New Milk Analysis Technologies to Improve Dairy Cattle Performance

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INTRODUCTION

Two years ago we introduced the application of new mid-infrared (**mid-IR**) for rapid milk fatty milk analysis (Barbano, et al., 2014) and reported positive correlations of bulk tank milk fat test with a higher proportion and concentration of de novo fatty acids in bulk tank milk. The form of the fatty acid data from the mid-IR was structured to provide information on the relative proportions of de novo (C4 to C14), mixed origin (C16:0, C16:1, C17:0), and preformed (C18:0 and longer) fatty acids in milk. We can also provide that information in units of grams per 100 grams of milk. Since that time, we have continued to collect data on milk fatty acid variation in bulk tank milk and it's relationship to feeding and farm management. A field study of 20 Holstein and 20 Jersey farms (Melissa paper #1) was completed in 2014 (Woolpert et al., 2016) and a follow up study of 40 Holstein farms was completed in 2015 (Woolpert, 2016) with the objective of determining farm feeding and management practices relate to milk fatty acid compostion and bulk tank milk fat and protein concentration. Starting in February of 2016, information on milk fatty acid composition of bulk tank milk was provided to the individual producers of the St Albans Cooperative (Vermont) along with their payment test data on the same milk samples.

In addition, in the last 2 years we have expanded our milk analysis research on fatty acid analysis to individual cow milk samples at Cornell and in collarboration with Miner Institute in Chazy, NY. Additional work is in progress in collaboration with Penn State and Michigan State Universities. Today, I will focus on the use of milk fatty acid (**FA**) information for feeding management of dairy cows at the bulk tank level and report the status of our work on individual cow data, particularly transition cows.

EXPERIMENTAL APPROACH

Prior to the current study a group of partiail least squares (**PLS**) chemometric prediction models were developed from mid-IR spectra. The spectra of modified milk calibration samples (Kalylegian et al., 2006a,b), bulk tank milks, and individual cow milks were used in combination with chemical reference chemistry for fat (AOAC, 2000; method 989.05; 33.2.26), total protein (AOAC, 2000; method 991.20; 33.2.11 and nonprotein nitrogen (AOAC, 2000; method 991.21; 33.2.12) with true protein calculated by difference, anhydrous lactose (Lynch et al., 2007) and gas liquid chromatography (Barbano and Sherbon, 1980; Lynch et al., 1992) for FA analysis using a Varian CP-SIL88 capillary column [(100m x 0.25 mm x 0.2 µm film thickness), ID code # CP7489; Varian, Inc., Lake Forest, CA], installed in a Hewlett Packard 6890 GC System equipped with an automatic liquid sampler and a flame ionization detector (Hewlett Packard Co., Wilmington, DE). A more complete descripiton of the fatty acid analysis methods and PLS model for fatty acid prediction model development was reported by Wojciechowski and Barbano (2016).

A library of chemometric prediction models for the major components in milk and milk FA composition for use on a Lactoscope FTA and Lactoscope CombiScope FTIR 600/300 (Delta Instruments, Drachten, The Netherlands) has been developed. A variety of individual FA and groups of FA were measured. The following individual FA were measured by mid-IR: C16:0; C18:0; C18:1 cis9, cis12; C18:1 trans 10; andC18:1 trans 11. The following groups of FA were measured: total FA; DeNovo (C4:0 to C14:0), mixed origin (C16:0, C16:1, C17:0), preformed (C18:0 and longer); total unsaturated FA, total cis FA; total trans FA; mono unsaturated FA; and poly unsaturated FA. All FA measures produce results from the IR in grams of FA per 100 grams of milk. Some researchers have used the grouping of FA as short, medium, and long chain FA but the exact definition of those groups varies among researchers. The group definitions of de novo, mixed origin, and preformed FA are much more clear and consistent because they are based on the biochemical pathways for FA synthesis and have better potential to be correlated with the biology, metabolism, and feeding of dairy cows.

In addition to the measures of FA concentrations, two fat concentration independent measures of FA structure were also done on each sample: mean FA chain length (expessed as mean carbon number per FA) and mean FA unsaturation (expressed as double bonds per FA). The measure of total FA (not fat) in g/ 100 g of milk is used as a new basis for a more

accurate measurement of total fat content in the milk. This approach eliminates most of the weakness of traditional measurses of fat by IR using the Fat A (C=O stretch) and Fat B (C-H stretch) because it compenstates sample by sample for differences in FA composition when trying to estimate the total fat content of the milk in comparison to ether extraction (Kaylegian et al., 2009a,b). The relative proportion of the total FA in milk that are represented by an individual or group of FA can be expressed on a relative basis as a precent of total FA in the sample. Thus, it is possible to produce a simulated gas chromatograph FA analysis of milk fat directly from the same (IR spectra) of milk tested on the IR for fat, protein, and lactose concentration.

The calibration adjustment of the fat, true protein, anydrous lactose and all FA measures on the IR milk analyzer is done once per month using a set of 14 modified milks described by Kaylegian et al. (2006a,b) that has reference values in (g FA per 100 g of milk) for each of the individual or groups of FA measured. The set of calibration samples is produced monthly at Cornell and was used to check the calibrations during the month.

RESULTS

2014 Farm Study (Woolpert et al., 2016)

This study investigated the relationship of management practices, diet characteristics, milk composition, and lactation performance with de novo fatty acid (FA) concentration in bulk tank milk from commercial dairy farms with Holstein, Jersey, and mixed breed cows. It was hypothesized that farms with higher de novo milk FA concentrations would more commonly use management and nutrition practices known to optimize rumen conditions that enhance de novo synthesis of milk FA. Farms (n = 44) located in Vermont and northeastern New York were selected based on a history of high de novo (HDN; 26.18 ± 0.94 g/100g FA; mean ± SD) or low de novo (LDN; 24.19 ± 1.22 g/100g FA) FA in bulk tank milk. Management practices were assessed during one visit to each farm in March or April, 2014. Total mixed ration samples were collected and analyzed for chemical composition using near infrared spectroscopy. There were no differences in days in milk at the farm level.

Yield of milk fat, true protein, and de novo FA per cow per day were higher for HDN versus LDN farms. The HDN farms had lower freestall stocking density (cows/stall) than LDN farms. Additionally, tiestall feeding frequency was higher for HDN than LDN farms. No differences between HDN and LDN farms were detected for dietary dry matter, crude protein, neutral detergent fiber, starch, or percentage of forage in the diet. However, dietary ether extract was lower for HDN than LDN farms. The difference in income per cow would depend on the actual milk price at any point in time. However, the average fat and protein price for the Federal Milk Order No. 1 for March and April 2014 was $4.62 and $10.17 per kg, respectively. Therefore, at 25 kg of milk per cow per day, the average HDN farm earned a gross of $5.50 and $7.72 per cow for fat and protein, respectively. The average LDN farm at 25 kg milk per cow per day earned a gross of $5.26 and $7.29 per cow for fat and protein, respectively. These differences for fat and protein between HDN and LDN herds at 25 kg of milk per 100 cows per year would result in a gross income difference of $8,544 for fat and $15,695 for protein. This research indicated that overcrowded freestalls, reduced feeding frequency, and greater dietary ether extract content are associated with lower de novo FA synthesis and reduced milk fat and true protein yields on commercial dairy farms.

2015 Farm Study (Woolpert, 2016)

The objective of this study was to evaluate the relationship of management practices and dietary factors with de novo fatty acid concentration in bulk tank milk from commercial dairy farms milking Holstein cows. Farms were selected based on de novo fatty acid concentration during the 6 mo previous to the farm visit and were categorized as high de novo (HDN; 24.61 ± 0.75 g/100 g of FA, mean ± standard deviation; n = 19) or low de novo (LDN; 23.10 ± 0.88 g/100 g of FA; n = 20). Farms were visited once in February, March, or April, 2015 and evaluated based on management and facility design known to affect cow behavior, physical and chemical characteristics of the diet, and the ration formulation and forage analyses obtained from the farm's nutritionist. The mean milk composition for HDN and LDN farms is shown in Table 1.

No differences in milk, fat, and true protein yields were detected between HDN and LDN farms, but milk fat and true protein content were higher ($P < 0.01$) on HDN farms (Table 1). This positive relationship between de novo FA and milk fat and true protein percentage agrees with previous results of Barbano et al. (2014) who evaluated bulk tank milk composition on over 400 commercial dairy farms. De novo FA expressed as g/100 g of FA and as g/100 g milk were higher ($P < 0.01$) on HDN farms, and preformed FA expressed as g/100 g of FA and as g/100 g milk were lower ($P < 0.01$ and $P = 0.02$, respectively) on HDN farms. These results are consistent with previous research (Woolpert et al., 2016) that indicated that HDN farms have higher milk fat and true protein con-

tent in bulk tank milk. De novo FA yield, expressed as g/d, was higher (P < 0.01) for HDN farms with no difference detected in milk yield (P = 0.91) suggesting that cows on HDN farms synthesized more de novo FA. However, milk weights per cow were not measured directly, but were estimated indirectly based on the number of cows milking on the day of the farm visit and the average bulk tank milk shipped per day during the month of the farm visit. Thus, the uncertainty in milk weight data was higher than the uncertainty in milk composition data. Consequently, further research is needed under conditions where milk weight per cow per day can be accurately measured, along with milk composition, to determine whether greater de novo FA synthesis is always associated with greater milk fat and true protein yields.

There were no differences in farm size, time away from the pen for milking, days in milk, or body condition score for HDN versus LDN farms. No differences between HDN and LDN farms in milk, fat, or true protein yield were detected; however, milk fat and protein content and de novo fatty acid yield per day were higher for HDN farms, as was gross income per unit of milk sold.

Table 1. Least squares means of milk composition factors for high de novo (HDN) and low de novo (LDN) farms for the month of the farm visit.

Item	HDN	LDN	SEM	*P* value
Milk yield, kg/d	31.9	32.1	0.9	0.91
Fat, %	3.98	3.78	0.04	<0.01
Fat, kg/d	1.27	1.21	0.03	0.25
De novo fatty acids[1]				
g/100 g milk	0.99	0.86	0.01	<0.01
g/100 g FA	25.99	23.78	0.22	<0.01
g/d	315.6	276.2	9.5	<0.01
Mixed fatty acids[2]				
g/100 g milk	1.48	1.35	0.02	<0.01
g/100 g FA	38.86	37.36	0.37	<0.01
g/d	472.0	434.2	15.2	0.08
Preformed fatty acids[3]				
g/100 g milk	1.32	1.38	0.02	0.02
g/100 g FA	34.60	38.21	0.50	<0.01
g/d	419.0	439.3	10.4	0.17
True protein, %	3.19	3.08	0.02	<0.01
True protein yield, kg/d	1.02	0.99	0.03	0.44
MUN, mg/dL	12.1	12.9	0.5	0.25
Anhydrous lactose, %	4.65	4.66	0.02	0.66
Anhydrous lactose, kg/d	1.46	1.51	0.05	0.51

[1] C4 to C14.
[2] C16, C16:1, and C17.
[3] Greater than or equal to C18.

The relatoinships between various milk fatty acid parameters across 40 farms and bulk tank milk fat test are shown in the Figures 1 thru 5 below.

Figure 1. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of de novo fatty acids in milk. In general, a farm needs to have a concentration of de novo fatty acids higher than 0.85 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

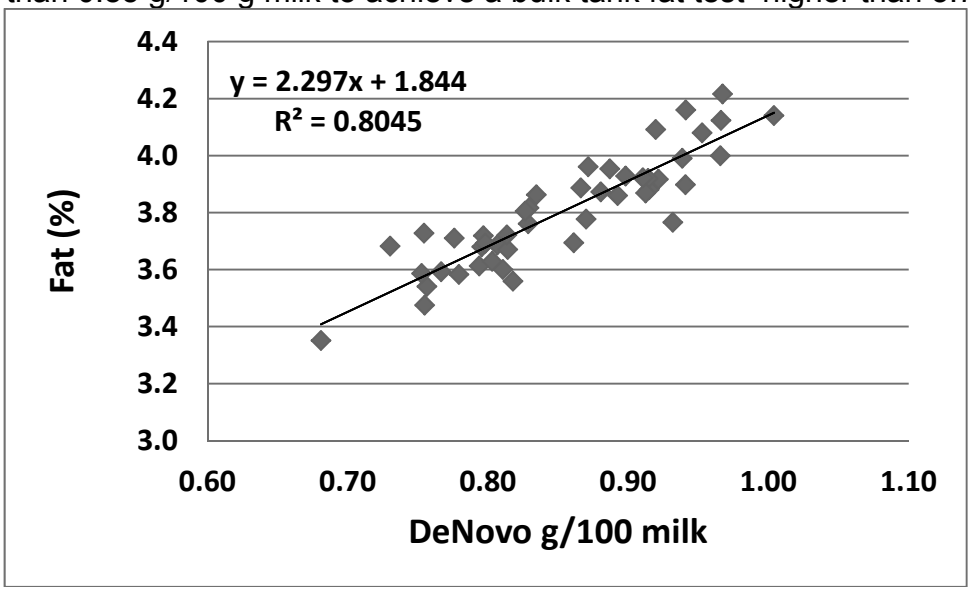


Figure 2. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of mixed origin fatty acids in milk. In general, a farm needs to have a concentration of de novo fatty acids higher than 1.40 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

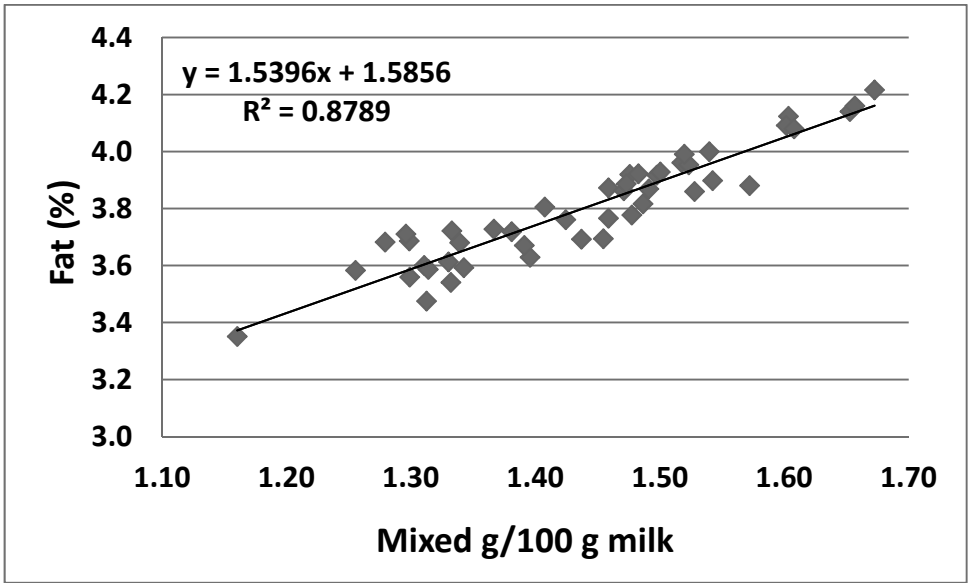


Figure 3. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of preformed fatty acids in milk. In general, the variation in preformed fatty acid concentration in Holstein

herds is less than de novo and mixed origin fatty acids and is not well correlated with bulk tank milk fat test.

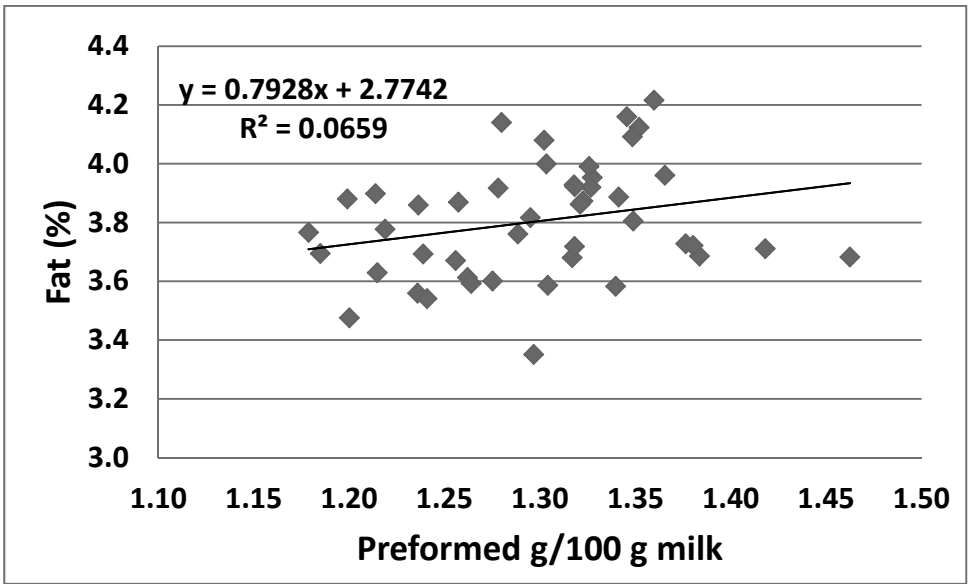


Figure 4. Relationship of bulk tank milk fat fatty acid unsaturation to fatty acid chain length. As fatty acid chain length increases, unsaturation increases and this appears to be due mostly to an increase in oleic acid (C18:1 cis 9).

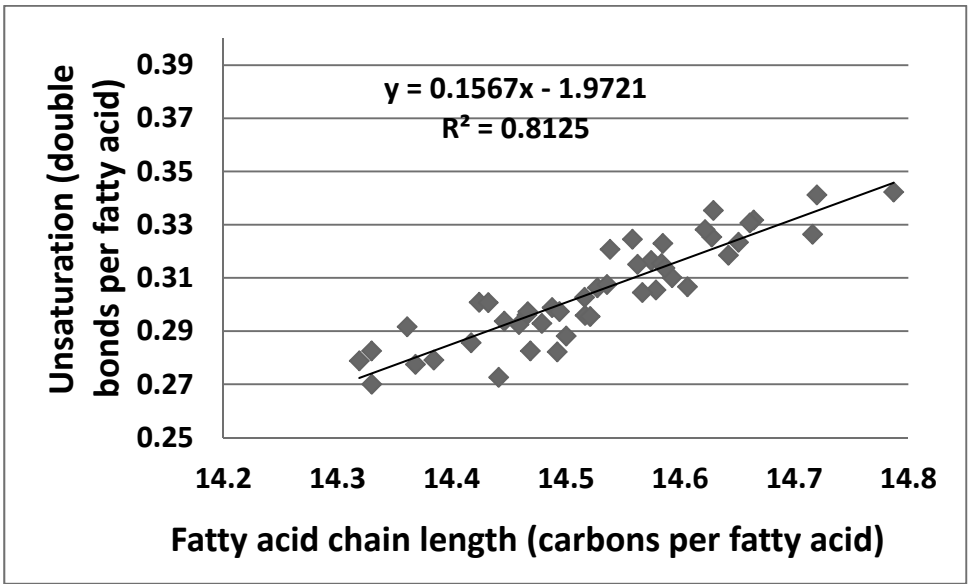
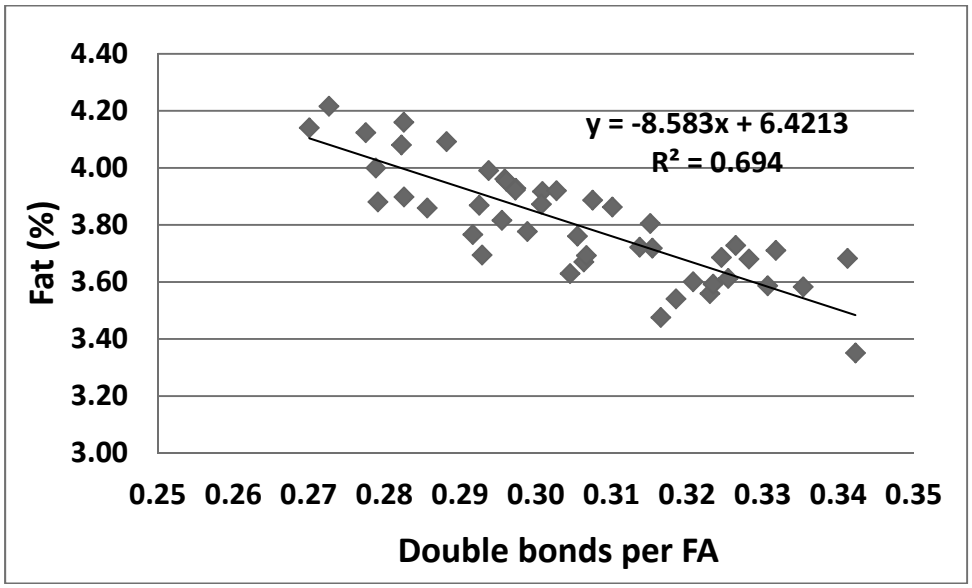
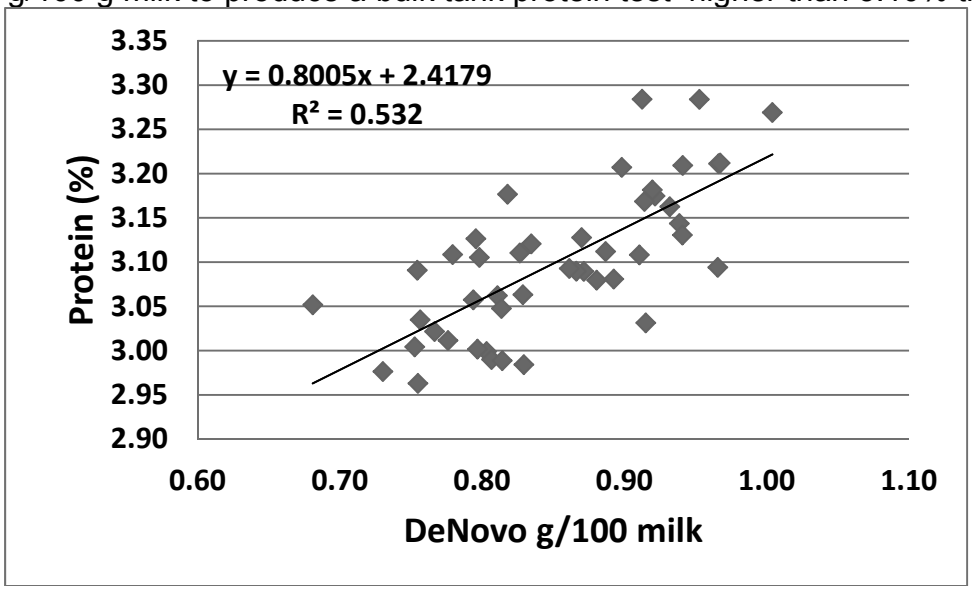


Figure 5. Relationship of bulk tank milk fat fatty acid unsaturation with bulk tank milk fat test. As double bonds per fatty acid increases the bulk tank milk fat test decreases. To achieve a 3.75 % fat test a farm needs to have a double bond per fatty acid of less than 0.31. The double bonds per fatty acid may be an indirection of the rumen unsaturated fatty acid load (RUFAL) and the rate of unsaturated fat release from forage sources (e.g., corn silage, distiller grains, and oil seeds) in the rumen. The double bonds per fatty acid may be an index of the level of milk fat depression in a dairy herd.



The relationship between de novo milk fatty acid concentration across 40 farms and bulk tank milk protein test is shown in the figure below.

Figure 6. Relationship of bulk tank milk protein test to concentration (g/100 g milk) of de novo fatty acids in milk. In general, a farm needs to achieve a concentration of de novo fatty acids > 0.85 g/100 g milk to produce a bulk tank protein test higher than 3.10% true protein.



It is hypothesized that when de novo fatty acid production is high, the the biomass of rumen microflora is high and this provides a higher level of essential amino acids produced in the rumen. When double bonds per fatty acid increase bulk tank milk protein test decreases (data not shown).

The difference in income per cow between HDN and LDN herds would depend on the actual milk price at any point in time. However, the average fat and protein price for Federal Milk Order No. 1 for February through April, 2015 (US Department of Agriculture, 2015) was $4.19 and $5.74 per kg, respectively. Therefore, at 30 kg of milk per cow per day, the average HDN farm earned a gross of $5.00 and $5.49 per cow for fat and protein, respectively. The average LDN farm at 30 kg milk per cow per day earned a gross of $4.75 and $5.30 per cow for fat and protein, respectively. These differences for fat and true protein between HDN and LDN herds at 30 kg of milk would result in a gross income difference of $9,125 for fat and $6,935 for true protein per 100 milking cows per year. High de novo farms tended to be more likely to deliver fresh feed twice versus once per day, have a freestall stocking density less than or equal to 110%, and provide greater than or equal to 46 cm of feed bunk space per cow. There were no detectable differences in forage quality or ration dry matter, crude protein, or starch content. However, ether extract was lower and physically effective neutral detergent fiber was higher for HDN compared with LDN farms. The results of this study indicate that feeding management, stocking density, dietary ether extract content, and the physical characteristics of the diet are related to de novo fatty acid, fat, and protein concentration in bulk tank milk from high-producing Holstein dairy farms.

SUMMARY OF BULK TANK MILK TESTING

The key FA parameter that was positively correlated with bulk tank milk fat and true protein concentration was DeNovo FA (g/100 g milk). Structural parameters of FA chain length (carbon number) and total unsaturation (double bonds /FA) were negatively correlated with fat and protein (g/100 g milk). This was true for both Jersey and Holstein. In general, a Holstein farm needs to have a concentration of de novo fatty acids higher than 0.85 g/100 g milk and a concentration of mixed origin fatty acids higher than 1.35 g/100 g milk to achieve a bulk tank fat test higher than 3.75%. As double bonds per fatty acid increase both fat and protein will decrease. Double bonds per fatty acid may be an index of effective RUFAL level in diet. Keeping the milk double bonds per fatty acid at 0.3 or lower produce higher milk and protein. Over crowding of cows in pens was correlated with lower de novo and mixed origin fatty acids and lower milk fat and protein test. Generally, when de novo fatty acid production is higher milk production per cow will be equal to or higher than when de novo is lower, but both milk fat and protein test (g/100 g of milk) will be higher. This will increase the income per unit of milk produced.

Milk Testing for Individual Cows (Barbano et al., 2015)

As the milk production per cow has increased, there is more demand placed on the physical and metabolic system of each individual dairy cow. More attention through automated information collections systems to the metabolic and physical condition of each cow is needed to keep each cow healthy and productive. Because each cow makes an individual contribution to both farm costs and income, it becomes a management challenge particularly in large dairy herds, to make each cow a “cow-of-interest” and make correct decision about health and reproduction to achieve improved overall performance of the dairy herd.

To achieve a focus on individual cow status, measurement of de novo, mixed origin, and preformed fatty acids in milk is also useful for individual cow milk testing, particularly during the transition period. The changes in de novo fatty acids as a relative percentage of total fatty acids reflects the energy balance status of the cow. Recently, we have developed a new milk mid-IR test that produces an estimate of blood NEFA level by testing the milk. This testing would be done on the same milk sample at the same time as the fat, protein, lactose, solids, MUN and fatty acid analysis using the mid-IR milk analyzer.

High blood NEFA indicates that a cow is mobilizing body fat and increases the risk of metabolic disorders. Milk and blood samples were collected from 60 lactating Holsteins once per week for the first 3 weeks of lactation. Cows were milked 3 times per day. Within + or – one milking of the time of blood collection, a milk sample was analyzed using a Delta Instruments (model FTA) mid-IR milk analyzer. A Wako NEFA HR test kit was used as an in vitro enzymatic colorimetric method for the quantitation of NEFA in blood serum and these values were used as reference values for development PLS regression model to predict blood NEFA from the mid-IR milk spectra. There are no NEFA in milk, so a model to predict blood NEFA from a milk sample uses differences in the milk spectra from sample to sample that are correlated with changes in blood NEFA. The final PLS model had 9 factors, used wavelengths in the following ranges (3000 to 2800, 1800 to 1700, 1585 to

1000 cm-1) with a standard error of cross validation of 172 µEq/L. Validation milk and blood sample pairs (n = 53) were collected from Holstein cows from a different herd. The mean value for the blood reference test was 713 µEq/L of serum and the mean value for the milk based blood NEFA prediction was 703 µEq/L of serum with a standard deviation of the difference (SDD) of 218 µEq/L for the 53 validation samples. Blood NEFA measured on blood is a snapshot of the NEFA concentration at an instant in time, while blood NEFA predicted from milk analysis represents a time average for the total time between milkings. The FTIR milk analysis to estimate blood NEFA is rapid (about 10 seconds), done simultaneously with all other milk component and fatty acid measures, and uses no reagents. This approach could be useful for rapid evaluation of risks of ketosis, displaced abomasum and possibly reproductive disorders. The relationship between the milk estimated blood NEFA level and the change in de novo milk fatty acids may have predictive power to provide an advanced warning that a cow is going to have a displaced abomasum.

Concepts for integration of mid-IR milk analysis directly into the milking systems on large farms are being considered. The combination of milk weight and the component concentrations (i.e., fat, protein, lactose, and milk NPN/Urea content) will allow calculation of energy output in the milk and in combination with feed input data will allow an estimate of energy and protein balance of individuals or groups of cows within the herd.

Some other measures that we have developed for use in individual cow milk testing are predicted blood NEFA for ketosis prediction, in addition to milk BHB and acetone concentrations. We are developing a milk estimated blood BHB method currently. The measurement and rate of change of blood NEFA estimated by milk analysis during the early transition period will provide a view of the metabolic status combined with energy balance estimates. Indirect measurement of rumen pH through milk analysis is in development and might provide insight into how a cow is interacting the complex mixture of nutrients in the rumen, as that impacts the chemistry of the milk.

Combinations of individual parameters that provide more predictive indices of feed efficency, ketosis, and probability of successful breeding may be derived from the current PLS models for milk analysis. In the future, development of models to determine pregnancy status and loss of pregnancy will bring further benefit in the applications of mid-IR milk testing for real-time farm management milk testing.

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What do Clues in Milk Composition Parameters Tell us About Herd Performance?

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A continuing effort in analyzing fat composition has opened a new frontier in evaluating herd performance (Woolpert, et al, 2016). For a long time, bulk tank milk has been analyzed for total fat content. Herd managers soon realized that feeding too much grain or putting cows on pasture would cause a depression in milk fat. These causes of milk fat depression were dogma until it was demonstrated that a potent unsaturated fat (C18:2 trans-10, cis-12) was responsible for many cases of milk fat depression (Bauman and Griinari, 2003).

In 1978, total protein was added to routine bulk tank milk analysis which was later refined to be true protein (January 2000) in most Federal Milk Marketing Orders, although California (not part of the Federal Orders) remains on the total protein system. Interest in milk protein increases in areas with multiple component pricing and when the price of protein is high (> $2.00/lb). About the same time, milk urea nitrogen (MUN) was added to routine milk analysis but has not been incorporated into a payment system. MUN is generally a measure of excess urea in the rumen. As a guideline, a MUN value of 5-6 has been shown to indicate a rumen that is short of available nitrogen while values above 15 usually indicate excessive nitrogen excretion.

