do they have the same digestion behavior as forages?
- What are the time-points?

Digestion curve of Soy Hulls



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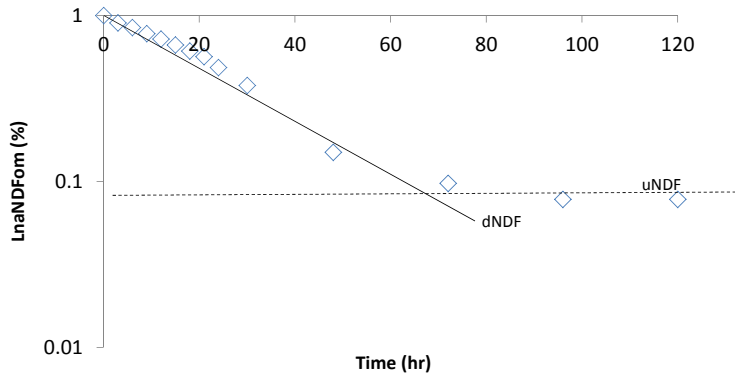
Digestion curve of Soy Hulls



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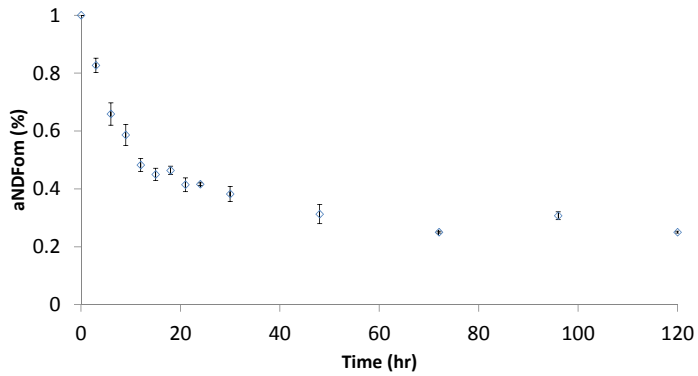
Digestion curve of Wheat Middlings



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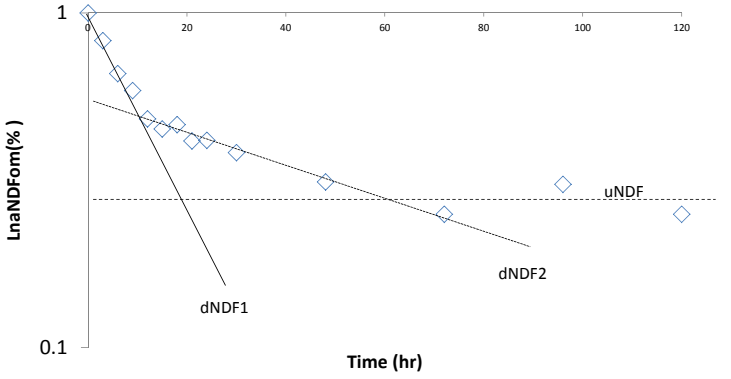
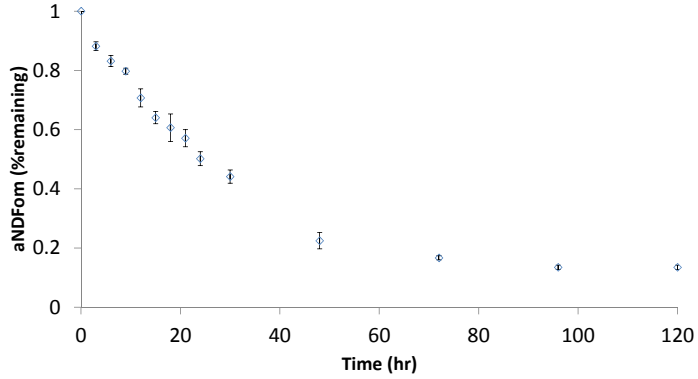
Digestion curve of Wheat Middlings

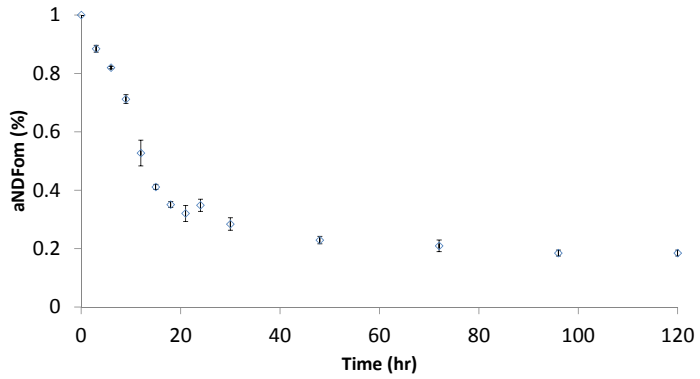


Alessandro Maria Zontini, 2014

Digestion curve of Defatted Corn Gluten Feed



Beet Pulp



Degradation behavior

Feed	1 dNDF	2 dNDF
Beet Pulp	x	
Canola Meal	x	
Citrus Pulp	x	
Corn Gluten Feed	x	
Corn Distiller	x	
Corn Germ	x	
Flaked Corn	x	
Rice Hulls	x	
Soy Plus	x	
Soy Hulls	x	
Wheat Distiller	x	
Wheat Midds		x

Observations

1) uNDF is best estimated at 120 of in vitro fermentation

2) Non-forages feeds are best characterized using a two pools model (dNDF + uNDF)

Which time points are most appropriate to estimate the decay?

Selecting time-points

TP/|1-Slope|	24-48-96	15-48-96	15-48-72	12-48-72	9-48-96	12-72-96	12-72-120	12-48-120
Beet Pulp	0.0477	0.0418	0.0676	0.0731	0.0962	0.0459	0.0510	0.0443
Canola Meal	0.0002	0.0099	0.0699	0.0709	0.0023	0.0479	0.0492	0.0706
Citrus	0.0036	0.0247	0.0130	0.0068	0.0420	0.0074	0.0076	0.0593
Corn Gluten	0.0672	0.0315	0.0810	0.0810	0.0315	0.0315	0.0122	0.0595
Corn Distiller	0.0748	0.0649	0.0729	0.0827	0.0868	0.0578	0.0538	0.0695
Corn Germ	0.0335	0.0334	0.0505	0.0722	0.0943	0.0786	0.0786	0.1096
Rice Hulls	0.2391	0.1962	0.1545	0.1384	0.1850	0.1621	0.1227	0.1469
Soy Bean Meal	0.0428	0.0454	0.0442	0.0398	0.0548	0.0705	0.0661	0.0351
Soy Hulls	0.0643	0.0825	0.0843	0.0655	0.0789	0.0566	0.0605	0.0544
Soy Plus	0.0818	0.0555	0.1089	0.1113	0.0555	0.0805	0.0579	0.0391
Wheat Distiller	0.0137	0.0343	0.0626	0.0554	0.0030	0.0342	0.0356	0.0259
Wheat Midds	0.0677	0.0398	0.0333	0.1162	0.0690	0.0115	0.0132	0.0885
Average	0.0614	0.0550	0.0702	0.0761	0.0666	0.0570	0.0507	0.0669
STD	0.0625	0.0483	0.0365	0.0350	0.0491	0.0406	0.0321	0.0343

Selecting time-points

TP/Intercept	24-48-96	15-48-96	15-48-72	12-48-72	9-48-96	12-72-96	12-72-120	12-48-120
Beet Pulp	0.033	0.004	0.012	0.042	0.092	0.023	0.027	0.022
Canola Meal	0.040	0.049	0.047	0.038	0.086	0.023	0.026	0.038
Citrus	0.021	0.001	0.017	0.000	0.054	0.018	0.016	0.009
Corn Gluten	0.037	0.028	0.039	0.028	0.035	0.033	0.026	0.022
Corn Distiller	0.039	0.031	0.032	0.032	0.064	0.018	0.015	0.027
Corn Germ	0.020	0.101	0.004	0.133	0.201	0.080	0.072	0.094
Rice Hulls	0.242	0.192	0.153	0.128	0.177	0.151	0.111	0.138
Soy Bean Meal	0.024	0.002	0.006	0.030	0.011	0.014	0.017	0.036
Soy Hulls	0.022	0.026	0.035	0.049	0.023	0.035	0.033	0.031
Soy Plus	0.050	0.010	0.042	0.033	0.024	0.013	0.004	0.012
Wheat Distiller	0.023	0.062	0.075	0.043	0.025	0.045	0.047	0.006
Wheat Midds	0.044	0.040	0.009	0.012	0.038	0.034	0.036	0.022
Average	0.050	0.045	0.039	0.047	0.069	0.041	0.036	0.038
STD	0.061	0.054	0.041	0.041	0.061	0.039	0.029	0.039

uNDF of Non-forage Fiber Sources

1) uNDF is best estimated at 120 of in vitro fermentation
2) Concentrates feeds are best characterized using a two pools model (dNDF + uNDF)
3) 0, 12, 72, and 120h are the time points to use for non-forage feeds

Comparison of three methods of estimation of uNDF - 120 hr fermentation, Chandler equation and the Conrad equation

Feed	aNDFom (%DM)	uNDF (%aNDFom)	2.4 x ADL (%aNDFom)	$ADL^{2/3}/NDF^{2/3}$ (%aNDFom)
Beet pulp	47	19	28	24
Canola meal	29	41	73	45
Citrus pulp	25	20	19	53
Corn gluten feed	37	14	15	4
Corn distiller	41	16	26	23
Corn germ	63	29	23	21
Flaked corn	13	14	26	23
Soybean meal	9	1	23	21
Soy hulls	72	9	10	7
Wheat distillers	38	26	29	22
Wheat middlings	45	31	17	23

uNDF Study @ Miner Institute

- What does it mean and how do we take advantage of the information?

Composition of diets used in uNDF study at Miner Institute.

	Diet			
Ingredient % of ration DM	LF-LD (Low CS)	HF-LD (High CS)	LF-HD (Low BMR)	HF-HD (High BMR)
Conventional corn silage	39.2	54.9	---	---
Brown midrib corn silage	---	---	36.1	50.2
Hay crop silage	13.4	13.4	13.3	13.3
Corn meal	17.3	1.6	20.4	6.3
Grain mix	30.1	30.1	30.2	30.2
Chemical composition				
Crude protein, % of DM	17.0	17.0	16.7	16.7
NDF,% of DM	32.1	35.6	31.5	35.1
Starch, % of DM	28.0	21.2	27.8	23.8
24-h NDF digestibility, %	56.3	54.0	62.0	60.3
peNDF, % of DM	17.3	23.1	18.5	21.5

uNDF study – Miner Inst.

	High CCS	Low CCS	High BMR	Low BMR
DMI lb/d	58.43	63.95	64.39	64.61
SCM lb/d	92.17	99.67	100.77	102.31
Efficiency	1.58	1.56	1.57	1.58

uNDF Intake, Rumen content and Fecal excretion

	High CCS	Low CCS	High BMR	Low BMR
uNDF Intake lb/d	5.80	5.27	4.87	4.48
uNDF Rumen lb	9.17	8.42	7.63	7.06
uNDF Fecal lb /d	5.80	5.27	4.87	4.48

Can we use this to better predict DMI?

	High CCS	Low CCS	High BMR	Low BMR
uNDF, %DM	9.92%	8.24%	7.57%	6.93%
uNDFi : uNDFf	1.00	1.00	1.00	1.00
uNDFi : uNDFr	0.63	0.63	0.64	0.63

uNDFi, uNDF Intake
uNDFf, uNDF Fecal
uNDFr, uNDF Rumen content

Interpretation

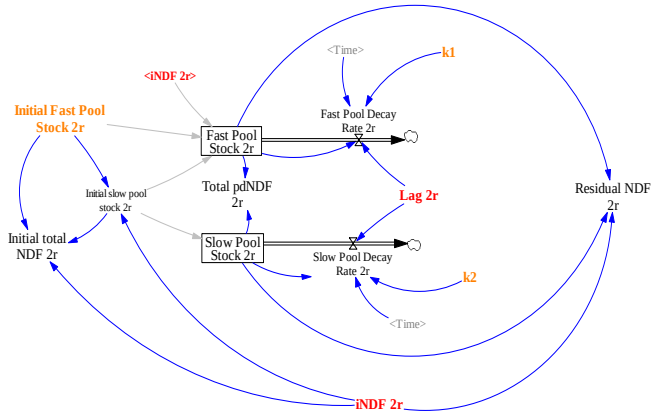
- Need to understand what changes uNDF Rumen content
  - 4.48 – 5.80 lbs. or 7% - 10% DMI is significant
  - Rumen content appears to determine intake and fecal output of uNDF
  - What causes variation of uNDF Rumen content?
- "Working hypothesis": the disappearance of the fast and slow pools of pdNDF determines volume of uNDF Rumen content and capacity along with the "ballast and rumen fill of the slow and uNDF fractions.

Perspective

	High CCS	Low CCS	High BMR	Low BMR	Median
uNDF, %DM	9.92%	8.24%	7.57%	6.93%	**7.90%**
uNDF Intake lb	5.80	5.27	4.87	4.48	**5.07**
uNDF Rumen, lb	9.17	8.42	7.63	7.06	**8.03**
uNDF Fecal/d	5.80	5.27	4.87	4.48	**5.07**
uNDFi:uNDFf	1.00	1.00	1.00	1.00	**1.00**
uNDFi:uNDFr	0.63	0.63	0.64	0.63	**0.63**

Take into account current uNDF% and intake while rebalancing diet. If you know current capacity based on current feeds you should be able to optimize better diet.

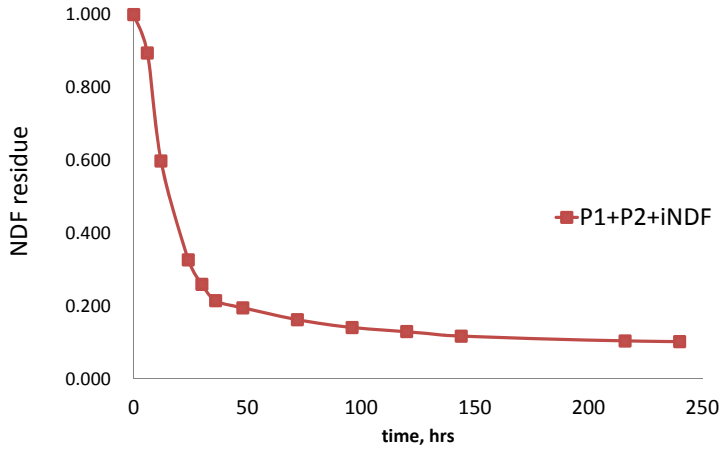
Calculation of rates and pool sizes using in-vitro 30, 120 and 240 hr NDFD data



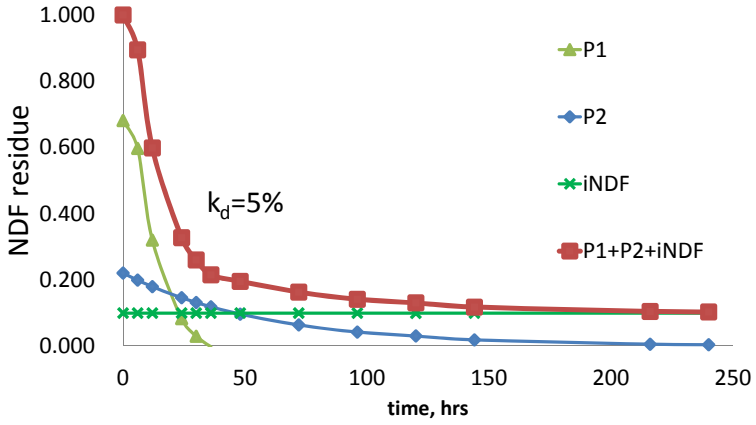
Parameters in orange are the those to be optimized

Raffrenato et al. 2011

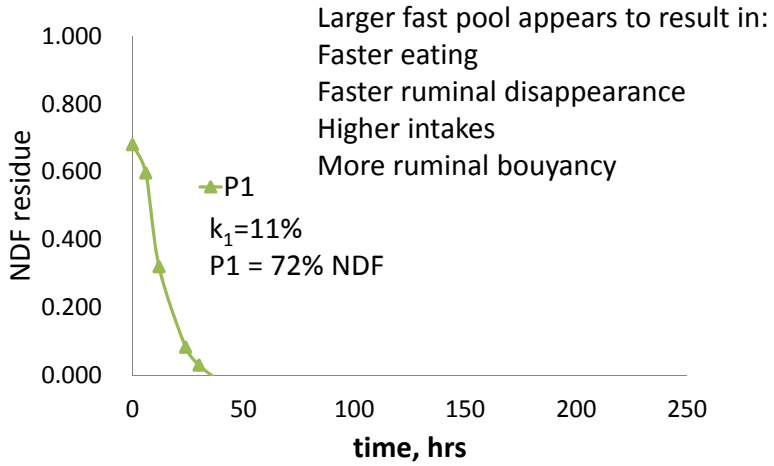
Corn silage example: NDF$_{digestibility}$