The latest addition to milk component analysis is the identification of specific milk fatty acids (Woolpert, et. Al., 2016). Because milk fat is in the form of triglycerides, there is a glycerol backbone that constitutes approximately 5.5% of the total fat weight. Therefore, fatty acids represent approximately 94.5% of the milk fatty acid weight. There are two main sources of fatty acids. The first is de novo synthesis by the mammary cell which can fatty acids from 4 to 16 carbons long. This occurs as a result of elongating acetate and butyrate (produced in the rumen and transported to the mammary cell via the blood) into fatty acids. However, butyrate is the foundation for nearly all de novo synthesis. Preformed fatty acids, originating from either the diet or from adipose stores in the body, are transported into the mammary cell. Preformed fatty acids are 16 carbons and longer. These two sources overlap in the 16 carbon fatty acids; therefore, this category is called mixed. Together, these fatty acid categories represent the total fatty acid content of milk resulting in the following relationship:

Milkfat = glycerol + fatty acids (de novo + mixed + preformed)

Under normal circumstances, 25% of the fatty acids are only synthesized de novo, 37.5% are in the mixed category, and 37.5% are in the preformed category (Woolpert, 2016). However, a 1 unit change in any fatty acid category results in about the same change in milk fat. For example, when milk with 3.8% fat has a decline in de novo content from 0.9 to 0.7, the fat content will decrease to 3.6%. As diet changes, these proportions of each category also change suggesting that they can provide insight in cow performance.

The balance of de novo fatty acids and preformed fatty acids in milk changes dramatically during the post calving transition period. After calving the preformed fatty acids are a high proportion of the total fatty acids in the milk fat (e.g., 50% or higher) and the de novo fatty acids are about 20% of the total fatty acids. When cows come into positive energy balance, the portion of de novo and preformed fatty acids in the milk should stabilize. The point at which they stabilize and move up and down during the remainder of lactation will be function of management practices (e.g., stocking density) and feed nutritional characteristics and quality.

Another feature of milk fat from ruminants is that it is highly saturated. Double bonds in milk fat come from two sources. First, there can be a high percentage of unsaturated fat reaching the mammary cell. This is usually detrimental as native unsaturated fat that escapes the rumen suggests incomplete biohydrogenation of dietary fat. Incomplete biohydrogenation can result from a heavy load of unsaturated fat, extremely high passage rates, or from impaired rumen function. Another scenario is that unsaturated fatty acids (particularly C18:1) may be provided

in the diet as rumen protected fatty acids. Being rumen protected, these unsaturated fatty acids have minimal effects on the rumen environment but can still affect the mammary cell when high levels are incorporated into milk fat.

Unsaturated fatty acid can also be produce in the mammary cells by the enzyme stearyl CoA desaturase. This enzyme converts C18:0 fatty acid (stearic acid) to C18:1 cis-9 fatty acid (oleic acid). This enzyme may play a role in maintaining the fluidity of milk fat. Milk fat needs to have a melting point lower than the body temperature of the cow for secretion.

The issue of chain length (carbons/fatty acids) and degree of unsaturation (double bonds per fatty acid) appears to be a fluidity issue. If chain length increases without a corresponding increase in double bonds, the fluidity of the milk fat would decrease. Likewise, an increase in double bonds decreases fluidity. For example, if there are a lot of unsaturated preformed fatty acids, de novo synthesis will be reduced (Barbano and Sherbon,1980). Remember that melting point goes down with the addition of double bonds and with shorter chain lengths.

Recently, 68 Holstein herds were analyzed for milk fatty acid composition by Cornell University. Some of these herds also conducted a TMR fatty acid analysis through Cumberland Valley Analytical Services. These are not randomly selected herds as they were submitted by herd consultants and nutritionists who were interested in learning more about the milk fatty acid profile of their herds. The mean, min and max values for 68 bulk tank samples are in Table 1.

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Table 1. Mean, min and max milk composition values for 68 Holstein herds.

	Protein	Fat	FA	de novo	Mixed	Preformed	carbon #	DB/FA	Fluidity
	g/100 g milk						FA CL	FA Unsat	C/DBL
Mean	3.06	3.64	3.43	0.81	1.30	1.31	14.65	0.32	46.07
Min	2.80	3.09	2.89	0.65	1.08	1.13	14.42	0.28	42.58
Max	3.34	4.16	3.94	1.00	1.52	1.57	14.90	0.35	52.08

Table 1 demonstrates that the large range in milk fat (1.07 units) is accompanied with large ranges in the individual fatty acid composition (*de novo*, 0.35; mixed, 0.44; preformed, 0.44 units). Milk protein content had a much narrower range (0.54 units) compared to total milk fat. This suggests that there are effects on milk fat that are much larger than the effects on milk protein. The column labeled Carbon # (FA CL; fatty acid chain length) refers to the average number of carbons per fatty acid. Fatty acid chain length is very much dependent on proportions of fatty acids in each category. As the proportion of preformed fatty acids increases, the chain length will increase. Since longer fatty acids have higher melting points, a longer chain length will decrease fluidity. The range in Table 1 for Carbon # was 14.42 to 14.90. DB/FA (FA Unsat) refers to the number of double bonds per fatty acid. As double bonds are added to fatty acids, melting point decreases which increases fluidity. The range in Table 1 for DB/FA was 0.28 to 0.35. Because there is an inverse relationship between carbon # and DB/FA relative to fluidity, a fluidity index of (carbon #)/(DB/FA) has been developed. Across the dataset, fat percent increases as the fluidity index increases (fat percent = 0.84 + .06*fluidity; R^2 = 0.30).

As a point of reference, Table 2 contains the milk component values for an example herd with very good milk composition based on the high level of *de novo* milk fatty acids and typical ratios of mixed and preformed fatty acids.

Table 2. An example herd with excellent milk components.

Protein	Fat	FA	de novo	Mixed	Preformed	carbon #	DB/FA	Fluidity
g/100 g milk						FA CL	FA Unsat	C/DBL
3.13	3.89	3.75	0.87	1.41	1.46	14.50	0.28	52

The 68 herds fall into several categories:

High levels of dietary unsaturated fat affecting the mammary gland. CLA (C18:2 *trans*-10:*cis*-12) has been shown to be a potent inhibitor of *de novo* milk fat synthesis in the mammary cell. These herds will have normal rumen function, but *de novo* synthesis will be down regulated. In this example herd (Table 3), fat is depressed to 3.46% but milk protein is near normal (3.1%). This suggests that the rumen is producing sufficient metabolizable protein to support high levels of milk protein production. However, *de novo* fatty acid synthesis is impaired. In this case, *de novo* milk fat synthesis is low (0.72 vs 0.78). The preformed fatty acids were high, as there was added fat in the diet. The increased percentage of preformed fatty acids led to increased average chain length; however, the number of double bonds is exceedingly high resulting in a more fluid fat. The fluidity index is low which is suggesting an imbalance in chain length and unsaturated fat.

Table 3. Example herd exhibiting normal milk protein, low milk fat, low de novo fatty acids, and a high level of unsaturated fat.

Protein	Fat	FA	de novo	Mixed	Preformed	carbon #	DB/FA	Fluidity
g/100 g milk						FA CL	FA Unsat	C/DBL
3.10	3.46	3.23	0.72	1.20	1.31	14.90	0.34	43
Expected			0.78	1.23	1.23			

1) **High levels of dietary unsaturated fat affecting both the rumen and the mammary gland**. Unsaturated fat can impair ruminal fiber digestion which will reduce ruminal protein production in addition to providing a high level of unsaturated fat directly to the mammary gland. In the example herd (Table 4), roasted soybeans were included in the diet resulting in an abnormally high level of dietary unsaturated fat. Both protein and fat content of the milk are depressed with a lowered *de novo* milk fatty acid synthesis (0.70 versus 0.80 g/100 g milk). Added fat in the diet is raising both the mixed and preformed categories. With the lowered *de novo* synthesis, fatty acid chain length is longer but again the amount of double bonds is higher than expected given this increase in chain length with a low fluidity index.

Table 4. Example herd with low milk protein, low milk fat, low *de novo* fatty acids and high level of unsaturated fatty acids.

Protein	Fat	FA	de novo	Mixed	Preformed	carbon #	DB/FA	Fluidity
g/100 g milk						FA CL	FA Unsat	C/DBL
2.94	3.57	3.34	0.70	1.27	1.37	14.82	0.34	44
Expected			0.80	1.27	1.27			

1) **A shortage of de novo milk fatty acids without a high degree of unsaturated fat**. These herds appear normal except that the milk fat is depressed. In the example herd (Table 5), fat is slightly depressed while protein and amount of unsaturated fatty acids are near normal. Herds such as this appear to have a shortage of substrate for *de novo* synthesis rather than an inhibition of *de novo* synthesis. It is widely recognized that acetate and butyrate are the building blocks of *de novo* fat synthesis in the mammary gland with much of the focus on acetate. However, butyrate may play a more important role than previously recognized. For example, 36 mole% of triglycerides contained C4 (butyrate) or C6 (butyrate + acetate) (Jensen, 2002). All the C4 and 90% of the C6 fatty acids were on the *sn*-3 position (the third leg of the triglyceride). Numerous rumen microflora produce butyrate, however, a primary substrate used in producing butyrate may be sugar (glucose and sucrose).

Table 5. An example herd with low fat with near normal protein and low amount of unsaturated fatty acids.

Protein	Fat	FA	de novo	Mixed	Preformed	carbon #	DB/FA	Fluidity
g/100 g milk						FA CL	FA Unsat	C/DBL
3.06	3.50	3.31	0.78	1.27	1.25	14.71	0.31	48
Expected			0.80	1.27	1.27			

1) **Excessive levels of palm fat in the diet**. High levels of palm fat (C16:0) in the diet can mask other fat production issues. In the herd shown in Table 6, protein and total fat are slightly

reduced. In this example, the level of mixed fatty acids is high (1.46 vs 1.30 g/100g milk). If the mixed fatty acids were not elevated, the actual fat content would be closer to 3.45% as opposed to the observed 3.62%. For most corn based diets, C16:0 represents about 20% of the total fatty acids. In this herd, the TMR fatty acid report (Figure 1) showed 35% of the total fatty acids were C16:0. Clearly, a C16:0 supplemental product is being added to the diet. Milk fatty acid composition for this herd suggests that more *de novo* synthesis is needed, probably dependent on sugar availability for ruminal butyrate synthesis, along with more total energy to spare the preformed fatty acids. Since the degree of unsaturation is low, adding more corn would be appropriate.

Table 6. Example herd with high levels of C16 fatty acids due to supplemental palm fat.

Protein	Fat	FA	de novo	Mixed	Preformed	carbon #	DB/FA	Fluidity
g/100 g						FA CL	FA Unsat	C/DBL
2.96	3.62	3.42	0.80	1.46	1.16	14.64	0.29	51
Expected			0.82	1.30	1.30			

Figure 1. TMR fatty acid levels for an example herd with high levels of palmitic acid. Typical corn based diets usually contain 20% of the fatty acids as C16:0 with no supplemental fat.

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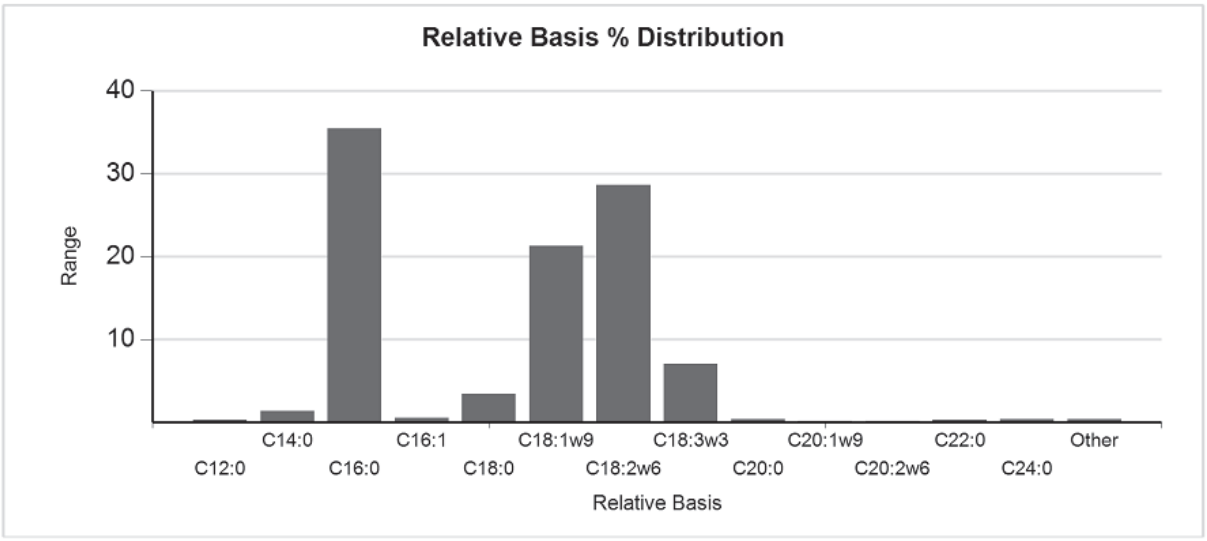

Leaky Gut's Contribution to Inefficient Nutrient Utilization

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Introduction

There are a variety of situations in an animal's life when nutrient utilization is reprioritized from productive towards agriculturally unproductive purposes. Two well-known examples that markedly reduce production are heat stress and ketosis. Decreased feed intake, experienced during both diseases, is unable to fully explain decreases in productivity. Additionally, both diseases are characterized by negative energy balance, body weight loss, inflammation, and hepatic steatosis. While the metabolism of ketosis and heat stress have been thoroughly studied for the last 40 years, the initial insult in the cascade of events ultimately reducing productivity in both heat-stressed and ketotic cows has not been identified. To that end, we have generated preliminary data strongly implicating a metabolic disruptor, endotoxin, as the etiological culprit in each case.

Heat Stress

Heat stress negatively impacts a variety of production parameters and is a significant financial burden (~$900 million/year for dairy in the U.S. alone; St. Pierre et al., 2003). Heat-stress affects productivity indirectly by reducing feed intake; however, direct mechanisms also contribute as we have shown reduced feed intake only explains approximately 35-50% of the decreased milk yield during heat stress (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). Direct mechanisms contributing to heat stress milk yield losses involve an altered endocrine profile, including reciprocal changes in circulating anabolic and catabolic hormones (Bernabucci et al., 2010; Baumgard and Rhoads, 2012). Such changes are characterized by increased circulating insulin concentration, lack of adipose tissue lipid mobilization, and reduced adipocyte responsiveness to lipolytic stimuli. Hepatic and skeletal muscle cellular bioenergetics also exhibit clear differences in carbohydrate production and use, respectively, due to heat stress. Thus, the heat stress response markedly alters post-absorptive carbohydrate, lipid, and protein metabolism through coordinated changes in fuel supply and utilization across tissues in a manner distinct from commonly recognizable changes that occur in animals on a reduced plane of nutrition (Baumgard and Rhoads, 2013). The result of HS is underachievement of an animal's full genetic potential.

Ketosis

The periparturient period is associated with substantial metabolic changes involving normal homeorhetic adaptations to support milk production. Unfortunately, a disproportionate amount of herd culling occurs before cows reach 60 days in milk (Godden, 2003). Ketosis is defined as an excess of circulating ketone bodies and is characterized by decreases in feed intake, milk production, and increased risk of developing other transition period diseases (Chapinal et al., 2012). Epidemiological data indicate about 20% of transitioning dairy cows clinically experience ketosis (BHBA > 3.0 mM; Gillund et al., 2001) while the incidence of subclinical ketosis (>1.2 mM BHBA) is thought to be much higher (> 40%; McArt et al., 2012). Ketosis is a costly disorder (estimated at ~$300 per case; McArt et al., 2015) and thus it represents a major hurdle to farm profitability. Traditionally, ketosis is thought to result from excessive adipose tissue mobilization (Baird, 1982; Grummer, 1993; Drackley, 1999) which in turn contributes to fatty liver (hepatic steatosis) and excessive ketone body synthesis (Grummer, 1993).

Heat stress etiology

Mechanisms responsible for altered nutrient partitioning during HS are not clear; however, they might be mediated by HS effects on gastrointestinal health and function as we and others have demonstrated HS compromised intestinal barrier function (Lambert et al., 2002; Dokladny et al., 2006; Pearce et al., 2013; Sanz-Fernandez et al., 2014). During HS, blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat leading to intestinal hypoxia (Hall et al., 1999). Enterocytes are particularly sensitive to hypoxia and nutrient restriction (Rollwagen et al., 2006), resulting in ATP deple-

tion and increased oxidative and nitrosative stress (Hall et al., 2001). This contributes to tight junction dysfunction and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013). As a result, HS increases the passage of luminal content into portal and systemic blood (Hall et al., 2001; Pearce et al., 2013). Endotoxin, otherwise referred to as lipopolysaccharide (LPS), is a glycolipid embedded in the outer membrane of Gram-negative bacteria, which are abundant and prolific in luminal content, and is a well-characterized potent immune stimulator in multiple species (Berczi et al., 1966; Giri et al., 1990; Tough et al., 1997). Activation of the immune system occurs when LPS binding protein (LBP) initially binds LPS and together with CD14 and TLR4 delivers LPS for removal and detoxification, thus LBP is frequently used as a biomarker for LPS infiltration (Ceciliani et al., 2012). For a detailed description of how livestock and other species detoxify LPS see our recent review (Mani et al., 2012). Endotoxin infiltration during HS into the bloodstream which was first observed by Graber et al. (1971), is common among heat stroke patients (Leon, 2007) and is thought to play a central role in heat stroke pathophysiology as survival increases when intestinal bacterial load is reduced or when plasma LPS is neutralized (Bynum et al., 1979; Gathiram et al., 1987). It is remarkable how animals suffering from heat stroke or severe endotoxemia share many physiological and metabolic similarities to HS, such as an increase in circulating insulin (Lim et al., 2007). Infusing LPS into the mammary gland increased (~2 fold) circulating insulin in lactating cows (Waldron et al., 2006). In addition, we intravenously infused LPS into growing calves and pigs and demonstrated >10 fold increase in circulating insulin (Rhoads et al., 2009; Stoakes et al., 2015a; Kvidera et al., 2016). Interestingly, increased insulin occurs prior to increased inflammation and the temporal pattern agrees with our previous in vivo data and a recent in vitro report (Bhat et al., 2014) suggesting LPS stimulates insulin secretion, either directly or via GLP-1 (Kahles et al., 2014). The possibility that LPS increases insulin secretion likely explains the hyperinsulinemia we have repeatedly reported in a variety of heat-stressed agriculture models (Baumgard and Rhoads, 2013). Again, the increase in insulin in both models is energetically difficult to explain as feed intake was severely depressed in both experiments.

Transition period inflammation

Recently, the concept that LPS impacts normal nutrient partitioning and potentially contributes to metabolic maladaptation to lactation has started to receive attention. Although LPS itself has not been the primary causative focus, general inflammation has been the topic of investigations. Increased inflammatory markers following parturition have been reported in cows (Ametaj et al., 2005; Bertoni et al., 2008; Humblet et al., 2006; Mullins et al., 2012). Presumably, the inflammatory state following calving disrupts normal nutrient partitioning and is detrimental to productivity (Loor et al., 2005; Bertoni et al., 2008), and this assumption was recently reinforced when TNFα infusion decreased productivity (albeit without overt changes in metabolism; Yuan et al., 2013; Martel et al., 2014). Additionally, in late-lactation cows, injecting TNFα increased (>100%) liver TAG content without a change in circulating NEFA (Bradford et al., 2009). Our recent data demonstrates increased inflammatory markers in cows diagnosed with ketosis only and no other health disorders. In comparison with healthy controls, ketotic cows had increased circulating LPS prior to calving and postpartum acute phase proteins such as LPS-binding protein, serum amyloid A, and haptoglobin were also increased (Fig. 1; Abuajamieh et al., 2015). Endotoxin can originate from a variety of locations, and obvious sources in transitioning dairy cows include the uterus (metritis), mammary gland (mastitis) and the gastrointestinal tract (Mani et al., 2012). However, we believe intestinal permeability may be responsible for inflammation 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 order to further investigate the effects of intestinal permeability on production and inflammation, we intentionally induced intestinal permeability in mid-lactation dairy cows using a gamma secretase inhibitor (GSI), a compound that specifically inhibits crypt stem cell differentiation into enterocytes via disrupting Notch signaling (van Es et al., 2005). We anticipated feed intake of GSI administered cows would decrease, so we pair-fed controls in order to eliminate the confounding effect of feed intake. Treatment with GSI decreased feed intake and altered jejunum morphology consistently with characteristics of leaky gut (shortened crypt depth, decreased villus height, decreased villus height to crypt depth ratio). Circulating insulin and LBP were increased in GSI cows relative to controls. Interestingly in our GSI model, acute phase proteins serum amyloid A and haptoglobin increased for both treatments over time, indicating inflammation was occurring in pair-fed controls as well (Kvidera et al., 2017a). This is not surprising, as pair-fed controls were receiving ~20% of their ad libitum intake and decreased feed intake has been shown to increase intestinal permeability in feed restricted rodents and humans (Rodriguez et al., 1996; Welsh

et al., 1998) and we have also observed this in pigs (Pearce et al., 2013; Sanz-Fernandez et al., 2014). Recently, we confirmed the detrimental effects of feed restriction in mid-lactation cows by demonstrating a linear increase in circulating acute phase proteins and endotoxin with increasing severity of feed restriction. Furthermore, cows fed 40% of ad libitum intake had shortened ileum villous height and crypt depth, indicating reduced intestinal health (Stoakes et al., 2015b). In summary, inflammation is present during the transition period and likely contributes to changes in whole-animal energetics.

Metabolism of inflammation

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

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

Energetic cost of immune activation

An activated immune system requires a large amount of energy and the literature suggests that glucose homeostasis is markedly disrupted (Leininger et al., 2000) during an endotoxin challenge. Upon immune system activation, immune cells switch their metabolism from oxidative phosphorylation to aerobic glycolysis, causing them to become obligate glucose utilizers in a phenomenon known as the Warburg Effect (Vander Hiden et al., 2009). Our group recently employed a series of LPS-euglycemic clamps to quantify the energetic cost of an activated immune system. Using this model, we estimated approximately 1 kg of glucose is used by the immune system during a 12 hour period in lactating dairy cows. Interestingly, on a metabolic body weight basis the amount of glucose utilized by LPS-activated immune system in lactating cows, growing steers and growing pigs were 0.64, 1.0, and 1.1 g glucose/kg BW0.75/h, respectively; Stoakes et al., 2015a; Kvidera et al., 2016, 2017b). Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake: this coupling of enhanced nutrient requirements with hypophagia obviously decrease the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus, wool). We and others have now demonstrated that both heat-stressed and ketotic animals have increased circulating markers of endotoxin and inflammation. We believe that the circulating LPS in both maladies originates from the intestine and thus both likely have an activated immune system. This activated systemic immune response reprioritizes the hierarchy of glucose utilization and milk synthesis is consequently deemphasized.

Nutritional strategies: the role of chromium (Cr) supplementation

Potential mitigation strategies during inflammatory conditions are currently of great interest; one such strategy is dietary Cr supplementation. Chromium is a nutrient well known for its role in improving insulin action. While the exact mechanism is not fully understood it appears to work intimately with the oligopeptide low-molecular-weight Cr-binding substance or chromodulin. In response to increasing insulin, Cr ions enter insulin dependent cells and in combination with chromodulin bind to insulin receptor sites amplifying the signaling cascade (Vincent, 2015). Subsequently, translocation of the insulin dependent glucose transporter GLUT-4 to the plasma membrane is improved and cellular glucose uptake is increased (Chen et al., 2006). Glucose availability to activated leukocytes impacts apoptotic rate, formation of reactive oxygen species, and adhesion interactions (Calder et al., 2007; Maclver et al., 2008). Previous studies have shown improved immune function (e.g., phagocytosis, blastogenic response, antibody production) of activated leukocytes supplemented with Cr (Moonsier-Shageer and Mowat, 1992; Chang

et al., 1996; Lee et al., 2000), while others observed no effect (Kegley et al., 1997a). Several studies have demonstrated increased markers of cytokine production in mice, steers, and cows (Burdick et al., 2011; Yuan et al., 2014; Jin et al., 2016). Additionally, we and others have shown an impact of Cr on neutrophil proliferation (Horst et al., unpublished data; Kafilzadeh et al., 2012; Yasui et al., 2014; Mayorga et al., 2016). However, the direct influence of Cr on bovine immunity remains poorly understood and warrants further investigation.

The benefits of Cr are not limited to its influence on immunity, but extend to metabolism and production parameters as well. Previous studies in cattle have demonstrated that Cr supplementation improved glucose clearance rate and decreased circulating insulin levels following an intravenous glucose tolerance test (Bunting et al., 1994; Kegley et al., 1997b; Stahlhut et al., 2006; Summer et al., 2007; Spears et al., 2012); suggesting enhanced tissue insulin sensitivity in animals fed Cr compared to those not supplemented. Additionally, supplemental Cr consistently increases feed intake in a variety of species (Mooney and Crowell, 1997; Sahin et al., 2003; Al-Saiyadi et al., 2004; MacNamara and Valdez, 2005; Toghyani et al., 2006; Hung et al., 2014). Further, beneficial effects of dietary Cr on milk production, average daily gain, carcass traits, and reproductive parameters have been documented within the literature (Page et al., 1993, Lindemann et al., 1995; Hayirli et al., 2001; Bryan et al., 2004; Smith et al., 2005, 2008; Sadri et al., 2009; Yasui et al., 2014, Liu et al., 2017). A summary of the effects of Cr supplementation on production parameters across different species is presented in Table 1.

Conclusion

Ketosis and heat stress are two of the most economically important pathologies which severely jeopardize the competitiveness of animal agriculture. Heat stress and ketosis affect herds of all sizes and every dairy region in country. The biology of ketosis and heat stress has been studied for almost a half century, but the negative impacts of both are as severe today as they were 30 years ago. We suggest, based upon the literature and on our supporting evidence, that LPS is the common culprit etiological origin of both metabolic disorders. Taken together, our data and the literature suggest that LPS markedly alters nutrient partitioning and is a causative agent in metabolic disruption during heat stress and ketosis. Identifying nutritional strategies to improve animal welfare and performance is critically important. The use of dietary Cr as a supplement may represent a practical avenue to maximize the animal response under these circumstances.

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Table 1. Effects of Cr supplementation on production parameters and metabolism in various species

Variable	Species	Response	Reference
Feed Intake	Cattle	=/↑	3,22/1,4,10,19,20, 21
	Pigs	=/↓/↑	7,13,12,27/11/6,14
	Poultry	↓/↑	24/16,17,23
Average Daily Gain	Cattle	↑	8,9
	Pigs	=/↑	2,12,15/5,13,26
	Poultry	↑	17,18,23
Milk yield	Cattle	=/↑	3,25/1,4,10,19,21

[1]Al-Saiyadi et al., 2004
[2]Amoikon et al., 1995
[3]Bryan et al., 2004
[4]Hayirli et al., 2001
[5]Harper et al., 1995
[6]Hung et al., 2014
[7]Jackson et al., 2009
[8]Kegley et al., 1997a
[9]Kegley et al., 1997b
[10]MacNamara and Valdez, 2005
[11]Mathews et al., 2001
[12]Mathews et al., 2003
[13]Mooney and Crowell.1995
[14]Mooney and Crowell, 1997
[15]Page et al., 1993
[16]Sahin et al., 2002
[17]Sahin et al., 2003
[18]Sands and Smith, 1999
[19]Smith et al., 2005
[20]Smith et al., 2008
[21]Soltan et al., 2010
[22]Spears et al., 2012
[23]Toghyani et al., 2006
[24]Uyanik et al., 2002
[25]Yasui et al., 2014
[26]Zhang et al., 2011

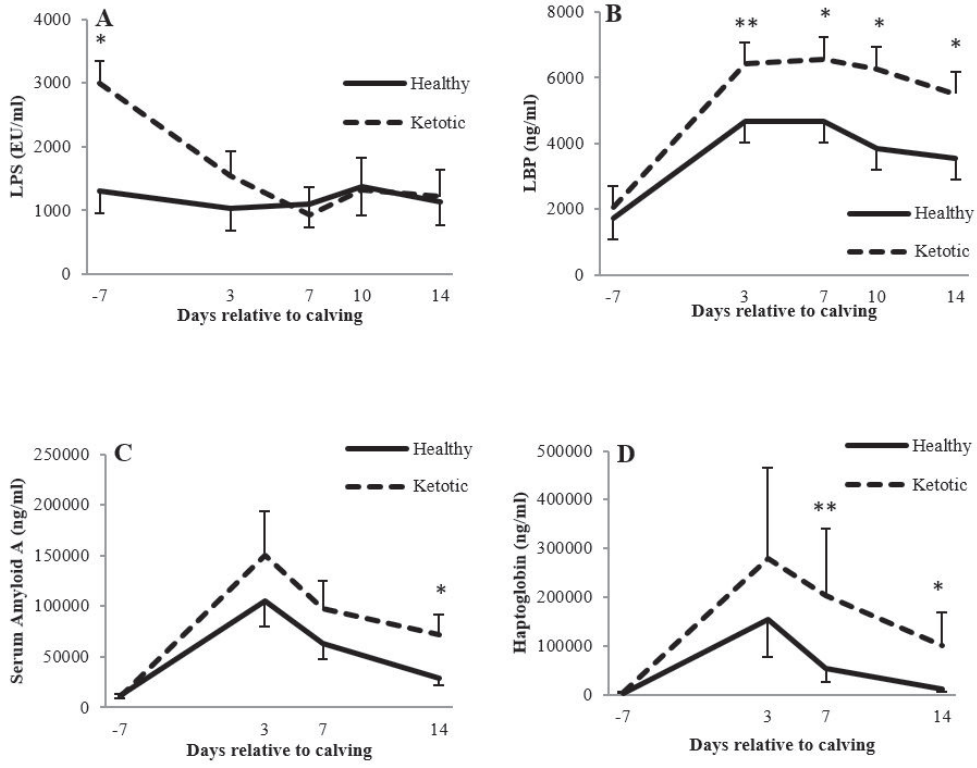

Figure 1. Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows.

How Supplementing Methionine During the Transition Period Can Improve Metabolic Health, Boost ECM Yield and Enhance Reproduction

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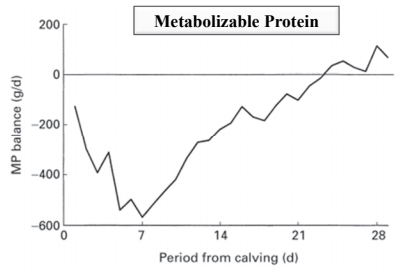
TAKE-HOME MESSAGES

- Transition cows experience increased protein mobilization, reduced liver and immune function, and increased inflammation and oxidative stress
- Increasing metabolizable protein (MP) to early fresh cows increases ECM yield but does not increase milk protein concentrations
- Supplemental Lys and Met increases milk protein concentrations
- Methionine is first limiting for protein synthesis when diets are balanced for Lys in MP
- Methionine is required for synthesis of S-adenosylmethionine (SAM), major methyl group donor in the body participating in many different methylation reactions
- Methionine is needed for synthesis of taurine and glutathione, two potent antioxidants that scavenge reactive oxygen metabolites (ROM) and free radicals, thereby reducing oxidative stress and associated tissue damage that can render cows more susceptible to health disorders
- Supplementing methionine during the transition period has been shown to:
  1. Increase DM intake
  2. Increase milk yield and milk protein concentrations
  3. Increase serum albumin concentrations
  4. Increased blood plasma glutathione and taurine concentrations
  5. Increase biomarkers indicative of improved liver function and reduced oxidative stress, and decreased biomarkers of inflammation
  6. Reduce incidence of ketosis
  7. Affect gene expression of embryos
  8. Increase amniotic vesicle and embryo size in multiparous cows
  9. Increase lipid content of embryos
  10. Reduce pregnancy loss in multiparous cows
- Appears that the benefits of AA balancing for transition cows are far reaching and extend well beyond improvements in milk production
- A further understanding of the benefits of AA balancing for transition cows awaits guidance from advancements in nutritional modeling and determination of AA requirements

How Supplementing Methionine During the Transition Period Can Improve Metabolic Health, Boost ECM Yield and Enhance Reproduction

Chuck Schwab, Schwab Consulting, LLC, Boscobel, WI and Professor Emeritus of Animal Sciences, University of New Hampshire

Negative protein balance in early postpartum cows has received little attention



MP is being mobilized because its needed!

Question:

Are milk protein and non-mammary functions of AA being negatively affected?

Average calculated MP balances in postparturient cows (n = 80) fed a ration containing 17.8% CP and 1.7 Mcal/kg of NEL. Individual values were calculated from daily measurements of CP intake and milk yield, and weekly measurements of milk composition. From Bell et al. (2000).

Numerous metabolic changes occur in transition cows

1. Increased glucocorticoids
2. Increased fat **and** protein mobilization
3. Increased plasma NEFAs and BW loss
4. Increased liver uptake of FA (often exceeding capacity for oxidation)
5. Increased ketone production **(ketosis)** and liver TG storage **(fatty liver)**
6. Reduced liver function (e.g., decreased glucose production)
7. Depressed immune function (e.g., decreased blood neutrophil-killing capacity)
8. Increased inflammation [characterized by increased synthesis of positive acute-phase proteins (e.g., ceruloplasmin and serum amyloid A) and decreased synthesis of negative acute-phase proteins (e.g., albumin)]
9. Increased oxidative stress (created by an imbalance between production of ROM and the neutralizing capacity of antioxidant mechanisms in tissues and blood)

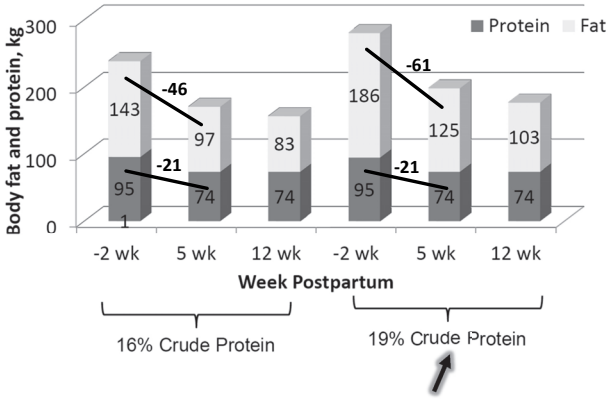
Abomasal infusion of casein protein to postpartum transition cows increases milk yield[1,2,3]

Item	4 DIM		15 DIM		29 DIM	
	CTRL	CAS	CTRL	CAS	CTRL	CAS
DM intake	14.3	14.9	18.8	17.8	22.1	19.9
MP, g/d	1,250	1,964	1,795	2,114	2,226	2,115
Milk, kg/d	27.4	36.9**	37.6	46.8**	41.0	48.9**
ECM, kg/d	32.0	43.5**	38.5	46.3*	40.0	43.2
Protein, %	4.4	4.5	3.7	3.4	3.2	3.0
Fat, %	4.8	4.9	4.1	3.9	3.9	3.3
Urea, *mM*	3.2	4.8**	3.0	4.3**	3.1	4.0*
BW change, kg	-29	-28	-52	-55	-52	-68

[1] Larsen et al. (2014)
[2] Primary feeds in prepartum diet: corn silage, grass-clover silage, barley straw, wheat grain, soybean meal, molasses and vegetable fat (DM intake averaged 10.1 kg/d for both groups). Primary feeds in postpartum diets: corn silage, grass-clover silage, wheat grain, soybean meal, rapeseed meal, sugar beat pulp, and vegetable fat
[3] Infused casein protein was planned to supply 360 g/d at 1 DIM, 720 g/d at 2 DIM, followed by daily reductions of 19.5 g/d ending at 194 g/d at 29 DIM. Infusion were initiated 6 h after calving and averaged 696, 490 and 212 g/d at the 4, 15, and 29 DIM sampling days

** P ≤ 0.01, * P ≤ 0.05

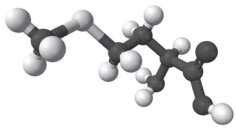
Both body fat and protein are mobilized

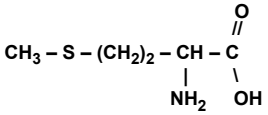


Komaragiri and Erdman (1997)

Increased milk by 3 kg/d with no change in intake

Why focus on methionine?





- ✓ Methionine and lysine are most limiting AA in MP for milk protein synthesis (NRC, 2001)
- ✓ Methionine is first-limiting for milk protein synthesis when MP is balanced for lysine
- ✓ Methionine is precursor for synthesis of other sulfur-containing AA such as cysteine, homocysteine, taurine and glutathione
- ✓ Methionine needed for synthesis of *S*-adenosylmethionine (SAM), major methyl group donor in the body participating in many different methylation reactions

Lys and Met concentrations in milk, rumen microorganisms and feedstuffs (% of AA or CP), relative to estimated ideal concentrations in MP

	Lys	Met		Lys	Met
Milk	7.7	2.7	**Brewer's grains**	4.1	1.7
Fluid associated bacteria[1]	7.7	2.4	**Canola meal**	5.6	1.9
Particle associated bacteria[1]	7.5	2.3	**Corn DDGS**	2.2	1.8
Protozoa[1]	10.8	2.1	**Corn gluten feed**	2.7	1.6
Estimated "ideal" in MP	7.2	2.5	**Corn gluten meal**	1.7	2.4
			Cotton seed	4.3	1.7
Alfalfa silage	4.4	1.4	**Linseed meal**	3.7	1.8
Corn silage	2.5	1.5	**Soybean meal**	6.3	1.4
Grass silage	3.3	1.2			
			Blood meal	9.0	1.2
Barley	3.6	1.7	**Feather meal**	2.6	0.8
Corn	2.8	2.1	**Fish meal**	7.7	2.8
Wheat	2.8	1.6	**Meat meal**	5.4	1.4

[1] Sok et al. 2017 (in press)

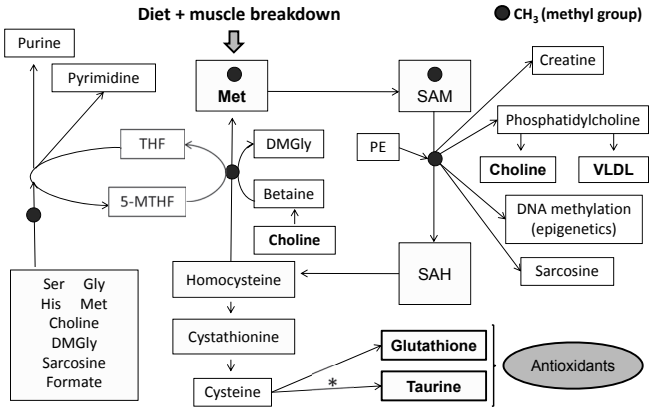
Plasma free methionine concentrations were lower in dairy cows with developing fatty liver than those not exhibiting fatty liver[1]

	Fatty livers Week -1		Fatty livers Week 1		Fatty livers Week 2		Fatty livers Week 4	
Plasma AA (*u*M/L)	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**
Met	**41.2***	**24.3**	**36.2**	**27.0**	**21.8***	**16.5**	**24.0**	**22.9**
Lys	71.2	73.5	89.4	84.6	45.0	56.3	62.9	55.4
TAA	2025	1762	1772	1841	1733	1691	2100	2225

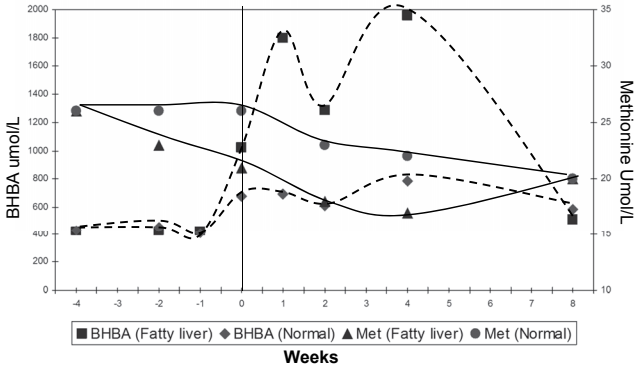
[1] Ten dry cows of Bohemian Black Pied breed with BCS 4.5-5.0 were subjected to feed restriction (no concéntrate) at the end of the first week after calving. Liver biopsies were performed on day 4 of feed restriction and analyzed for neutral fat. According to results, 7 cows experienced various stages of fatty liver and 3 exhibited no evidence of fatty liver.

Pechova et al. (2000) (Acta Vet. Brno. 69:93-99)

Interactions of methionine cycle, transulfuration pathway, and folate cycle



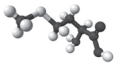
BHBA and serum Met in 4 "healthy" cows and 4 cows with "severe fatty liver"[1]



[1] Cows were identified from 35 cows with similar calving dates on a farm with 250 cows. Cows were fed legume and grass hays with a concéntrate containing brewers grains, soybean meal, and soy sauce cake

Shibano and Kawamura (2006) (J. Vet. Med. Sci. 68:393-396) Courtesy of Drackley, 2014

Unique roles of methionine in metabolic regulation



- ✓ Via SAM: methylation of proteins and DNA; synthesis of creatine, epinephrine and polyamines; regulation of gene expression; one-carbon-unit metabolism (methylation reactions)
- ✓ Via homocysteine: oxidant; inhibition of nitric oxide synthesis
- ✓ Via taurine: antioxidant; anti-inflammatory agent; regulator of intracellular osmolality; conjugation with bile acids (modulates digestion and absorption of fat and fat-soluble vitamins)
- ✓ Via glutathione:
  1) Antioxidant - scavenges free radicals and other reactive oxygen species (e.g., hydroxyl radical, lipid peroxyl radical, peroxynitrite, H_2O_2)
  2) Metabolism (e.g., synthesis of prostaglandins)
  3) Metabolic regulation [e.g., signal transduction, gene expression, cell proliferation (including hepatocytes, lymphocytes, intestinal epithelial cells), cytokine production and immune response, and function and integrity of cell membranes and mitochondria

Study 1

(Osorio et al., 2013, 2014a,b)

	Close-up (15.1%CP)	Lactation (17.5% CP)
Corn silage	35.9	33.0
Alfalfa silage	8.2	5.0
Alfalfa hay	3.5	4.0
Wheat straw	15.4	4.0
Cottonseed	---	3.5
Wet brewers grains	6.0	10.0
Ground shelled corn	13.0	22.2
Soy hulls	4.0	4.0
SBM, 48% CP	3.1	3.3
Expeller SBM	2.0	6.2
SoyChlor	3.8	---
Blood meal	1.0	0.3
Urea	0.3	0.14
Rumen-inert fat	---	1.0

Frequency of occurrence of health problems

	Diet		
	CON	MS	SM
Assigned cows	24	15	17
Cows removed[1]	10	3	4
Twins	2	0	1
Ketosis	**6**	**1**	**2**
Displaced abomasum	3	2	2
Retained placenta	0	1	1
Cows completing study	14	12	12

[1] Four of the 17 cows that were excluded from the experiment were diagnosed with 2 clinical diseases

Osorio et al. (2013)

A summary of early lactation cow RPLys and Met supplementation experiments

7 experiments that measured production responses to increasing Met, Lys, or both in MP after calving

---

+ 0.70 kg/d milk
+ 0.16% units milk protein
+ 79 g/d milk protein
+ 0.02% units milk fat
+ 48 g/d milk fat

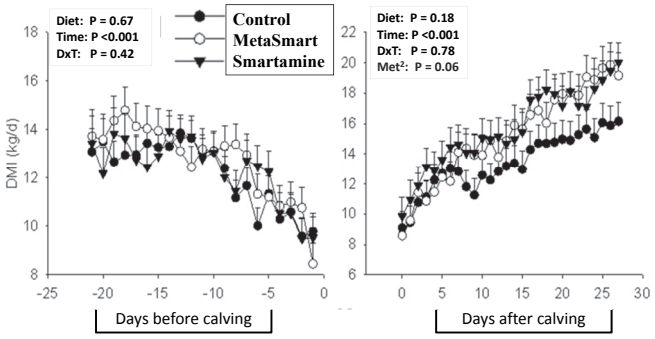
5 experiments that measured production responses to increasing Met, or Met + Lys in MP starting before calving

---

+ 2.30 kg/d milk
+ 0.09% units milk protein
+ 112 g/d milk protein
+ 0.10% units milk fat
+ g/d milk fat

Garthwaite et al. (1999)

DM intake pre- and postpartum

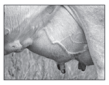


[1] Osorio et al. (2013)
[2] Contrast statement of CON versus MS + SM

A summary of some early Ajinomoto lactation cow experiments

Ave. milk response = 3.8 kg

Week of lactation	RPAA used	Conducted by	Milk, kg/d		
			Cont	Trt-1	Trt-2
0 - 8	**LM**	**Julien et al. (1999)**	**45.7**	**50.3**	
0 - 6	**LM**	**Robinson et al. (1996)**	**33.8**	**35.8**	
0 - 4	**LM**	**Sniffen et al. (1999)**	**43.4**	**47.9**	
0 - 6	**L, LM**	**Sniffen et al. (1999)**	**42.9**	**45.3**	**49.4**
0 - 6	**L**	**Nocek et al. (1999)**	**37.1**	**41.1**	
0 - 4	**LM**	**Chalupa et al. (1999)**	**32.6**	**35.5**	
0 - 10	**LM**	**Harrison et al. (1995)**	**34.7**	**38.1**	**39.0**



Better performance with Met supplementation during the postpartum period[1]

	Control	MetaSmart[2]	Smartamine M[2]
DMI, kg/d	13.3	15.2	15.6
Milk, kg/d	35.7[b]	38.1[ab]	40.0[a]
Milk protein, %	3.04[b]	3.26[a]	3.19[ab]
Milk fat, %	4.27	4.68	4.09
ECM, kg/d	41.0[b]	44.8[a]	45.0[a]

[1] Osorio et al. (2013)
[2] Fed in amounts to achieve a predicted Lys/Met ratio in MP of 2.80/1

Selected blood metabolites, liver composition and whole-blood leukocyte phagocytosis[1]

	Diet			*P* -value
	CON	MS	SM	Met[2]
NEFA, mEq/L	0.432	0.494	0.420	0.43
BHBA, nmol/L	0.687	0.697	0.645	0.82
TAG, mg/dL	301	327	300	0.66
VLDL, ug/uL	0.43	0.45	0.47	0.65
Liver, % wet wt				
Total lipid	10.55	9.53	8.66	0.24
TAG	4.27	4.55	3.14	0.50
Phagocytosis[3], %	38.5	55.1	45.8	**0.07**

[1] Osorio et al. (2014a)
[2] Contrast statement of CON versus MS + SM
[3] Percent of immune cells able to engulf pathogens

Selected blood metabolites, liver composition and whole-blood leukocyte phagocytosis[1]

	Diet			*P* -value
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NEFA, mEq/L	0.432	0.494	0.420	0.43
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TAG, mg/dL	301	327	300	0.66
VLDL, ug/uL	0.43	0.45	0.47	0.65
Liver, % wet wt				
Total lipid	10.55	9.53	8.66	0.24
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Phagocytosis[3], %	38.5	55.1	45.8	**0.07**

[1] Osorio et al. (2014a)
[2] Contrast statement of CON versus MS + SM
[3] Percent of immune cells able to engulf pathogens

Summary and Conclusions

Supplementation with MetaSmart or Smartamine M, when Lys was adequate:

- Increased milk production and milk protein content
- Increased post-calving DM intake
- Reduced liver lipid accumulation
- Increased blood phagocytosis (leukocyte-killing capacity)
- Resulted in a tendency for lower incidence of ketosis
- Increased biomarkers reflective of improved liver function
- Decreased biomarkers of inflammation
- Increased biomarkers reflective of reduced oxidative stress

Author conclusions: The beneficial effect of feeding MS or SM on improved milk production was due, at least in part, to increased voluntary DMI, better immuno-metabolic status, and perhaps by optimizing the use of body lipid reserves

Osorio et al. (2013, 2014ab)

Biomarkers of liver function, inflammation and oxidative stress[1]

	Diet			*P* -value	
	CON	MS	SM	Diet	Met[2]
Liver function					
Carnitine, nmol/g tissue	37.5	98.2	66.0	0.01	**<0.01**
Albumin, g/L	35.1	36.1	35.7	0.28	0.15
Inflammation					
Ceruloplasmin, umol/L	3.02	2.68	2.71	0.03	**0.009**
Serum amyloid A, ug/mL	61.0	40.7	43.5	0.17	0.06
Oxidative stress					
ORAC, mol/L	11.9	12.9	12.4	0.05	**0.04**
Glutathione, mM	1.27	1.55	1.73	0.15	0.07

[1] Osorio et al. (2014a)
[2] Contrast statement of CON versus MS + SM

Study 2
(Zhou et al., 2016a,b,c)

	(-21 to calving)	Fresh (0-30)
Corn silage	36.4	33.4
Alfalfa silage	8.3	5.1
Alfalfa hay	4.3	3.0
Wheat straw	15.6	3.0
Cottonseed	-	3.6
Wet brewers grains	4.3	9.1
Ground shelled corn	12.9	23.9
Soy hulls	4.3	4.2
SBM, 48% CP	**2.6**	**2.4**
Expeller SBM	**2.6**	**6.0**
SoyChlor	**3.9**	**-**
ProvAAIAdvantage (Blood based)	**0.9**	**1.5**
Urea	**0.3**	**0.2**

Transcriptome profiling of genes associated with Met and glutathione metabolism as well as components of the inflammation, oxidative stress, growth hormone/insulin-like growth factor-1 axis[1]

Conclusions:

- ✓ Supplementation with Smartamine M or MetaSmart to cows during the peripartal period can affect hepatic expression of Met, glutathione metabolism, inflammation, oxidative stress, and DNA methylation-related genes
- ✓ Production of glutathione could be increased by Met supplementation
- ✓ Sustained supply of Met within the liver could increase synthesis of antioxidants (e.g., glutathione and taurine) and also alter tissue-wide DNA methylation
- ✓ As such, inflammation, oxidative stress and genome-wide transcription of genes could be altered

[1] Osorio et al. (2014b)

RP-Met and RP-Choline during the transition period

	Close-up				Fresh			
	CON	MET	CHO	MIX	CON	MET	CHO	MIX
Lys, %MP	6.63	6.60	6.65	6.62	6.21	6.20	6.22	6.20
Met, %MP	1.87	**2.39**	1.87	**2.35**	1.81	**2.18**	1.81	**2.18**
MP-Lys, g	81	81	80	80	143	142	142	142
MP-Met, g	23	**29**	23	**28**	42	**50**	41	**50**
Lys/Met	3.52/1	**2.79/1**	3.48/1	**2.86/1**	3.40/1	**2.86/1**	3.46/1	**2.84/1**

MET = 0.08% Smartamine M in diet DM to achieve 2.8% Lys/Met in MP
CHO = 60 g/d ReaShure

Zhou et al. (2016c)

Frequency of occurrence of health problems of cows completing the experiment

	Control	Methionine	Choline	Methionine plus choline
Cows	20	21	20	20
Ketosis[1]	**8**	**3**	**5**	**4**
Displaced abomasum	2	0	4	1
Retained placenta[2]	3	2	5	1
Endometritis	0	0	0	1
Mastitis	0	1	0	0

8/20 **4/20**

[1] Defined as cows have moderate (~40 mg/dL) or large ketone concentration (< 80 mg/dL) in urine, as detected using a reagent strip and treated by veterinarians with oral propylene glycol or intravenous dextrose

[2] Defined as fetal membranes retained >24 h

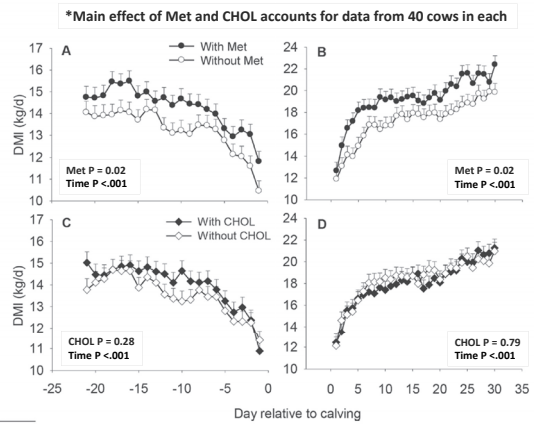
Zhou et al. (2016c)

Effects of RPM and choline on milk production and composition

	Treatment group					
Item	**No RPM**	**RPM**	***P***	**No RPC**	**RPC**	***P***
Milk, kg/d	**40.0**[b]	**44.0**[a]	**0.03**	42.5	41.5	0.56
ECM, kg/d	**40.7**[b]	**44.7**[a]	**<0.01**	43.1	42.3	0.57
Protein, %	**3.13**[b]	**3.32**[a]	**<0.01**	3.19	3.26	0.32
Protein, kg/d	**1.24**[b]	**1.43**[a]	**<0.01**	1.35	1.33	0.70
Fat, %	3.75	3.74	0.92	3.74	3.77	0.84
Fat, kg/d	**1.43**[b]	**1.58**[a]	**0.02**	1.52	1.50	0.76
MUN, mg/dL	12.87	12.89	0.96	12.68	13.08	0.29

Zhou et al. (2016c)

Greater DMI prepartum and postpartum with Met

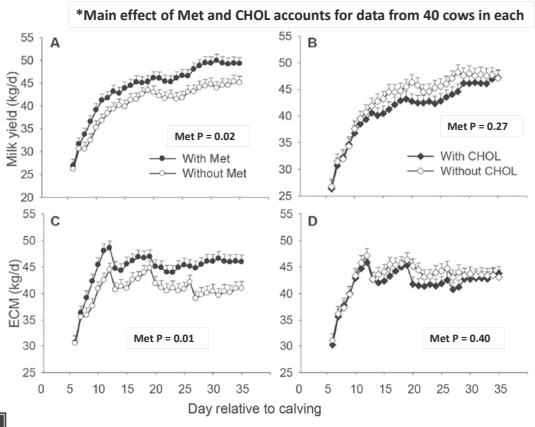


Effects of RPM and choline on pre- and post-partum blood and liver biomarkers

	Treatment group					
Blood	**No RPM**	**RPM**	***P***	**No RPC**	**RPC**	***P***
Glucose, mmol/L	3.78	3.80	0.68	**3.71**	**3.87**	**0.02**
NEFA, mmol/L	0.61	0.62	0.87	0.64	0.60	0.53
BHBA, mmol/L	0.88	0.92	0.66	0.97	0.83	0.12
Insulin	0.53	0.57	0.53	**0.47**	**0.63**	**0.01**
Glucose:insulin	10.65	9.52	0.29	11.14	9.03	**0.05**
NEFA:insulin	1.70	1.78	0.79	**1.88**	**1.60**	0.39
Liver TAG, % of wet tissue	2.91	2.81	0.83	2.75	2.97	0.62

Zhou et al. (2016c)

Greater milk yield and ECM with Met



Effects of RPM and choline on liver function biomarkers[1]

	Treatment group					
Parameter	**No RPM**	**RPM**	***P***	**No RPC**	**RPC**	***P***
Bilirubin, umol/L	4.38	4.22	0.82	4.44	4.16	0.69
AST, U/L	100.11	100.91	0.84	99.23	101.79	0.53
Cholesterol, mmol/L	3.31	3.62	0.11	3.41	3.53	0.55
GGT, U/L	21.58	24.03	0.79	23.41	25.20	0.38
Paraoxinase[2], U/ml	84.54	93.09	0.07	88.45	89.18	0.87

[1] Met x choline interaction was not significant for any of the parameters
[2] Synthesized in the liver, released into blood stream where it is associated with HDL and prevents it from oxidative damage. Cows with high concentrations (92 vs. 54 U/ml produced more milk (Bionaz et al., 2007)

Zhou et al. (2016a)

Effects of RPM and choline on inflammation and acute-phase proteins (APP)[1]

	Treatment group					
Parameter	**No RPM**	**RPM**	***P***	**No RPC**	**RPC**	***P***
Albumin, G/L	**35.53**	**36.55**	**0.04**	36.21	35.83	0.50
Ceruloplasmin, umol/L	2.84	2.73	0.45	2.74	2.82	0.56
Haptogloblin, g/L	0.47	0.35	0.08	0.41	0.41	0.94
IL-1*B*, pg/mL	7.12	4.98	0.14	6.25	5.86	0.79
IL-6, pg/mL	**835**	**1,086**	**0.03**	964	958	0.96

[1] Met x choline interaction was not significant for any of the parameters

Zhou et al. (2016a)

Effects of RPM and choline on blood neutrophil and monocyte phagocytosis and oxidative burst

	Treatment group					
Parameter	**No RPM**	**RPM**	***P***	**No RPC**	**RPC**	***P***
Monocyte phagocytosis	43.03	45.28	0.28	42.62	45.69	0.15
Neutrophil phagocytosis	**54.69**	**61.05**	**0.01**	57.60	58.14	0.81
Monocyte oxidative burst[1]	21.35	23.99	0.15	21.96	23.38	0.43
Neutrophil oxidative burst	**49.28**	**57.27**	**0.03**	52.20	54.34	0.54

[1] Values for control, RPM, RPC and RPM+RPC were 17.72^{b}, 26.19^{a}, 24.99^{a}, 21.78^{ab} (P<0.05)

Zhou et al. (2016a)

Effects of RPM and choline on biomarkers of oxidative stress[1]

	Treatment group					
Parameter	**No RPM**	**RPM**	***P***	**No RPC**	**RPC**	***P***
ROM[2], mg of H_2O_2 /100 mL	13.71	13.42	0.60	13.46	13.66	0.72
FRAP[3], umol/L	135.84	135.54	0.95	135.02	136.36	0.80
Total glutathione, umol/g of protein	**23.32**	**62.83**	**0.01**	46.55	39.61	0.64
Reduced glutathione, umol/g of protein	**22.81**	**62.10**	**0.01**	45.87	39.04	0.65

[1] Met x choline interaction was not significant for any of the parameters
[2] ROM (reactive oxygen metabolite)
[3] FRAP (ferric-reducing ability of plasma)

Zhou et al. (2016a)

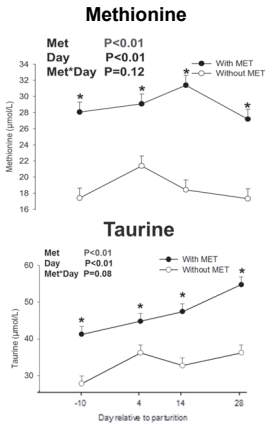
Effects of RPM and choline on choline metabolism[1]

	Treatment group					
Parameter	**No RPM**	**RPM**	***P***	**No RPC**	**RPC**	***P***
Choline metabolism						
Plasma choline, mg/dL	38.04	37.38	0.69	37.25	38.16	0.58
Plasma PC[2], mg/mL	111.56	115.96	0.41	112.79	114.73	0.72
Milk choline, mg/L	26.78	27.13	0.88	27.43	26.47	0.68

[1] Met x choline interaction was not significant for any of the parameters
[2] PC (phosphatidylcholine)

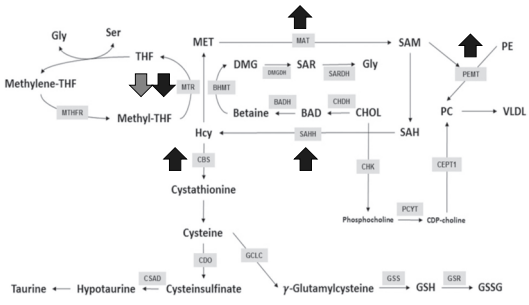
Zhou et al. (2016a)

Plasma methionine and taurine concentrations during the transition period



Zhou et al. (2016b)

Effect of RPM and RPC on transcription of key enzymes



Zhou et al. (2016b)

Effect of improved methionine nutrition on reproduction

Effect of dietary methionine supplementation in early lactation cows

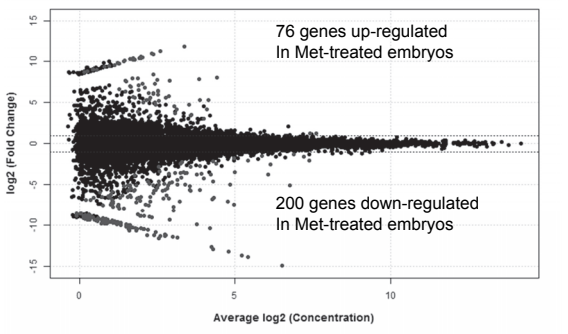
1. Embryo quality
2. Gene expression

- 72 Holstein cows
- Dry period - housed in a single pen and fed same basal diet
- Calving to 70 days in milk - housed in tie stalls and milked twice daily
- At calving, blocked by parity and calving date and randomly assigned to 2 dietary treatments differing in Met adequacy

6.8% Lys and 1.9% Met in MP
6.8% Lys and 2.4% Met in MP

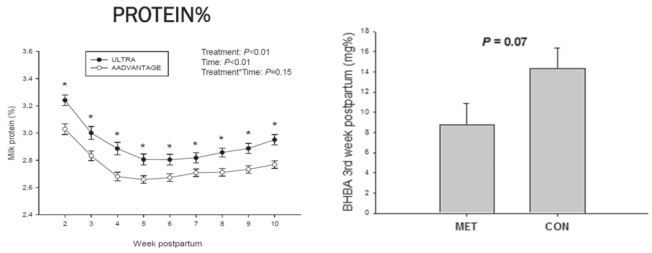
Souza, Carvalho, Dresch, Vierira, Hackbart, Luchini, Bertics, Betzol, Wiltbank, Shaver

Effect of improved methionine nutrition on reproduction



Souza, Carvalho, Dresch, Vierira, Hackbart, Luchini, Bertics, Betzol, Wiltbank, Shaver

Effect of improved methionine nutrition on reproduction



Souza, Carvalho, Dresch, Vierira, Hackbart, Luchini, Bertics, Betzol, Wiltbank, Shaver

Effect of improved methionine nutrition on reproduction

Evaluate effect of top-dressing Smartamine M (21 g/d) on embryo development

- 309 Holstein cows in free stall barn (138 primiparous, 171 multiparous)
- Dry period - fed same basal diet
- At calving, blocked by parity and randomly assigned to 2 dietary treatments differing in Met adequacy

6.9% Lys and 1.9% Met in MP **(50 g DDGS)**
6.9% Lys and 2.3% Met in MP **(29 g DDGS + 21 g Smartamine M)**

Toledo et al. *unpublished*

Effect of improved methionine nutrition on reproduction

	Smartamine M	Control	
Number of super-ovulated cows	**35**	**37**	***P*-value**
CL number	17.0 ± 1.3	17.7 ± 1.5	0.90
Total ova/embryos recovered	9.1 ± 1.4	6.8 ± 1.0	0.18
Number of fertilized ova	6.5± 1.1	5.5 ± 0.9	0.56
% Fertilized ova	74.7 ± 5.6	82.2 ± 3.8	0.27
Number of transferable embryos	5.0 ± 0.9	4.3 ± 0.1	0.57
% Transferable embryos	56.3 ± 6.5	62.5 ± 6.0	0.49
Number of degenerate embryos	1.5 ± 0.4	1.3 ± 0.4	0.75
% Degenerate embryos	18.5 ± 4.6	19.7 ± 4.7	0.83

Souza, Carvalho, Dresch, Vierira, Hackbart, Luchini, Bertics, Betzol, Wiltbank, Shaver

Effect of improved methionine nutrition on reproduction

Table 1. Amniotic vesicle size

	n	Volume (mm^3)
Primiparous		
Control	31	610.6
RPM	36	596.0
P value		0.71
Multiparous		
Control	34	472.3
RPM	45	592.1
P value		**0.05**

Toledo et al. *unpublished*

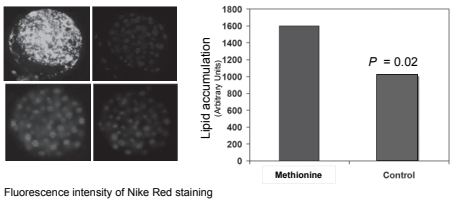
Effect of improved methionine nutrition on reproduction

Table 2. Embryo size				
	n	Crown-rump length (mm)	Abdominal diameter (mm)	Volume (mm^3)
Primiparous				
Control	35	10.4	5.6	169.6
RPM	38	10.9	5.7	191.9
P value		0.10	0.54	0.21
Multiparous				
Control	36	10.5	5.3	160.5
RPM	44	11.0	5.9	209.3
P value		0.27	**0.03**	**0.01**

Toledo et al. ***unpublished***

Effect of Methionine Supplementation from -21 DIM to 72 DIM on Lipid Accumulation of Preimplantation Embryos

Embryos (n= 37) harvested 7 d after timed AI at 63 DIM from cows fed a control diet or the control diet enriched with rumen-protected methionine.