Corn silage example: P1+P2+iNDF



Corn silage example: fast pool



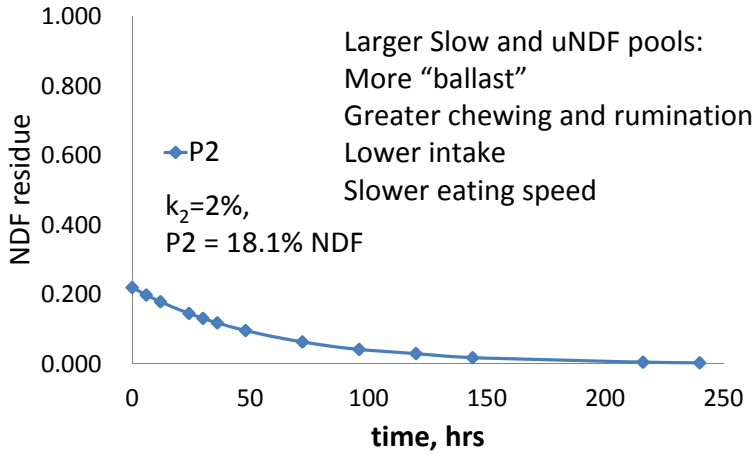
Larger fast pool appears to result in:
Faster eating
Faster ruminal disappearance
Higher intakes
More ruminal bouyancy

Undigested NDF residues of CS, Grass silage and Hay Busted Straw 47h in situ followed by washing machine and NDF processing in Ankom 10x20 dacron bags using Ankom fiber analyzer
Miner 2014

Corn silage uNDF residue after 47h in situ, laundered and NDF assay

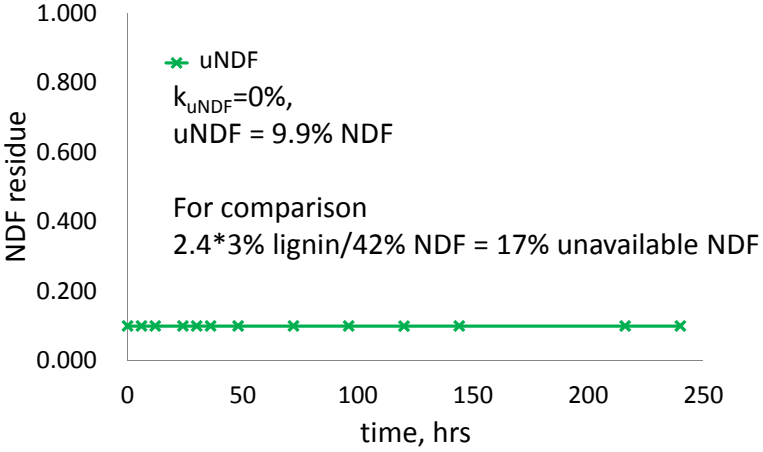
Grass silage uNDF residue after 47h in situ, laundered and NDF assay

Corn silage example: slow pool

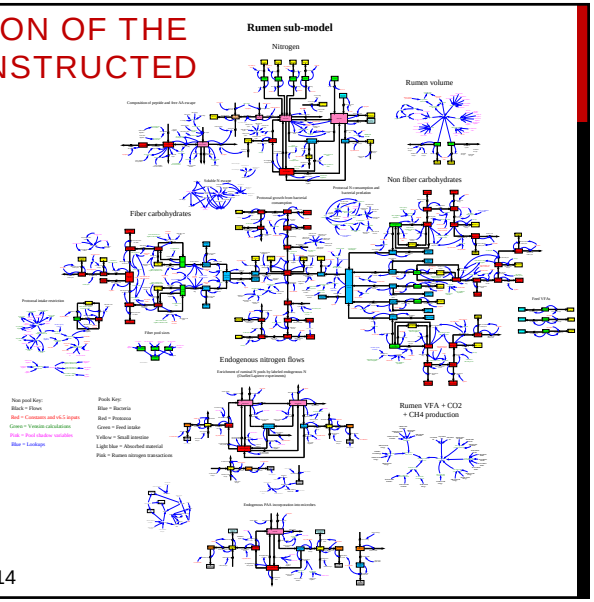


Straw (HB) uNDF residue after 47h in situ, laundered and NDF assay

Corn silage example: iNDF



NEW VERSION OF THE CNCPS CONSTRUCTED IN VENSIM



Ryan Higgs, Ph.D. 2014

Application of a technology to improve NDF digestibility

Ingredients	lb DM	% Diet
Corn Silage Processed 35 DM 49 NDF Medium	22.9	38.8%
Alfalfa Silage 17 CP 46 NDF 20 LNDF	11.5	19.4%
Corn Grain Ground Fine	15.4	26.1%
Soybean Meal 47.5 Solvent	0.0	0.0%
Soy Pass	4.4	7.5%
Blood Meal Average	1.5	2.5%
Energy Booster 100	1.0	1.7%
MinVit	2.2	3.7%
Urea	0.1	0.2%
Total	58.9	100%

Chemical composition of the diets

Crude protein, %DM	**15.6**
SolP (% CP)	**39.5**
Ammonia (% SP)	**8.5**
ADIP (% CP)	**6.7**
NDIP (% CP)	**15.7**
%NFC	**36.4**
Sugars	**2.4**
Starch	**27.2**
NDF	**35.4**
peNDF	**55.8**
Lignin (% NDF)	**10.0**
Ether extract	**4.7**
Ash	**8.2**
Forage % DM	**58.3**

Chemical analyses of the control and treatment forages using the three pool approach for NDF

Feed name	Fast Pool NDF (% NDF)	Slow Pool NDF (% NDF)	uNDF (%NDF)	kd 1 (%/hr)	kd 2 (%/hr)
Control corn silage	54.2	27.2	18.6	9.7	1.4
Treatment corn silage	62.5	25.3	12.2	6.1	1.9
Control alfalfa silage	32.3	29.4	38.3	5.2	1.5
Treatment alfalfa silage	50.5	12.4	37.1	9.0	1.8

Predicted rumen pools sizes and expected DM intake – g/d

	Control	Control lower intake	Technology treatment
B3 Fast CHO	1849	1624	2578
B3 Slow CHO	3082	2732	2174
C CHO	5082	4587	4203
Total rumen NDF	10013	8943	8955
DMI (lbs)	59.1	51.4	59.1

Dry matter intake on the control example was reduced to a level where the total rumen NDF pool was equivalent to the treatment example (indicated in red). Based on this example intake might be expected to be different by 7.7 lbs. The diet modeled is high forage and high NDF and probably represents the situation with the greatest opportunity to achieve an intake response.

Conclusions and implications

- The use of 240 hr NDFD better describes the undigestibility of the forage for use in cattle
- A better description of NDF undigestibility can be implemented by commercial laboratories – especially for undigested NDF – will have to build new NIR calibrations
- Working to develop a larger data set to explain the variation in NDF pool sizes and rates for all NDF containing feeds
  - Within forage group information is linked to agronomic and environmental conditions but not well described

Opportunity with uNDF

- Improve predictions of energy from forages – more biologically appropriate measurement
- Eliminate the need for ADF and lignin measurements
  - Only do ADF to get to lignin
  - Only use lignin to calculate relationships to NDF (either CNCPS approach or Weiss et al 1992)
- Helps improve predictions of intake and rumen function – microbial production, etc

Thank you for your attention.

mev1@cornell.edu

The Metabolism and Productive Responses to Heat Stress: Potential Nutritional Strategies

Lance Baumgard and Rob Rhoads
baumgard@iastate.edu
Iowa State University
Virginia Tech University

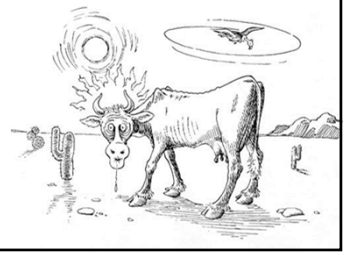
Department of Animal Science

Heat Stress is not Fever

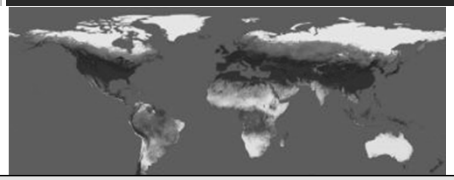
When environmental temperature nears the animal's body temperature, the animal's cooling mechanisms are impaired.

Fever vs. Hyperthermia

Very different biology

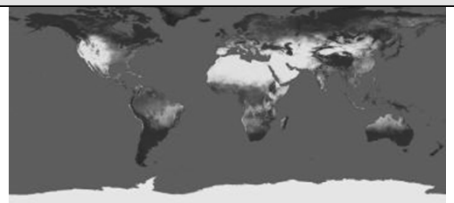


Heat Stress is a Global Problem



January 2003, NASA

40% of W. Canadian summer days THI > 72
Ominski et al., 2002



July 2003, NASA

Heat Stress: Economics and Food Security

- Cost: (lost productivity, mortality, product quality, health care etc.)
  - American Agriculture: > $4 billion/year
  - Global Agriculture: > $100 billion/year
- Heat abatement is the primary strategy to mitigate heat stress
  - But most developing countries and small stake-holders lack the resources to afford cooling technology
- Heat stress is the largest impediment to efficient animal agriculture (even in developed countries)
- Threatens global food security
- Regionalizes animal agriculture

St. Pierre et al., 2003; Baumgard and Rhoads, 2013

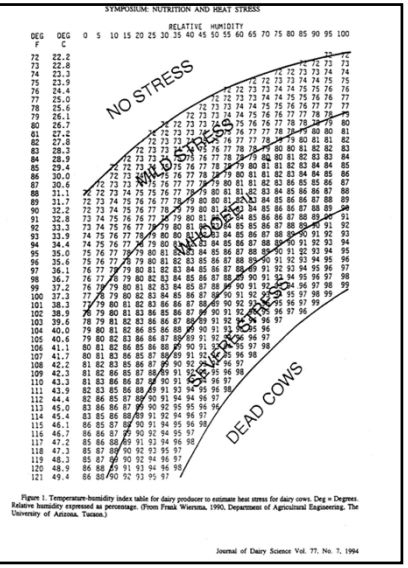
Heat Stress will Become More an Issue in the Future if:

- Climate change continues as predicted
- Genetic selection continues to emphasis lean tissue accretion, milk synthesis, etc..
  - Heat producing processes
- Developing countries become more affluent
- Human population continues to migrate towards the equator
  - Animal agriculture will migrate with the consumer

Baumgard & Rhoads, 2013

Temperature Humidity Index (THI)

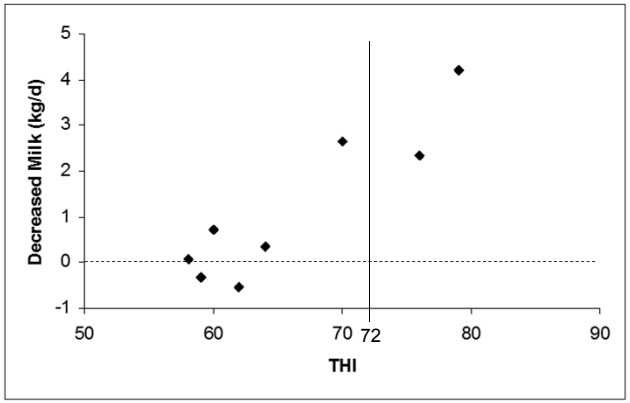
- Easy way to measure and evaluate heat stress
- Based on cows only under shade..solar radiation is incredibly potent
- 72 thought to be when cows become susceptible
- Based on 60 year old data when cows were producing 10-15 kg/d



Time to Re-Evaluate THI?

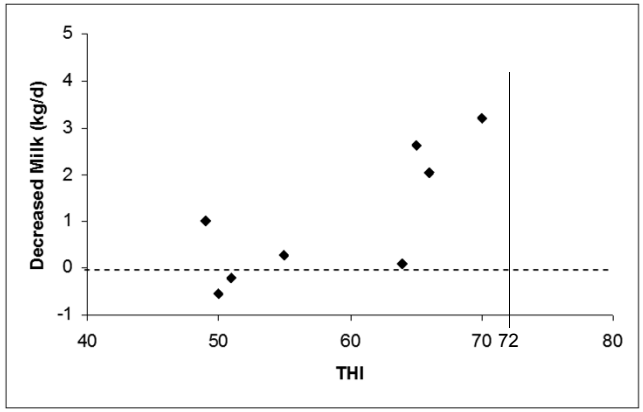
- When do modern dairy cows begin to experience heat stress?
- When should dairymen initiate cooling systems?
- Is it peak daily heat, average daily THI or minimum daily THI that is most indicative of heat stress?

Temperature Humidity Index Daily Average



Zimbelman et al., 2009

Temperature Humidity Index Daily Minimum



Zimbelman et al., 2009

THI Summary

- Modern high producing cows begin to experience heat stress at a THI of 65-68
  - Much lower than the traditional 72
- As milk production continues to increase, the THI at which cows become “stressed” will continue to decrease
- Pasture based cows will become heat-stressed sooner than those under shades…….solar radiation

Heat Stressed Cow

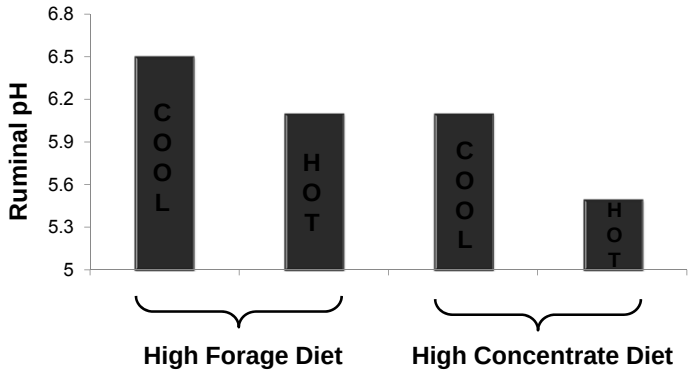
Results of Heat Stress

- Decrease in production (milk and growth)
- Reduced body condition
- Acute health problems
- **Rumen acidosis**
- Significant drop in pregnancy rate
  - See Albert DeVries webinar
- High incidence of abortions
- High death loss

Added all up … costly!

Effect of Heat Stress on Ruminal pH of Holstein Cows

(Mishra et al., JAS 30:1023)



Heat Stress Induced Rumen Acidosis

- Originates via:
  1) Altered respiration
     - Loss of systemic buffering capacity
  2) Changes in feed and feeding behavior
     - Reduced feed intake
     - Increased concentrates
     - "sorting"
     - "bout/slug" feeding
     - Drooling
     - Less saliva production

Increased Respiration Rate

- Body requires 20:1 ratio of HCO_3:CO_2 in blood
- Increased expired CO_2
- To compensate, the kidney dumps HCO_3
- Therefore less HCO_3 to buffer the rumen

Summary

- ↑ Respiration = ↓ blood HCO_3 = ↓ saliva HCO_3
- ↓ Feeding = ↓ rumination = ↓ saliva production
- ↑ Drooling = wasted saliva
- Altered feeding habits and "hotter" rations
- Accumulated effects = rumen acidosis

Heat Stress and Rumen Acidosis, Avoid It!

Metabolism Review

- Ad Libitum Intake
  - ↑ Insulin
  - ↓ NEFA
  - ↓ catabolic hormones
- Restricted Intake
  - ↓ Insulin
  - ↑ NEFA
  - ↑ catabolic hormones

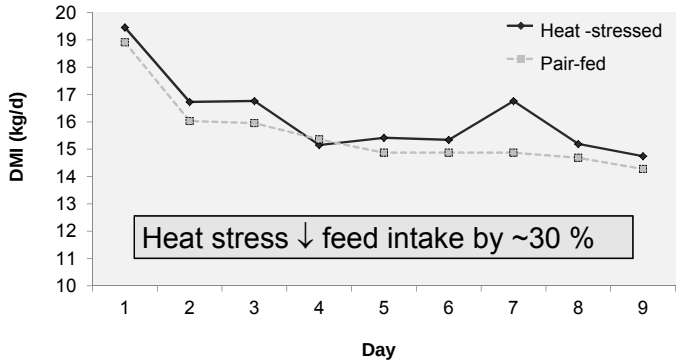
Heat Stress Questions??

- Does the decrease in feed intake explain the reduced milk yield during heat stress?

  Indirect vs. direct effects of heat

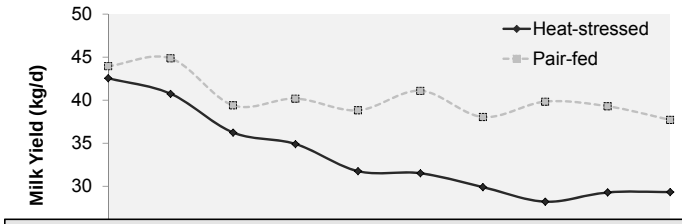
- If we have a better understanding of the biological reasons WHY heat stress reduces production, we'll have a better idea of how to alleviate it.