Fluorescence intensity of Nike Red staining

Acosta et al. (2016)

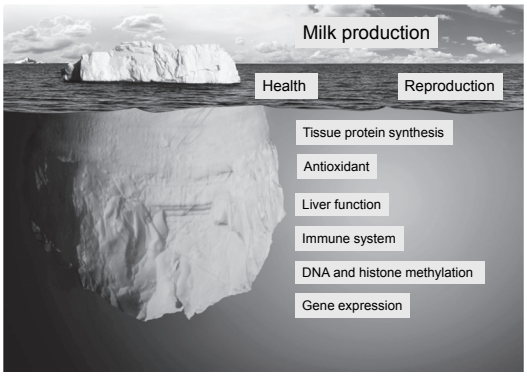
University of Illinois at Urbana-Champaign

Effect of improved methionine nutrition on reproduction

Table 3. Pregnancy loss						
	Primiparous			Multiparous		
Interval	Control	RPM	*P*	Control	RPM	*P*
28-61 d	12.8% (5/39)	14.6% (6/41)	0.37	**19.6%** (10/51)	**6.1%** (3/49)	**0.03**

Toledo et al. ***unpublished***

Methionine has functions beyond being a building block for protein synthesis



Summary and conclusions regarding improved Met nutrition on reproduction

- Methionine supplementation of the dam did not alter fertilization or embryo quality as determined by gross morphology, or early embryonic development
- Methionine supplementation of the dam during early embryo development changed gene expression in the embryo…most genes were down-regulated
- Still unknown how these changes in gene expression caused by supplemental Met will affect later pregnancy and calf physiology
- **For multiparous cows, Met supplementation:**
  - ✓ **Increased amniotic vesicle volume and embryo size**
  - ✓ **Reduced pregnancy loss**

Summary and Conclusions

1. There is no doubt that supplemental Met, when a limiting AA, has profound effects on the production and nutritional health of transition cows, as well as subsequent reproduction
2. The benefits of AA balancing for transition cows are far-reaching and appear to extend beyond the benefits realized by cows during the current lactation
3. A further understanding of the benefits of AA balancing for transition cows await:
   - ✓ Guidance from further advancements in nutritional modeling and determination of the "ideal balance" of absorbed AA for transition cows
   - ✓ Availability of a greater assortment of RPAA supplements with established bioavailability values for research and field evaluation work
   - ✓ Continued research on effects of improved AA nutrition on intermediary metabolism

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Feeding and Managing a Herd for 100 Pounds of Milk/Day - Thinking Outside the Normal Paradigm

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If you want your dairy herd to produce 100 pounds of milk per day, then you must consider the following principles.

1. Each additional pound of peak milk will yield 239 pounds of milk over a 305-day lactation.
2. First-lactation animals need to produce 75% of the expected mature cow production.
3. Second lactation animals need to produce 90% of the expected mature cow production.
4. First-lactation animals should make up only 35% of the milking herd.
5. Third and greater lactation cows need to average 115 pounds of milk.
6. Second lactation cows need to average 103 pounds of milk.
7. First lactation cows need to average 80 pounds of milk.
8. Dry matter (DM) intake of the close- up will impact DM intake after calving. Maximize intake prior to calving, so that DM intake is maximized in the first 28 days in milk.

The goals that are set for your herd will depend on the demographics of the herd. What percentage of the herd are mature cows is an important factor. The mature cows are the engine of the herd, pulling the rest of the herd with them. The mature cows are able to produce more than 100 pounds per day and they will make up for the lower production of the first lactation animals. Consider the following group of cows. To make the math easy to follow assume that we have 100 milking cows and 35% of them are 1st lactation cows. Mature cows make up 40% of the group. Second lactation cows make up 25% of the group. Given these demographics, you can calculate production goals to reach 100 pounds of milk. Milk production of the mature cows needs to be 115 pounds of milk. Their contribution to the daily milk production is (115 x 0.40) = 46 pounds or percent. Milk production of the second lactation cows needs to be 103 pounds. Their contribution to daily milk production would be (103 x 0.25) = 26 pounds or percent. The first lactation cows need to contribute 28 pounds or 28% to daily milk production. Since first lactation cows make up 35% of the group, they need to produce (28/0.35) = 80 pounds of milk. If you add 46 + 26 + 28 = 100 pounds of milk. The objective of this exercise was to illustrate that each herd has their own unique demographics. If your herd contains 40% first-lactation cows, then the older cows in the herd are going to have to give more milk then in our example, if you want to reach 100 pounds.

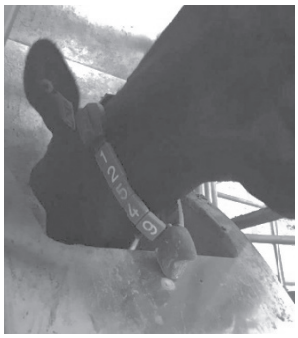
To set production goals for your herd you need to repeat this exercise. You should start with a realistic projection of first-lactation cow milk production. Since you already know what percentage of your herd is first-lactation cows, you can estimate their contribution to daily milk production. When you know the contribution of the first lactation animals, then you can set goals for the second-lactation and older cows. Since first-lactation cows will be allocating approximately 20% of their nutrient intake toward growth, they will only produce about 75% of mature cow milk production. If you want to average 100 pounds of milk per cow, you need to focus on getting high milk production from your older cows. To optimize milk production of the older cows, you need to start with their dry cow program. Getting mature cows to consume more than 30 pounds of dry matter during the close-up period will help them eat more after calving. This will reduce body condition loss during the first 30 days of lactation. Excessive loss of body condition during the first two weeks of lactation can lead to fat accumulation in the liver. This accumulation of fat in the liver will reduce glucose production by the liver. What is observed is sluggish appetite and poor start-up milk in these cows. If a herd is to maintain milk production of 100 pounds per day, you cannot have poor start-up milk and sluggish appetite in fresh cows.

Optimizing Dry Matter Intake of Transition Cows

Field trials on commercial dairies has shown that feeding a low starch, high sugar and soluble fiber diet

to close-up cows has increased dry matter intake. In a mixed pen of first-lactation and mature cows, dry matter intake was increased 1.7 pounds per day when cows received a low starch, high sugar and soluble fiber pre-fresh diet (Dort College Trial). After calving, cows were split by parity into two groups, first-lactation cows and mature cows. Both groups received a high sugar and soluble fiber diet through 30 DIM. Dry matter intake during the first 30 days in milk was increased 2.6 pounds in the mature cows and 4.5 pounds in the first-lactation cows compared to the pre-treatment period. Paramount dairy in Michigan was already getting good dry matter intake in their pre-fresh cows. During the pre-treatment period, pre-fresh cows consumed 32 pounds of dry matter. The pre-fresh diet during this period contained 9 pounds of chopped wheat straw. When pre-fresh cows were put on a low starch, high sugar and soluble fiber diet during the treatment period, dry matter intake was increased to 35 pounds with 11 pounds of chopped straw in the diet. These two fields trials demonstrate that feeding QLF liquid supplement during the close-up period at four to five pounds as fed (2.5 – 3.0 lbs. DM) stimulates dry matter intake in close-up cows. These trials did not have a control group but there was a control group in the trial at Swisslane dairy. At Swisslane dairy, after calving there were three treatments, control (no liquid supplement), high sugar and soluble fiber (QLF) and high sugar and soluble fiber plus NutriTek (QLFNT). Both QLF molasses-based liquid supplements and Diamond V yeast-based product NutriTek have been shown to boost dry matter intake and milk yield in transition cows. In addition, NutriTek contains bioactive fermentation compounds, including antioxidants and polyphenols, which may enhance the immunity of transition cows and help them better cope with metabolic stress and inflammation. Anytime stress can be reduced on cows in the transition period, it is a good thing.

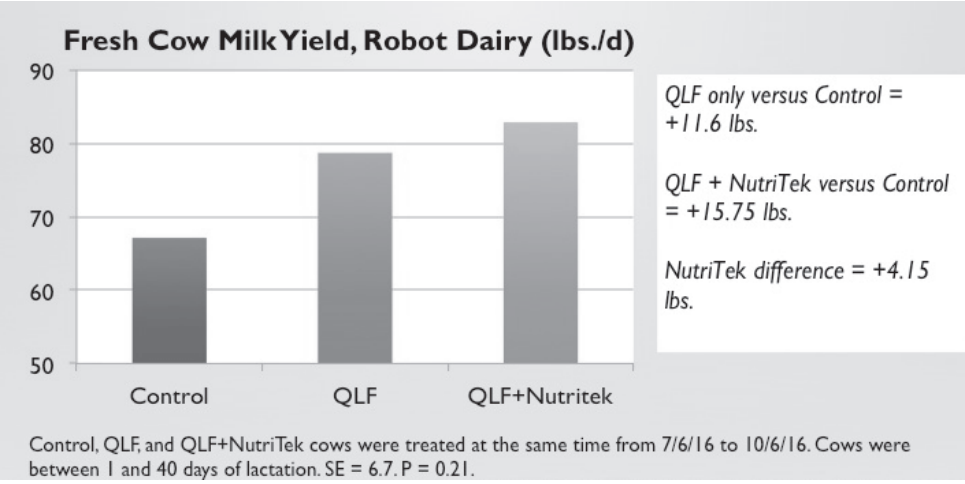
Experimental Protocols at Swisslane Dairy:
Swisslane Dairy located at Alto, MI with a herd size of 2450 cows, has both conventional and robot operations. The robot operation milks 480 cows with eight Lely robot stations. The trial was conducted from July 6, 2016 through Jan. 31, 2017. A QLF liquid supplement was formulated to supply 19 g of Diamond V NutriTek when fed at 4 pounds as fed. This liquid supplement contained 6% crude protein and 27% total sugar on an as-fed basis. All close- up mature cows received on a dry matter basis, a low starch (16%), high sugar (8.6%) and high soluble fiber (6.5%) diet. This diet was fed for 21 days pre-calving. After calving, early lactation mature cows in the robot herd were randomly assigned to either the control, QLF or QLFNT treatments. The treatments were delivered into the feeding station on the Lely robotic milking pod. Treatments, 4 pounds of liquid supplement as fed were dropped on top of the pellets being fed to the cows. Individual cows were milked by robots about 3 times per day, so QLF and QLF with NutriTek was targeted to be delivered by the pumps to feeding pans at 1.33 lbs. as-fed per milking visit (see images below). Treatments were fed through 100 DIM.

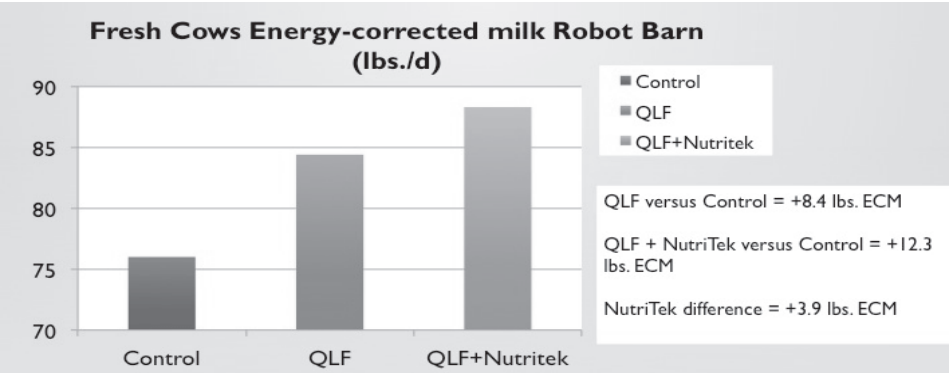

Results: Swisslane Dairy Robot Herd:
Compared with Control, feeding QLF during early lactation increased milk yield by 11.6 lbs., and QLF+NutriTek increased milk yield by 15.75 lbs. Energy-corrected milk was increased by 8.4 lbs. with QLF, and 12.3 lbs. by QLF+Nutritek. Rumination time, an indicator of rumen and cow health, increased 25 min per day with QLF and 33 min per day with QLF+NutriTek. What may have contributed to the increase in milk was the increase in dry matter in the close-up cows. When close-up cows received a low starch, low sugar diet, they consumed 29 pounds of dry matter intake. When close-up cows received a low starch, high sugar, high soluble fiber diet, the cows consumed 35.9 pounds of dry matter. Total cases of fresh cow diseases were similar among the 3 treatments.

Economic Analysis:
Does it pay to feed QLF or QLF+NutriTek during the transition period? Using the observed milk response from the well-controlled study at Swisslane Dairy Robot Herd, feeding QLF+NutriTek generated a net return of $0.88 cow/day at $16 hundredweight milk price. In the Dordt College field trial, the net return even after accounting for the additional dry matter

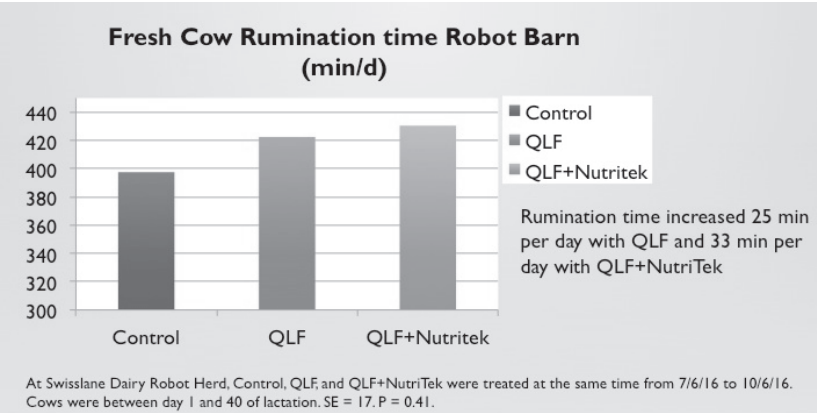
intake pre-fresh and post-fresh was $1.27 per cow/day at $16 hundredweight milk price. From the perspective of improved fresh cow health, data from Paramount Dairy showed that QLF + NutriTek generated a 3.85 return on investment, which was a saving of $25,978 for every 1000 cows. Feeding a low starch, high sugar and soluble fiber diet pre-fresh, which was followed by a moderate starch (24 – 26%, high sugar (7 – 8%), high soluble fiber (6 -8%) using QLF and QLF +NutriTek generated highly positive return on investment for the dairy producers. More importantly, these positive impacts on start-up lactation should continue to carry over throughout the entire lactation and provide long-term positive returns.



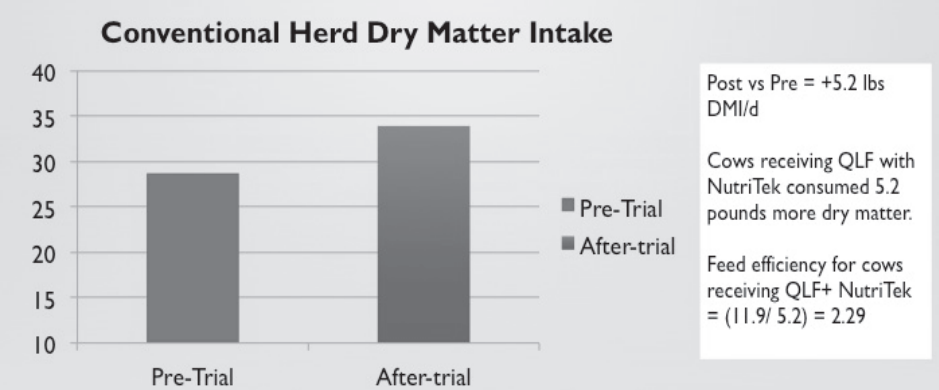
Control, QLF, and QLF+NutriTek cows were treated at the same time from 7/6/16 to 10/6/16. Cows were between 1 and 40 days of lactation. SE = 6.7. P = 0.21.



Control, QLF, and QLF+NutriTek cows were treated at the same time from 7/6/16 to 10/6/16. Cows were between day 1 and 40 of lactation.



At Swisslane Dairy Robot Herd, Control, QLF, and QLF+NutriTek were treated at the same time from 7/6/16 to 10/6/16. Cows were between day 1 and 40 of lactation. SE = 17. P = 0.41.



QLF+NutriTek was fed from 625/16 to 10/6/16. The milk yields from 3/1/16 to 7/6/16 (without QLF+NutriTek) were used as comparisons. Cows were between day 1 and 40 of lactation. SE =0.39 P < 0.0001

Does it pay to feed QLF + NutriTek during the Transition Period Based on Reduction in Death Loss, Metritis and DA?
Close- up period = 21 days, Post- fresh period = 30 days
Cost of NutriTek = $0.15/cow/day

	No QLF+ NutriTek	QLF+ NutriTek	Cost/Cow, $
Pre-Fresh Diet, $/Day	4.04	4.26	(0.22 X 21) = $4.62
Lactating Cow Diet, $/Day	6.69	6.84	(0.15 X 30/) = $4.50
Total Cost, $/Cow			$9.12
Cost to Feed 1,000 Transition Cows, $			$9,120
Reduction in Metritis per 1,000 Cows		74 less	(74X$304) = $22,498
Reduction in DA per 1,000 Cows		15 less	(15X$340) = $5,100
Reduction in Death Loss /1,000 Cows		3 less	(3X$2500) = $7,500
Return on Investment			($35,098/$9120) = 3.85:1

Conclusions

If you want to attain 100 pounds per day in your dairy herd, it is all about maximizing dry matter intake beginning with the pre-fresh period through the fresh cow period and continuing through 150 DIM. The feeding strategy presented in this paper increased dry matter intake in pre-fresh cows and early lactation cows. This was responsible for these cows reaching higher peak milk, having fewer fresh cow issues and having stronger start-up milk. What makes this strategy work is feeding less starch and more sugar and soluble fiber. This creates a healthy environment in the rumen to enhance fiber digestion. By enhancing fiber digestion, rumen-fill is reduced and cows are able to consume more dry matter intake. This program begins with high quality forage and the dairy should use technology that improves the quality and fermentation of silage and use technology that increases dry matter intake and glucose supply in high producing cows. It is also necessary to address ration sorting on the farm. By eliminating ration sorting by using a molasses-based liquid supplement, the cow will consume more rumen effective fiber. This will result in a healthier rumen environment with less risk of SARA and better digestion of fiber. The impact of better fiber digestion will be higher milk components. The goal is to ship a minimum of 6.5 pounds of components. This will require a 3.6% fat and a 3.0% protein at 100 pounds of milk. To achieve this goal requires removing the bottlenecks on the dairy to high dry matter intake and putting up high quality digestible forages.

Feeding and Managing for 35,000 Pounds of Production: Diet Sorting, Dry Cow Strategies and Fiber Digestion

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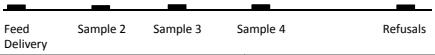
Sorting Behavior of Dairy Cows: Commercial TMR Survey

- 50 Freestall Dairies - Minnesota
- Univ. of MN Study
- Sorting Measured in High Production Group (117 ± 51 cows) TMR Sampled 5 times during feeding period



# Cows	>150
Feeding Frequency	70% feed 1x/ daily
Frequency of Feed Pushup	3 – 12 x daily
Linear Feed bunk space/ cow	18"
Daily Milk Yield/ cow	88 lbs.

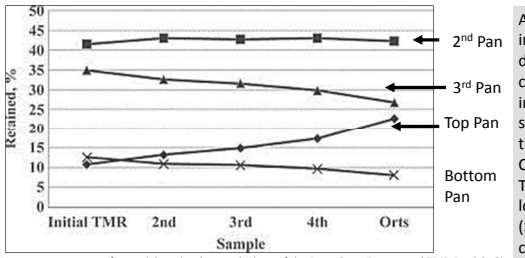
JDS 93:822-829

Goals for Getting to 100 Pounds of Milk

35% First lactation animals in herd
65% pregnant by 120 days in milk
Average 150 – 155 DIM
Peak Milk Mature Cows = 130 pounds
Peak Milk 2nd Lactation Cows = 117 pounds
Peak Milk 1st Lactation Cows = 98 pounds

32 -35 pounds DMI in pre-fresh cows
Eliminate sorting of the pre-fresh and lactating cow diets
Feed a low starch (12 – 14%), high sugar (7.5 – 8.5%), high soluble fiber (7 – 9%) pre-fresh diet.
Use technology that reduces fresh cow diseases.
Use technology that improves forage quality and increases feed intake.

Particle Distribution Change Over Time



Average percentage of material retained on each sieve of the Penn State Forage and TMR Particle Size Separator over time [top (♦;>19mm), second (■;>8mm), third (>1.18mm), and bottom (×;<1.18mm pans] for 50 freestall herds in Minnesota. Samples represent the initial TMR collected at feed delivery, the second, third, and fourth samples collected every 2 to 3h after feed delivery; and the orts.

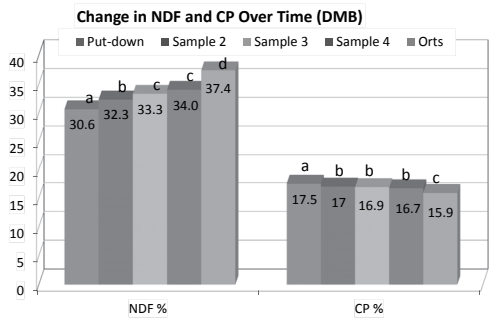
JDS 93:822-829

A straight line indicates that cows did not sort. A line curving up indicates that cows sorted against those particles. Cows sorted the TMR and left the long particles (>19mm) and consumed more of the short and fine particles (3rd pan and bottom pan).

Our Goal is to Ship 6.5 Pounds of Components per cow/day

- Example: 100 pounds of milk with a 3.6% fat test and a 3.0% protein test = 6.6 pounds of components per day/cow.
- Must drive dry matter intake in transition cows and high cows without depressing fiber digestibility.
- Think outside the normal paradigm.
- Traditional paradigm: Need to feed high starch diets to make milk and can't make milk on high forage diets.
- New paradigm: Feed a low to moderate starch diet with high sugar (7 – 8%) and high soluble fiber (6 -9%) and feed a minimum of 50% forage.
- This works because sucrose and glucose sugars increase fiber digestion compared to starch.

Change in NDF and CP Over Time (DMB)



JDS 93:822-829

NDF content of the TMR increased by 22% and CP decreased by 9% due to sorting.

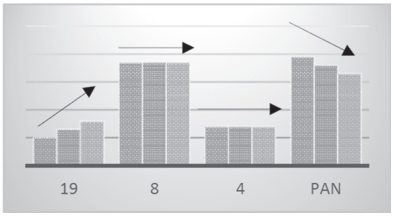
It is ration sorting that is causing lower than desired milk fat and milk protein.

Must Eliminate Sorting of the TMR

- All Cows Sort Their Ration

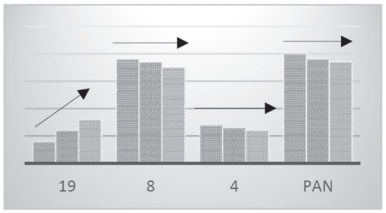
  - Cows sort against long particles in the diet (>19 mm).
  - Cows dig holes in the TMR to reach the short and fine particles.
  - A short or fine particle is anything smaller than 8 mm.
  - First Lactation Cows Sort More than Mature Cows.
  - Jersey cows are more effective sorters of the TMR than Holstein Cows.
  - Excessive sorting of the ration can increase the risk of SARA.
  - Sorting of the TMR reduces the intake of forage NDF.

Pattern 1	**Eat the goodies, leave the hay**
Description	This is the classical view of sorting. Cows are sorting against the long particles and sorting for the short particles.
To Watch	Particle length of top screen, Molasses liquid product like QLF, TMR DM%



Leads to SARA and lower milk component yields

Pattern 2	**Leave the hay, can't find the goodies**
Description	In this scenario the cows are sorting against the top screen but cannot sort for the pan. The bottom three layers disappear equally.
To Watch	Particle length of top screen

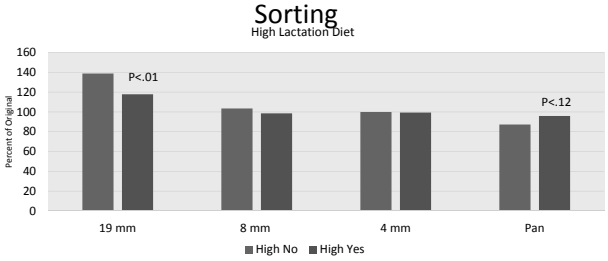


Leads to reduced milk fat and milk protein yields

Pre-fresh diet for swisslane dairy

Ingredient	DM, lbs.	As fed, lbs.	Nutrients	
Grass Hay	6.0	6.75	Crude Protein, %	13.0
Wheat Straw	2.2	2.70	Metabolizable Protein, grams	**1300**
Corn Silage	9.0	28.0	Starch, %	**16.1**
Wet Beet Pulp	5.0	19.2	Sugar, %	**8.65**
Pre-fresh grain	2.7	3.0	Soluble Fiber, %	**6.5**
Ground corn	1.1	1.3	Potassium, %	1.38
Wheat midds.	2.0	2.3	Sodium, %	0.14
Soybean meal	2.5	2.8	Chloride, %	1.33
QLF dairy transition 6	**2.4**	**4.0**	Sulfur, %	0.54
Total	32.9		NEL, mcal/lb.	0.66

Liquid Inclusion in the Diet: Effect on Sorting

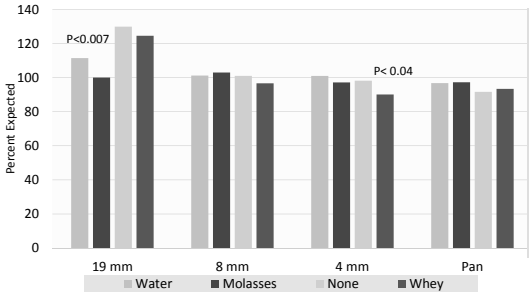


Value = 100 indicates no sorting. Values > 100 indicate sorting against those particles in the TMR. Inclusion of liquid in the TMR reduces the sorting of the top screen and cows consume more long particles. Adding liquid prevented sorting for fine particles.

High cow diet SwissLane dairy Robot Barn, No QLF Supplement.

Ingredient	DM, lbs.	As fed, lbs.	Nutrient	
Corn Silage	18.0	56.3	Crude Protein, %	16.30
Alfalfa silage	12.0	23.0	Starch + Sugar + Sol. Fiber, %	41.6
Dairy Hay	1.25	1.4	Starch, %	**27.84**
Whey Permeate	1.0	5.5	Soluble Fiber, %	**8.15**
Wet Beet Pulp	2.0	7.7	Sugar, %	**5.6**
Propel CHO	4.5	5.0	ME Milk, Pounds	109.0
Robot Pellet	12.6	14.5	Forage, %	46.9
Soy hulls/ Wheat Midds.	4.4	5.25	peNDF, %	16.75
Soybean meal	1.0	1.11	peNDF, lbs.	11.2
RUP Protein + Mineral	5.8	6.2	Methionine, grams	70
Bergafat T 300	0.32	0.33	Lysine, grams	226
Total	66.6		Fat, %	3.84

Effect of Type of Liquid on Sorting: Not All Liquids Eliminate Sorting of the TMR



Whey did not prevent the sorting of the long particles in the TMR. Whey encouraged the consumption of short particles at the expense of the long particles. Adding water was not as effective as adding molasses to reduce sorting

HIGH COW DIET SWISSLANE DAIRY ROBOT BARN, WITH QLF SUPPLEMENT

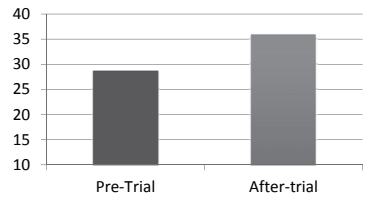
	DM, lbs.	As fed, lbs.	Nutrient	
Corn Silage	18.0	56.3	Crude Protein, %	16.27
Alfalfa silage	12.0	23.0	Starch + Sugar + Soluble Fiber, %	42.4
Dairy Hay	1.25	1.4	Starch, %	**26.0**
Whey Permeate	1.0	5.5	Soluble Fiber, %	**8.77**
Wet Beet Pulp	2.0	7.7	Sugar, %	**7.65**
Propel CHO	4.5	5.0	ME Milk, Pounds	109.0
Robot Pellet	12.0	13.8	Forage, %	46.1
Soy hulls/ Wheat Midds.	4.75	5.25	peNDF, %	16.7
Soybean meal	1.0	1.11	peNDF, lbs.	11.1
RUP Protein + Mineral	5.75	6.1	Methionine, grams	70
QLF dairy transition 6	**2.4**	**4.0**	Lysine, grams	226
Total	64.5		Fat, %	3.24

Optimizing Dry Matter Intake of Transition Cows: Feeding to Enhance Fiber Digestion and Reduce Diet Sorting

- Case Studies:

1. Swisslane Dairy
2. Dort College Trial
3. Paramount Dairy

Pre-fresh mature cow dry matter intake (lb./d)



Pre-Trial DMI = 28.7 lbs.
Post-Trial DMI = 35.9 lbs.
Post vs Pre = +7.2 lbs.

QLF+NutriTek was fed from 7/6/16 to 1/31/17. The dry matter intake from 3/1/16 to 7/6/16 (without QLF+NutriTek) was used as comparison. SE = 5.2. ***P < 0.001.***

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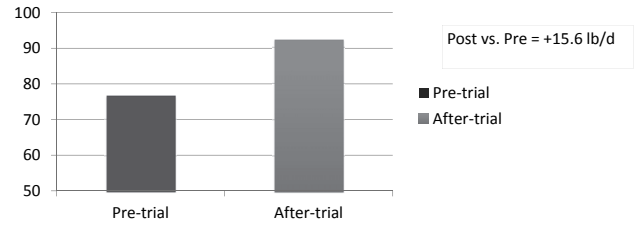
Conventional barn fresh cow milk yield (lb/d)



QLF+NutriTek was fed from 7/6/16 to 1/31/17. The milk yields from 3/1/16 to 7/6/16 (without QLF+NutriTek) were used as comparisons. Cows were between day 1 and 200 of lactation. SE = 8.0. ***P < 0.001.***

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Introduction

- Farm: Commercial Dairy, 400 cow Holstein Herd
- Issues dairy producer wanted to have fixed.
  1. Sorting of pre-fresh and post-fresh diet.
  2. Low Milk Fat Test in early lactation.
  3. Desire higher peak milk.
- QLF Dairy Transition 6 was fed at 4 pounds/cow pre-fresh and post-fresh (first 30 DIM).
- NutriTek from Diamond V was in the dry mineral.
- Diet adjusted to be iso-caloric and iso-nitrogenous to diet prior to QLF addition
- QLF liquid supplement replaced some corn and fat in the diet.

Average production data for all lactations based on entire RAW DATA (DIM 1 – 200) from Robot Barn

	Control	QLF	Difference
Milk yield, lbs./d	90.3	98.6	+8.3 lbs.
Milk Fat, %	3.57	3.64	
Milk Protein, %	3.13	3.12	
Milk Fat Yield, lbs./d	3.22	3.59	**+0.37**
Milk Protein Yield, lbs./d	2.83	3.08	**+0.25**
Energy-corrected milk, lbs./d	90.7	99.8	+9.1

Pounds of components shipped per cow = (3.59 + 3.08) = 6.67 when QLF supplement was fed. Pounds of components shipped for control cows = (3.22 + 2.83) = 6.05

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Dry Matter Intake, (lbs./day) Pre-QLF and during the QLF feeding period

	Pre-QLF Feeding	QLF Feeding Period	Difference
Pre-Fresh Mature Cows and 1st Lactation Cows	24.88	26.56	+ 1.7 lbs.
Fresh 1st Lactation Cows, DIM 1-30	34.06	38.58	+ 4.5 lbs.
Fresh Mature Cows, DIM 1-30	43.43	46.01	+ 2.6 lbs.

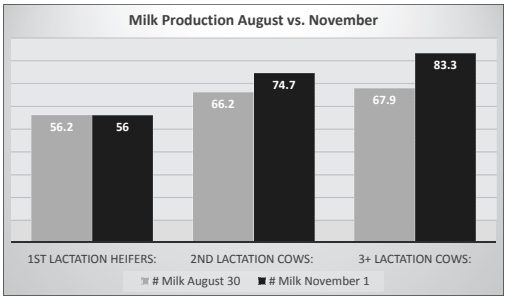
Pre-QLF period from Jan 1, 2016 through Sept. 15, 2016.
QLF feeding period from Sept. 16, 2016 through Nov. 18, 2016, 62 days.

Does it pay to feed QLF during the Transition Period and Lactation?

Close- up period = 21 days, Lactation Period: 1 - 200 DIM

	No QLF	QLF Dairy Transition 6	Difference, Cost/Cow, $
Pre-Fresh Diet, $/Pound of DM.	0.139	0.147	0.008
Pre-fresh DMI, Lb./d	29	35.0	($0.88 X 21) = $18.48
Lactating Diet, $/Day per lb. DM	0.1186	0.12	
Estimated DMI/d.	56.4	59.8	
Cost/Cow/day, $	6.69	7.18	(0.49 X 200 DIM) = $98
Breakeven Milk Response @ $16/cwt.			3.6 lb./day
Observed Milk Response All Lactations, 1- 200 DIM			+ 9.1 lbs. ECM milk/day
Net Return, (9.1 – 3.6) = (5.5 X 0.16)			**+ $0.88 per cow/day**

Results: Milk Production, lbs./cow



2nd lactation cows, + 8.5 pounds of milk.

3+ lactation cows, + 15.4 pounds of milk

Conventional Dairy Herd Trial Conducted by Students at Dordt College in Iowa.

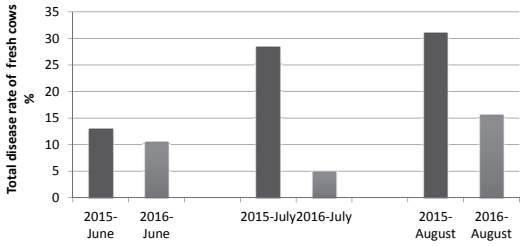
Nicholas Leyendekker, Imanuel Feodor, Ross Schreur
Senior Students, Dordt College

Economic Analysis of Dordt College Trial Accounting for Increased DMI when QLF and NutriTek were Fed

	Pre-QLF	QLF Feeding Period	Diff.
Pre-Fresh Diet, $/cow/day	3.00	3.45	
Cost for 21 days Pre-Fresh, $	63.00	72.45	+9.45
Fresh Cow Diet, $/cow/day	5.21	5.69	
Cost for 60 days of lactation, $	312.60	341.40	+28.80
Difference in cost for 81 days, $/cow			+38.25
Breakeven milk needed at $16/CWT			4.0
Actual Milk response, lbs.		(8.5 + 15.4)/2	11.95 lbs.
ROI at $18/CWT Milk Price		(11.95 – 4.0) X 0.16	$1.27/cow/d

PARAMOUNT DAIRY, CARO, MI

Total rates of Paramount Dairy fresh cow diseases



Average fresh cow disease rate for summer 2016 was 10.3%

Average fresh cow disease rate for summer 2015 was 24.1%

QLF+NutriTek was fed during June to August 2016. The disease rates in June to August 2015 (without QLF+NutriTek) were used as comparisons. Overall, QLF+NutriTek decreased total disease rates. DIM were between 1 and 30.

Ration

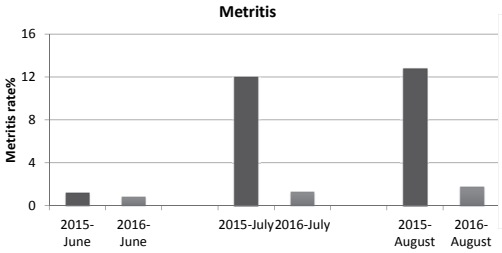
Dry and Pre-fresh cows	
Ingredient	**DM (lb./d)**
Dry cow mix	5.26
QLF-Nutritek	**3.02 (5 lbs. as fed)**
Straw	11.08
Canola	4.13
Corn Silage	9.52
Total	33.01

Started on May-5-2016

Fresh cows	
Ingredient	**DM (lb./d)**
Fresh cow mix	17.5
QLF-Nutritek	**3.02 (5 lbs. as fed)**
Straw	1.8
Haylage	8.0
Corn Silage	18
Total	48.32

Started on May-25-2016

Metritis rates of Paramount Dairy fresh cows



Metritis rates in summer pre-QLF + NutriTek were 8.7%

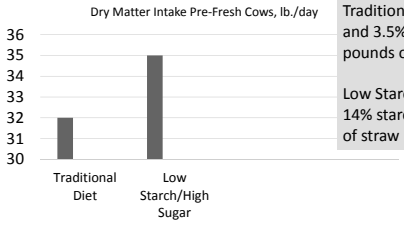
Metritis rates in summer with QLF + NutriTek were 1.3%

A reduction in Metritis rate of 85% (8.7-1.3/8.7)

Per 1000 cows that is 74 less cows with metritis. (87 – 13)

QLF+NutriTek was fed during June to August 2016. The disease rates in June to August 2015 (without QLF+NutriTek) were used as comparisons. DIM were between 1 and 30 of lactation.

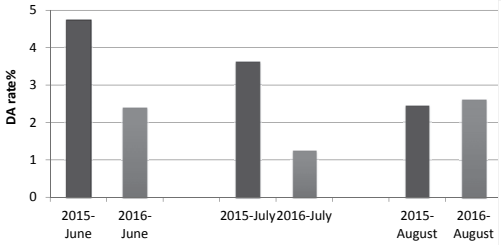
Impact of low starch, high sugar and soluble fiber diets on feed intake in close-up cows.



Traditional diet contained 16% starch and 3.5% sugar and contained 9 pounds of chopped straw.

Low Starch/High sugar diet contained 14% starch, 7.5% sugar and 11 pounds of straw

DA rates of Paramount Dairy fresh cows



DA rates in summer pre-QLF + NutriTek were 3.6%

DA rates in summer with QLF + NutriTek were 2.1%

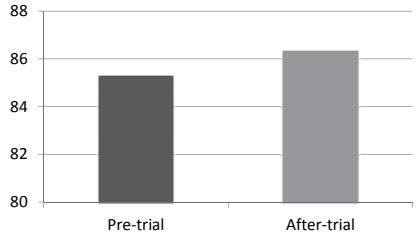
Per 1000 cows that is 15 fewer DA'S

A reduction in DA rate of 42% (3.6 – 2.1/3.6)

15 fewer DA's is a saving of $5,100

QLF+NutriTek was fed during June to August 2016. The disease rates in June to August 2015 (without QLF+NutriTek) were used as comparisons. DIM were between 1 and 30 of lactation.

Paramount Dairy Milk yield (lb./d)

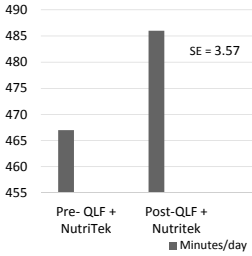


Heat Stress present in July and August 2016.

Milk increase was 1 pound but milk yield should have been lower due to heat stress.

QLF+NutriTek was fed from 5/25/16 to 8/31/16. The milk yields from 3/1/16 to 5/24/16 (without QLF+NutriTek) were used as comparisons. Cows were between day 1 and 40 of lactation. SE = 0.73. P = 0.28.

Rumination of Cows Pre- and Post QLF + Nutritek, Minutes/day

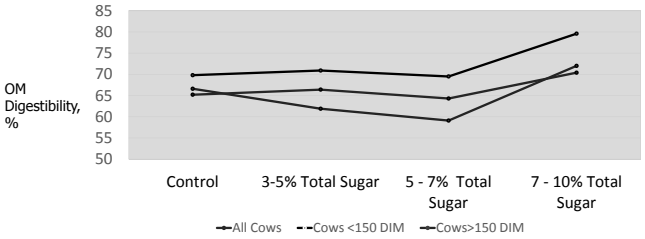


Difference was 19 minutes per day. This may indicate that QLF+NutriTek stimulated the intake of long particles in the diet, which require increased rumination.

Increased rumination is associated with increased saliva production, which may lead to better rumen health.

FIBER Digestibility and Sugar Feeding

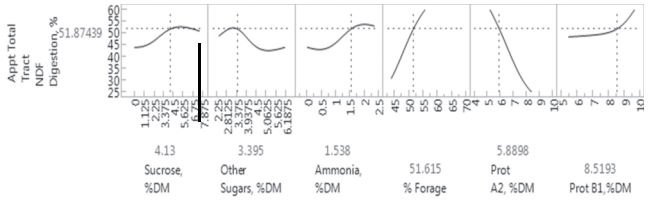
Impact of Sugar on Organic Matter Digestibility
n= 47



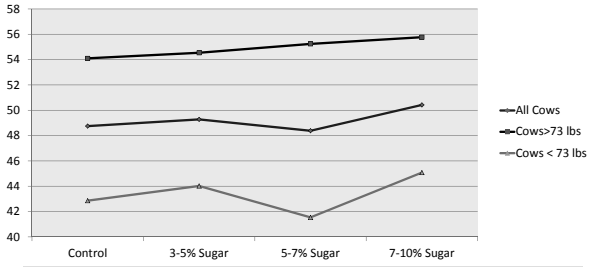
OM Digestibility increased 5 – 10 units when diet contained greater than 7% total sugar. Increased OMD leads to greater feed intake.

Impact of Sugars on the Digestibility of Fiber (NDF) in Lactating Dairy Cows

Key Points:
Fiber digestion optimized when the total sugar in the diet was between 7.0 – 7.5% (4.13 + 3.39)
Need at least 4.13% sucrose in the diet to optimize fiber digestion.
Optimum forage % = 51 – 57%

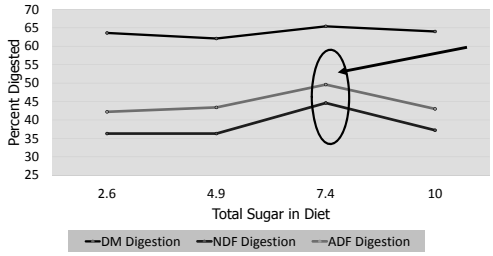


Net Result of Increased NDF and OM Digestibility is an Increase in Dry Matter Intake: Effect of Added Sugar on Dry Matter Intake, lbs./day.
Source: meta-analysis of 97 diet comparisons from 25 published trials



Overall Response to Added Sugar, was 1.7 to 2.1 pounds of DM Intake

Impact of Sugar on Diet Digestibility
Broderick and Radloff, J. Dairy Sci. (2004) 87:2997
60% Forage, Liquid Molasses Replaced High Moisture Corn

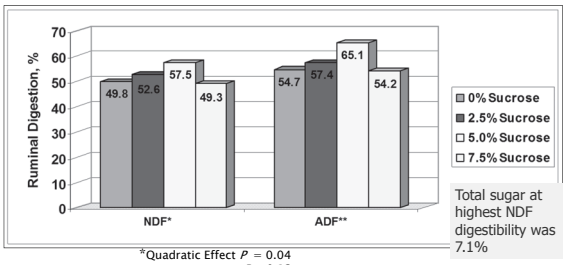


The Sweet Spot

Supplemental Sugar Recommendations to Optimize Dry Matter intake in Dairy Cows

- Supplement Enough! Aim for 7%- 7.5% Total Diet Sugar in lactating cow diets.
- Aim for 7.5 – 8.5% total sugar in dry cow diets
- Focus on Higher Producing Cows
- Provide Enough Rumen Degradable Protein (10-11%)
- Provide Adequate Rumen Effective Fiber, minimum 20% peNDF
- Monitor Cow Response
  - Measure DM Intake – DM intake should increase in dry cows and fresh cows.
  - Watch MUN's – MUN's should decrease
  - Watch Manure – should see less undigested fiber in manure
  - Watch TMR-Sorting – TMR sorting should decrease within 7 days.

Impact of Sucrose on Fiber Digestion
60% Forage Diet- Sugar Source Sucrose peNDF > 21%



Total sugar at highest NDF digestibility was 7.1%

*Quadratic Effect $P = 0.04$
**Quadratic Effect $P = 0.13$

Source: Broderick & et al. 2008. JDS:91:4801.

DCAD, It's Not Just For Dry Cows

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So what is DCAD? (Dietary Cation Anion Difference)

Element	% of DM	g/kg	Atomic Wt, g	Eq/kg	mEq/kg
K	1.06	12.0	39.1	0.271	271
Na	0.23	2.3	23.0	0.100	100
Cl	0.24	2.5	35.5	0.067	67

Mongin(1981)DCAD = mEq K + mEq Na + mEq Cl
DCAD = 271 + 100 + 67
DCAD = 304 mEq per kg DM
= 30.4 mEq per 100g DM

- With elements that are not monovalent, valence is accounted for
  - –Sulfur has a -2 valence, Atomic Wt =32, 1 Eq = 32/2 = 16

DCAD, It's not just for Dry Cows

J. Dairy Sci. 98:8973–8985
http://dx.doi.org/10.3168/jds.2015-9949

Intake, milk production, ruminal, and feed efficiency responses to dietary cation-anion difference by lactating dairy cows

M. E. Iwaniuk and R. A. Erdman[1]
Animal and Avian Sciences Department, University of Maryland, College Park 20742

- Work was part of Marie Iwaniuk's M.S. Thesis
- Marie is currently working on her PhD at Maryland
  - –Studying factors affecting feed efficiency in dairy cattle
  - –Spent last year as graduate intern at Purina Mills
  - –Marie is a pretty good statistician!

DCAD, 3 things you must know:

1) Balancing strong ion intakes in excess of requirements occurs by urinary excretion
2) SID (Strong Ion Difference) = $Na^+ + K^+ - Cl^-$
3) Urinary Strong Ion Excretion (Eq. Basis), The cations must equal the anions:

$$Na^+ + K^+ + H+ (NH_4^+) = Cl^- + OH^-(HCO_3^-)$$

Peter Stewart
(Strong Ion Theory)

DCAD related to the Strong Ions:
Sodium(Na), Potassium(K), & Chlorine (Cl)

- Osmoregulators:
  - – ~100% absorbed from diet
  - – Excess excreted in the urine, not feces
  - – Primary intracellular, extracellular, and rumen ions
- Acid-base balance(urine)
  - – High Cl/S diets: Acid urine (pH < 7)
  - – High K/Na diets: Alkaline urine (pH > 7)
  - – Ruminants have alkaline urine ($HCO3^-$)

Ion	Intra-cellular	Blood	Rumen Fluid
	------	mEq/L ------	
Na^+	12	145	84
K^+	139	4	27
Cl^-	4	116	8
HCO_3^-	12	29	6
Amino acids & proteins	138	9	(VFA's) 105
Mg++	0.8	1.5	4.2[1]
Ca++	<0.0002	1.8	3.5[1]
Osmoles	290	290	315[1]

[1]Bennick et al. (JDS, 1978)

DCAD: The Difference between Ruminants and Monogastrics

Mineral	2001 Dairy NRC Lactating Cows % of DM	2012 Swine NRC Lactating Sows % As Fed
Na	0.23	0.20
K	1.06	0.20
Cl	0.24	0.16
S	0.20	-
Ca	0.67	0.64
Mg	0.20	0.06
P	0.36	0.56
DCAD, mEq/kg	304	93

Simple DCAD Equation:
DCAD (mEq/kg) = Na + K - Cl

- Cows:
  - –High K Diet
  - –High DCAD
  - –Alkaline urine: pH 7.5-8
- Sows:
  - –Low K Diet
  - –Low DCAD
  - –Acid urine, pH = 6.5

DCAD and Milk Fever

- Ender, et al., (1962, 1971), Dishington (1975)
  - –Milk fever in dairy cows was reduced by:
    - –Reduced dietary cations (Potassium (K), Sodium (Na)
    - –Increased dietary anions (Chloride (Cl), Sulfur (S)
  - –Reduced blood pH increased blood calcium
- Series of experiments with "anionic salts" for preventing milk fever
  - –Elliot Block (McGill University), Jesse Goff and Ron Horst (USDA-ARS, Ames) and several others

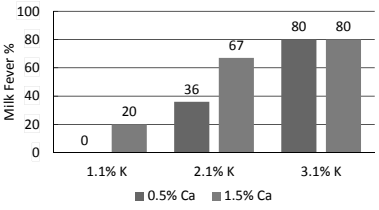
The Most Important DCAD Concept!

Feeding low DCAD diets in dry cows is GOOD!!

Feeding low DCAD diets in milking cows is BAD!!

DCAD and Milk Fever

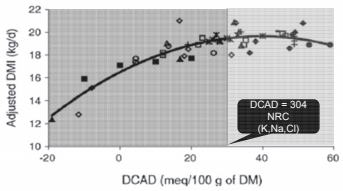
(Goff and Horst 1997 J Dairy Sci. 80:176-186)

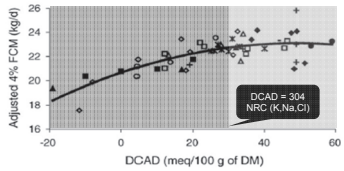


	DCAD, mEq/kg Diet Dry Matter	
Diet K	0.5% Ca	1.5% Ca
1.1	-98	-50
2.1	222	202
3.1	408	461

- Clearly high DCAD increased milk fever incidence!
- High calcium diets may exacerbate problem
- Milk fever can be prevented by feeding low DCAD, modest Ca diets

What about DCAD in Lactating cows?





- Meta-Analysis of:
  - – 12 papers
  - – 17 experiments
  - – 54 treatment means
- DCAD, mEq/100g DM = K + Na –Cl
- Suggested Max FCM and DMI at 40 and 34 mEq/kg, respectively.
- Many diets with added Cl supplements to reduce DCAD
  - THAT IS BAD!
- ~50% of data from diets with less than the implied NRC DCAD from minimum Na, K , & Cl requirements (304 mEq/100g DM)

(Hu and Murphy Meta-Analysis, 2004)

There are lots of DCAD Equations… Which One to Use?

Equation	Elements Included	DCAD, mEq/kg DM
Ender (1971)	Na + K - Cl - S	179
Mongin (1981)	Na + K - Cl	304
2001 Dairy NRC	(Na + K + 0.15 Ca + 0.15 Mg) – (Cl + 0.6 S + 0.5 P)	284
Goff et al. (2004)	Na + K – Cl – 0.6S	228

- Every equation gives a different value
- Ender (1971) (DCAD-S)used for milk fever prevention (most commonly used)
- Mongin (1981) used for monogastrics (simplest to use)
- Dairy NRC adjusted for absorption of all dietary cations an anions (never used)
- Goff et al. (2004) (S-coefficient based on urine and blood pH effects)
  - –Probably the most correct, S absorption is about 50 to 60% in cattle

DCAD in Lactating cows? The Impetus for Marie's Study

- Hu and Murphy's analysis:
  - –Very limited number of studies (12) and treatment means (54)
  - –That is what was available in 2004
- Lot's of published research on feeding buffers in dairy cattle (1960's to 1990's)
  - –Feeding buffers increases DCAD
  - –NaHCO3, Na2CO3, KHCO3, K2CO3

Why not use data from the buffer studies to expand the dataset?

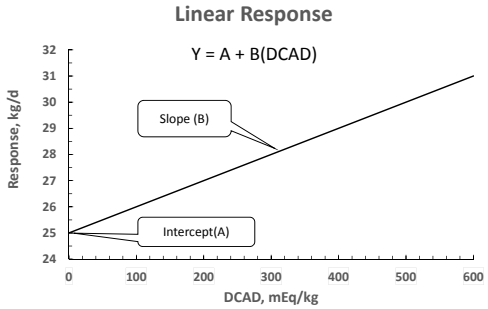
- Problem: Many studies had incomplete diet mineral analysis for DCAD
  - –Missing Cl
- Solution: Use the 2001 NRC Software to "fill in" the missing minerals

DCAD in Lactating Cows? Marie's Study

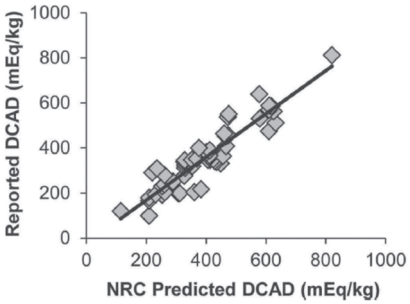
- Reviewed 53 articles where "buffers" were fed
  - Journal of Dairy Science and several others
- Study Inclusion Criteria
  - Complete Dietary Ingredient Composition
  - Must contain treatment means:
    - DMI
    - Milk Production
    - 3.5% FCM
    - Fat (% or yield)
- Also examined milk protein, rumen pH and VFA, DM, ADF and NDF digestibility
- We did not evaluate blood or urine acid-base indicators

DCAD in Lactating Cows: Evaluating the Responses

We used 2 different models:



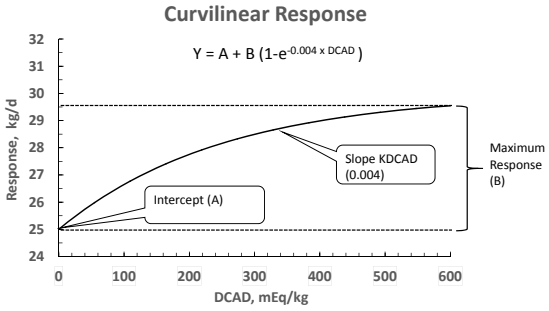
DCAD in Lactating Cows?



We found good agreement between measured and NRC Predicted DCAD

DCAD in Lactating Cows: Evaluating the Responses

We used 2 different models



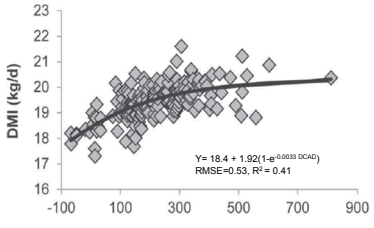
DCAD in Lactating Cows? Final Data Set

- 43 articles (Published Years 1965 to 2011)
- 196 dietary treatment means
- 89 treatment comparisons (Δ DCAD)
- DCAD-S Range -68 to +811 mEq/kg DM
  - Vast majority: 0 to 500 mEq/kg of diet DM
- Equations based on Ender Equation:

  DCAD, mEq/kg DM = K + Na + Cl -S
  - Also evaluated using Mongin Equation (K + Na - Cl)
    - Results were very similar
    - (Sulfur content among studies varied little)

DCAD in Lactating Cows? Dry Matter Intake Responses

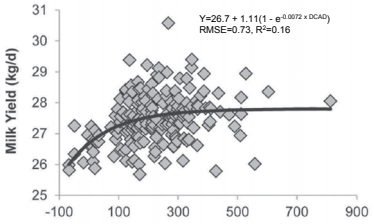
- Curvilinear DMI responses to DCAD
- Maximum DMI Response
  - 1.92 kg/d (4.2 lb/d)
- Response: % of Maximum
  - 66%, DCAD = 290
  - 80%, DCAD = 425
- Small responses to >425 mEq/kg DM



You could expect about a 3 lb/d increase in DMI by increasing DCAD from 0 to 400 mEq/kg

DCAD in Lactating cows? Milk Production Responses

- Curvilinear milk responses to DCAD
- Maximum milk response
  - 1.11 kg/d (2.4 lb/d)
- Response:% of Maximum
  - –66%, DCAD = 150
  - –80%, DCAD = 225
- Small responses to >225 mEq/kg DM
- Conclusion: Not much milk production response to DCAD



DCAD in Lactating cows? Other Responses

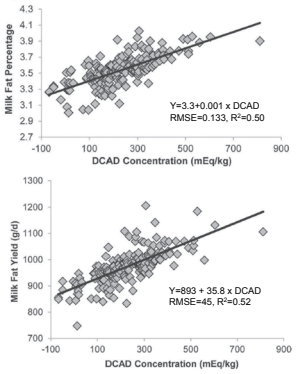
- No change in milk protein %
- Protein yield increased with milk yield (Non-significant)
- FE (Feed Efficiency, FCM per DMI)
- Increased 0.01 units per 100 mEq/kg DCAD
  - –FE = 1.39 @ 0 DCAD
  - –FE = 1.44 @ 500 DCAD
- Change in FE similar to what would be expected with a 3 kg/d increase in milk production

DCAD in Lactating cows? Milk Fat Percent and Yield

- Linear response:
  - Milk fat %
  - Fat yield
- Fat % (0.1%/100 mEq/kg DCAD)
  - – Fat = 3.3% @ 0 DCAD
  - – Fat = 3.8% @ 500 DCAD
- Fat yield (g/d) (38 g/d per 100 mEq/kg DCAD)

DCAD,mEq/kg	Fat yield
0	893g (2.0 lb/d)
500	1085 (2.4 lb/d

- Fat Yield:
  - Biggest production response to DCAD!



DCAD in Lactating cows? Summary Production Responses

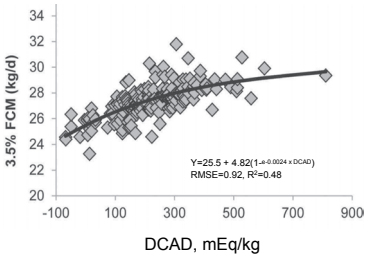
- Linear effect on fat % and yield (0.1% and 38 g/d) per 100 mEq/kg DCAD
- Curvilinear DMI, Milk, and FCM Responses

DCAD, mEq/kg

Item	Max Resp. kg/d	66% Max	80% Max	Hu & Murphy (DCAD-S)
DMI	1.92	290	425	275
Milk	1.11	150	225	215
FCM	4.82	450	675	No Max

DCAD in Lactating cows? 3.5% Fat Corrected Milk Response

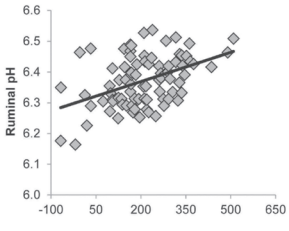
- Curvilinear FCM responses to DCAD
- Maximum FCM Response
  - 4.82 kg/d (10.6 lb/d)
- Response: % of Maximum
  - –66%, DCAD = 450
  - –80%, DCAD = 675
  - –(Outside the measured inference range)



FCM response reflects curvilinear increase in milk yield and the linear increase in fat yield

DCAD in Lactating cows? Rumen pH Responses

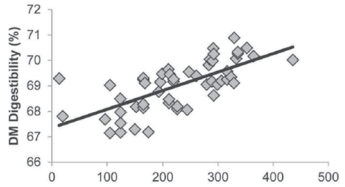
DCAD Responses-Rumen pH



- Rumen pH increased 0.003 units per 100 mEq/kg DCAD
  - –pH = 6.31 @ 0 DCAD
  - –pH = 6.46 @ 500 DCAD
- pH increase corresponds with milk fat responses
  - –Consistent with pH effects on rumen biohydrogenation of FA and milk fat depression
- Increased DCAD, More stable rumen environment
- Less fluctuation in feed intake
- Reduced Laminitis

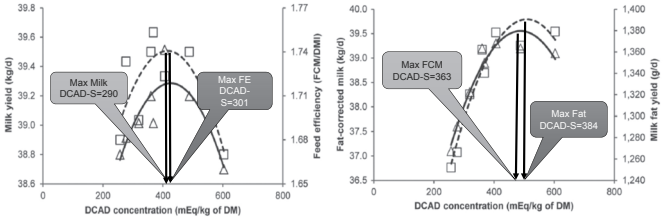
DCAD in Lactating cows? DM Digestibility Responses

- Linear for DM digestibility (n = 52)
- DM Dig increased 0.73 units per 100 mEq/kg increase in DCAD
  - DMDig = 67.4 @ 0 DCAD
  - DMDig = 71.1 @ 500 DCAD
- 4 units in DM Digestibility is huge response
- Big effects on DM intake



Practical Application of DCAD: At what DCAD should I feed?