Lactation: Effects of Heat Stress on Feed Intake



Rhoads et al., 2009

Effects of Environment on Milk Yield

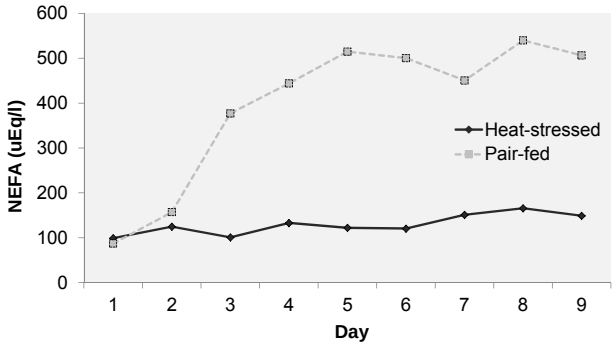


Heat stress ↓ yield ~45%
Pair-feeding ↓ yield by ~19%

Thus, ↓ feed intake only accounts for ~50% of the reductions in milk yield

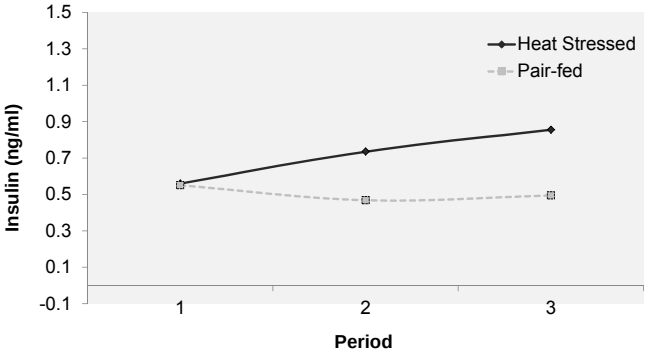
Rhoads et al., 2009
Wheelock et al., 2010

Effects of Heat Stress on Adipose Tissue Mobilization: Cattle



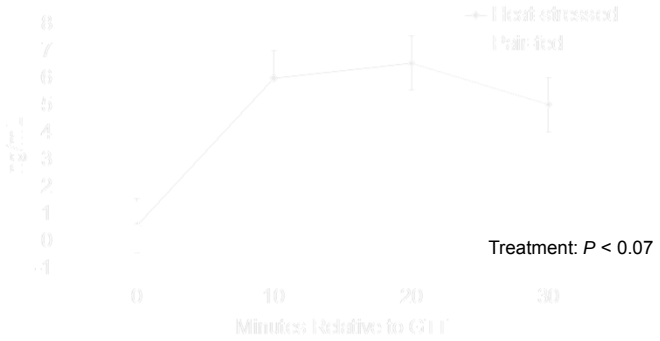
Rhoads et al., 2009

Circulating Insulin in Cattle



Wheelock et al., 2010

Insulin Response to the GTT

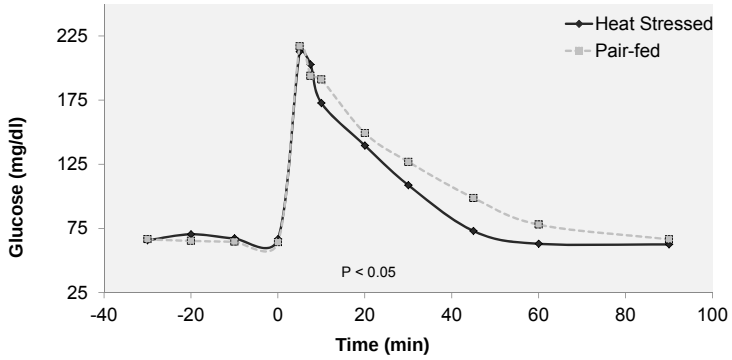


O'Brien et al., 2010

Potential Fuels for Ruminants

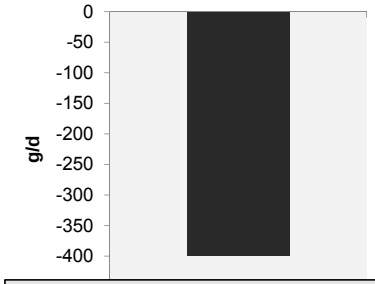
- VFA (acetate)
  - Contribution is presumably decreased b/c DMI is reduced
- NEFA
  - Do not increase during heat stress
- Amino Acids
  - Efficiency of capturing ATP is low
- Glucose
  - By process of elimination, glucose contribution to whole animal energetics may be increased?

Glucose Tolerance Test



Wheelock et al., 2008

Lactose: Heat Stress vs. Pair-Fed Thermal Neutrals

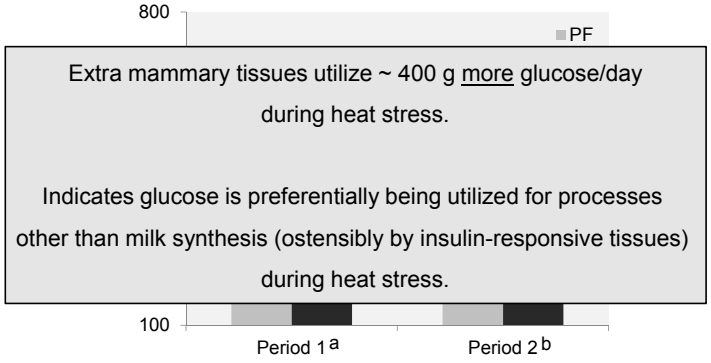


Heat Stress Cows Secrete ~400 g less lactose/day than Pair-Fed Thermal Neutral Controls

Is the liver producing ~ 400 g less glucose/day???? or is extra-mammary tissues utilizing ~400 g more/day

Rhoads et al., 2009
Wheelock et al., 2010

Whole Body Glucose Production



Extra mammary tissues utilize ~ 400 g more glucose/day during heat stress.

Indicates glucose is preferentially being utilized for processes other than milk synthesis (ostensibly by insulin-responsive tissues) during heat stress.

Period: $P < 0.05$

Wheelock et al., 2009 (ADSA abstract)

Energetic Summary

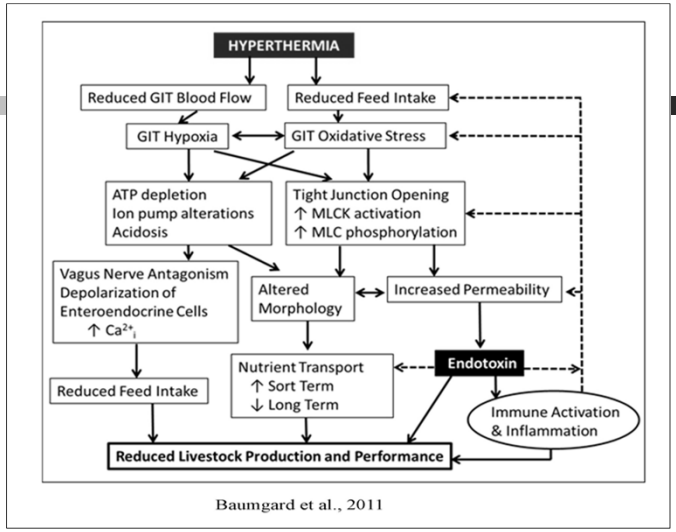
- Decreased feed intake only accounts for ~50% of the reductions in milk yield
- Tissue differences in sensitivity to catabolic and anabolic signals
- Heat-stressed cows have increased insulin action
  - Decreased NEFA
  - Increased glucose disposal
- Heat-stressed cows require extra energy
  - Especially glucose

Why Increased Insulin??

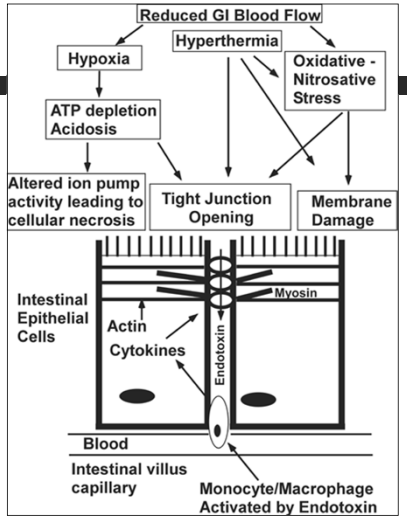
- Direct or Indirect effects of heat?
- Indirect: associated/caused by heat compromised gastrointestinal track barrier function?

Heat Stress and Gut Health

- Massive diversion of blood flow to skin and extremities
- Coordinated vasoconstriction in intestinal tissues
  - Reduced nutrient and oxygen delivery to enterocytes
  - Hypoxia increases reactive oxygen species (ROS)
- Reduced nutrient uptake increases rumen and intestinal osmolarity in the intestinal lumen
  - Multiple reasons for increased osmotic stress

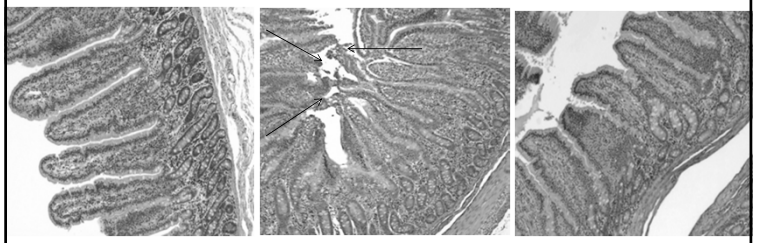


Baumgard et al., 2011



Lambert, 2009

Intestinal Morphology



Thermal Neutral | Heat Stress | Pair-fed

Pearce et al., 2011

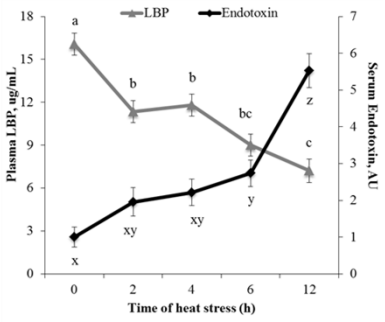
Heat Stress and Gut Integrity

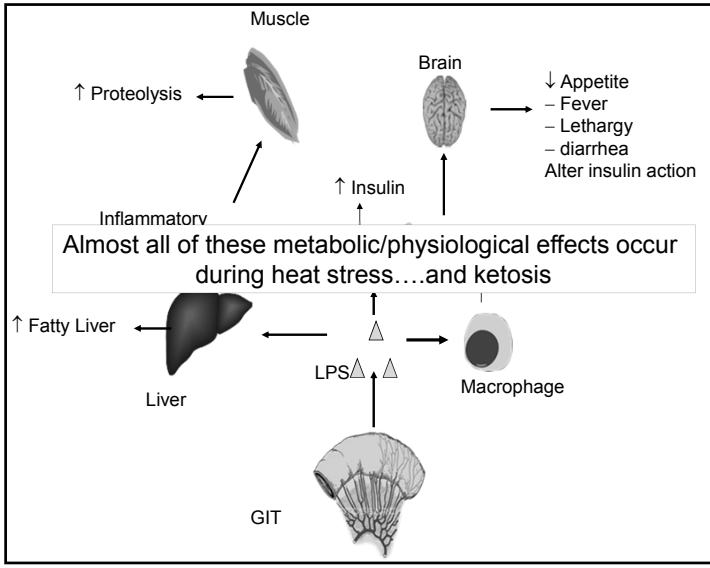
- Endotoxin (aka. Lipopolysaccharide: LPS)
- Component of bacteria cell wall
- When bacteria die, LPS is released into intestine
- Normally LPS is prevented from entering through GIT tight junctions
- During HS some LPS enters blood stream

Heat Stress and Gut Health

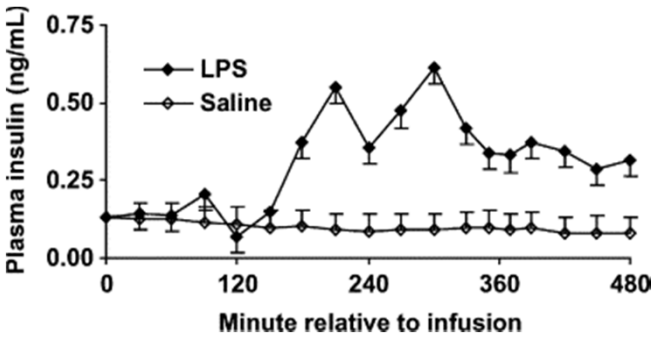
- LPS can cause liver damage
  - May impair gluconeogenesis capability
  - May impair ability to export VLDL (fatty liver)
  - May impair ability to secrete anabolic hormones
- LPS stimulates inflammatory cytokine production….catabolic condition
  - TNFα, IL-1 etc..
    - Reduced appetite
    - Stimulates fever
    - Causes muscle breakdown
    - Induces lethargy
    - ....reduces productivity

The effects are rabid!
Plasma LPS & LBP



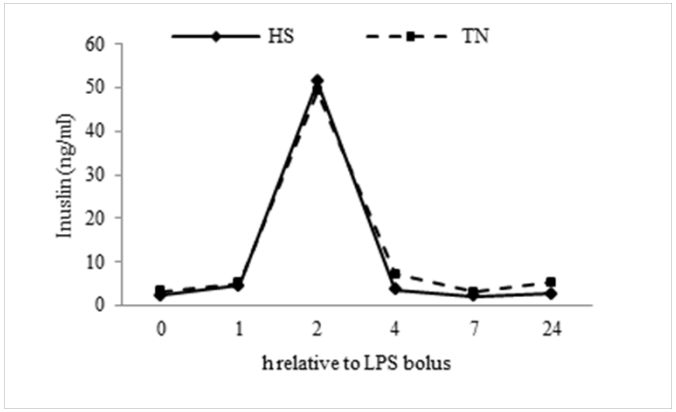


Mammary LPS Infusion Causes Increased Insulin Levels

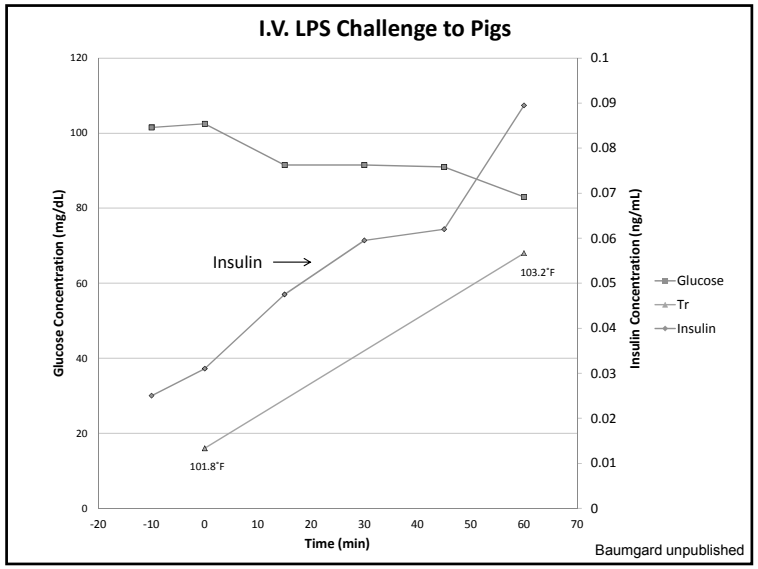


Matthew Waldron et al., 2006
University of Missouri

I.V. LPS Acutely Increases Insulin Secretion

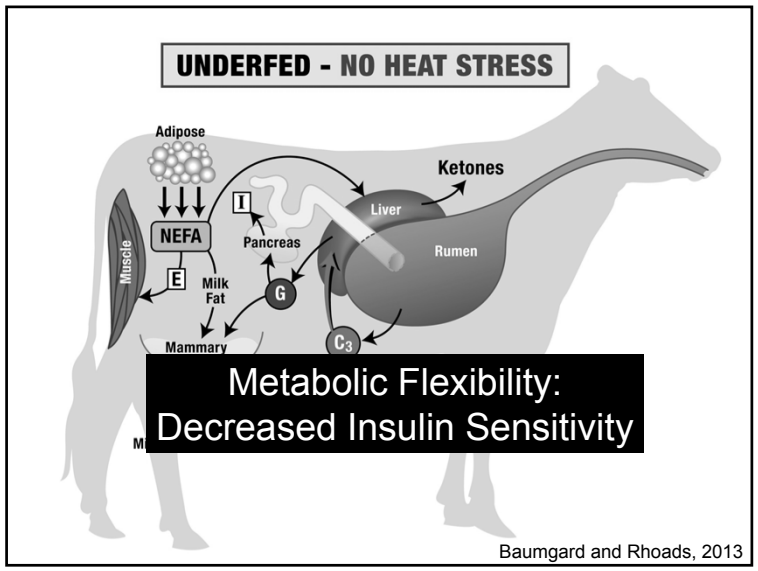


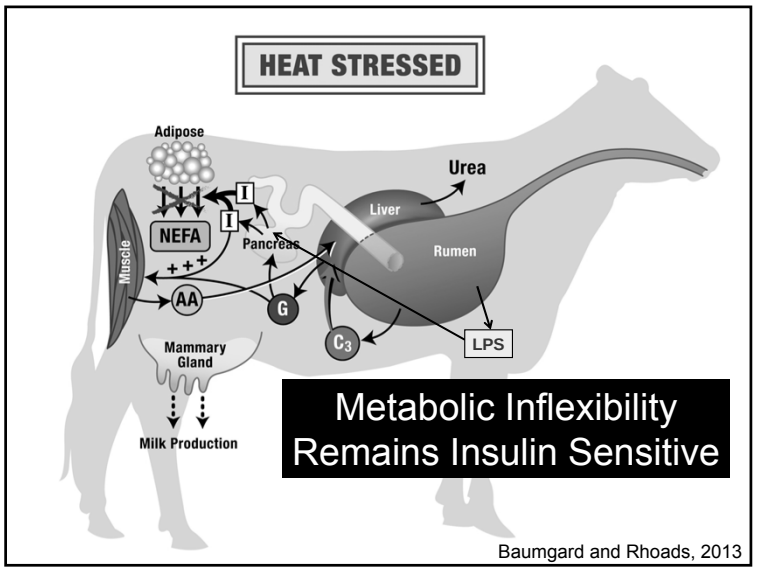
Rhoads et al., 2009 ADSA Abstract



Lactating Dairy Cow
Metabolic Adaptation to Heat Stress

Summary





Dietary and Management Options?

- Strategies recently evaluated by our group
  - Rumensin
    - Increases rumen propionate production
  - rbST
    - Partitions nutrients towards mammary gland
- BUT Heat Stress Abatement is the Key

Dietary and Management Strategies to Reduce the Negative Effects of Heat Stress

- Reduce walking distance
- Reduce time in holding pen
  - Ventilate and cool
- Exit lane cooling
- Don't "lock up or work" during mid day
- Feed early in the morning and late in the night
  - Push up often
  - Remove old feed
- Avoid vaccinations during the middle of the day
- At least provide shade for dry cows

Dietary and Management Strategies to Reduce the Negative Effects of Heat Stress

- Feed more frequently
  - Especially during the cooler parts of the day
- Fiber:
  - Avoid the temptation to reduce fiber content
  - Rumen acidosis
  - Production data: see J. Santos webinar
- Protein
  - Currently unknown if protein requirements change during heat stress
  - RDP about 10% of CP: see J. Santos webinar

Dietary and Management Strategies to Reduce the Negative Effects of Heat Stress

- Clean water tanks daily
  - Consider re-hydration therapies, especially in transition cows
    - Decreased rumen content of Na^+ and K^+ (Beede &Collier, 1982)
    - Electrolyte supplementation may be effective
  - Increased opportunity for dehydration
  - Medicate/supplement the water?
- Dietary HCO3
  - Helps prevent rumen acidosis
    - Heat stress cows are already prone to rumen acidosis
    - Can increase to 300-400 g/head/d during the summer

Dietary and Management Strategies to Reduce the Negative Effects of Heat Stress

- Dietary Fat (by-pass)
  - Additional energy without the heat increment of fermentation
    - Heat stressed cows are in negative energy balance dietary fat should help maintain milk yield and body condition
    - Can go up to 7-8% of dietary dry matter
- Potassium
  - Cows use potassium to sweat, thus there is an increased potassium need during heat stress
  - Can increase to 1.7% of ration dry matter
  - Consider $K^{+}HC0^{3}$……consider the costs
  - Be careful of a positive DCAD in dry cows

Dietary and Management Strategies to Reduce the Negative Effects of Heat Stress

- Betaine:
  - Not for methyl donor reasons
  - But for GIT integrity reasons
  - Used extensively in the Asian poultry and swine industries during heat stress
- Niacin
  - Increases skin vasodilatation and decreases body temperature: Whether small decreases in rectal temperature translates into improved production remains to be determined

Dietary and Management Strategies to Reduce the Negative Effects of Heat Stress

- Chromium
  - Appears to improve productivity, likely due to increased DMI
- DCAD:
  - Keep in 30-40 meq/100 g of DM
  - No apparent improvements of going higher
- Direct fed microbials/yeast
  - Products that increases rumen digestion, stabilizes pH, increases propionate and increases DMI should benefit a heat stressed cow
    - The inconsistencies in the literature regarding these variables is of interest

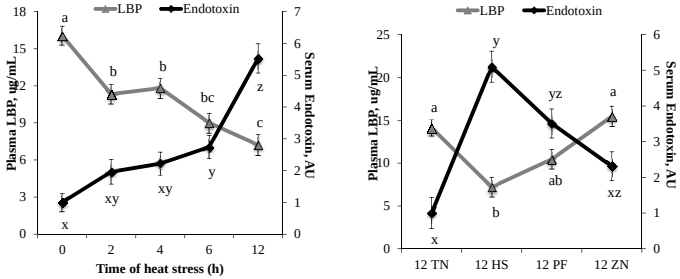
Potential nutritional strategies to ameliorate intestinal permeability

Supplement	Presumed Mechanism of Action
Bicarbonate	Acidosis prevention
Glutamine	↑ intestine integrity
Zinc	↑ intestine integrity, antioxidant
Dairy Products	↑ intestine integrity
Vitamin A	Antioxidant
Vitamin C	Antioxidant
Vitamin E	Antioxidant
Selenium	Antioxidant
Dexamethasone	↑ intestine integrity
Betaine	Osmotic regulation; CH_3 donor
Conjugated Linoleic Acid	↑ Energy balance
Chromium	↑ Feed Intake
Yeast, yeast extract/DFM	Acidosis prevention & ↑ Feed Intake
Ionophores	Acidosis prevention
β-glucan	Immune modulation
Mannanoligosaccharide	↑ intestine integrity
Rehydration therapy	↑ intestine integrity & ↑ Feed Intake
Butyrate	↑ intestine integrity
Mycotoxin binders	↑ intestine integrity

Gut Health and Zinc

- Alam et al., 1994. Enteric protein loss and intestinal permeability changes in children during acute shigellosis and after recovery: effect of zinc supplementation
- Rodriguez et al., 1996. Intestinal paracellular permeability during malnutrition in guinea pigs: effect of high dietary zinc. Gut. 39:416-422.
- Sturniolo et al., 2001. Zinc supplementation tightens “leaky gut” in Crohn’s disease. Inflamm. Bowel Dis. 7:94-98.
- Finamore et al., 2008. Zinc deficiency induces membrane barrier damage and increases neutrophil transmigration in Caco-2 cells. J. Nutr. 138:1664-1670
- Peterson et al., 2008. Moderate zinc restriction affects intestinal health and immune function in lipopolysaccharide-challenged mice. J. Nutr. Biochem. 19:193-198.
- Mahmood et al., 2009. Zinc carnosine, a health food supplement that stabilizes small bowl integrity and stimulates gut repair processes. Gut 56:168-175

Zinc: Plasma LPS & LBP



Gabler and Baumgard, unpublished

Heat Stress Abatement

BUT the primary strategy to improve production during heat stress is management!

Shade, soakers, misters, fans, etc.., even in humid Environments

Elanco Heat Abatement Management Guide
Ask your Elanco Rep for a copy or see URL
www.elanco.us/pdfs/usdbunon00147_heat_guide.pdf

Summary

- Concentrate on maintaining healthy rumen pH
  - It will pay dividends during late Summer and Fall
- Heat stress markedly effects metabolism independent of reduced nutrient intake
  - Can in large-part be explained by increased insulin action
  - Maximizing glucose synthesis will improve production
- There is no dietary magic pill
- Dietary and management modifications
  - Ionophores, rbST etc…
  - Fat feeding makes sense
  - Consult with your nutritionist

Questions?

Acknowledgments

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  - # 2008-35206-18817
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- Zinpro Inc.
- Elanco Animal Health
- Midwest Dairy Association
- National Pork Board
- Iowa Pork Producers
- TechMix
- Form A Feed
- Kemin Industries
- ViCor Corp
- Murphy Brown
- Nutriad Inc.

USDA NIFA United States Department of Agriculture National Institute of Food and Agriculture

- Victoria Sanz-Fernandez
- Sarah Pearce
- Jay Johnson
- Rebecca Boddicker
- Amir Nayeri
- Nathan Upah
- Anna Gabler
- Sam Lei

Heat Increment of Feed

- Heat produced from fermentation AND post-absorptive metabolism
- Very difficult to determine
- Rumen
  - Acetate fermentation creates more heat
  - Fat has very little heat of fermentation
- Post-absorptive
  - Depends upon metabolic fate
    - Stored or oxidized
  - Acetate utilization is less efficient than propionate

Heat Increment of Common Feed Ingredients

Feed Ingredient	DM (%)	NDF % of DM	TDN % of DM	NE_L (Mcal/Kg)	HI (Mcal/ton)	HI/NE_L (Kcal/Mcal)
Haylage	35.0	53.0	59.0	1,326	277.32	658
Corn Silage	38.3	48.0	66.1	1,500	321.85	617
Grass Hay	88.0	53.0	55.0	1,228	672.10	684
Alfalfa Hay	89.9	47.5	60.0	1,350	718.59	651
Whole Cottonseed	93.0	49.0	87.0	2,453	801.15	386
Corn	87.0	10.0	88.0	2,035	886.23	550
SBM, 48%	90.0	14.0	81.0	1,866	857.54	562
Palm Oil (FA)	100.0	0.0	170.1	5,676	1,103.96	214
Prill (FA)	100.0	0.0	170.1	6,776	1,314.23	214
Tallow	99.0	0.0	191.3	6,402	1,228.81	214

Adapted from Chandler, 1994. Values for heat increment where derived from a multiple linear regression model: $y=a+bx+cx^2$. Where y= Kcal HI/Mcal NE_L and x=TDN solved constants are a= 1350.812, b= -17.1496, and c= 0.091517

Effects of Supplemental Dietary Fat on Body Temperature Indices and Production Parameters in Lactating Cows

Reference	Fat Type	RT	RR	DMI	FE	MY	MF	MP	Metabolites
1	SFA/UFA	↑	↑	↓	↑	↔	↑	↔	↑ NEFA
2	SFA	↓	↔	↔	↑	↑	↑	↑	↓ NEFA
3	SFA	NM	NM	↔	↔	↑	↓	↑	NM
4	LCFA	↔	↔	↔	↑	↑	↔	↓	↓ NEFA
5	SFA	NM	NM	↔	↔	↑	↑	↑	NM
6	SFA	↔	↔	↔	↔	↔	↔	↔	NM
7	LCFA/Tallow	↔	↔	↔	↔	↔	↔	↔	NM
8	SFA	NM	NM	↔	↔	↑	↔	↔	↔
9	SFA/UFA	↔	↔	↔	↔	↔	↔	↔	↔

NM: Not Measured
↑: Increase
↓: Decrease
↔: No Change
SFA: Saturated Fatty Acids
UFA: Unsaturated Fatty Acids
LCFA: Long-Chain Fatty Acids

RT: Rectal Temperature
RR: Respiratory Rate
DMI: Dry Matter Intake
FE: Feed Efficiency
MY: Milk Yield
MF: Milk Fat
MP: Milk Protein
NEFA: Non-Esterified Fatty Acids

1 Moallem et al., 2010
2 Wang et al., 2010
3 Warntjes et al., 2008
4 Drackely et al., 2003
5 Gallardo et al., 2001
6 Chan et al., 1997
7 Knapp and Grummer, 1991
8 Skaar et al., 1989
9 Moody et al.,1967

Feeding Dietary Fat

- Milk yield responses are variable
  - About 50% (better than most feed supplements)
- Does not appear to improve body temperature indices
  - Small decreases may be difficult to detect at specific but limited time points
  - Would be of interest to measure body temp continuously in additional fat-fed heat-stressed fat fed cows
- Dry matter intake can sometimes decrease in thermal neutral cows
  - This does not happen during heat stress

HEAT STRESS: WHAT'S THE GUT GOT TO DO WITH IT?

M.V. Sanz-Fernandez[1], S.K. Stoakes[1], J.S. Johnson[1], M. Abuajamieh[1], J.T. Seibert[1], S.C. Pearce[1], N.K. Gabler[1], R.P. Rhoads[2] and L.H. Baumgard[1]

[1]Iowa State University, Ames, Iowa
[2]Virginia Tech. State University, Blacksburg, Virginia

TAKE HOME MESSAGE

Heat stress (HS) is a global problem which jeopardizes animal welfare, profitability, and global food security. Indirect effects of HS such as reduced feed intake contribute to, but do not fully explain, decreased productivity. Heat stressed animals initiate metabolic changes that do not reflect their plane of nutrition. This indicates that HS directly effects metabolism and productivity independent of reduced feed intake. In a variety of species, environmental hyperthermia compromises the intestinal barrier function resulting in increased permeability to luminal content including bacteria and bacterial components. Presumably, heat stress causes leaky gut in ruminants as well. The leakage of luminal content into the portal and ultimately the systemic circulation elicits an inflammatory response that may facilitate the detrimental effects of HS on animal agriculture. Identifying flexible management strategies (i.e. nutritional supplementation) to immediately decrease HS susceptibility without negatively influencing production traits would be of great value to global animal agriculture.

INTRODUCTION

Economic Impact

Heat stress negatively impacts a variety of dairy production parameters including milk yield, milk quality and composition, rumen health, growth and reproduction, and is a significant financial burden (~$900 million/year for dairy, and > $300 million/year in beef and swine in the U.S. alone; St. Pierre et al., 2003; Pollman, 2010). When the ambient temperature and other environmental conditions create a situation that is either below or above the respective threshold values, efficiency is compromised because nutrients are diverted to maintain euthermia as preserving a safe body temperature becomes the highest priority, and product synthesis (milk, meat, etc.) is deemphasized. Advances in management (i.e. cooling systems; VanBaale et al., 2005) and nutritional strategies (West, 2003) have partially alleviated the negative impacts of HS on cattle, but productivity continues to decline during the summer. The detrimental effects of HS on animal welfare and production will likely become more of an issue in the future if the earth's climate continues to warm as predicted (IPCC 2007) and some models forecast extreme summer conditions in most U.S. animal producing areas (Luber and McGeehin, 2008). A 2006 California heat wave purportedly resulted in the death of more than 30,000 dairy cows (CDFA, 2006) and a recent heat wave in Iowa killed at least 4,000 head of beef cattle (Drovers Cattle Network, 2011). Furthermore, almost 50% of

Canadian summer days are environmentally stressful to dairy cows (Ominski et al., 2002). This illustrates that most geographical locales, including temperate and northern climates, are susceptible to extreme and lethal heat. Thus, for a variety of aforementioned reasons, there is an urgent need to have a better understanding of how HS alters nutrient utilization and ultimately reduces animal productivity. Defining the biology of how HS jeopardizes animal performance is critical in developing approaches (genetic, managerial, nutritional and pharmaceutical) to ameliorate current production issues and improve animal well-being and performance. This would help secure the global agricultural economy by ensuring a constant supply of animal products for human consumption.

Direct and Indirect Effects of Heat Stress

Reduced feed intake during HS is a highly conserved response among species and presumably represents an attempt to decrease metabolic heat production (Baumgard and Rhoads, 2012). It has traditionally been assumed that inadequate feed intake caused by the thermal load was responsible for decreased milk production (Beede and Collier, 1986; West, 2003). However, our recent results challenge this dogma as we have demonstrated disparate slopes in feed intake and milk yield responses to a cyclical heat load pattern (Shwartz et al., 2009). To test this, we employed the use of a thermoneutral pair-fed group in our experiments which allowed us to evaluate thermal stress while eliminating the confounding effects of dissimilar nutrient intake. Our experiments demonstrate that reduced feed intake only explains approximately 35-50% of the decreased milk yield during environmental-induced hyperthermia (Rhoads et al., 2009a; Wheelock et al., 2010; Baumgard et al., 2011). This indicates that HS directly effects nutrient partitioning beyond that expected by reduced feed intake.

An appreciation of the physiological and metabolic adjustments to thermoneutral negative energy balance (NEBAL; i.e. underfeeding or during the transition period) is prerequisite to understanding metabolic adaptations occurring with HS. Early lactation dairy cattle enter a unique physiological state during which they are unable to consume enough nutrients to meet maintenance and milk production costs and typically enter NEBAL (Baumgard and Rhoads, 2013). Negative energy balance is associated with a variety of metabolic changes that are implemented to support the dominant physiological condition of lactation (Bauman and Currie, 1980). Marked alterations in both carbohydrate and lipid metabolism ensure partitioning of dietary and tissue derived nutrients towards the mammary gland, and not surprisingly many of these changes are mediated by endogenous somatotropin which naturally increases during periods of NEBAL. One classic response is a reduction in circulating insulin coupled with a reduction in systemic insulin sensitivity. The reduction in insulin action activates adipose lipolysis, leading to the mobilization of non-esterified fatty acids (NEFA; Bauman and Currie, 1980). Increased circulating NEFA are typical in transitioning cows and represent (along with NEFA derived ketones) a significant source of energy (and precursors for milk fat synthesis) for cows in NEBAL. Postabsorptive carbohydrate metabolism is also altered by reduced insulin action during NEBAL resulting in reduced

glucose uptake by systemic tissues (i.e. muscle and adipose). Reduced nutrient uptake coupled with the net release of nutrients (i.e. amino acids and NEFA) by systemic tissues are key homeorhetic (an acclimated response vs. an acute/homeostatic response) mechanisms implemented by cows in NEBAL to support lactation. The thermoneutral cow in NEBAL is metabolically flexible, and can depend upon alternative fuels (NEFA and ketones) to spare glucose. Glucose can then be utilized by the mammary gland to copiously produce milk (Bauman and Currie, 1980).