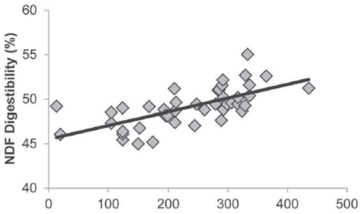
(Iwaniuk et al. (2014)JDS 98:1950)



Optimal DCAD depends on feed costs, value of increased milk, and more importantly: Milk fat (Milk fat yield is the main economic response)

DCAD in Lactating cows? NDF Digestibility Responses

- Linear increase in NDF digestibility (n = 46)
  - NDFDig increased 1.5 units per 100 mEq/kg increase in DCAD
  - NDFDig = 45.4 @ 0 DCAD
  - NDFDig = 53.1 @ 500 DCAD
- 2/3's of DM Digestibility response was due to change in NDF Dig



- Oba and Allen (1999) suggested that a 1 percentage unit increase in NDF Dig resulted in 0.17 and 0.25 kg/d increases in DMI and FCM, respectively
- 75% of DMI and 55% of FCM responses to DCAD could be attributed to increased NDF Digestibility

Practical Application of DCAD: Balancing for DCAD begins with ingredient selection

mEq/kg DM

Feed Ingredient	K	Na	Cl	S	DCAD	CP, %	NDF, %
Corn	107	9	-23	-63	31	9.4	9.5
DDGS	281	130	-28	-275	109	29.7	38.8
SBM	775	13	-155	-244	389	53.8	9.8
Canola Meal	361	30	-11	-456	-76	37.8	29.8
Corn Silage	307	4	-82	-88	142	8.8	45.0
Alfalfa Hlg	775	13	-155	-188	445	22.8	36.3
Grass Silage	795	22	-181	-131	505	18.0	49.9
Barley Silage	621	57	-203	-106	369	12.0	56.3

Comments:

- High K feeds are high DCAD feeds
- Nontraditional protein supplements (Canola, DDGS) will lower DCAD compared with SBM (High S)
- High NDF feeds:
  - Alfalfa, grasses, small grain silages
  - High DCAD feeds
- IE adding forage not only increases fiber, it also increases DCAD

Practical Application of DCAD: At what DCAD should I feed?

Item	Max Resp. kg/d	DCAD-S, mEq/kg (Iwaniuk, 2015) 66% Max	80% Max	Hu & Murphy (DCAD-S)
DMI	1.92	290	425	275
Milk	1.11	150	225	215
FCM	4.82	450	675	No Max

- There is no NRC DCAD requirement!
  - Suggested DCAD based on NRC requirement for Na, K, Cl, S
    - DCAD(Na,K,Cl) = 304 mEq/kg (30.4 mEq/100 g)
    - DCAD-S(Na,K,Cl,S) = 179 mEq/kg (17.9 mEq/100 g)
- That's too low!
- Practical minimum is 300(DCAD-S)and 425(DCAD) mEq/kg

Practical Application of DCAD: Increasing Fiber also increases DCAD

Example: Substituting Grass Silage for Corn

Feed Ingredient	DCAD, mEq/kg	NDF %
Grass Silage	505	49.9
Ground Corn	31	9.5
Difference	+474	+40.4

If Current Diet has: 25% Ground Corn, 5% grass silage
and Contains: 28% NDF, DCAD= 200mEq/kg (NDF too low)

If New Diet has: 20% Ground Corn, 10% grass silage
Then it Contains: **30% NDF, DCAD= 237mEq/kg**

Remember, increasing fiber also increases DCAD!

Practical Application of DCAD:

After ingredient selection, your choices to increase DCAD are with either Na or K Supplements

Mineral Supplement	K%	Na%	Cl%	DCAD, Eq/kg	DCAD
Salt (NaCl)	0.0	39.3	60.7	0	Neutral
Potassium Chloride (KCl)	52.4	0.0	47.6	0	Neutral
Potassium Carbonate (K_2CO_3)	52.4	0.0	0.0	1340	Positive
Sodium Bicarbonate ($NaHCO_3$)	0.0	27.7	0.0	1203	Positive
Sodium Sesquicarbonate ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$)	0.0	30.5	0.0	1325	Positive

Comments:

- NaCl and KCl are DCAD neutral
  - Addition has no effect on DCAD
- K_2CO_3, $NaHCO_3$,$Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$ have similar DCAD effects on a weight basis

Adding:
0.75% Potassium Carbonate, 0.83% Sodium Bicarbonate, or 0.75% Sodium Sesquicarbonate to diet DM increases DCAD by 100 mEq/kg

Practical Application of DCAD: Will DCAD Pay?

Herd: 50 lb/d dry matter intake (DMI)
80 lb/day milk
3.6% fat
DCAD-S=200 mEq/kg

The response from increasing DCAD to 300 mEq/kg

DCAD: mEq/kg	200	300	Diff.	Unit Value (Cost)	Added Income (Cost)
Income					
Milk, lb/d	80.0	80.3	0.30	$ 0.17	$ 0.05
Milk Fat, lb/d	2.88	2.97	0.09	$ 2.30	$ 0.21
Costs					
DMI, lb/day	50.0	50.6	0.60	$ 0.11	$ (0.07)
lb NaHCO3 (.8%)	0.00	0.40	0.40	$ 0.25	$ (0.10)
Net Return					**$ 0.09**

Yes, there is a return but barely… with today's milk prices
Remember, increased fat test is the primary return

DCAD Summary

- Feed High DCAD to Milking Cows (low DCAD is BAD)
- No NRC requirement for DCAD
  - Practical minimum: ~300 mEq/kg diet
- Adjust DCAD by:
  - Ingredient selection (High K Feeds)
  - Adding Na and K bicarbonate, carbonate and sesquicarbonate salts
- Milk fat is the primary economic response

Water for Dairy Production: Where Does it Go and Why Does Quality Matter?

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Introduction

It has been estimated that that 122 gallons of water are required to produce one pound of milk (1, 020 L/kg of milk) (Hoekstra, 2012). This estimate includes water of three categories 1) surface and groundwater, 2) rainwater, and 3) the volume of water needed to dilute pollutants. It is also estimated that most (95-99 %) of this water used to produce milk is needed to produce feed, while less than 1 % of this water is used for drinking (Owusu-Sekyere et al., 2016). Despite the fact that such little water is used for drinking, water plays an important role in milk production; this is because water's importance for sustenance only follows oxygen in the ranking elements of importance for sustenance. Illustrating this are two studies conducted by (Little et al., 1980) who restricted water for 4 and 14 d at a rate of 50% and 90 % of expected voluntary intake. These restrictions resulted in reductions in milk yields by 3 and 74%, respectfully. Because the quality of water varies greatly, and the consumption of water is vital to both life and production, it should be of little surprise that water quality is of critical importance to the commercial dairy industry. The objectives of this work are to review the flux of water through the lactating dairy cow and to review the major factors affecting the quality of drinking water.

Water Intake and Loss

An old rule of thumb is that cows need to drink 2 times more water than the volume of milk they produce, but, in practice, this is likely a bit of an underestimation (Holter and Urban, 1992). As much as 25 % of a cow's total water needs may come from feed. Additionally free water intake (FWI) is also known to be positively associated with feed intake, sodium and potassium content, and increasing humidity and environmental temperatures (NRC, 2001; Appuhamy et al., 2016). Table 1 and Figure 1 summarizes the results of a French study that investigated water intake, excretion and partitioning in Holstein cows placed in climatic chambers either at or above thermoneutrality (Khelil-Arfa et al., 2014). As predicted, the greatest losses of water occurred through fecal routes, followed by losses due to milk production and urine excretion. Increasing the temperature resulted in an increase in evaporated water from 5 to 9 gallons per day or 17 to 32 % of the total water intake (TWI). When expressed per unit of DMI, investigators also observed that increases in evaporative water losses were compensated for by increases in FWI. This increase in FWI is believed to be an adaptive reaction to ameliorate heat stress (Silanikove, 2000).

Water is a source of nutrients. Water can also be an important contributor to the daily intake of minerals in cattle. This is illustrated in a study conducted by in Merced County California in which mineral intake from both water and feed were estimated (Castillo et al., 2013). Of the total minerals analyzed, the proportion coming from water averaged 4 %, but ranged from 0.30 – 20 %. Although this source of minerals is arguably cheap, it may be problematic especially when trying to reduce overall whole-farm mineral balances and also when balancing diets based on dietary cation-anion difference (DCAD); (Beede, 2006; Elrod et al., 2013). Dr. Dave Beede of Michigan State University has created an excellent resource to estimate DCAD intake that accounts for contributions from both the diet and water supplies. This electronic calculator and resource can be found at the following website: https://msu.edu/~beede/extension.html .

Water Quality

High quality water is often easy to spot. It is generally clear and colorless, but it is also easy to overlook the fact that water contains more than just oxygen and hydrogen. Water may also contain pollutants, dangerous microorganisms, and many different types of minerals, all of which affect water quality and possibly production and the health of the lactating dairy cow. The Earth's water moves dynamically above, on, and below the earth's surface. When water moves, it comes into contact with various geological, biological and artificial surfaces that affects its chemical composition (Petersen et al., 2015). It is also important to remember that the composition of drinking water

is not only under natural influence but septic tanks, milk-house wastes and industrial drainage or drilling practices (Vidic et al., 2013) may also contribute to these composition problems. It is generally recommended that the water supply for cattle should be evaluated several times a year for coliforms, pH, minerals, nitrate and nitrites, and total bacteria. Expected levels and potential benchmarks of concerns for common water quality tests are given in Table 2.

Source of Problematic Minerals and Compounds. Troubleshooting water problems are not easy, but below is a listing and brief description of problems that may be encountered by a commercial dairy farm. Table 2 is a practical list of average, expected and possible problem concentrations of analytes in drinking water for dairy cattle (Beede, 2012). For a more in-depth review of mineral tolerance and toxicity, readers are referred to the National Research Councils report from Committee on Minerals and Toxic Substances in Diets and Water for Animals (NRC, 2005). Chapter 35 of this publication entitled, "Water as a Source of Toxic Substances," is an excellent summary, while other discussions of water and minerals can be found throughout the publication. The publication notes that sulfur, sodium, iron, magnesium, selenium and fluoride are the minerals that are most likely to reach toxic concentrations in drinking water. Additionally, copper zinc, bromine, bismuth and some rare-earth elements may be added to feed and found in water, and these sources together may resulting in a potential toxicities. When interpreting these guidelines two important points should be made. Firstly, controlled research studies on how these minerals affect animal performance, health, and the foods they produce, is lacking. As a result, except for copper, (0.5 ppm is recommended for livestock, which is lower than the 1.3 ppm in the human drinking water standards) many of these recommendations are made with observations not tested across species. Furthermore, those mineral estimates transposed from human drinking water standards may be conservative when applied to livestock. Secondly, reactivity and toxicity of mineral elements is highly influenced by the chemical form in which they exist. This is a challenge because water analyses and reports focus on the total concentration of a mineral and usually do not report on data related to speciation.

Total Dissolved Solids (TDS) and Salinity. Total dissolved solids (TDS), sometimes referred to as "salinity," is an estimate of inorganic constituents dissolved in a sample of fresh water. Dairy cattle may tolerate some degree of salinity so some caution when interpreting Table 2 and applying results is recommended. The world's growing need for water has brought about greater interest in water desalination (Yermiyahu et al., 2007), while only a small number of studies have sought to evaluate the effects of desalination techniques on dairy cattle. Solomon et al. 1995 reported that desalination increased free water intake by almost 3 gallons, and daily milk yield by over 4.5 pounds. Based on recommendations of the NRC (2001), it is generally believed that water containing 5000 to 7000 ppm of TDS is "reasonably safe" for heifers and dry cows, but producers should avoid offering this water to pregnant or lactating (or both) cattle as production may be impaired. The publication also notes that waters > 7000 ppm of TDS should not be fed to cattle in any stage of production.

Sulfates. Sulfates in ground water usually originate from sulfate-bearing minerals in soils and rocks. The upper safe limit for SO4 is believed to be around 50 ppm with the maximum upper concentration is 300 ppm. A recent study of water samples from the Northern Great Plains observed that 37 % of the samples exceed this upper concentration (Petersen et al., 2015). Sulfates found in drinking water usually include calcium, iron, magnesium, manganese, and sodium salts. Although all of these forms have laxative effects, sodium sulfate is believed to be the most potent. Iron sulfate has been shown to negatively affect free water intake. A major concern with high concentrations of sulfate in drinking water is that in the reducing environment of the rumen. Specifically, most sulfur originating from salts will be reduced to sulfide, and the combined sulfur from feed and water may tie up trace minerals, particularly copper and selenium, making them unavailable to the animal (Socha et al., 2003; NRC, 2001).

Iron. Iron in water is usually found in the ferrous (Fe+2) rather than ferric (Fe+3) form. Recently, Genther and Beede (2013) tested changes in iron concentration, valances [ferrous (Fe+2) or ferric (Fe+3)], and sources (salts) on FWI. Results of this research can be summarized by the three following observations: 1) when the concentration of iron was increased (0, 4 or 8 ppm) with the addition of ferrous lactate [Fe(C3H5O3)2] FWI was reduced at the highest concentrations of iron (8 ppm); 2) valence of iron source was not observed to affect free water intake at concentrations up to 8 ppm; and 3) increasing the concentrations (0, 8 or 12. 5 mg/L) of different salts of Fe [ferrous chloride (FeCl2) or ferric chloride (FeCl3)] did not affect FWI. Consumption of high concentrations of iron may interact with other minerals (i.e. copper and zinc). For example, consumption of high amounts of iron (250 – 1200 ppm) from ferris carbonate has been shown to reduce the absorption of copper in mature cattle (Spears et al., 2003). An additional concern with cows consuming high concentrations of

iron, especially in the reduced form, is the increased potential to contribute to oxidative stress. This may of be particular concern in animals with compromised immune systems, like the periparturient cow (Konvičná et al., 2015).

Nitrates. Neither nitrate (NO3-) or nitrite (NO2-) are required by animals. Nitrate poisoning can occur through the consumption of feed or water containing high concentrations (Jones, 1972). Dairy producers should be mindful that rumen microbes reduce nitrates to nitrites; hence, ruminants are more sensitive to toxicities associated with nitrate than are monogastric animals. When absorbed into the bloodstream, nitrites reducing the oxygen carrying capacity of blood. In a survey of 128 Iowa dairy farms, an elevation in the nitrate concentration of drinking water was correlated with increasing calving intervals (Ensley, 2000). The Dairy NRC (2001) recommends that nitrate-nitrogen (NO3-N) not exceed 10 mg/L and nitrate (NO3-) not exceed 44 mg/L. Water testing results which include nitrate and nitrite in mg/L can be converted to nitrogen values by dividing these values by 4.43 and 3.29, respectively (NRC, 2005). It is also known that ruminant animals may adapt to consuming high amounts of nitrate because, in some circumstances, rumen microbes may metabolize it completely rather than convert it to nitrite. This is presumably because ruminants have greater numbers of nitrate metabolizing microbes in the rumen (Lin et al., 2013).

Summary

Drinking water is vital to both the vitality and production of the lactating dairy cow. Although much remains to be learned about water quality and concentration, it is important to test water. By obtaining estimates for water intake, these data may help nutritionists further understand the mineral consumption and DCAD levels of the herd. Furthermore, water testing may indicate problematic constituents of water.

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Table 1. Summary of results of a study in which ambient temperature was increased and the observed effects on water intake and excretions (Khelil-Arfa et al., 2015)

	Thermoneutral[1]	**High temperature[2]**
Dry matter intake, lbs/d	46.9	41.7
Milk Yield, lbs/d	68.1	63.7
% Fat	3.96	3.81
% Protein	3.00	2.79
Body weight, lbs	1408	1384
Free Water Intake, gal	20.4	22.6
Water in feed, gal	8.17	7.25
Total water intake, gal	28.6	29.8
Free Water Intake, lbs	170	188
Water in feed, lbs	68	60
Total water intake, lbs	238	249
Urine output, gal	4.7	5.4
Fecal output, gal	12.6	10.4
Milk Output, gal	7.1	6.7
Evaporated water, gal	5.1	9.1
Metabolic, gal	1.2	1.2
Retained, gal	0.2	-0.4
Free Water Intake, % TWI[3]	71.3	75.8
Water in feed, % TWI[3]	28.6	24.3
Urine output, % TWI[3]	16.4	18.1
Fecal output, % TWI[3]	44.1	34.8
Milk Output, % TWI[3]	24.9	22.0
Evaporated water, % TWI[3]	17.7	30.5
Metabolic, % TWI[3]	4.2	3.9
Retained, % TWI[3]	0.79	-1.34
Free Water Intake, % DMI[4]	3.6	4.0
Water in feed, % DMI[4]	1.5	1.3
Urine output, % DMI[4]	0.8	1.0
Fecal output, % DMI[4]	2.2	1.8
Milk Output, % DMI[4]	1.3	1.2
Evaporated water, % DMI[4]	0.9	1.6
Metabolic, % DMI[4]	0.2	0.2
Retained, % DMI[4]	0.0	-0.1

[1] ambient temperature, relative humidity and unadjusted temperature humidity index (THI) was 60°F, 54.3%, and 59.4% respectively.

[2]ambient temperature, relative humidity and unadjusted THI was 83°F, 28.9%, and 73.2%, respectively.

[3] Total water intake, gallons/gallons

[4]pounds/pounds

Table 2. Average, expected and possible problem concentrations of analytes in drinking water for dairy cattle (*as presented by Beede, 2012*) values are derived from analyses in which most of the water samples were from farms with suspected animal health or production.

Measurement	Average[1]	Expected[2]	Possible problems, or caution[3]
pH, cows	7.0	6.8-7.5	< 5.1 or > 9.0
		Units are mg/L or ppm	
Total dissolved solids, TDS	368	< 500	> 3, 000
Total alkalinity	141	0-400	> 5, 000
Carbon dioxide	46	0-50	-
Chloride[4]	20	0-250	-
Sulfate	36	0-250	> 2,000
Fluoride	0.23	0-1.2	> 2.4
Phosphate	1.4	0-1.0	-
Total hardness	208	0-180	-
Calcium	60	0-43	> 500
Magnesium	14	0-29	> 125
Sodium	22	0-3	> 20 veal calves; > 150 cows
Iron	0.8	0-0.3	> 0.3 (taste, veal)
Manganese	0.3	0-0.05	> 0.05 (taste)
Copper	0.1	0-0.06	> 0.6-1.0
Silica	8.7	0-10	-
Potassium	9.1	0-20	-
Arsenic	-	0.05	> 0.20
Cadmium	-	0-0.01	> 0.05
Chromium	-	0-0.05	-
Mercury	-	0-0.005	> 0.01
Lead	-	0-0.05	> 0.10
Nitrate as NO_3 [5]	34	0-44	> 100
Nitrate as NO_2	0.28	0-0.33	> 4.0-10
Hydrogen sulfide	-	0-2	> 0.1
Barium	-	0-1	>10
Zinc	-	0-5	> 25
Molybdenum	-	0-0.068	-
Total bacteria/100 ml	336,300	< 200	> 1 million
Total coliform100 ml	933	< 1	> 1 calves; > 15-50 cows
Fecal coliform/100 ml[6]	-	< 1	> 1 calves; > 10 cows
Fecal streptococcus/100 ml	-	< 1	> 3 calves; > 30 cows

[1] For most measurements, averages are from about 350 samples; most samples are taken from water supplies in farms with suspected animal health or production problems.

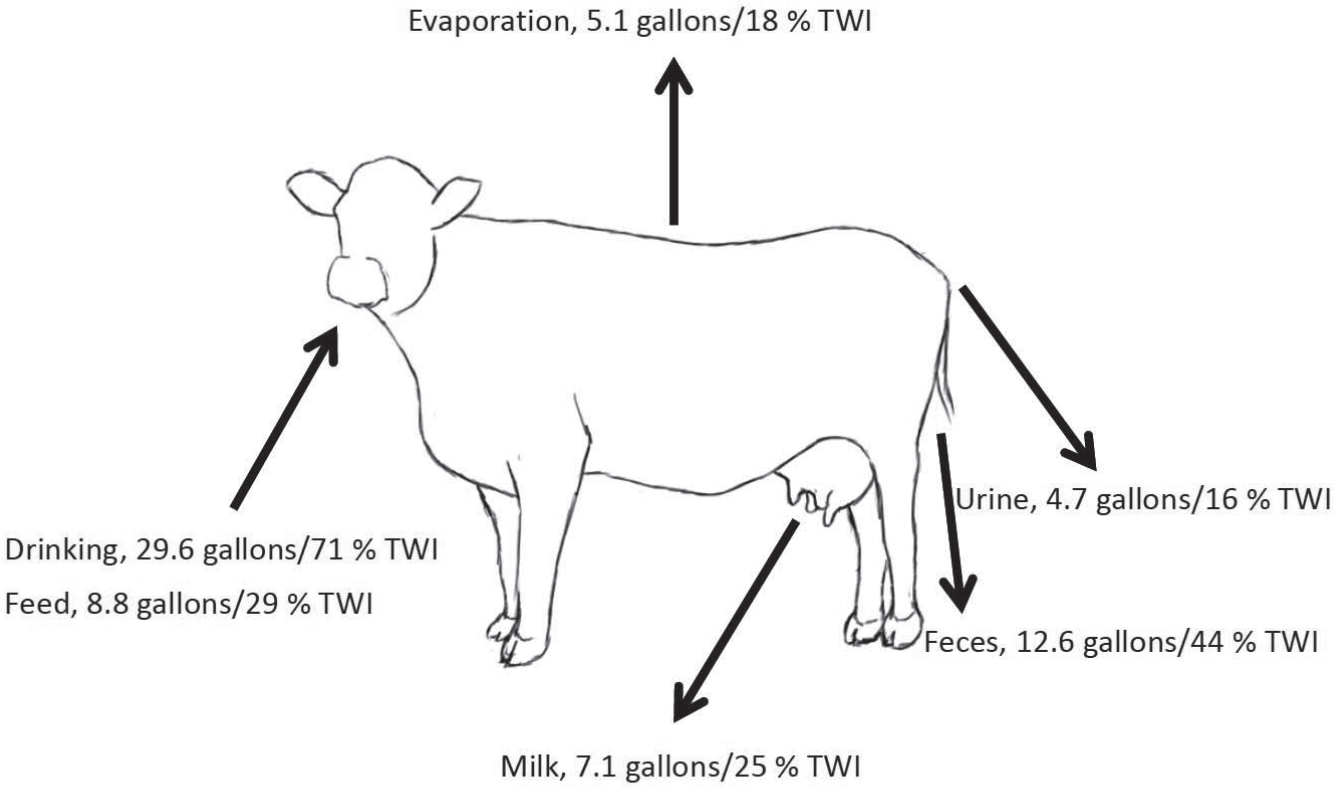
[2] Based primarily on criteria for water acceptable for human consumption.

[3] Based primarily on research literature and field experiences.

[4] Field observations suggest free or residual chlorine concentrations up to 0.5 to 1.0 ppm may not affect ruminants adversely. Municipal water supplies with 0.2 to 0.5 ppm have been used

successfully. Swimming pool water with 1.0 ppm, or 3 to 5 ppm chlorine in farm systems with short contact time have caused no apparent problems for cattle.

Figure 1. Measured flow of water (in gallons per day or as a % of total water intake (TWI)) estimated in cows consuming 47 pounds of dry matter and producing 68 pounds of milk that contained 3.96 % fat and 3.00 protein (Khelil-Arfa et al., 2014).



Does TMR Sampling Have Value?

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Summary

Sampling and analyzing a TMR has several potential uses including evaluating the nutrient content of the diet that was actually fed and to estimate manure excretion of nutrients via mass balance calculations. The consistency of ration delivery can be evaluated by sampling the TMR and it can be used to determine whether the ration that was delivered to the cows is the same as the diet that was formulated. However, for any of these uses to be valid, the TMR sample must accurately reflect the diet that was delivered. Previously we found that sampling variation was substantial for TMR samples. This was investigated further by sampling three different TMR (one had silages and concentrate, one had silages, concentrate and hay, and one had silages, hay, whole cottonseed, and concentrate) using two different sampling protocols. One protocol was simple and consisted of taking several handfuls of TMR across the feed bunk. The other protocol consisted of putting trays in the feedbunk prior to feed delivery and then removing the trays filled with TMR, mixing, and sampling from the trays. Sampling protocol had very little effect on sampling variation or on the accuracy of the sample. Samples of TMR did not accurately estimate the true mineral concentrations (sodium, phosphorus and copper) of the TMR. A single sample of TMR (using either protocol), however generally gave an accurate estimate of the true concentration for DM and CP in the TMR. For NDF, a single sample had a high risk of being wrong (i.e., inaccurate), but taking duplicate samples and averaging the analytical results were generally accurate. TMR sampling can be accurate for macronutrients but care must be taken when sampling and often duplicate samples will be required.

Introduction

Proper sampling of ration ingredients and submitting those samples for nutrient analysis to a good lab are essential components of diet formulation. The relative importance of sampling, analytical, and real variation on overall variation in nutrient composition data of ingredients has been discussed previously (Weiss et al., 2012; Weiss et al., 2014). Sampling variation was an equal or greater source of variation than was real month to month variation for corn silage over a 12 month period. Although real variation over a 12 month period was the greatest source of variation for hay crop silage, sampling variation was still an important source of variation. The overall conclusion from all those data is that averages of duplicate samples, not single sample data, should be used for ration formulation. Ingredients are sampled and analyzed mainly to provide data for diet formulation. Total mixed rations (TMR) are sampled and analyzed for other reasons including monitoring consistency both within a feedbunk and day to day and to evaluate the feeder and TMR mixer. Because of the different use of TMR composition data, sampling protocols developed for ingredients may not fit TMR sampling.

Why Sample a TMR ?

1. Assessing within bunk variation in nutrient delivery. Ideally, the nutritional composition and physical form of a TMR is consistent across the feedbunk within a pen. Numerous factors affect consistency of TMR delivery (Oelberg, 2015), but will not be discussed here. When evaluating consistency of delivery, samples are taken at various locations across the bunk, analyzed for something and then the variation is calculated. This measure of variation is compared to a benchmark to determine whether the TMR is consistent across the pen. A basic premise of this approach is that the variation between samples is caused by location and not sampling. Sampling variation refers to the difference between two samples taken in the same location within a feed bunk. If that variation was similar to the variation between samples taken at different locations within the feed bunk, you would not know whether diet delivery was inconsistent (i.e., location in the bunk really affects composition) or if the sampler was not very good at taking representative samples. Therefore, if your objective is to evaluate consistency, multiple samples at multiple locations within the feed bunk should be taken so variation caused by sampling and location can be partitioned.

2. Assessing day to day consistency of TMR delivery. Whether day to day variation in nutrient composition of TMR is important is unclear at this time. In a survey-type experiment (Sova et al., 2014), herd

average milk production was negatively correlated with day to day variation in NEL concentration (i.e., high variation was association with lower herd average production). However in controlled experiments, substantial day to day variation in NDF, forage to concentration ratio and fatty acids has not had any major effects on cow productivity (McBeth et al., 2013; Weiss et al., 2013; Yoder et al., 2013). Nonetheless if your objective is to evaluate day to day variation in nutrient delivery, sampling variation must be separated from variation caused by day. To do this, multiple samples must be taken each day over multiple days. This will allow you to determine whether day is the source of variation or if the observed variation is simply an artifact of sampling.

3. Determining whether the delivered ration matches the formulated one. The nutrient composition of commonly fed forages and many concentrates exhibit substantial within farm variation (Weiss et al., 2012; St-Pierre and Weiss, 2015). Sampling and monitoring TMR composition could be used to suggest when the nutrient composition of a feed has changed indicating it is time to re-sample and re-formulate. The nutrient composition of a TMR also reflects the recipe that was actually delivered to the pen on that day. Sampling TMR can be used to troubleshoot feed delivery. Because of feeder and scale errors the delivered diet may differ markedly from the formulated diet even when the nutrient composition of the individual ingredients has not changed. Sampling a TMR, if the results accurately reflect the delivered diet, could help a nutritionist identify nutrient deficiencies or feed delivery problems. To make valid conclusions regarding the nutrient composition of the delivered diet, the sample results must accurately reflect the composition of the TMR delivered to the pen. If sampling error is high, a nutritionist may conclude that the delivered TMR is not what was formulated and spend time trying to identify the reason why that occurred when in reality the TMR was correct; it was the sample that was bad. Conversely, a bad sample could suggest that the TMR is matching specifications when really it does not.

4. Monitoring nutrient management plans. On some dairy operations, the amount of phosphorus and nitrogen excreted in manure must be monitored to ensure compliance with environmental regulations. Accurate sampling of manure is extremely difficult and calculated nutrient balance offers an alternative approach (Castillo et al., 2013). Intake of P or N can be calculated by multiplying TMR delivery to the herd by its concentration of P and N and sampling milk and analyzing that for P and N and then subtracting milk secretion from intake. The remainder is an estimate of the amount of N and P excreted in manure. Measuring P and N (i.e., CP) in a TMR sample can be used to estimate intake of those nutrients. However, if the sample does not accurately reflect the TMR, the actual nutrient application to soil may exceed a farm's nutrient management plan.

Using TMR composition data to evaluate diets and troubleshoot nutritional problems has great potential; however, for TMR data to be useful the nutrient composition of the sample must accurately reflect what was delivered to the pen. The recurring theme for all the possible uses of TMR sampling data is that sampling error must be known for you to reach valid conclusions regarding the data.

Is sampling error a concern for TMR ?

Sampling error (or sampling variation) simply means that if you take multiple samples from the same population, you obtain different values (ignoring analytical variation). A population could be a truck load of distiller grains, a pile of silage that will be fed to a group of cows today or a TMR that was delivered to a pen of cows. With respect to feeds and TMR, sampling error occurs because different particles have widely different nutrient composition. A TMR is comprised of particles that vary in density, size, shape, and nutrient composition. A stem of hay is light long and is generally high in fiber whereas a grain of salt is heavy and small and has no fiber but lots of sodium. From a field study of about 50 dairy farms across the U.S., sampling and analytical variation (because of the design of the experiment, these two sources of variation could not be separated) accounted for 36 to 70% of the total within farm variation in TMR composition (the range represents different nutrients) over a 12 month period (St-Pierre and Weiss, 2015). Sampling error was great enough to have a substantial impact on interpretation of results (Table 1). For example, based on Table 1, you have a 10% chance that a single sample of TMR could have a CP concentration <16% when the true concentration was 17.1%. These large sampling errors reflect the heterogeneous nature of TMRs and the ease at which poor samples can be taken.

Improper sampling techniques could result in a sample having fewer small particles than the actual TMR. Small particles are often rich in starch, minerals, or protein. Because of the wide disparity between particles with respect to size and density, particle gradients can develop within a pile of TMR in the feed bunk. With mechanical movements, large light

particles (such as pieces of hay) tend to rise to the top of a stack and dense small particles tend to sink. This means that a handful of TMR taken from the top of the pile may have higher NDF, and a handful taken from the bottom of the pile may be enriched in starch, protein and minerals.

TMR Sampling Project

To determine whether sampling method affected the accuracy (i.e., how close the nutrient composition of a TMR sample came to the true composition of the TMR) and precision (how much variation was observed among samples) of TMR sampling and to determine the overall accuracy of TMR sampling a study was conducted at OARDC in Wooster. Three different pens with TMR that differed greatly in ingredient components (Table 2) were sampled for 3 consecutive days and then sampled again for 3 consecutive days the following week. Each TMR was sampled using two different sampling methods (discussed below) and a duplicate independent sample was taken each day from each method. Each sample was then assayed in duplicate for DM, NDF and CP using standard wet chemistry methods at the OARDC Dairy Nutrition Lab. Dry ground samples were sent to Rock River Laboratory (Watertown, WI) and analyzed in duplicate for major and trace minerals using standard wet chemistry methods. This protocol allowed us to determine sampling error for 3 different types of TMR and whether sampling method could affect accuracy and precision. This paper will discuss mostly accuracy rather than precision.