Well-fed ruminants primarily oxidize acetate (a rumen produced VFA) as a principal energy source. During NEBAL, cattle increase their energy dependency on NEFA. However, despite the fact that heat stressed cows have marked reductions in feed intake and are losing considerable amounts of body weight, they do not mobilize adipose tissue (Rhoads et al., 2009a; Wheelock et al., 2010). Therefore, it appears that heat stressed cattle experience altered post-absorptive metabolism compared to thermoneutral counterparts, even though they are in a similar negative energetic state (Moore et al., 2005; Rhoads et al., 2013). The unusual lack of NEFA response in heat stressed cows is probably in part explained by increased circulating insulin levels (O'Brien et al., 2010; Wheelock et al., 2010), as insulin is a potent anti-lipolytic hormone. Increased circulating insulin during HS is unusual as malnourished animals are in a catabolic state and experience decreased insulin levels. We have recently demonstrated that heat stressed growing pigs undergo similar metabolic adaptations (Pearce et al., 2013a), suggesting that this is a well conserved response vital for the acclimation to HS. Increased insulin action may also explain why heat stressed animals have greater rates of glucose disposal (Wheelock et al., 2010). Therefore, during HS, preventing or blocking adipose mobilization/breakdown and increasing glucose "burning" is presumably a strategy to minimize metabolic heat production (Baumgard and Rhoads, 2013). The enhanced extra-mammary glucose utilization during HS creates a nutrient trafficking problem with regards to milk yield. The mammary gland requires glucose to synthesize milk lactose which is the primary osmoregulator determining overall milk volume. Therefore, the mammary gland may not receive adequate amounts of glucose resulting in reduced mammary lactose and subsequent milk production. This may be a primary mechanism accounting for additional reductions in milk yield beyond the portion explained by decreased feed intake.

LEAKY GUT: RESPONSIBLE FOR THE DIRECT EFFECTS OF HEAT STRESS?

Mechanisms responsible for altered nutrient partitioning during HS are not clear, however, they might be mediated by HS effects on gastrointestinal health and function (Figure 1). The small intestine is one of the first tissues up-regulating heat shock proteins during a thermal load (Flanagan et al., 1995), demonstrating a higher sensitive to heat damage (Kregel, 2002). During heat stress, blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat (Lambert et al., 2002), leading to intestinal hypoxia (Hall et al., 1999). Enterocytes are particularly sensitive to hypoxia and nutrient restriction (Rollwagen et al., 2006), resulting in ATP depletion and increased oxidative and nitrosative stress (Hall et al., 2001). This contributes to tight

junction dysfunction, and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013b). As a result, HS increases the passage of luminal content as lipopolysaccharide (LPS) into the portal and systemic blood (Hall et al., 2001; Pearce et al., 2013b). Further, endotoxemia is common among heat stroke patients (Leon, 2007) and it is thought to play a central role in heat stroke pathophysiology, as survival increases when intestinal bacterial load is reduced (Bynum et al., 1979) or when plasma LPS is neutralized (Gathiram et al., 1987). It is remarkable how animals suffering from heat stroke or severe endotoxemia share many physiological and metabolic similarities such as an increase in circulating insulin (Lim et al., 2007). Infusing LPS into the mammary gland increased (~2 fold) circulating insulin in lactating cows (Waldron et al., 2006). In addition, we intravenously infused LPS into growing calves and pigs and demonstrated >10 fold increase in circulating insulin (Rhoads et al., 2009b; Stoakes and Baumgard, unpublished). Again, the increase in insulin in both models is energetically difficult to explain as feed intake was severely depressed in both experiments.

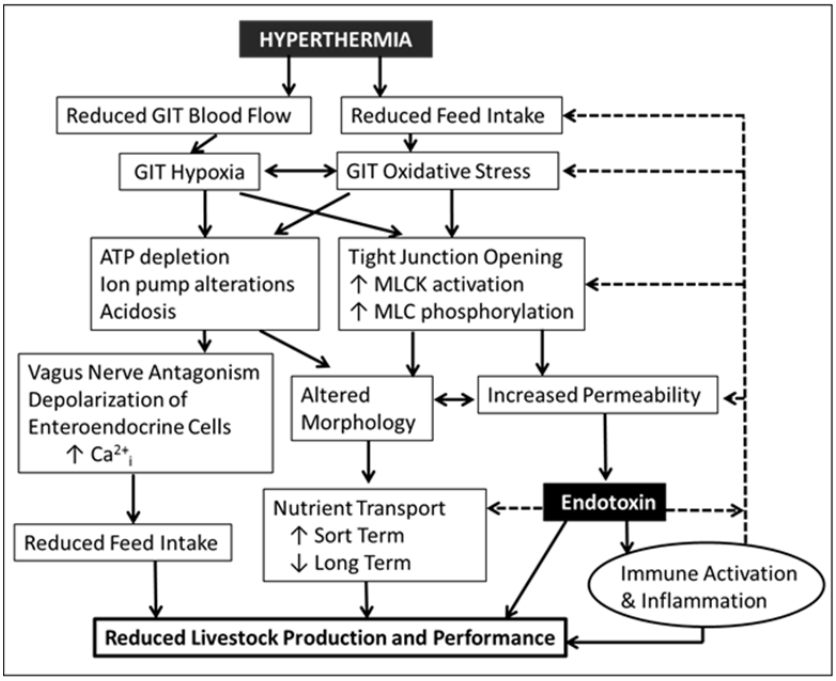

Figure 1: Etiology of heat stress induced leaky gut

Intestinal Integrity & Steatohepatitis

Interestingly, a variety of diseases associated with increased intestinal permeability such as heat stress and stroke, Crohn's disease, inflammatory bowel disease, Celiac disease, and alcoholism are often associated with increased plasma LPS concentrations and an inflammatory acute phase response (Bouchama et al., 1993; Pearce, et al. 2013b; Draper et al., 1983; Parlesak et al., 2000; Ludvigsson et al., 2007). There is increasing evidence that translocation of gut microbiota contributes to hepatic inflammation (Bieghs and Trautwein, 2013) which might impair liver function leading to fat accumulation and ultimately steatohepatitis (Ilan, 2012; Dumas et al.,

2006; Solga and Diehl, 2003; Farhadi et al., 2008; Miele et al., 2009). The association between leaky gut and fatty liver is of particular interest in the ruminant animal who is already an inefficient exporter of hepatic lipids. There is reason to believe that similar breakdown of gut integrity may be responsible for hepatic disorders (e.g. fatty liver and ketosis; Figure 2) frequently observed in the transition dairy cow. A transitioning dairy cow undergoes a post-calving diet shift from a mainly forage based to a high concentrate ration. This has the potential to induce rumen acidosis which can compromise the gastrointestinal tract barrier (Khafipour et al., 2009). In addition, calving is a physically stressful event and cytokines released from the damaged reproductive tract may have an impact on the liver's ability to export lipids. Preliminary data has shown an increase in plasma lipopolysaccharide binding protein (LPSBP), an acute phase protein which binds LPS to stimulate an immune response, in cows that required treatment for clinical ketosis compared to healthy transition cows (Nayeri et al., 2012). Nevertheless, the effects of the transition period on the intestinal barrier function and its role in the development of fatty liver and ketosis among other transition disorders remain unknown and require further investigation.

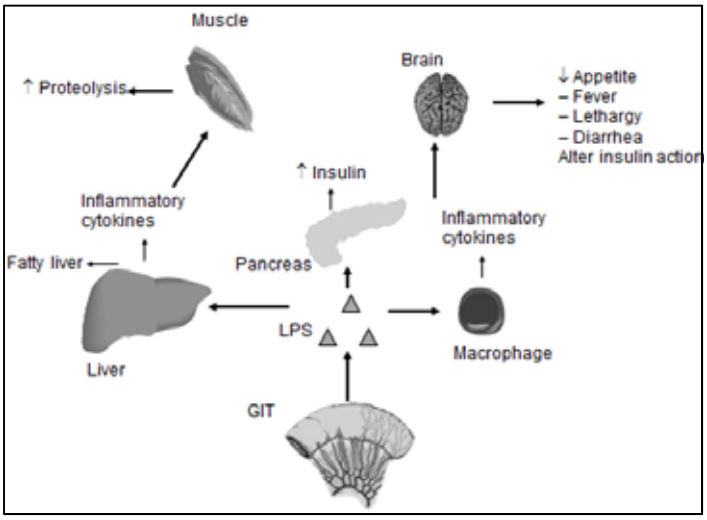

Figure 2: LPS induced metabolic alterations

NUTRITIONAL STRATEGIES TO PREVENT LEAKY GUT

Bicarbonate

Acidosis may exacerbate intestinal issues (Khafipour et al., 2009) as rumen pH is lowered during the summer months (Mishera et al., 1970). This may be explained by increased respiration rate which decreases blood carbon dioxide (CO_2) and increases the need for the kidney to secrete bicarbonate to maintain a healthy 20 to 1 ratio of bicarbonate to CO_2 in the blood. Increased secretion of bicarbonate by the kidney reduces the amount available to be used in the saliva to buffer rumen pH. In addition, reduced feed intake results in reduced rumination time which is a key stimulator of saliva production. Thus, the increased susceptibility of heat-stressed cattle to rumen acidosis might be prevented by dietary bicarbonate supplementation.

Glutamine

Glutamine is a conditionally non-essential amino acid as it can be formed from ammonia and and glutamate. It is a primary energy source for intestinal cells (Singleton and Wischmeyer, 2006) and supplemental dietary glutamine has demonstrated improvement in intestinal barrier function in malnourished children (Lima et al., 2005). A potential mechanism of action for glutamine's beneficial effects is the enhanced expression of heat-shock protein 70 (Singleton and Wichmeyer, 2006). Glutamine supplementation to high producing thermoneutral cows did not improve milk yield (Metcalf et al., 1996). However, a study by Caroprese and co-workers (2013) demonstrated that during HS, glutamine supplementation improved milk, fat, protein, and casein yields. Caroprese and colleagues also observed improvement in cell mediated immune response which was likely responsible for the observed lower somatic cell count, possibly indicating a role for glutamine in the alleviation of mastitis.

Zinc

Dietary zinc is essential for normal intestinal barrier function (Alam et al., 1994), and supplemental zinc is beneficial in a variety of animal models and human diseases characterized by increased intestinal permeability (Alam et al., 1994; Zhang and Guo, 2009). We have recently demonstrated that supplemental zinc can partially alleviate the effects HS on intestinal integrity in acute and chronically heat-stressed growing pigs (Sanz-Fernandez et al., 2014; Pearce et al., 2013b). The mechanisms by which zinc improves intestinal integrity are not well understood, but might include: the up-regulation of tight junction proteins (Zhang and Guo, 2009), a role as antioxidant via induction of metallothioneins (Wang et al., 2013), and/or increasing the expression of antimicrobial substances as β-defensins (Mao et al., 2013).

Dairy Products

Dietary dairy products (e.g. colostrum and whey protein) have been also demonstrated to improve intestinal health under different types of challenges (Playford et al., 1999 and 2001; Khan et al., 2002; Prosser et al., 2004). Interestingly, dietary dairy products have demonstrated alleviation of HS effects on the intestinal barrier function both *in vivo* (Prosser et al., 2004) and *in vitro* (Marchbank et al., 2011). Once again their mechanisms of action are not well understood but both colostrum and whey protein are rich in antimicrobial proteins (e.g. glucomacropeptides, lactoferrin), immunoglobulins, growth factors (e.g. Transforming Growth Factor-β), and certain amino acids (glutamine, cysteine, and threonine; Krissansen, 2007). Further, dietary dairy products have shown to up-regulation heat-shock protein 70 (Marchbank et al., 2011) and tight junction proteins (mediated by TGF-β; Hering et al., 2011), and increase mucin production (mediated by threonine and cysteine; Sprong et al., 2010); which might explain their beneficial effect on intestinal health.

Inhibiting milk fat synthesis during HS may attenuate or eliminate the negative energy balance. As a result of the extra available energy, synthesis of other milk and milk components may increase (i.e., lactose and protein). In addition to enhancing milk yield, inhibiting milk fat synthesis and thus improving energy balance may improve animal well-being and reproductive success (Bauman et al., 2001). We utilized conjugated linoleic acid (CLA) in an attempt to strategically improve energy balance during HS, but did not detect any noticeable improvement in production variables (Moore et al., 2005).

Antioxidants

Hypoxia of the small intestine during HS can lead to oxidative stress and production of free radicals (Hall et al., 1999). In addition, intestinal inflammation leads to loss of antioxidant capacity (Buffinton and Doe, 1995b). Therefore, supplementation of antioxidants such as selenium and vitamins A, E, and C during HS is of great interest.

Vitamin A can mitigate the effects of induced mucosal damage (Elli et al., 2009) and deficiency can have negative effects on immunity and integrity in the gut (Yang et al., 2011; Thurnham et al., 2000). This was the case of vitamin A-deficient beef calves that suffered reduced intestinal integrity and were more susceptible to a secondary E. coli infection (He, et al., 2012). Dietary vitamin A has the potential to improve weight gain and feed efficiency in HS broilers and this effect was amplified when vitamin A was combined with zinc (Kucuk et al., 2003). In addition, cows supplemented with β-carotene during hot months had increased milk yield and pregnancy rates (Aréchiga et al., 1998).

Table 1. Potential nutritional strategies to ameliorate intestinal permeability

Supplement	Presumed Mechanism of Action
Bicarbonate	Acidosis prevention
Glutamine	↑ intestine integrity
Zinc	↑ intestine integrity
Dairy Products	↑ intestine integrity
Vitamin A	Antioxidant
Vitamin C	Antioxidant
Vitamin E	Antioxidant
Selenium	Antioxidant
Dexamethasone	↑ intestine integrity
Betaine	Osmotic regulation; CH_3 donor
Conjugated Linoleic Acid	↑ Energy balance

Vitamin E supplementation has reduced gut bacterial translocation and increased survival in radiation induced intestinal injury (Singh et al., 2012). Supplementation also increases vitamin A serum concentrations, suggesting a protective role for vitamin E on vitamin status (Sahin et al., 2002b). Sahin and coworkers (2002a) also demonstrated improved production performance in Japanese quails supplemented with vitamin C and

E during HS. Dairy cows administered 3000 IU of vitamin E during two consecutive summers had similar pregnancy rates compared to controls (Ealy et al., 1994), however little research has examined its effects on production and immune status in dairy cows.

Vitamin C is decreased in inflammatory bowel disease patients (Buffinton and Doe, 1995a) as well as heat stressed lactating cows (Padilla and Matsuia, 2006). Supplementation has demonstrated positive effects during HS by reducing tocopheroxyl radicals back to the active form of vitamin E (Sahin, 2002b).

Selenium is part of selenoproteins such as glutathione peroxidase, which is a major free radical scavenger system in the cell (Loeb et al., 1988). Selenoproteins also play an important role in cell growth as deficiency has been linked to DNA damage and poor cell cycle control (Rao et al., 2001) which may be pertinent to intestinal integrity due to high enterocyte turnover rate. In patients with celiac disease, characterized by small intestine damage, selenium deficiency is a risk factor due to poor absorption which can lead to increased reactive oxygen species and inflammation (Stazi and Trinti, 2008; Barrett et al., 2013). Supplementation with selenium has the potential to reduce lipid peroxidation and epithelial damage to intestinal mucosa, and prevent bacterial translocation (Baldwin and Wiley, 2002; Oztürk et al., 2002). Sheep injected with selenium during HS lost less weight compared to their HS control counterparts (Alhidary et al., 2012).

Many of the antioxidant compounds listed above have synergistic effects with one another or with minerals like zinc (Kucuk et al., 2003; Sahin et al., 2002a, 2002b). Research demonstrating effects of supplemental antioxidant on production parameters during HS is scarce and further research is needed to allow for the development of supplementation recommendations, particularly in ruminants.

Dexamethasone

Dexamethasone is a synthetic corticosteroid with anti-endotoxic and anti-inflammatory properties. Previous research has demonstrated a marked increase in corticosteroids in response to HS (Collier et al., 1982; Baumgard and Rhoads, 2013). Dexamethasone prevented the increase in plasma aspartate transaminase and alanine aminotransferase (both markers of hepatic health), IL-6 and LPS in a rat model of heat stroke, probably by blocking endotoxemia (Lim et al., 2007). Also in a heat stroke model, primates injected with corticosteroid had reduced endotoxemia as well as an increased survival rate (Gathiram et al., 1988a, 1988b). Further research is needed within the livestock industry to explore potential pharmacological roles of dexamethasone in heat stress abatement practices.

Betaine

Betaine, also known as trimethylglycine, is an osmotic regulator and methyl donor which may exhibit several beneficial effects in heat-stressed animals including the potential to protect against osmotic stress by decreasing sodium potassium pump activity (Cronje, 2007).

Betain supplementation improves intestinal integrity in both healthy and coccidian infected birds (Kettunen et al., 2001). In addition, betaine ameliorated the effects of HS on weight gain, immunity and body temperature indices in rabbits (Hassan et al., 2011). Supplemented thermoneutral mid-lactation dairy cows experienced an increase in milk yield, a decrease in milk protein percent, and altered milk fatty acid profile (Peterson et al., 2012). However, no differences were observed in milk production parameters in HS cows (Hall et al., 2012). Lack of sufficient evidence in support of or against betaine's role in HS alleviation warrants the need for further investigation.

MANAGEMENT STRATEGIES

Despite increased efforts to combat HS through nutritional strategies, cooling technology and management practices still represent the main approach to relieve HS. Providing shade, ventilation, and cooling as well as reducing walking distance can be strategies implemented to reduce the harmful effects of HS. Increasing milking frequency is strategy that has not been thoroughly evaluated during HS, but is a well-described lactogenic stimulant during thermal neutral conditions (Fitzgerald et al., 2007). Controlling the timing of feeding is also beneficial, as early morning and late night feeding helps to push the peak heat of fermentation to cooler parts of the day. Pushing up feed often so cows consume several small meals instead of a few large meals will aid in acidosis prevention and reduce steep increases in metabolic heat caused by consuming a large meal. Stressors of any kind (i.e. vaccinations) should be avoided during hotter parts of the day as the combination of HS and handling stress is unfavorable. Administration of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) should be avoided as they may exacerbate gastrointestinal integrity issues.

CONCLUSION

High ambient temperatures have a negative effect on animal health and performance, costing billions of dollars in losses to global animal agriculture. Gut integrity is compromised by HS and the resultant systemic inflammation might partially explain its negative effects on production. Nutrition is an example of an easily adjustable tactic to ameliorate the detrimental effects of environmental hyperthermia. For instance, heat-stressed animals shift energy metabolism toward carbohydrate usage and reduce lipid oxidation. Therefore, diets or nutritional supplements promoting glucose production (i.e. ionophores) and utilization may be useful. In addition, intestinal health improvement via dietary supplementation might be advantageous. Finally, cooling management practices such as shade, evaporative cooling, and strategic timing of farm activities aid in the mitigation of the adverse effects of HS. Even in today's most

well managed dairies, HS remains a problem. In order to resolve current HS production issues and develop better mitigation strategies, a better understanding of the biology and mechanisms of how HS threatens animal health is essential.

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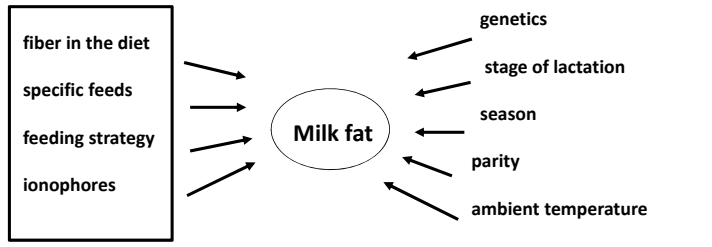
PENNSTATE

Fatty Acid Nutrition and Milk Fat Depression

Kevin Harvatine, Ph.D.
Assistant Professor of Nutritional Physiology
Penn State University
kjh182@psu.edu

2015 Herd Health and Nutrition Conferences

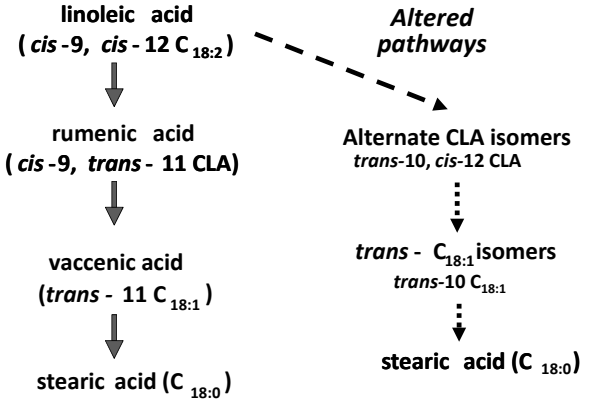
Milk Fat Affected by Many Factors



Diet Induced Milk Fat Depression (MFD)

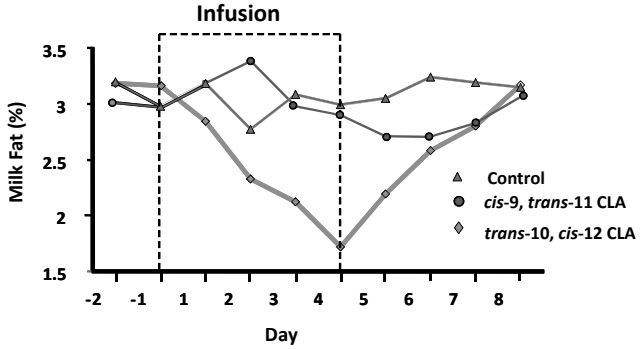
= Specific inhibition of milk fat

Rumen Biohydrogenation



Griinari and Bauman, 1999

The Mammary Gland is Acutely Responsive to CLA



Baumgard et al., 2000

Dietary Risk Factors

- **Associative Effects**
  - Dietary fatty acid level and profile
  - Availability of fatty acids
  - Rumen modifiers- ionophore
  - Dietary carbohydrate profile
  - Rate and extent of fermentation
  - Effective fiber
  - Ruminal N balance
  - Feeding strategies/management
  - Silage fermentation/quality
  - Forage types
  - Individual cow effect (level of intake etc)

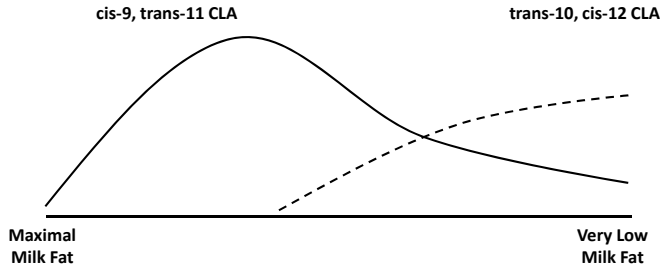
RUFAL: Rumen Unsaturated Fatty Acid Load (but C18:2 most important)

High producing cows normally most susceptible

Risk Factors Cause Milk Fat Depression

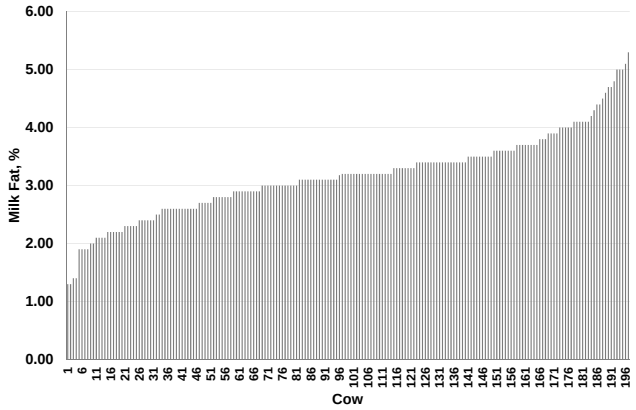
- Rarely is low milk fat caused by a single factor on a farm
- We can't eliminate all the risk factors
- We don't want to eliminate all the risk factors!!!

There is a Continuum From "High" to "Low" Milk Fat



Each herd and each cow is somewhere on this continuum!!

Variation in an Example Herd Group



What is the Time-Course of Induction of and Recovery from Milk Fat Depression?

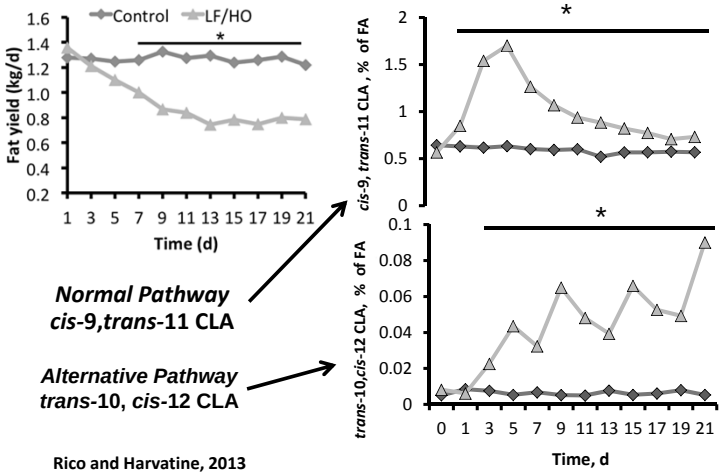
- **When MFD occurs……when did the problem originate?**
- **When correcting the diet……when do we expect to see improvements???**

Characterizing Recovery From MFD

- **TMR diets:**
  - **1) Low forage/High Oil (LF/HO)**
    29.5% NDF, 27% starch, 6.9% FA (including 3% soy oil)
  - **2) Control**
    36.9% NDF, 18% starch, 2.6% FA
- Milk sampled every other day

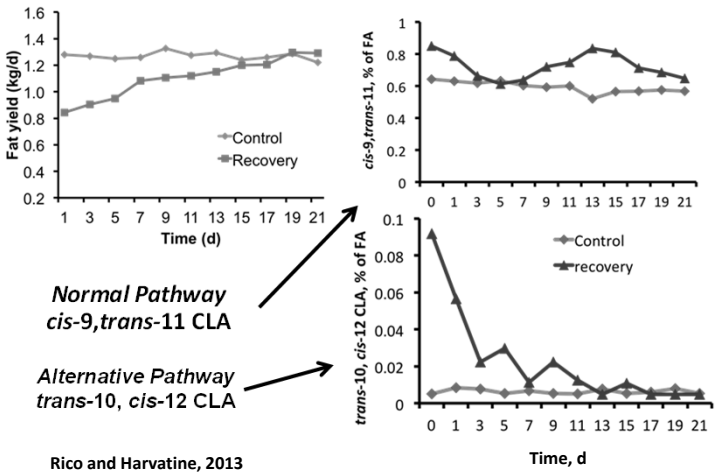
Rico and Harvatine, 2013

Time Course of induction of MFD



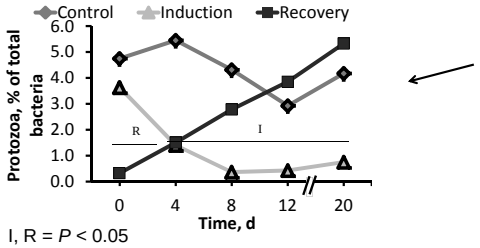
Rico and Harvatine, 2013

Time Course of Recovery of MFD



Rico and Harvatine, 2013

Microbes During Induction and Recovery



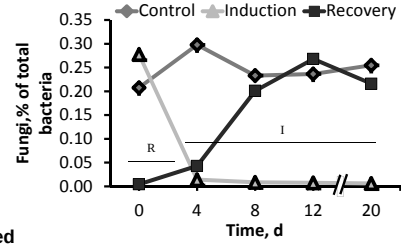
Ciliate protozoa

	P value
Trt	<0.001
Trt x time	<0.001
SEM	1.26

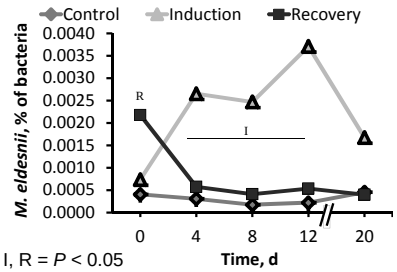
I, R = $P < 0.05$

Total Fungi

	P value
Trt	<0.001
Trt x time	<0.001
SEM	1.18



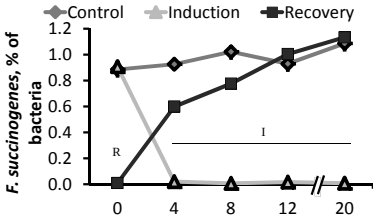
Rico et al. Unpublished



Megasphaera eldesnii
(Lactate user)

	P value
Trt	0.02
Trt x time	0.01
SEM	0.002

I, R = $P < 0.05$
i, r = $P < 0.10$



Fibrobacter succinogenes
(Fibrolytic)

	P value
Trt	<0.001
Trt x time	<0.001
SEM	0.13

Time Course of Milk Fat Depression: Key Messages

- **Following a dietary adjustment-**

  A lag of 7 to 10 days is expected to see milk fat depression

- **Following diet corrections-**

  It will take 10 to 14 days to rescue milk fat synthesis

Can We Accelerate Recovery?

Is it more important to change diet fermentability or unsaturated fat level?

Correcting Unsaturated Fat vs Diet Fermentability During Recovery

- **Milk fat reduced with low NDF and high oil diet** (26% NDF, 31.3% starch, 7.1% FA, 3.3% C18:2)
- **Recovery diets tested**

1) Control

31.8% NDF, 24.0% starch, 4.2% FA, and 1.7% C18:2

2) High Oil (HO)

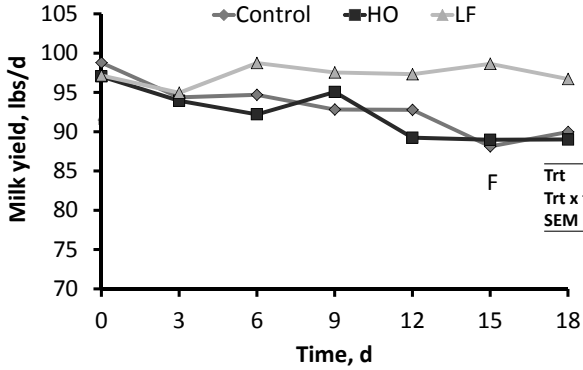
31.3% NDF, 21.6% starch, **6.7% FA, and 3.0% C18:2**

3) Low NDF (LF)

28.4% NDF, 29.0% starch, 4.3% FA, and 1.7% C18:2

Rico et al., Unpublished

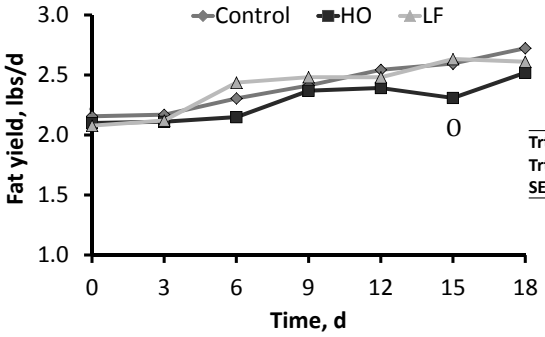
Correcting PUFA vs NDF: Milk Yield



P value	
Trt	0.09
Trt x time	0.24
SEM	2.98

Rico et al., Unpublished

Correcting PUFA vs NDF: Fat Yield (lb)

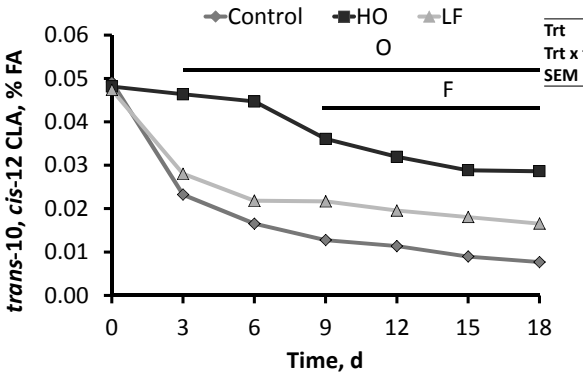


P value	
Trt	0.21
Trt x time	0.18
SE	0.08

O, F = $P < 0.05$

Rico et al., Unpublished

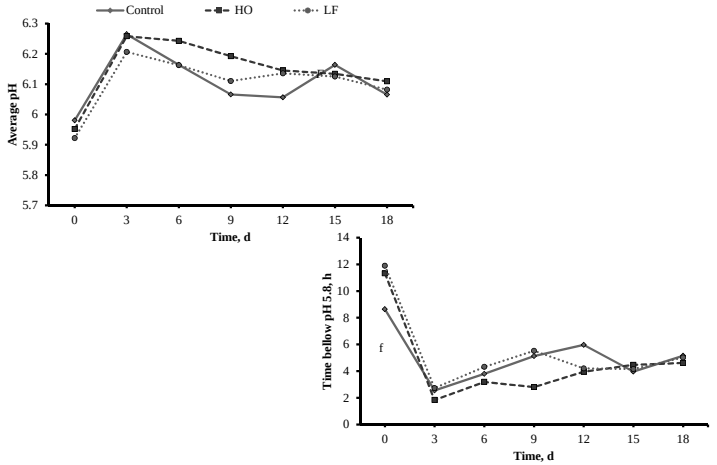
Correcting PUFA vs NDF: Milk *trans*-10,*cis*-12 CLA



P value	
Trt	<0.001
Trt x time	<0.001
SEM	0.002

Rico et al., Unpublished

Ruminal pH During Recovery



Rico et al., Unpublished

Correcting PUFA vs NDF: Key Messages

- **Dietary unsaturated fatty acids are the most important factor to correct.**
- **Correcting fermentability provides an additional benefit, but may lose milk.**

Do you have to remove Rumensin to Recover?