Sampling protocols. Both protocols were performed immediately after the TMR was delivered to the pen. The simple protocol consisted of taking 1 handful of TMR every approximately 10 feet of the feed bunk yielding about 6 handfuls per pen. The top, middle, and bottom third of the TMR was sampled alternatively as the sampler walked the feed bunk. The handfuls were placed into a large plastic bag. The handfuls were collected with the palm facing upward to reduce loss of small particles. That process was immediately repeated to yield a duplicate sample. The complex sampling protocol consisted of placing 4 trays (2 ft wide x 3 ft long x 8 inches tall in the manger just before TMR delivery. The trays were equally spaced across the bunk (Tray 1 was at the south end, then 2, 3, and 4). Immediately after feed was delivered, the 4 trays filled with TMR were pulled to the center aisle. The contents of Tray 1was emptied onto a clean sheet of plastic and mixed using a scoop. The contents was sectioned and 2 approximately 1/8 sections was removed with a scoop and placed into an empty, clean tray. That process was repeated with Tray 3. The subsample from Trays 1 and 3 were combined, thoroughly mixed and a section was removed with a scoop and placed into a bag. The duplicate sample was obtained by repeating this process using the contents of Tray 2 and 4. The 4 samples per pen (2 sampling methods in duplicate) were brought to the lab and analyzed.

Determining accuracy. Each day the TMR were sampled, all TMR ingredients (silages, hays, concentrate mixes, and cottonseed) were sampled in duplicate and analyzed in duplicate using standard wet chemistry methods. Ingredient inclusion amounts were recorded electronically using commercially available TMR software. Multiplying inclusion rate by assayed composition (mean of the duplicate samples and duplicate assays) yielded what we considered the actual or true composition of the TMR.

Effect of sampling protocol

The effect of sampling protocol (simple vs. complex) on sampling variation was not consistent across the different TMR or across nutrients. For the majority of TMR and nutrients, protocol had no effect on sampling variation. The complex protocol had greater sampling variation than the simple method for DM concentration in TMR-1 (contained hay and cottonseed) and for NDF concentrations for TMR-2 (contained hay) and TMR-3 (contained only silage and concentrate). Conversely, the complex protocol had statistically lower sampling variation for NDF concentration of TMR-1, for CP and Na in TMR-2, and Na in TMR-3. We hypothesized that for the most variable matrix (TMR-1 that contained silage concentrate, hay, and cottonseed) the complex sampling method would be more consistent, and for the simplest matrix (TMR-3 with just silages and concentrate) sampling protocol would not have any effect on sampling variation. With respect to sampling variation, the simple protocol was generally just as good (and much easier and faster) than the complex method.

We also statistically tested whether sampling protocol affected nutrient concentrations. This does not evaluate accuracy (e.g., the protocols could give similar numbers but both could be wrong). For most nutrients and TMR types, sampling protocol did not affect analytical results. The only meaningful difference between sampling protocols was for NDF concentration of TMR-3 (silage and concentrate only). The simple method yielded a mean of 46.1% whereas the complex method had a mean of 43.2 (Table 3). If this was a consistent finding across TMR types (i.e., the simple method had higher NDF concentrations) it would likely mean that the protocol resulted in loss of small particles, but since this was only found with one TMR type it may be just a spurious finding.

Accuracy has a flexible definition depending on how good is good enough. If you were constructing a nuclear submarine, tolerances might be expressed in nanometers but if you are digging a hole for a fence post, tolerances may be several inches. For TMR accuracy we decided that if a sample result was within 5% of the real value, the sample was accurate. Accuracy was evaluated for major nutrients (DM, NDF, and CP), phosphorus (because it can be used in nutrient management plans, sodium (because most sodium is from salt) and copper (as an example trace mineral). To evaluate accuracy we calculated the deviation of the real value from each sample result and we also calculated the mean of the duplicate samples (within each protocol) and calculated the deviation of the real value from that mean.

Minerals

About half the copper in the three TMR were from mineral supplements and about half was from basal ingredients. Taking a single sample using either protocol from any of the 3 types of TMR had absolutely no value in estimating the true concentration of copper. Of the 72 individual TMR samples (3 types of TMR x 6 days x 2 protocols x 2 duplicate samples = 72), only 8 (11%) of the samples were within 5% of the true value and 39 samples (54%) were more than 20% different from the true value. Across sampling protocols and TMR types, samples usually had lower concentrations of Cu than the actual TMR. The samples for TMR-3 (silage and concentrate) were slightly less inaccurate compared with the other two types of TMR. The average deviation for TMR-1 and TMR-2 was about 25% (averaged across sampling protocol) and about 18% for TMR-3. Taking duplit-ween the TMR types. A single sample to assess the NDF concentration of a TMR was less reliable than for CP. Only 50% of the single samples were within 5% of the true concentration for NDF and almost 20% of the samples differed by more than 10% (Table 3). Using means of duplicates increased the chance of being within 5% of the mean (60% of the means were within 5% of the true values), but more importantly, means greatly reduced the chances of obtain extreme deviations (only 10% of the means were more than 10% different from the true value.

Conclusions

Using a simple, yet good sampling technique for obtaining TMR samples was generally accurate for macronutrients (DM, NDF, and CP), however using results from a single sample had a high risk of being very wrong (>10% different) with respect to NDF. Taking duplicate samples and averaging reduced the risk of being wrong but did not greatly increase overall accuracy. Sampling TMR did not accurately assess mineral delivery.

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Table 1. Sampling variation in TMR samples taken from 49 farms (one pen per farm) over a 12 month period (St-Pierre and Weiss, 2015).

Nutrient	Mean	Sampling +analytical variation	
		SD	80% range[1]
DM, %	48.3	2.91	44.6 – 52.0
NDF, %	32.9	1.81	30.6 – 35.2
CP, %	17.1	0.89	16.0 – 18.2
P, %	0.41	0.030	0.37 – 0.45
Na, %	0.42	0.091	0.30 – 0.54
Cu, ppm	23	5.1	16.5 – 29.5

[1] Assuming a normal distribution, 80% of the samples should fall within this range. 10% of the samples would be higher than the highest value and 10% would be lower than the lowest value.

Table 2. Ingredient composition of three types of TMR (% of DM).

	TMR-1	TMR-2	TMR-3
Corn silage	43	19	22
Alfalfa silage	8	32	0
Mixed silage	0	21	58
High quality grass hay	8	0	0
Low quality grass hay	0	9	0
Whole cottonseed	10	0	0
Concentrate[1]	31	19	20

[1] A different concentrate mix was fed in each TMR but the primary ingredients were ground corn, soybean meal and minerals. The concentrate was fed as a meal.

Table 3. The true nutrient concentrations of 3 TMR (measured over a 6 day period) and concentrations obtained from sampling the TMR using a simple or complex protocol. All values are on a DM basis[1].

	True Concentration[2]		Simple Protocol[3]		Complex Protocol[3]	
DM, %	Mean	Range	Mean[1]	Range[2]	Mean[1]	Range[2]
TMR-1	55.5	55.4 – 57.5	55.1	53.9 – 56.5	54.6	48.6 – 56.9
TMR-2	52.1	50.8 – 54.2	51.3	49.8 – 53.5	51.7	50.0 – 53.1
TMR-3	49.7	48.5 – 50.7	48.7	46.7 – 50.9	49.5	48.1 – 51.2
NDF, %						
TMR-1	32.4	31.2 – 34.2	31.5	28.4 – 35.0	32.2	29.7 – 35.3
TMR-2	41.8	41.2 – 43.0	43.7	41.1 – 48.6	42.4	39.2 – 46.4
TMR-3	45.8	44.8 – 47.4	46.1	42.5 – 50.3	43.2	39.7 – 47.2
CP, %						
TMR-1	16.4	15.8 – 16.8	15.7	14.5 – 16.6	15.3	15.8 – 16.8
TMR-2	13.1	13.0 – 13.2	12.9	11.6 – 13.5	13.0	12.3 – 13.4
TMR-3	12.5	12.2 – 13.0	12.4	11.9 – 13.1	12.8	12.1 – 13.2
P, %						
TMR-1	0.38	0.35 – 0.40	0.32	0.28 – 0.34	0.32	0.28 – 0.35
TMR-2	0.29	0.28 – 0.30	0.23	0.21 – 0.26	0.23	0.20 – 0.25
TMR-3	0.27	0.25 – 0.29	0.24	0.21 – 0.26	0.23	0.19 – 0.27
Na, %						
TMR-1	0.12	0.10 – 0.13	0.14	0.11 – 0.18	0.14	0.12 – 0.17
TMR-2	0.07	0.06 – 0.08	0.06	0.06 – 0.08	0.06	0.06 – 0.08
TMR-3	0.12	0.09 – 0.14	0.13	0.11 – 0.15	0.13	0.11 – 0.15
Cu, ppm						
TMR-1	14.6	13 – 17	11.6	8 - 16	12.6	7 – 16
TMR-2	19.2	18 – 20	14.5	9 - 19	13.8	8 – 17
TMR-3	15.8	13 – 19	14.7	9 - 19	15.6	10 – 21

[1] TMR-1 contained silages, hay, whole cottonseed and concentrate; TMR-2 contained silages, hay and concentrate; TMR-3 contained silages and concentrates.

[2] True concentrations were determined using composition data of the TMR ingredients and actual inclusion rates. The range represents concentrations over a 6 day period.

[3] The simple protocol consisted of taking handfuls of TMR across the feed bunk. The complex protocol consisted of putting trays in the feed bunk prior to feed delivery and sampling from the trays. The mean was calculated across 6 days and duplicate samples each day (within sampling protocol). Range represents the lowest and highest value for a sample.

Don't Forget the Strong Ions

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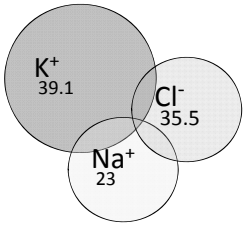

Don't Forget the Strong Ions

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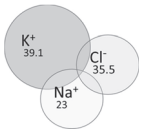
The Strong Ion's Role in Osmoregulation

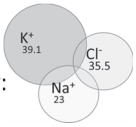
(Normal Osmotic Pressure: 300 mOsm)



Ion	Intra-cellular	Blood	Rumen Fluid
	------ mEq/L ------		
Na^+	12	145	84
K^+	139	4	27
Cl^-	4	116	8
HCO_3^-	12	29	6
Amino acids & proteins	138	9	(VFA's) 105
Mg++	0.8	1.5	4.2[1]
Ca++	<0.0002	1.8	3.5[1]
Osmoles	290	290	315[1]

[1]Adapted from Bennick et al. (JDS, 1978)

What are the Strong Ions?

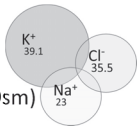


Strong Ion Term coined by Peter Stewart in paper:

- "Strong Ion Theory of Acid-Base Balance" (*Respiration Physiology* (1978) *33, 9-26)*
- Cations and Anions that are completely soluble in biological fluids
  - Cations: Sodium (Na), Potassium (K) Magnesium (Mg
  - Anions: Chloride (Cl), Sulfate, Lactate, Volatile Fatty acids, Beta-hydroxy butyrate.
- I will focus on the 3 principal mineral elements: K, Na, and Cl


Peter Stewart

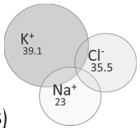
Understanding Strong Ions



- Osmotic effects, Ionic Strength (milliosmoles/Liter, mOsm)
  - Measure of the number moles in solution
- Electrical Charge, millequivalents/Liter (mEq) (Moles + Charge)
  - Corresponding valences for K, Na, Cl and SO_4 are +1, +1, -1, and -2, respectively
- Role of Strong Ions is better understood when diet concentrations are reported as:
  - Millequivalents (mEq) per kg or per 100 g Diet DM
  - Not as percentages in the diet
  - Very Important when disposed of in urine

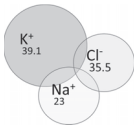
Ion	% of Diet DM	g/kg	Equivalent Wt. grams	mEq/Kg	mEq/100 grams
Sodium (Na)	0.230	2.30	23.0	100	10.0
Potassium (K)	0.391	3.91	39.1	100	10.0
Chloride (Cl)	0.355	3.55	35.5	100	10.0

What are the Strong Ions?



- Primary Functions:
  - Active Transport of Nutrients (glucose, amino acids)
  - Osmoregulation- (Na-K ATPase): Water balance across tissues, digesta, kidney, cell membranes etc.
- Highly available, Nearly 100% absorbed from diet
  - This is true in nearly all animal species
- There are minimal reserves for the cow to draw on
  - Deficiencies manifest themselves quickly (1-2 days)
  - Common symptoms of K, Na, and Cl deficiencies include decreased feed and water intake, dry manure
- Excess Strong Ions are excreted in the urine, Not feces

Dietary Strong Ions are not Expensive to Supplement



The Relative Costs of Increasing Diet K, Na, and Cl by 100mEq/kg (98, 58, and 89 g/d, respectively)[1,2]

	Added Cost, $ per Cow/Day		
Mineral Supplement	K	Na	Cl
Salt		$0.02	$0.02
Potassium Chloride	$0.10		$0.10
Potassium Sesquicarbonate	$0.25		
Sodium Bicarbonate		$0.13	
Sodium Sesquicarbonate		$0.09	

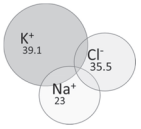
[1]Cow consuming 25 kg (55 lb) DM per day
[2]Dietary K, Na, and Cl increase by 0.39, 0.23, and 0.35%, respectively

Ruminants Evolution on Forages

	------- % of DM -------			------- mEq/kg DM -------		
Forage	K	Na	Cl	K	Na	Cl
Corn Silage	1.20	0.01	0.29	307	4	82
Alfalfa Haylage	3.03	0.03	0.55	775	13	155
Grass Silage	2.81	0.05	0.64	795	22	181
Barley Silage	2.42	0.13	0.72	621	57	203
Rye Silage	3.34	0.05	0.90	854	22	253
Orchardgrass	3.58	0.04	0.67	916	17	188

Comments:
- Nutritional environment: High K, Low Na, and Moderate CL
- Ruminant are equipped to get rid of excess K
- Their kidneys should function well with alkaline urine

What Regulates Urine pH?



- When there are Excess Cations (K,Na) to Excrete

  $Na^+ + K^+ + H+ (NH_4^+) = Cl^- + OH^-(HCO_3^-)$

  Urinary: Bicarbonate ↑ , pH ↑

- When there are Excess Anions (Cl) to Excrete

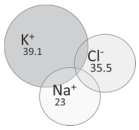
  $Na^+ + K^+ + H+ (NH_4^+) = Cl^- + OH^-(HCO_3^-)$

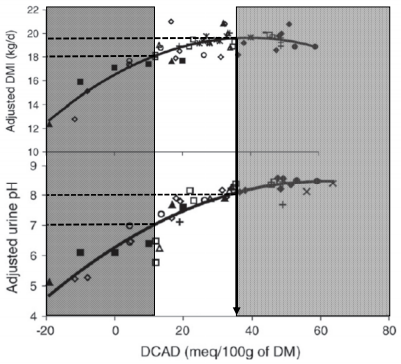
  Urinary: NH_4^+↑, Bicarbonate ↓, pH ↓

Too much Cl in relation to K and Na → Acid Urine

Your job is to make sure that the cow has just enough Cl and more than enough K and Na to have an Alkaline Urine

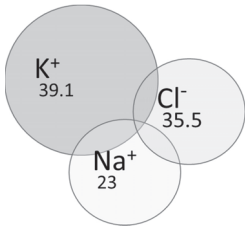
Dairy Cows Like to Operate with an Alkaline Urine (pH=7.5 to 8)





- Peak DMI occurs when urine pH is about 8
- That point is reached with DCAD of ~ 37.5 mEq/100 g
- Dry Matter Intake (DMI) falls off rapidly as urine pH drops to 7 or below.
- Low DCAD diets reduce urine pH (dry cows)
- Don't feed lactating cows for low urine pH!

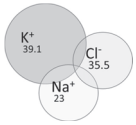
Mongin DCAD (K + Na –Cl)
Adapted from Hu and Murphy, 2004, J. Dairy Sci. 87:2222



Excess Strong Ions Drive Cows to Drink!!

(OK, Not that kind of drink)

What Regulates Urine pH?



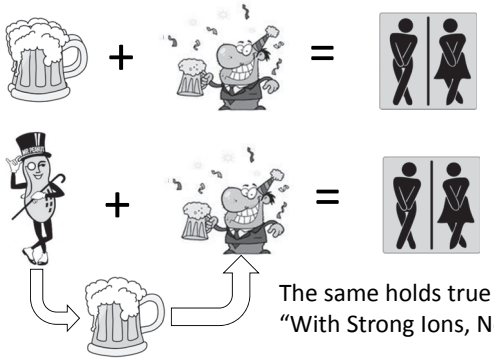
1) Strong Ion intakes in excess of requirements are eliminated in the kidney (urine)
2) SID (Strong Ion Difference) = $Na^+ + K^+ - Cl^-$
   - DCAD is a Proxy for Urinary SID
3) Urinary Strong Ion Excretion (Eq. Basis), The cations must equal the anions:

   $Na^+ + K^+ + H^+ + (NH_4^+) = Cl^- + OH^-(HCO_3^-)$

   Cations Anions

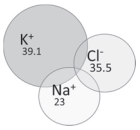
The Beer Example: An Excellent Source of Water?? (That must be eliminated)

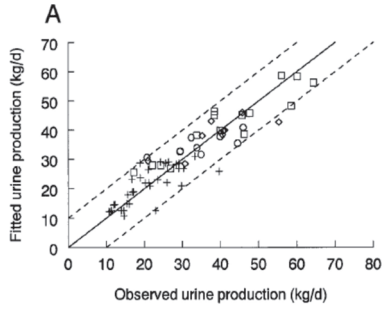
Mechanism



The same holds true with cows!
"With Strong Ions, Not Beer"

Strong Ion Intake Drives Urine Output in Dairy Cows





Urine Volume, kg = 0.115 Na Intake, g/d + 0.058 K Intake, g/d

(R^2 = 0.848, SE = 5.16)

From Bannink et al., 1999, J. Dairy Sci 82:1008

9 g/day Excess Na
or
17 g/d Excess K

1 Liter Extra Urine

Is this the:

- Push Mechanism?
- Pull Mechanism?

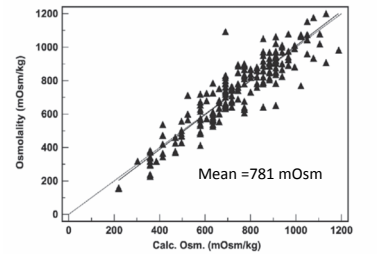
Summary: Strong Ion Effects on Water Intake, Urine Output and pH

- Excess K and Na, Increases
  - Water Intake
  - Urine Output
  - Urine pH
- If you want cows to drink more, increase Diet K and Na
  - More Water Intake, More Watering Space
- Feeding excess Na & K increases urine output
- Excess Cl:
  - Decreases urine pH (Cows like an alkaline, NOT AN ACID Urine)
  - Requires more Na and Cl

Dumping extra strong ions in the diet has consequences! Pay attention, especially to Cl

There is a Limit to how Concentrated Urine can be

Observed Range in Urine Concentration (Milliosmoles/Liter)



Bannink et al. 1999

Projected Urine Conc.

K = 881 mOsm

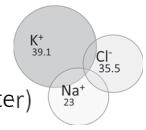
Na = 756 mOsm

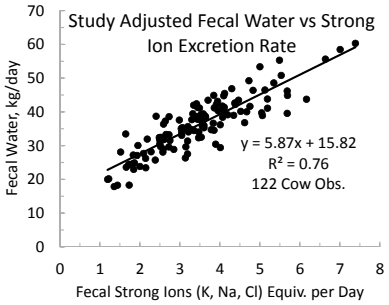
This suggests that the cow is minimizing the amount of water lost in order to get rid of excess K and Na

From Alcántara-Isidro et al. (2015) RRJVS 1:34

Cow Manure is 85% Water

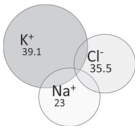
(Something has to hold that water)





- Just like other body fluids:
  - Fecal water is related osmotic pressure
  - Strong ion (K, Na, and Cl) contents
- Implied Strong Ion Osmotic Effect
  - 170 mOsm (more than 50% of total (300)
  - Other Fecal Minerals (Ca, P, Mg), VFA contribute
- This suggests a metabolic fecal strong ion requirement to maintain a constant fecal water

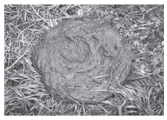
Add First Glance, Added K Appears to Improve N Use (lower MUN)



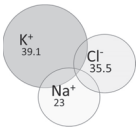
	-------- Added Potassium, mEq/kg DM ---------			
Milk	0	125	250	375
MUN, mg/dL	15.5	14.0	13.6	12.0
Protein %	2.95	2.99	2.95	2.92
Protein, g/d	1143	1174	1158	1124

[1]Iwaniuk et al., 2015 J. Dairy Sci. 98:1950

- But, there was no change in protein % or yield.
- Don't be fooled: Milk MUN went down because urine volume went up to get rid of excess K.
- Same amount of Urea-N excreted in a larger urine volume



Cow Manure is 85% Water



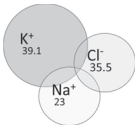
Preliminary Lucas Plot (Regression) Analysis

Apparently Absorbed Ion = Dietary Ion (both as g/kg Diet DM)

Strong Ion	Intercept (Met. Fecal) g/kg DM	Slope (Abs. Coeff.)	RMSE g/kg DM	*P* <
K	-2.48	1.02	0.27	0.001
Na	-1.45	0.98	0.53	0.001
Cl	-1.11	0.92	0.52	0.001

- Implied Absorption Coefficients-Very High
  - ~100 % for K and Na, 92% for Cl
- Most Fecal K, Na, and Cl is Metabolic
  - 2.48, 1.45, and 1.11 g/kg Diet DM, respectively
- Consistent with maintaining constant fecal H_2O

What Do Cows Need? Milk production



Strong Ion	2001 NRC	Castillo et al., 2013[1]	Difference, g/kg	% Change
K, g/kg milk	1.50	1.54	+0.04	+2.6
Na, g/kg milk	0.65	0.41	-0.24	-37.1
Cl, g/kg milk	1.15	1.03	-0.12	-10.4

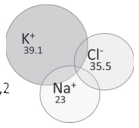
[1]Castillo et al., 2013. J. Dairy Sci. 96 :3388; 39 herds averaging 787 cows per herd

Potassium concentrations seem fine.

More recent data suggests Na = 0.40 and Cl = 1.0

Why is milk Cl and especially Na so much lower now?

What Do Cows Need? 2001 NRC Maintenance + Milk Requirements (g/d)[1,2]



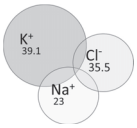
Ion	End. Fecal-Urinary	Met. Fecal	Heat Stress	Total Maint	Milk	Total	% Diet DM	DCAD, mEq/kg
K	29	153	3	185	83	268	1.07	273
Na	29	--	4		32	64	0.26	113
Cl	16	--	--		58	72	0.29	81

[1]1540 lb (700 kg) Cow consuming 55 lb (25 kg) DMI
[2]Assumes true absorption coefficient of 90% for each strong ion

Diet DCAD = 304

How many people feed diets with those concentrations of K, Na, and Cl?

Why are milk Na and Cl so much lower today?



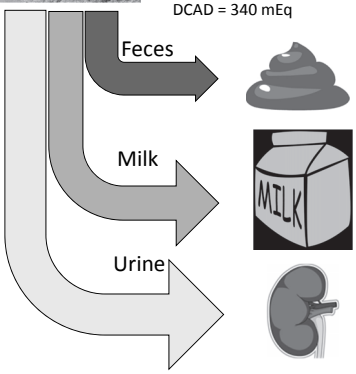
Strong Ion	2001 NRC	Normal Milk[1]	Milk with High SCC[1]	% of Normal
K, g/kg	1.50	1.73	1.54	91
Na, g/kg	0.65	0.57	1.05	184
Cl, g/kg	1.15	0.91	1.47	161

[1]From Review by Harmon, 1994, J. Dairy Sci 77:2103

- 2001 NRC values based on 1965 British estimates
- Mastitis increases milk Na and Cl
- How much has milk SCC has declined in the last 50 years?

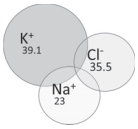
Ratios of Strong Ions in feed: Not the ratio ends up in urine

1540 lb Cow, 55 lb DMI,
110 lb milk,
Diet K, Na, Cl:
1.2, 0.3, and 0.35%
DCAD = 340 mEq



Ion	g/d	Eq/d	Eq Ratio, K:Na:Cl
K	300	7.7	3.1
Na	75	3.2	1.3
Cl	88	2.5	1.0
Minus			
K	63	1.6	1.7
Na	36	1.6	1.6
Cl	34	1.0	1.0
Minus			
K	75	1.7	1.2
Na	20	0.9	0.6
Cl	50	1.4	1.0
Equals			
K	162	4.1	36.7
Na	19	0.8	7.3
Cl	4	0.1	1.0

What Do Cows Need? 2001 NRC Maintenance Req.



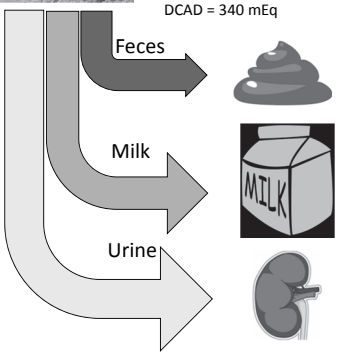
Strong Ion	Endogenous Fecal & Urinary, g/kg BW	Metabolic Fecal (g/kg Diet DM)	Severe Heat Stress g/100 kg BW
K	0.038	6.1 (2.6)	0.40
Na	0.038	--	0.50
Cl	0.0225	--	--

Comments:

- Endogenous Urinary Excretion-Impossible to Measure
  - Dependent on the relative excess of other strong ions
- Metabolic fecal usually expressed per unit diet DM
- Heat stress values not large or well defined

If we increased diet CL to 0.6%

1540 lb Cow, 55 lb DMI,
110 lb milk,
Diet K, Na, Cl:
1.2, 0.3, and 0.35%
DCAD = 340 mEq



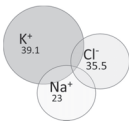
0.35% Dietary Cl
Urinary Bicarbonate:
= K + Na – Cl
= 4.1 + 0.8 – 0.1
= 4.8 equivalents (288 g HCO_3^-)

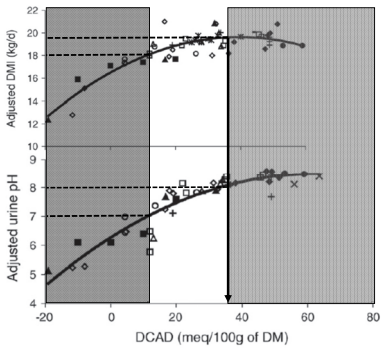
0.60% Dietary Cl (+63 g, +1.8 Eq)
Urinary Bicarbonate:
= 4.1 + 0.8 – 1.9
= 3.0 equivalents (188 g HCO_3^-)

Small change in Cl, Big impact on urinary HCO_3^- and pH

Ion	g/d	Eq/d	Eq Ratio, K:Na:Cl
K	162	4.1	2.4
Na	19	0.8	0.4
Cl	67	1.9	1.0

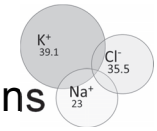
Always Remember: Cows Like an Alkaline Urine





- Urinary cations (K, Na) need to exceed anions (Cl)

Summary: Don't Forget the Strong Ions



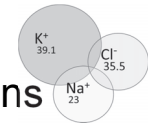
<u>Feed for an alkaline urine (pH ~ 7.5 to 8)</u>

- Remember High DCAD is only a proxy for Urinary SID
- Cows need much more urinary K/Na than Cl
- Adding more NaCl or KCl to diet won't help you!

<u>Watch Cl, Do Feed Analysis!</u>

- Feed enough to meet milk and maintenance needs
- Not too much in excess, leads to lower urine pH
- Small grain and grass silages, can be fairly high in Cl
- <u>If too Cl is too high</u>
  - Add Na or K Carbonate/Sesquicarbonate instead of NaCl or KCl

Summary: Don't Forget the Strong Ions



<u>Water Intake</u>

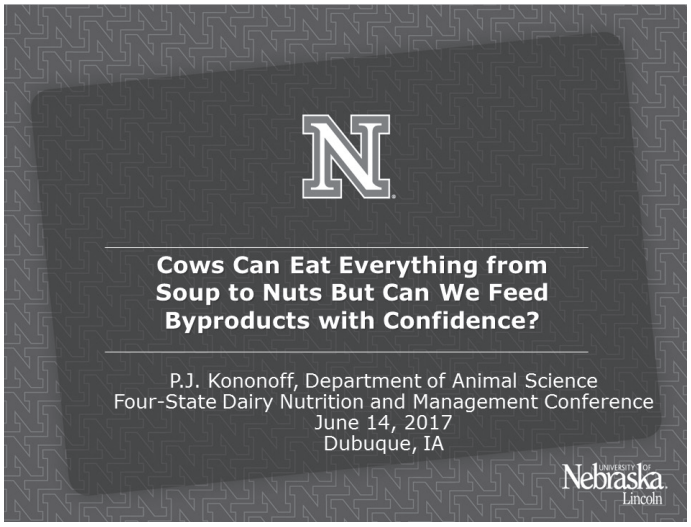
- 9 grams extra Na, 17 grams extra K increase H2O by 1L.
- If want to increase H2O intake:
- Add dietary K, Na
- Make sure that you have good quality water, adequate watering space

Finally, Pay Attention:

"Dumping extra strong ions in the diet has consequences. The cow can handle extra K and Na, but not Cl."

Cows Can Eat Everything from Soup to Nuts But Can We Feed Byproducts with Confidence?

P.J. Kononoff, Department of Animal Science
University of Nebraska



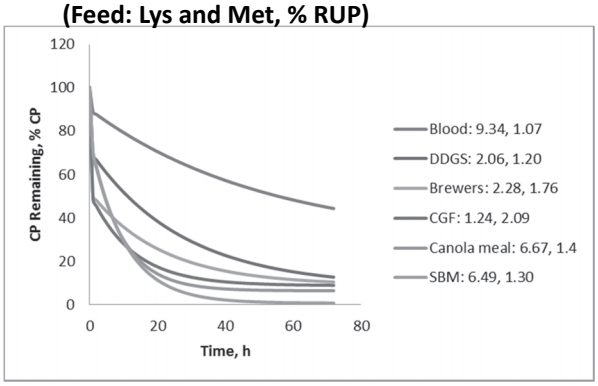
By-Products Defined

- "… secondary products produced in addition to the principle product (AAFCO, 2016)."
- originate from a wide range of industries including the food, fiber, beverage, and bioenergy industries.