- **Milk fat reduced with low NDF and high oil diet** (25.3% NDF, 30.6% starch, 6.9% FA, 3.2% C18:2)
- **Recovery diet**

31.2% NDF, 24.6% starch, 4.3% FA, and 1.7% C18:2

1) Control

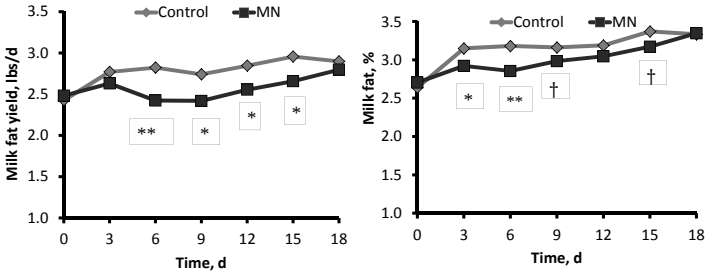
Rumensin removed

2) Monensin (MN)

Rumensin remained in diet

Rico et al., 2014

Effect of Monensin on Recovery: Milk Fat



* = $P < 0.05$

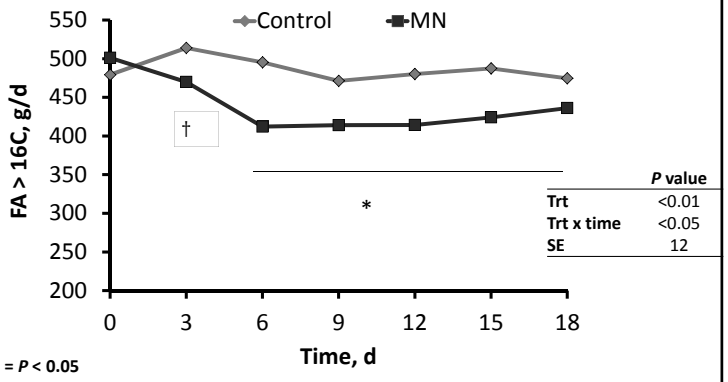
† = $P < 0.1$

	P value
Trt	0.02
Trt x time	0.15
SEM	0.74

	P value
Trt	0.01
Trt x time	0.05
SEM	0.06

Rico et al., 2014

Effect of Monensin on Recovery: Preformed FA



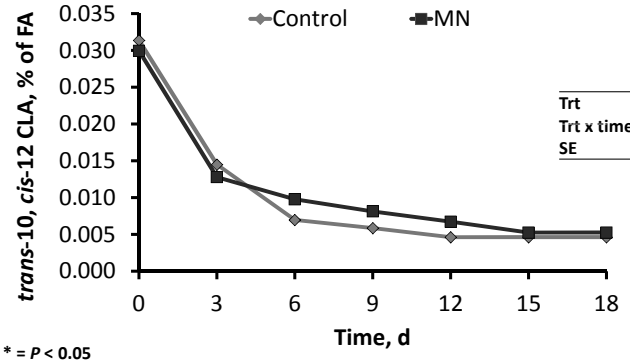
	P value
Trt	<0.01
Trt x time	<0.05
SE	12

* = *P* < 0.05

† = *P* < 0.1

Rico et al., 2014

Effect of Monesin on Recovery: *trans*-10 *Cis*-12 CLA



	P value
Trt	0.66
Trt x time	0.77
SE	0.001

* = *P* < 0.05

† = *P* < 0.1

Rico et al., 2014

Key Messages

- Milk fat synthesis can be rapidly rescued in Rumensin supplemented diets by correcting unsaturated fatty acid concentration and diet fermentability.
- In some cases, milk fat can be rescued before you run out of the current mineral?
- Monensin is a risk factor and its removal may help if you can cannot correct other things!

Can Rumen Available Methionine Decrease Risk of MFD

- **15 high and 15 low producing cows fed Alimet (25 g/d) or Control (No Alimet)**
- **Three Dietary Phases**

1) Low Risk Diet

33.5% NDF and no added oil

2) Moderate Risk Diet

31% NDF and 0.75% soybean oil

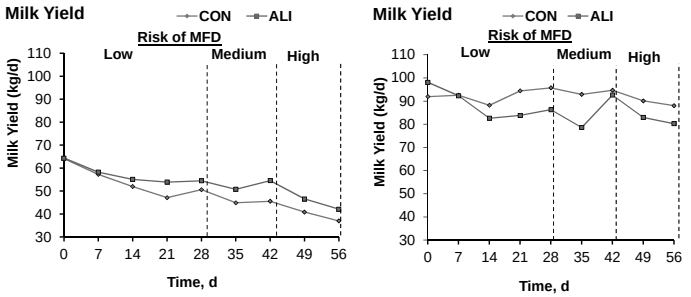
3) High Risk Diet

28.5% NDF and 1.5% soybean oil

Baldin et al., 2014 Unpublished

Effect of Alimet on Risk of MFD: Milk Yield

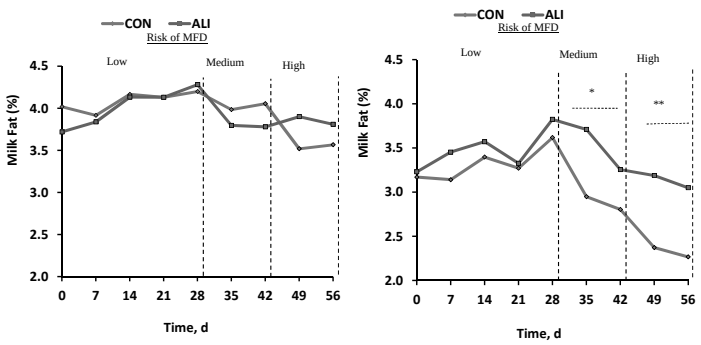
Low Cows **High Cows**



Baldin et al., 2014 Unpublished

Effect of Alimet on Risk of MFD: Fat %

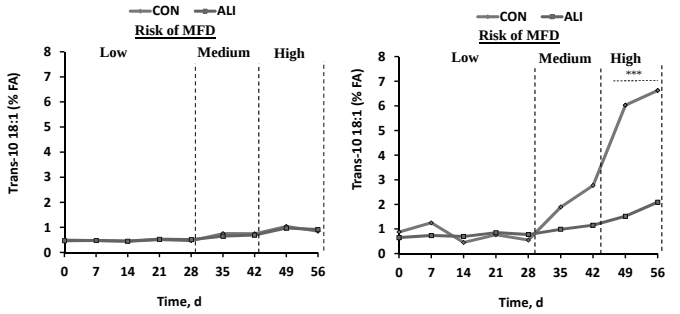
Low Cows **High Cows**



Baldin et al., 2014 Unpublished

Effect of Alimet on Risk of MFD: *trans*-10 C18:1

Low Cows **High Cows**



Baldin et al., 2014 Unpublished

Alimet and Risk of MFD: Key Messages

- **High producing cows are generally at the highest risk for MFD**
- **Alimet appears to reduce the risk of MFD in high producing cows and while feeding high risk diets**
- **Many cows in a "normal" milk fat herd are milk fat depressed**
- **This may take some of the "bumps out of the road"**

Baldin et al., 2014 Unpublished

What is the Mechanism??

- **We don't have the answer yet!!**
- **Definitely strong rumen mechanism, but cannot exclude a post-absorptive mechanism**
- **Most likely due to stabilizing rumen environment or altering rumen microbial population**
  - **Increased microbial mass may allow more biohydrogenation**
  - **May stabilize or increase microbial populations important to biohydrogenation**

What are the Sources of Variation in Corn FA Profile?

- **Environment**
  - Seems to be a very small impact of environmental factors on fatty acid profile of corn grain
- **Genetics**
  - Greatest impact
  - Literature is not near as deep as soybean FA profile
  - There was some interest in high oil and oleic corn ~15 years ago

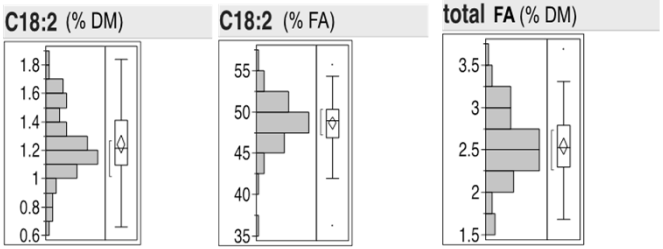
Why are High Corn Silage Diets Higher Risk for Milk Fat Depression??

- **More rapidly fermented starch?**
- **Lower effective fiber?**
- **Difference in fiber digestibility/rates?**
- **Level and rate of C18:2 availability??**
- **Low in fat, but cows eat a large amount**

Where are the PUFA in Corn Silage?

	DM	Total FA	C18:1	C18:2	C18:3
	---------Percent of Total in Plant--------				
Kernels	44.0	80.3	96.8	92.4	17.1
Leaves	13.3	11.9	0.9	2.0	71.3
Stalk	31.4	5.1	0.7	3.3	9.7
Cob	7.6	1.7	1.1	1.6	1.0
Husk&Shank	3.6	1.0	0.6	0.8	0.9

67 Corn Silages from Two Test Plots (2013)

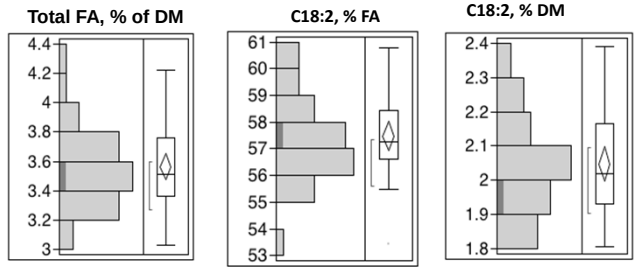


Quantiles		
90.0%		1.60384
75.0%	quartile	1.4094
50.0%	median	1.2167
25.0%	quartile	1.0954
10.0%		0.93576

Quantiles		
90.0%		52.2013
75.0%	quartile	50.2998
50.0%	median	48.875
25.0%	quartile	46.89
10.0%		45.2388

Quantiles		
90.0%		3.10991
75.0%	quartile	2.79108
50.0%	median	2.53337
25.0%	quartile	2.3032
10.0%		2.05506

36 Corn Grains From PSU Plots

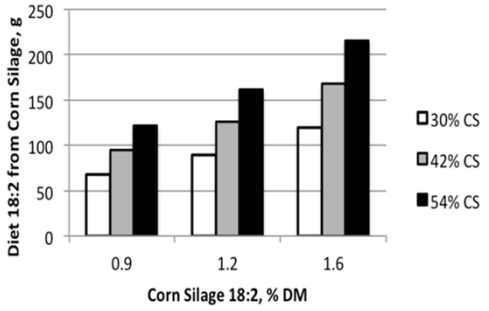


Total FA, % of DM		
97.5%		4.22363
90.0%		3.9327
75.0%	quartile	3.76238
50.0%	median	3.51523
25.0%	quartile	3.36032
10.0%		3.27073
2.5%		3.02912

C18:2, % FA		
97.5%		60.8029
90.0%		60.0147
75.0%	quartile	58.4559
50.0%	median	57.2509
25.0%	quartile	56.6012
10.0%		55.7298
2.5%		53.429

C18:2, % DM		
97.5%		2.39002
90.0%		2.24764
75.0%	quartile	2.16401
50.0%	median	2.02082
25.0%	quartile	1.93132
10.0%		1.85445
2.5%		1.80728

Intake of C18:2 in diets that contain 30, 42, or 54% corn silage (% DM) from hybrids that contain 0.9, 1.2, or 1.6% C18:2 (% of DM)



~60 to 90 g/d difference in C18:2 intake just in the corn silage

Nutrition is best practiced as an "Experiment in Progress"!!

- **When milk fat is <u>Acceptable</u>**
  - **Inclusion of risk factors acceptable and may be advantageous to production and efficiency**
- **When milk fat is <u>Low</u>: Look For a Reason**
  - **When did it start and what happened ~7-10 d prior?**
  - **Is it a certain string or group of cows?**
    - **High Producing cows are normally more susceptible**
  - **What season is it?**
  - **Is the sample a daily average?**

The Experiment in Progress

1. Diet Polyunsaturated Fatty Acids

- Concentration of C18:2
- Source of C18:2

  Very different rates of rumen release

  - Ca Salts are more slowly released, but are not inert

**Best First Step- Reduce diet PUFA

- Least risk of losing milk in the short-term
- Replace unsaturated fat supplements with saturated fat supplements

- Monitor milk yield and milk fat over time

2. Diet Fermentability

- Analyze carbohydrate profiles and effective fiber
- Experience with similar diets in the region is important
- Start to titrate down starch and increase fiber
- Switch rapidly fermentable sources for less rapidly fermentable sources
- Increase forage NDF and effective fiber

**Careful….. May Lose Milk!!

3. Rumen Modifiers

- Rumensin
  - Risk factor, but does not cause MFD by itself
  - Can be synergistic with other risk factors for induction
- Yeast & Direct Fed Microbials
  - May reduce incidence of MFD in some cases
  - Have not tested their effect on recovery
- DCAD
  - Increases DCAD decreases MFD
- HMTBa
  - Reduces the risk of MFD

**Remember we are dealing with many interactions!

4. Feeding Strategies

- Number of feeding times per day
- Slick bunks before feeding?
- Feeding times

* You can slug feed TMR!

5. Saturated Fat Supplements

- No risk for induction of milk fat depression
- High palmitic acid (C16:0) supplements may increase milk fat in some cases
- Milk fat depression will reduce the effectiveness of high palm supplements

Monitor milk yield and milk fat over time!!!

****Set Expectations for the Time Required**

Conclusions

- **MFD is caused by unique fatty acids originating from ruminal biohydrogenation**
- **Rumen environment is critical and involves interactions of numerous dietary, cow, and environmental factors**
- **Induction occurs in ~7 to 10 d and recovery requires 10 to 18 d**
- **Unsaturated FA are the 1st issue to consider**
- **Alimet reduces risk of MFD in high risk situations**

Constant "Experiment in Progress" to maximize energy intake, milk yield, and milk fat yield

Lab Members:

Daniel Rico, Michel Baldin, L. Whitney Rottman, Mutian Niu, Natalie Urrutia, Andrew Clark, Liying Ma, and Jackie Ying

Collaborator:

Dr. Dale Bauman, Cornell University

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Thank You

FATTY ACID NUTRITION AND MILK FAT DEPRESSION

K.J. Harvatine, Ph.D.
Department of Animal Science
The Pennsylvania State University

INTRODUCTION

Milk fat concentration is variable and very responsive to many factors including genetics, season of the year, and physiological state, but is especially responsive to diet. Synthesis of milk fat is an energy demanding process, but also represents a significant portion of the economic and nutritional value of dairy products. First described over one and a half centuries ago, diet-induced milk fat depression (**MFD**) is characterized by a decrease in milk fat yield of up to 50% with no change in milk yield or yield of other milk components. MFD is classically observed in ruminants fed highly fermentable diets or diets high in plant oils. Varying levels of MFD are commonly experienced today in both intensively and extensively managed dairy herds, and this represents a level of milk fat production below the genetic potential of the cow. MFD is also a useful variable for evaluating herd management; in many cases onset of diet-induced MFD is an indication of modified ruminal fermentation and in more pronounced cases this can be associated with ruminal acidosis and reduced efficiency. Therefore, maintaining optimal milk fat synthesis has value beyond the milk fat sold. Although we know extensively the cause of MFD we continue to experience MFD because of the high-energy requirements of cows and the desire to maintain optimal milk production. Numerous dietary factors commonly interact to cause MFD making prediction difficult. Recently we have investigated the time course of induction and recovery of MFD that provides insight into identifying causative factors and setting expectations for correction of MFD.

Historical Theories of Milk Fat Depression

The investigation of diet-induced MFD has a rich history that has included many theories to explain reduced milk fat synthesis. Most of these theories postulated that limitations in substrate supply for milk fat synthesis caused MFD, generally based on changes in absorbed metabolites as a consequence of alterations in ruminal fermentation. For example, the alterations in the ruminal environment typically include decreased pH and decreased acetate to propionate molar ratio (Bauman and Griinari, 2001). This formed the basis for one of the most widely known substrate supply limitation theories that proposed that acetate supply was limiting milk fat synthesis. However, the reduced ratio of acetate to propionate with highly fermentable diets is predominantly due to increased ruminal production of propionate (Bauman and Griinari, 2001, 2003), and ruminal infusion of acetate to cows that during MFD has only a marginal impact on milk fat yield (Davis and Brown, 1970). Overall, several decades of research has tested numerous theories based on substrate limitations and found little to no evidence in their support (extensively reviewed by Bauman and Griinari, 2003 , Shingfield and Griinari, 2007, Bauman et al., 2011).

Davis and Brown (1970) recognized that *trans*-C18:1 fatty acids (**FA**) were increased in milk fat of cows with low-milk fat syndrome. They suggested that these *trans*-FA originated from incomplete ruminal biohydrogenation of unsaturated FA and might contribute to the development of MFD. Subsequent studies have demonstrated a clear relationship between *trans*-FA and MFD (see reviews by Bauman and Griinari, 2003, Shingfield and Griinari, 2007, Bauman et al., 2011). Investigations over the past dozen years have clearly established that diet-induced MFD is associated with rumen production of unique FA from ruminal metabolism of dietary polyunsaturated fatty acids (**PUFA**). Referred to as the "biohydrogenation theory," the basis for diet-induced MFD relates to an inhibition of mammary lipid synthesis by specific FA that are intermediates in the biohydrogenation of dietary PUFA, and these are only produced under certain conditions of altered ruminal fermentation (Figure 1, Bauman and Griinari, 2003). *Trans*-10, *cis*-12 conjugated linoleic acid (CLA) was the first of these to be recognized and it has been extensively investigated at the whole animal and molecular level (reviewed in Bauman et al., 2011).

Ruminal Biohydrogenation

Ruminant diets are low in total fat, although forages, oilseeds, fat supplements, and some byproducts can result in a significant intake of PUFA. Dietary FA are metabolized in the rumen resulting in a large difference between the dietary FA pattern and the profile of FA absorbed from the small intestine. Most FA in the diet are esterified and these are hydrolyzed in the rumen and the resulting unsaturated FA are isomerized (double bond position changed) and biohydrogenated (double bond removed; Figure 1). The extent of biohydrogenation and the intermediates formed are determined by the properties of the fat source, retention time in the rumen, and characteristics of the microbial population (Allen, 2000, Palmquist et al., 2005). Dietary factors that modify ruminal fermentation (ex. high starch, high oil, rumensin) also modify ruminal FA metabolism through associative effects that presumably result in a microbial population that utilizes the alternative pathway of PUFA biohydrogenation.

Ruminal biohydrogenation may be simply described as a function of the available FA pool size, ruminal retention time, and bacterial biohydrogenation capacity (Harvatine and Bauman, 2007). Microbial biohydrogenation is a multi-step process for which the kinetics are not well documented. Harvatine and Allen (2006b) used the pool and flux method (Firkins et al., 1998) to observe *in vivo* ruminal FA kinetics of a cottonseed-based diet that included a fat supplement. Dietary FA had a slow ruminal passage rate (6.4 to 7.4%/h) indicating a long average rumen retention time. In contrast, the fractional biohydrogenation rate of linoleic acid was high (14.6 to 16.7%/h). Interestingly, the biohydrogenation of *trans* C18:1 FA was also very high (33.4 to 48.4%/h), although a decrease in the biohydrogenation rate of *trans*-C18:1 FA was associated with an increased duodenal flow of biohydrogenation intermediates and diet-induced MFD. *In vivo* ruminal FA kinetics clearly demonstrates that ruminal FA metabolism is responsive to associative dietary factors and that the long retention time

provides ample time for metabolism of fat sources that are not rapidly available in the rumen.

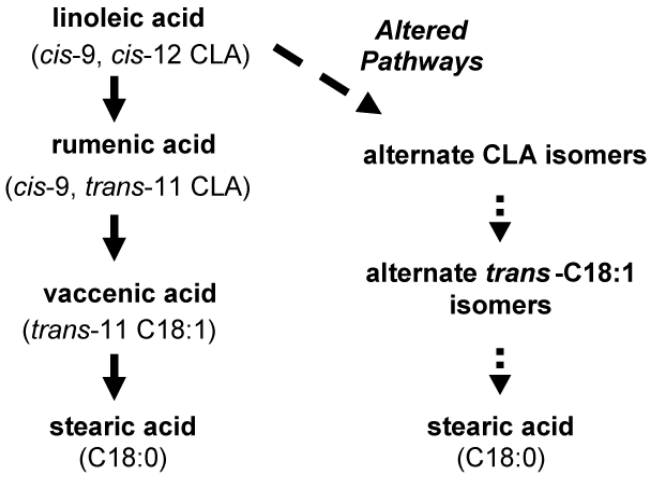

Figure 1. Biohydrogenation pathways during normal and altered ruminal fermentation. Adapted from Griinari and Bauman (1999)

DIETARY RISK FACTORS FOR MILK FAT DEPRESSION

Prediction of the occurrence of MFD is complex because it is not directly caused by a single dietary factor, rather is the result of numerous factors that reduce the rate of biohydrogenation and shift biohydrogenation to the alternate pathway. It is preferable to think of dietary "risk factors" that move a diet along a continuum from low to high risk. Below is a summary of major risk factors. This is not a complete list, but highlights the most important issues.

1. Diet Fermentability

The microbial population is driven by the substrate available and by the rumen environment and is directly dependent on the concentration of starch and NDF and the rates and extent of ruminal digestion. Maximizing fermentablity is important for energy intake, but care should be given to minimizing sub-acute ruminal acidosis. Milk fat depression more commonly occurs with corn silage compared to haylage based rations and with more rapidly digested starch sources such as high moisture corn compared to dry ground corn. Providing multiple sources of starch and fiber with overlapping rates of digestion is the safest approach. Additionally, sugar substituted for dietary starch reduces risk without loss of digestibility (Mullins and Bradford, 2010).

Low milk fat is commonly associated with sub-clinical and clinical ruminal acidosis, but MFD is frequently observed without a reduction in rumen pH (Harvatine and Allen, 2006a). Rumen pH is dependent on the VFA profile, rate of production, and rate of absorption, buffer secretion, and presence of dietary buffers and varies by

approximately 1 to 1.2 pH units over the day (Allen, 1997). It appears that the microbial shift causing MFD occurs before changes in a rumen pH are apparent, but may be related to more subtle changes such as the timing of low pH.

2. Diet Polyunsaturated Fatty Acids

Unsaturated fatty acids have a dual impact on ruminal biohydrogetion in that they modify the microbial population and increase the amount of substrate that must be biohydrogenated. It is important to know the total amount of unsaturated fat and also the source since this dictates the fatty acid profile and rate of ruminal availability. Fish oil has the greatest impact, but is not commonly found in diets in the USA. Cotton, soy, corn and many other plant oils are high in linoleic acid and incorporation of these grains, oils, and their byproducts increases the risk of MFD. The concept of Rumen Unsaturated Fatty Acid Load (RUFAL, Jenkins, 2011) is a simple and insight calculation that is complemented by consideration of the fat source. There are significant differences in the rate of ruminal availability, for instance cottonseed and whole roasted soybeans are expected to have a much slower release of fatty acids in rumen than distillers grains, ground sources, or oil supplements.

Fat is commonly supplemented to increase diet energy density and many protected fat supplements are available. Supplements that are high in saturated fat (palmitic and stearic) do not increase the risk of MFD, however calcium salts of fatty acids are available in the rumen and can reduce milk fat (Lundy et al., 2004, Harvatine and Allen, 2006b). The calcium salt slows the release of unsaturated fat in the rumen and does reduce the impact of these oils compared to free oil, but does not provide a high level of rumen inertness. The impact of calcium salts depends on the profile of the fat supplement and interaction with other factors. For instance, we have observed in two experiments that calcium salts of palm FA reduced milk fat in high producing cows, but not in low producing cows presumably because of differences in intake, passage rate, and rumen environment (Harvatine and Allen, 2006a, Rico and Harvatine, 2011).

3. Rumen Modifiers

Many supplements have a large impact on the rumen microbial population. Monensin is the most common rumen modifier associated with MFD (Jenkins, 2011). However, it is only a risk factor and can be safely used in many diets. Other rumen modifiers may reduce risk, although their effectiveness generally has not been specifically tested. We have ongoing work demonstrating that HMTBa (Alimet, Novus International) reduces the risk of milk fat depression in high risk situations, although the exact mechanism is not yet clear. Additionally, direct fed microbials have been shown to stabilize rumen biohydrogenation during a high diet fermentability challenge (Longuski et al., 2009), although a clear role for these supplements in preventing milk fat depression has not been well investigated.

4. Feeding Strategies

Slug feeding grains is commonly associated with sub-clinical rumen acidosis and MFD. Many assume that TMR feeding eliminates this issue since every bite has the same nutrient composition. However, the rate of intake of fermentable organic matter is very variable over the day due to sorting and variable rates of intake. Generally, cows sort for more fermentable feed particle early in the day, but also consume feed at approximately a three times higher rate after delivery of fresh feed. We recently compared feeding cows 1x/d or in four equal meals very six hours (Rottman et al., 2011, Rottman et al., 2014). The frequent feeding treatment decreased the concentration of alternate biohydrogenation FA and increased milk fat yield and concentration. This experimental treatment highlights the potential to increase milk fat through management of feed delivery.

HOW TO PREDICT THE OCCURANCE OF MILK FAT DEPRESSION

The complexity of predicting dietary fermentability and associative effects makes prediction of MFD difficult. It is arguably impossible to balance a diet that maximizes milk yield and energy intake without incorporation of numerous risk factors. Ruminant nutrition is best practiced as a continuous experiment that monitors cow response to diet modification (Allen, 2011). It is important to monitor nutrient concentrations and model predicted benchmarks that are applicable to your region and logical based on previous experience with similar diets. However, even with the best feed analysis, software, and experience the interaction of diet ingredients and effectiveness of the diet is best determined by the cow and observed by titration and observation.

Diet fermentability is much more extensively handled by feed analysis and software prediction than dietary fat. Dietary FA have typically been consolidated in ration balancing and simply reported as total ether extract or fat concentration. More recently the FA profile of feedstuffs has been included in feed libraries and a more detailed approach of FA nutrition has been taken (Moate et al., 2004). Effectively utilizing this information in diet formulation represents a challenge because of rumen alterations of dietary FA and the fact that individual FA isomers differ in their biological effect. Thus, based on the current understanding of bioactive FA, effective models must predict ruminal outflow of individual FA, including specific *trans*-FA isomers. Secondly, the metabolism of FA by rumen bacteria is extremely dynamic and difficult to integrate into prediction algorithms. Ruminal FA models must account for dietary associative effects that modify the predominant pathways and rates of ruminal biohydrogenation thereby altering the pattern of FA outflow. This may require a mechanistic rather than empirical approach to adequately model. Book values are expected to accurately represent the FA profile of forages and grains and testing of individual lots should not be required for most feedstuffs. However, more variability exists in byproducts, which may require frequent testing of FA concentration depending on the byproduct and source. An understanding and quantification of all factors that induce altered ruminal fermentation is not currently available and development of prediction equation that consider dietary risk factors will require further experimentation and more advanced modeling.

THE TIME COURSE OF INDUCTION AND RECOVERY

Dietary factors that cause low milk fat have almost exclusively been studied through induction of MFD. This is useful because it tells us what dietary factors cause MFD, but it does not directly tell how to recover or accelerate recovery once you have MFD. We recently conducted a high-resolution time course experiment to characterize the timing of induction and recovery of diet induced MFD (Rico and Harvatine, 2013). We induced milk fat depression by feeding a low fiber and high soybean oil diet and then recovered by feeding a higher fiber and low oil diet. We took milk samples every other day to observe milk fat change over time. Milk fat yield decreased progressively when the low fiber and high oil diet was fed and was significantly decreased after 7 days (Figure 2). When switched to the recovery diet, milk fat yield progressively increased and was not different from control until day 11. A key insight from the experiment is the expected lag between making diet adjustments and recovery of milk fat synthesis. Addition of a risk factor may cause milk fat depression in 7 to 10 days and elimination of a risk factor is expected to take 10 to 14 days to observe a benefit. Knowing the time course is very important to identify what may have caused milk fat depression and knowing how long to wait to determine if a diet correction has been effective in improving milk fat.

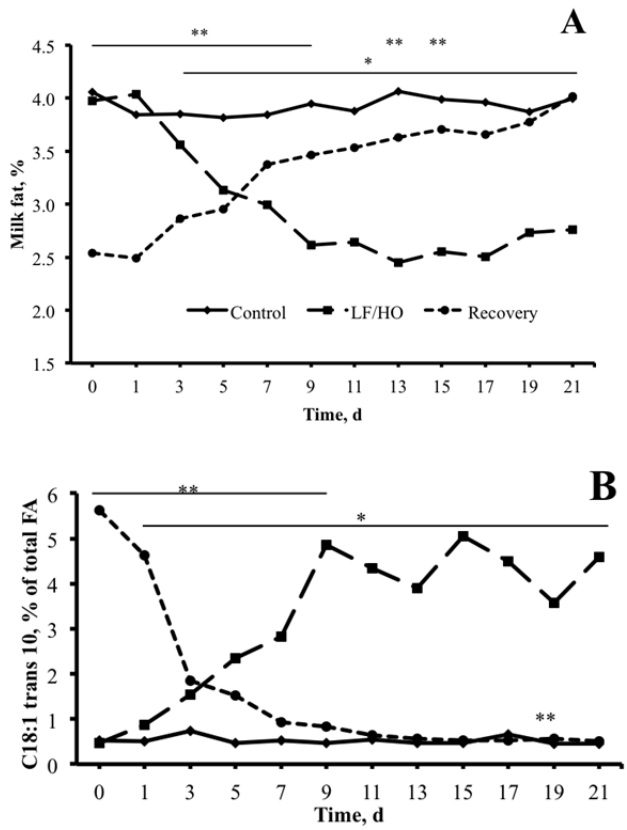

Figure 2. Temporal changes during induction of and recovery from milk fat depression. Panel A. Milk fat percent and Panel B. Milk fat concentration of the bioactive *trans*-10 C18:1 fatty acid.

RAPIDLY RECOVERING MILK FAT

When milk fat moves below the herds goal the logical approach is to systemically remove risk factors. The challenge is which risk factors to remove without loss of milk or energy intake. A multistep approach may be best. First, determine the diet polyunsaturated fat level and availability. In the short term, minimizing PUFA intake is the best first step and is expected to have little effect on milk yield. Secondly, determine if diet fermentability is higher than optimal. In some cases reducing fermentability may reduce sub-clinical acidosis and improve rumen function without loss of milk. If diet fermentability appears within safe limits a reduction may result in lost milk yield so monitor production closely after a diet modification. Lastly, determine if a rumen modifier can be added to stabilize fermentation. For example, if a direct fed microbial is not being used it may be a good opportunity to try a supplement in the herd. It is important to have reasonable expectations on the time-course of recovery. Dietary changes are expected to result in observable improvements in 10 to 14 d, but complete recovery will require nearly 3 weeks and maybe longer with more modest dietary changes.

OTHER IMPORTANT REGULATORS OF MILK FAT YIELD

Seasonal Variation in Milk Fat

Most dairymen and nutritionists recognize a seasonal change in milk fat that is commonly attributed to changes in forage sources, weather, or herd days in milk. A very repeatable seasonal pattern is observed in milk fat and protein concentration at the milk market level. Milk fat and protein concentration peak around December and January and reach a nadir around July and August. This highly repeatable pattern appears to be independent of year-to-year differences in forage quality and weather. A similar pattern is observed in milk marketing orders in different regions. This seasonal variation should be incorporated into the expected milk fat concentration when setting production goals and troubleshooting milk fat production.

Circadian Patterns

Circadian rhythms are changes that occur over the day and repeat every day. Dairymen commonly recognized that morning and evening milking differ in milk yield and composition. Gilbert et al. (1972) reported 0.65 kg higher milk yield at the morning milking, but 0.32 and 0.09 percentage unit higher milk fat and protein, respectively, at the evening milking in cows milked at 12 h intervals. More recently Quist et al. (2008) conducted a large survey of the milking-to-milking variation in milk yield and composition on 16 dairy farms. Milk yield and milk fat concentration showed a clear repeated daily pattern over the 5 days sampled in herds that milked 2 and 3 x/d. Surprisingly milk yield was highest and milk fat lowest in the AM milking of herds milked 2 x/d, but milk yield and milk fat concentration was lowest at the AM milking and highest at the night milking of herds milked 3 x/d. The difference in these rhythms may be due to differences in the length of time represented by each milking interval. However, their

data demonstrated a rhythm of milk and milk fat. We have recently observed milk yield and milk composition at each milking while milking every 6 h and feeding cows 1 x/d at 0800 h or in 4 equal feedings every 6 h. We observed an effect of time of day on milk and milk fat yield and milk fat and protein concentration in cows milked every 6 h. This high resolution and well-controlled experiment demonstrates the circadian pattern of milk synthesis and the interaction of the timing of nutrient intake in high producing dairy cows (Mean MY = 47.7 kg/d). This variation is commonly observed with AM/PM DHIA testing and on large herds shipping multiple tankers per day. We continue to explore nutritional opportunities based on these rhythms.

CONCLUSIONS

Milk fat depression results from an interaction between ruminal fermentation processes and mammary tissue metabolism. Investigation of milk fat synthesis over the past 100 years has resulted in numerous theories based on observational differences in dietary associations, alterations in ruminal fermentation, and adaptations in animal metabolism. To date, the biohydrogenation theory is the only proposed mechanism that has provided causative evidence and withstood rigorous examination. The mechanism by which biohydrogenation intermediates reduce milk fat synthesis has and will continue to provide insight into the regulation of milk fat synthesis. Milk fat depression continues to be a real-world condition that reduces the efficiency and productivity of dairy cows, but understanding its fundamental basis will allow for effective management and intervention strategies. Management of the risk factors associated with MFD is required to reach both milk and milk fat yield goals. The time course of induction and recovery can be utilized to both identify contributing factors and set expectations for recovery. Lastly, the seasonal and circadian pattern of milk fat synthesis explains variation observed between summer and winter and between milkings and should be considered in monitoring and setting production goal.

ACKNOWLEDGEMENTS

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