Dodge Co., WI

http://www.cnn.com/2017/01/19/health/spilled-skittles-road-trnd/

Key byproducts
Rumen Disappearance of CP ,
(Feed: Lys and Met, % RUP)



Animal Byproducts

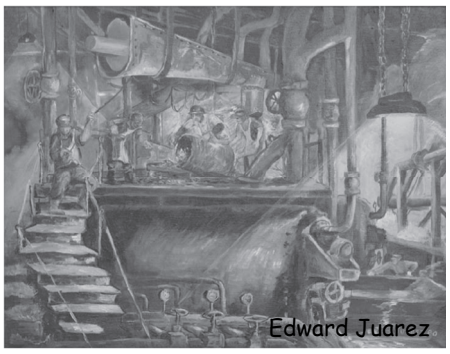
Slaughter numbers

Species	Number, million hd
Cattle	30.6
Swine	118
Chickens	8909
Turkeys	243

Byproduct production

Product	000 tons
Tallow	1, 954
White grease	564
Yellow grease	833
Meat and bone meal	2, 464
Poultry meal	1, 256
Feather meal	479

Animal Byproducts



Essential Rendering, all about rendering

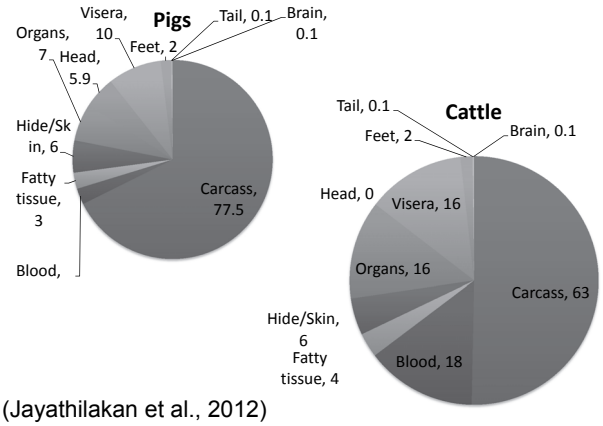
- D.L. Meeker, Editor

http://www.nationalrenderers.org/publications/essential-rendering/

Bloodmeal: Summary

Description	Notes
Nutritional aspects	
Key Nutrients	
(In)Digestibility	
Challenges	
Anti-nutritional	
Toxins	
Contaminants	
Logistical Aspects	
Other Notes	

By-products as a % of market live weight



(Jayathilakan et al., 2012)

Canola Meal, 41 % CP, 29 % NDF, 3.5% EE

- Canola meal is the meal remaining after the extraction of oil from *Brassica* seeds by either mechanical or solvent extraction methods (AAFCO, 2016).
- Canola is a trademarked name for rapeseed which contains < 2 % erucic acid in the oil and < 30 µmoles of alkenyl glucosinolates per gram of oil-free DM
- Glucosinolates: bitter, impair palatability, interfere with the synthesis of thyroid hormones by impairing the uptake of iodine (Woyengo et al., 2016).

Canola Meal

	CM	2/3 CM	1/3 CM	DDGS
Corn Silage	27.5	27.5	27.5	27.5
Alfalfa Hay	27.5	27.5	27.5	27.5
Ground Corn	34.9	33.9	33.0	31.8
Canola Meal	**6.63**	**4.6**	**2.3**	**0**
DDGS	**0**	**3.24**	**6.63**	**10.4**
RP Fat	**1.6**	**1.4**	**1.2**	**1.0**
Min/Vit	**1.25**	**1.25**	**1.25**	**1.25**
CP	15.1	15.0	15.1	15.1
NEL, Mcal/pd	0.72	0.72	0.71	0.71

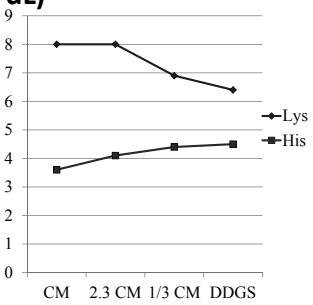
Mulrooney et al. (2009)

Canola Meal: Summary

Description	Notes
Nutritional aspects	
Key Nutrients	
(In)Digestibility	
Challenges	
Anti-nutritional	
Toxins	
Contaminants	
Logistical Aspects	
Other Notes	

Milk Production Plasma AA (uMol/dL)

	CM	2/3	1/3	DDGS
DMI, lbs	56.0	56.4	57.6	55.8
Milk, lbs	78.2	79.5	76.7	76.2
ECM, pds/d	81.6	85.3	81.3	79.3
Protein, %	3.05	3.06	3.06	3.01
Fat, %	3.81	4.05	3.97	3.87



J. Dairy Sci. 92:5669–5676
doi:10.3168/jds.2009-2276
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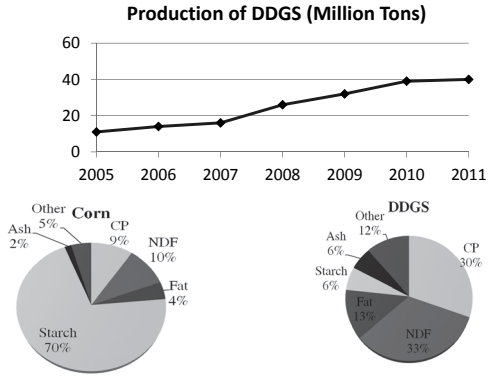
Canola meal replacing distillers grains with solubles for lactating dairy cows

C. N. Mulrooney, D. J. Schingoethe,† K. F. Kalscheur, and A. R. Hippen
Dairy Science Department, South Dakota State University, Brookings 57007-0647

Corn-Ethanol



Key Amino Acids (Schwab et al., 2005)

Item	His, % CP	Lys, % CP	Met, % CP
Milk	**2.7**	**7.6**	**2.7**
Bacteria	**2.0**	**7.9**	**2.6**
Alfalfa Silage	1.7	4.4	1.4
Corn Silage	1.8	2.5	1.5
Grass Silage	1.7	3.3	1.2
Barley	2.3	3.6	1.7
Oats	**2.4**	4.2	2.9
Wheat	**2.4**	2.8	1.6
Corn	**3.1**	2.8	2.1
DDGS	2.5	2.2	1.8
Brewers Grains	2.0	4.1	1.7
Canola Meal	2.8	5.6	1.9
SBM	2.8	6.3	1.4

DDGS Fuel-Ethanol

Limiting AA in diets with DDGS?

	Control	10 % DDGS	20 % DDGS	30% DDGS
Corn Silage	38	38.0	38.0	38.0
Alfalfa Hay	12.0	12.0	12.0	12.0
Ground Corn	17.6	17.3	16.8	16.3
RFDG, 3.5% EE	**0.0**	**10.0**	**20.0**	**30.0**
SBM, 44% CP	**8.1**	**5.3**	**2.7**	**0.0**
By-pass Soy	**9.3**	**6.2**	**3.1**	**0.0**
Soyhulls	**12.0**	**8.0**	**4.0**	**0.0**
Rumen Inert Fat	0.44	0.62	0.84	0.98
Vit/Min	2.52	2.59	2.65	2.73

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Lactation performance and amino acid utilization of cows fed increasing amounts of reduced-fat dried distillers grains with solubles[1]

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Limiting AA in diets with DDGS?

	Control	10 % DDGS	20 % DDGS	30% DDGS
Corn Silage	38	38.0	38.0	38.0
Alfalfa Hay	12.0	12.0	12.0	12.0
Ground Corn	17.6	17.3	16.8	16.3
RFDG, 3.5% EE	**0.0**	**10.0**	**20.0**	**30.0**
SBM, 44% CP	8.1	5.3	2.7	0.0
By-pass Soy	**9.3**	**6.2**	**3.1**	**0.0**
Soyhulls	12.0	8.0	4.0	0.0
Rumen inert Fat	0.44	0.62	0.84	0.98
Vit/Min	2.52	2.59	2.65	2.73
Chemical Composition				
CP, % DM	17.7	17.7	17.6	17.6
NDF, % DM	40.7	42.0	41.3	41.9
LYS, % MP	6.38	6.04	5.65	5.33
MET, % MP	1.72	1.73	1.73	1.75
LYS:MET, % MP	3.7	3.5	3.3	3.0

Modified DGS
DM = 45.6
NDF = 30.8
CP = 30.2
Fat = 13.5

WCGF
DM = 55.9
NDF = 36.9
CP = 23.1
Fat = 5.1

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Nitrogen utilization, nutrient digestibility, and excretion of purine derivatives in dairy cattle consuming rations containing corn milling co-products

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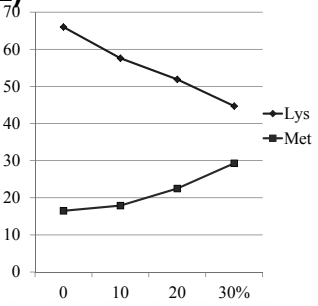
Milk Production Plasma AA (uMol/L)

	0 %	10%	20%	30%
DMI, lbs/d	50.4	51.1	52.7	49.3
Milk	76.7	77.3	78.9	78.2
ECM, lbs/d[1]	72.4	77.3	78.8	78.2
Protein, %[2]	2.99	3.06	3.13	2.99
Fat, %[1]	3.18	3.40	3.46	3.72
Protein Yield, lbs/d	2.29	2.38	2.44	2.36
Fat Yield, lbs/d	2.4	2.6	2.7	2.9

[1] Linear effect
[2] Quad effect



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Lactation performance and amino acid utilization of cows fed increasing amounts of reduced-fat dried distillers grains with solubles[1]

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†Dairyland Inc., Brookings, SD 57006

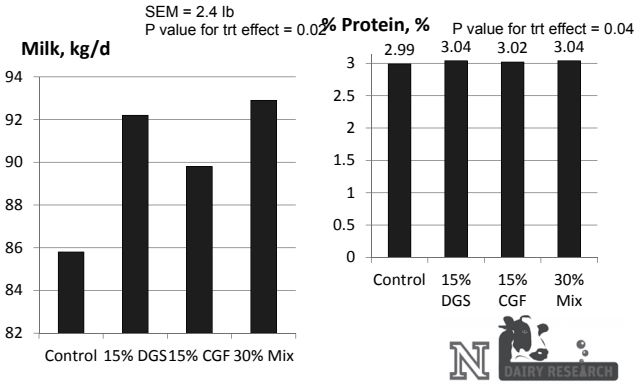
	Control	MWDGS	WCGF	30% Blend
Modified DGS	---	15.0	---	15.0
WCGF	---	---	15.0	15.0
Corn silage	28.0	25.5	23.0	24.0
Alfalfa haylage	9.8	9.0	8.0	3.5
Alfalfa hay	9.8	9.8	8.0	3.5
Brome hay	3.5	3.0	3.0	6.0
Ground corn	17.5	13.5	14.5	9.5
SBM	6.0	3.5	5.5	3.2
Soy Pass	6.0	4.0	4.5	3.5
Cottonseed	6.0	5.5	5.5	4.0
Soybean hulls	10.0	10.2	10.0	10.0
Tallow	1.0	---	1.0	---
Urea	0.24	---	---	---
Vitamins and minerals	2.1	2.0	2.1	2.8

DDGS: Summary

Description	Notes
Nutritional aspects	
Key Nutrients	
(In)Digestibility	
Challenges	
Anti-nutritional	
Toxins	
Contaminants	
Logistical Aspects	
Other Notes	

	Control	MWDGS	WCGF	30% Blend
CP	18.5	18.7	18.6	18.6
NDF	35.0	36.6	35.0	37.0
Starch	23.7	20.4	21.6	18.8
EE	4.0	5.8	4.0	5.6

Impact of DGS and CGG



Experimental Approach

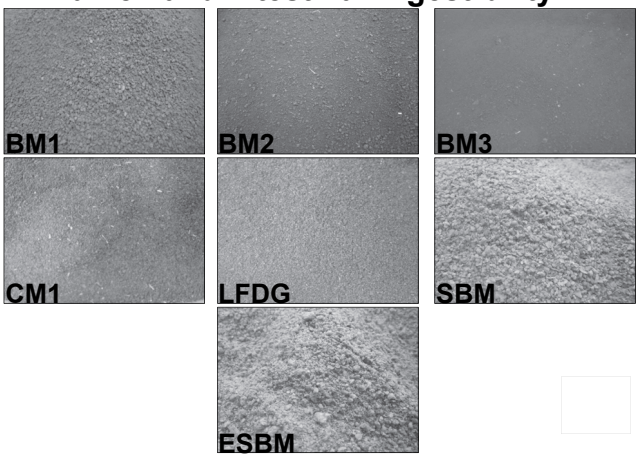
- 5 samples of each feed were collected during the summer of 2012.
- Analyzed for chemical composition (including AA)
- RUP determined using
  - the nylon bag (In Situ) procedure
    - Microbial correction with purines and DNA
  - In vitro (Cleale et al., 1987 and Ross et al., 2013)
- dRUP determined by mobile bag and invitro

(Paz and Kononoff, 2014)

Corn Gluten Feed: Summary

Description	Notes
Nutritional aspects	
Key Nutrients	
(In)Digestibility	
Challenges	
Anti-nutritional	
Toxins	
Contaminants	
Logistical Aspects	
Other Notes	

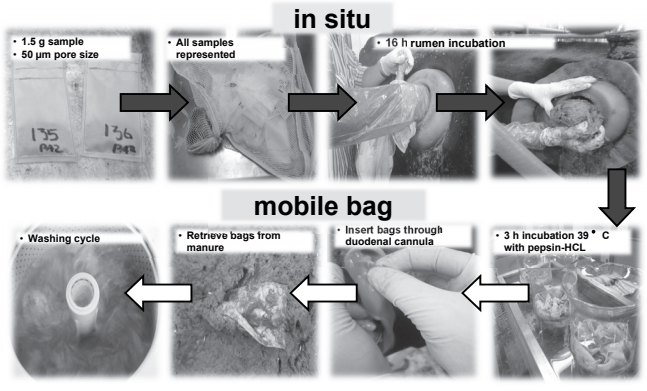
Rumen and Intestinal Digestibility



UNL Study on RUP supplies

- Determine ruminal degradation and intestinal digestibility of CP and AA of
- Blood meal
  - **BM1**
  - **BM2**
  - **BM3**
- Canola Meal **(CM)**
- DDGS **(LFDG)** low-fat distillers dried grains with solubles;
- Soybean Meal (48% CP) (**SBM**);
- Expeller SBM, (**ESBM**).

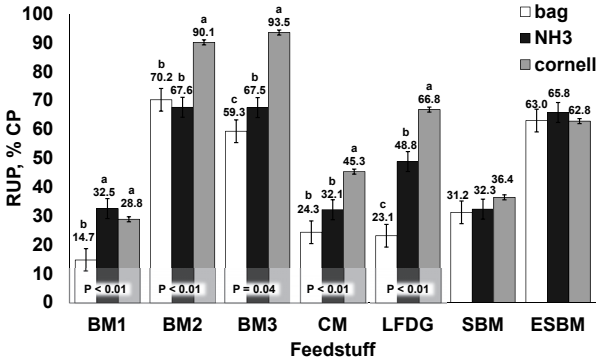
(Paz and Kononoff, 2014)



- **RDP** = $\frac{\text{initial CP in sample – final CP in residue}}{\text{initial CP in sample}} \times 100$
- **RUP** = 100 - RDP

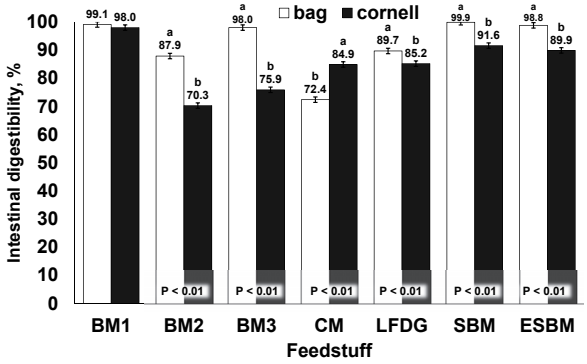
Hvelplund, 1985

rumen-undegradable protein



Effect – P-value					
feedstuff	< 0.01	procedure	< 0.01	feedstuff × procedure	< 0.01

intestinal digestibility RUP



Effect – P-value					
feedstuff	<0.01	procedure	<0.01	feedstuff × procedure	<0.01

Incorporating Diet and Pen Variation into Ration Formulation

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Summary

The composition of a diet fed to a group of cows varies batch to batch, and depending on the degree of variation, milk production and cow health could be negatively affected. If the batch to batch standard deviations (SD) for important nutrients are known, accurate diet safety factors can be calculated. For example, if you wish to reduce the risk of feeding a diet with inadequate fiber because of nutrient variation, you can use the SD to determine the formulation target for NDF (or any other nutrient). Within a pen, nutrient requirements vary cow to cow. Knowing the within pen SD in milk yields among cows can be used to determine nutrient specification for the diet. On average metabolizable protein allowable milk should be about 1 SD greater than mean milk yield for the pen (if the pen does not contain fresh cows). More emphasis should be placed on knowing variation in diet composition and requirements within a pen. This will require collating feed composition data within a farm so nutrient variation can be calculated and it will require means of obtaining individual cow milk yield data (e.g., milk meters or using DHI data). Incorporating variation in ration formulation should reduce feed costs, while maintaining high milk yields. It could also reduce the amount of nutrients excreted in manure which will reduce environmental issues. Overall farm efficiency could be increased.

Introduction

Most currently available ration software uses definite inputs and produces definite solutions as opposed to using stochastic inputs and producing stochastic results. Examples of definite inputs are the corn silage you are going to use has 41% NDF and the milk production for the pen you are formulating is 85 lbs. Stochastic inputs could be the corn silage averages 41% NDF but varies + 3 percentage units and the pen averages 85 lbs. of milk but milk production by cows within the pen varies by + 30 lbs. In reality, all inputs needed by ration software (e.g., nutrient composition, milk yield, milk composition, body weight, etc.) are not constant but vary and in some cases vary widely. The question is: Should that variation be taken into account when formulating diets?

Variation in nutrient composition

A primary reason to incorporate nutrient variation into diet formulation is to ensure (with a certain amount of uncertainty or risk), that the formulated diet provides adequate nutrients or the formulated diet does not provide excessive nutrients. The nutritionist must decide whether reducing the risk of under feeding or over feeding is more important.

The variation in nutrient composition of feeds can be determined by taking an adequate number of samples from the feed over time and using a spreadsheet or some other means to calculate the standard deviation (SD). The number of samples and the timing of samples varies depending on the feed. For corn silage, 5 or 6 samples taken over a period of a few weeks often is adequate for estimating SD. For alfalfa silage with multiple cuttings, more samples over a longer period of time may be needed. For most forages and wet feeds (e.g., high moisture corn, wet corn gluten feed, or wet brewers grains), an estimate of variation within the farm is needed because of large farm to farm variation (St-Pierre and Weiss, 2015). In other words, those feeds need to be sampled at each farm and the SD is calculated from those samples. You should not go to a national or regional database to obtain the SD. For many other feeds such as dry corn, soybean meal, dry corn gluten feed, and soyhulls, farm to farm variation is not large and you can use the SD from national or regional databases (e.g., www.nanp-nrsp-9.org). However, variation in nutrient composition of ingredients is not the same as variation in nutrient composition of a TMR. If care is followed when making the TMR (i.e., recipe is carefully followed and inclusion rates are adjusted for DM), the variation in nutrient composition of the TMR will almost always be less than the weighted average variation in the ingredients. For example if 70%, 18%, and 12% of the NDF in a diet is provided corn silage (SD=1.7), alfalfa silage (SD=2.0), and concentrate (SD= 0.48) respectively, the weighted average SD for

the TMR (SD has to be squared first, then averaged and then converted back to SD) = Square root of [0.7*(1.72) + 0.12*(0.482) + 0.18*(22)] = 1.66. This is not the correct method for calculating SD of a TMR because it assumes the variation within feeds are not independent. However if you sampled (accurately and precisely) the TMR, the SD may only be 60 or 70% as large as that value (these calculations will be discussed below). The reason why the variation in nutrient composition of a TMR is almost always less than the weighted average variation in ingredients is because the nutrient composition of feeds varies independently. For example on Monday, the NDF concentration of corn silage was higher than average but the NDF concentration in the alfalfa was lower than average so the overall deviation in diet NDF would be less than the deviation for either ingredient.

What ultimately matters is not the variation within ingredients but the batch to batch variation in TMR composition; however, obtaining the SD of TMR is difficult. You could sample TMR over several days, have the samples analyzed and calculate the SD, but sampling a TMR is problematic (discussed in another paper in this Proceedings). Another option is to use Monte Carlo simulation using inclusion rates of ingredients and their mean and SD for nutrient of interest. What this approach basically does is calculate expected concentrations of the nutrient (in this example NDF) for hundreds of diets incorporating expected ingredient variation into each calculated diet. The SD for NDF from those hundreds of simulations is then calculated. Spreadsheets such as Excel can do these simulations. Another option is to sample each TMR ingredient over multiple days and then calculate the expected TMR concentration each day using the daily concentration data and inclusion data (preferably actual inclusion as recorded by TMR software). The SD is then calculated from those daily calculated concentrations. We did an experiment to compare SD of TMR calculated as the weighted average SD from ingredients to SD of TMR calculated from daily delivery data (Table 1). For DM, the daily SD (which is the more correct value) ranged from 61 to 103% of the ingredient calculated SD; for NDF the range was 60 to 74% and for CP the difference ranged from 19 to 53%. Across TMR and nutrients the more correct estimate of TMR SD was about 65% of the value calculated from ingredient SD.

If you go to all the trouble of calculating an accurate estimate of batch to batch SD, you need to know what to do with the number. The effect of day to day variation (this reflects batch to batch variation for pens fed once daily) in TMR composition on cows has only recently been researched. We have conducted 4 studies to determine effects of day to day variation in dietary concentrations of CP, DM, NDF, and fat and in most cases we saw no or only very modest negative effects of substantial variation (McBeth et al., 2013; Weiss et al., 2013; Yoder et al., 2013; Brown and Weiss, 2014). However in an epidemiological type study (Sova et al., 2014) found a negative relationship between day to day variation in NEL concentration and milk yield across herds. At this point, data are equivocal, but modest day to day variation in many nutrients probably is not a major issue; hence setting benchmarks for day to day TMR SD and then striving to reduce variation to match the benchmark may not have a great pay off.

Another use of TMR SD is risk management. In other words, knowing the SD for TMR composition can be used to set farm specific safety factors for important nutrients. Underfeeding nutrients for a long enough time (this may be just a few days or several weeks depending on the nutrient) will negatively affect milk production and/or cow health and reproduction. Overfeeding nutrients often inflates feed costs, increases excretion of nutrients in manure, and depending on the nutrient and degree of overfeeding negatively affect health and production of cows. The nutritionist needs to set both upper and lower targets on nutrient composition and determine how much risk he or she is willing to accept. For example, a diet might be formulated to average 30% NDF, but because of past experiences with health issues, the nutritionist does wants the TMR to have <28% NDF (i.e., the target), 95% of the time (i.e., only 1 day out of every 20 days). If one knows the SD for TMR NDF, the formulated concentration of NDF for the diet can be calculated so that the diet is only below 28% NDF 5% of the time (Figure 1). Setting targets (both low and high) can be based on past experience, nutritional models (e.g., a model predicts a significant drop in milk yield if diet protein is less than 15%), feed costs, environmental regulations and other factors. Because of all these factors, these have to be set on a farm to farm basis; I cannot provide universal estimates.

Once the lower or upper target is set, the acceptable risk of feeding a diet greater than or less than that target must be determined. You may determine that a diet that is less than your lower target can be fed once a week without causing a problems (1 out of every 7 days or approximately 14% of the time). Or you may determine you only want to feed a diet that has less than the target value 1 day out of every 20 days (5% of the time). Determining an acceptable risk level is farm specific; however, reducing risk also comes with a cost. For example if you want to essentially eliminate the risk of feeding a ration that is inadequate in protein, you will need to formulate a diet that is extremely excessive in protein. In this case you will

probably never underfeed protein but feed costs will be high and a substantial amount of nitrogen will be excreted in manure potentially causing environmental problems. Overfeeding NDF reduces the risk of acidosis but increases the risk of reduced dry matter intake and lower milk yields. When setting risk levels and lower or upper targets the potential cost of over or underfeeding nutrients must be considered. Probabilities of being greater or less than a specific value can be calculated using the normal distribution curve (Figure 1). As good approximations:

15% of values will be < (mean – **1.0***SD); 15% of the values will be > (mean+1*SD)
10% of values will be < (mean – **1.25***SD); 10% of the values will be > (mean+1.25*SD)
5% of values will be < (mean – **1.65***SD); 5% of the values will be > (mean+1.65*SD)

Those risk coefficients (1.0, 1.25, and 1.65) in conjunction with the lower (or upper) Target value are used to calculate the concentration of the nutrient in the formulated diet:

When you are more concerned about a deficiency:
Formulated Concentration = Low Target + (Risk*SD)

Conversely if you are more concerned about excessive concentrations;
Formulated Concentration = High Target – (Risk*SD)

As an example, you determined that you do not want to feed a TMR with <28% NDF more than 5% of the time and the SD for NDF in the TMR is 2.0. Based on above factors, 5% risk = 1.65 SD units. Therefore the formulation target for NDF is 28 + (1.65*2.0) = 31.3% NDF (Figure 2). This means that if you formulate a diet for 31.3% NDF and the SD remains at 2.0, the TMR will have less than 28% NDF about once every 20 days. Conversely it will have more than 33.8% NDF about 1 day out of every 10 days. If you wish to be less conservative and set the risk level at 15% (i.e., 15% of the time your diet has <28% NDF) formulating the diet for 30% NDF is adequate (Figure 1). This calculation can be done for any nutrient if it approximates a normal distribution and you have an estimate of the SD for the TMR.

Variation in cow inputs within a pen

Incorporating within pen variation (i.e., cow to cow) into the diet formulation process should be useful in determining the desired nutrient specifications for a given diet. In this situation, variation over time is usually not a major concern because pen average milk yield, body weight, and milk composition probably does not change much day to day. However within a pen on a given day there may be cows producing 30 lbs. of milk and cows producing 150 lbs. of milk. The primary cow inputs into ration formulation software are body weight (BW), milk yield, milk composition and parity (as a proxy for body growth), and variation in those inputs create variation in calculated nutrient requirements. Increasing BW increases the energy (NEL) and metabolizable protein (MP) requirement of cows; however, the ranges observed in BW of cows within a herd (assuming a single breed) usually are not large enough to substantially affect nutrient requirements. This means that using pen average BW (or even breed average BW) is probably adequate when formulating a diet for the pen. Yields of milk, milk fat and milk protein can vary greatly among cows within a pen, and the observed range will depend on the grouping system used by the farm. Farms that group based on milk production will have smaller within pen ranges in milk yields than farms that group based on other criteria. Yields of milk and milk components have a substantial impact on energy and protein requirements and using a pen mean milk yield when formulating a diet will result in higher producing cows being underfed causing reduced production. To overcome this problem, most nutritionists choose a milk yield greater than pen average and formulate to that value. Often the selected value is rather arbitrary (e.g., , 10 lbs. above the average for the pen). Using the SD in yield of milk within a pen, rather than an arbitrary constant should result in more accurate diet formulation by reducing the risk of underfeeding high producing cows while minimizing the degree of overfeeding lower producing cows.

Not only does nutrient requirements vary within a pen, so does dry matter intake (DMI). Milk yield is positively correlated with DMI but the strength of the correlation depends on stage of lactation. If stage of lactation is not considered, then the correlation is relatively week (e.g., cows in early lactation may have high milk yield but low DMI). When early lactation data are excluded (generally <30 days in milk) the correlation between milk yield and DMI is about 0.7 (Kramer, 2009). This means that if the pen does not contain fresh cows, one should assume higher producing cows are eating more feed than lower producing cows. Therefore when a diet is balanced for an average cow in a pen, the diet will support greater than average milk yields because of greater intake. But when a diet is balanced for mean milk yield, will the greater intake by higher producing cows be adequate to maximize their milk yield? For cows past 30 or 40 days in milk (and assuming similar BW), a 10 lbs. increase in fat-corrected milk yield would be associated with a 3 to 3.5 lbs. greater DMI when cows were fed the same diet. On average, that increase in

DMI will not provide adequate NEL and MP as milk yields exceed mean milk by more than about 15 lbs./day when the diet is formulated to meet requirements for the average cow. For example, if a group of cows (all cows >30 DIM) averages 80 lbs. of milk/day expected DMI is about 54 lbs. (NRC, 2001), If the diet was formulated to exactly meet NEL and MP requirements for the average cow, a cow producing 95 lbs. of milk would be expected to eat about 60 lbs. of DM and that would provide enough NEL and MP to support about 90 lbs. of milk (using NRC, 2001 equations). If all the equations we use are perfectly accurate (which they most definitely are not) and if the high producing cow has similar digestive and metabolic efficiency as the average cow, then when you feed for the average cow, the cow that was producing 95 lbs. would start producing 90 lbs. This is greater than the mean but milk production was lost. Using the same assumptions, a cow that was producing 150 lbs. of milk/day would drop about 25 lbs./day when switched to the 80 lbs. diet. Clearly you do not want to formulate for the average.

Most nutrition models used today will calculate protein and energy allowable milk. These numbers simply mean that if a cow consumed the formulated diet at the stated DMI, she has enough MP and NEL to produce those allowable yields of milk. The optimal degree of overfeeding depends on feed costs and milk price (e.g., the degree of overfeeding should be reduced when feed costs are high and milk price is low). Assuming a typical feed cost to milk price ratio, based on simulations and assuming a normal distribution of milk yields within a pen, and that the pen does not contain cows <30 DIM, MP allowable milk should be about 1 SD above mean milk for the pen (Weiss, 2014; Cabrera, 2016). This is not the same as using the mean + 1SD to formulate the diet. Mean milk and mean DMI (or estimated DMI using mean milk) should be used when formulating but MP allowable milk should be 1 SD above what the diet was formulated for. This degree of overfeeding should not be applied to all nutrients. For most minerals and vitamins, a 20% safety factor is probably adequate (i.e., NRC requirement X 1.2). Overfeeding of NEL has to be evaluated very carefully. The lower producing cows in a pen fed a diet with moderately excess MP simply excrete the excess nitrogen and although this has an environmental and economic cost, it does not affect the cow greatly. However a cow fed excess NEL, if all the equations are correct, will gain BW and condition. In many cases this is desirable but cows may become excessively fat. In general, NEL should be overfed less than MP; however body condition score should be monitored and NEL adjusted to obtain the desired condition.

The main problem with using SD to determine the appropriate degree of MP overfeeding is that the SD in milk yields within a pen is not known on most farms. Based on very limited data (which means it is likely wrong), within pen SD for milk yields on farms that did not group by milk production averaged 16% of the average milk yield. Therefore if a herd averaged 85 lbs. and did not group by milk yield, an estimated SD would be 85 * 0.16 = 13.6 lbs. Using the above information, MP allowable milk for this pen should be approximately 85+13.6 = 98 or 99 lbs./day. When cows are grouped by milk yield the within pen SD should be markedly lower but I do not know how low it would be. The primary reason feed costs should be less when cows are grouped by production is because the degree of overfeeding is reduced (Table 2). If DHI or other production data are available, SD can be calculated and should be used in formulation.

The information above was limited to pens that contained cows >30 DIM. Dry matter intake of fresh cows (less than 3 or 4 weeks in milk) is low relative to milk yield so the above factors are not appropriate for a group of fresh cows. At this time, adequate data are not available to determine the degree of overfeeding for protein that should be applied to this group. Rather than meeting MP and NEL requirements, the primary goal when formulated a diet for this group is to maximize DMI.

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Table 1. Variation (SD) in DM, CP, and NDF for ingredients and TMR (each sampled 6 different days over a 2 week period)[1]

	Standard Deviation, % of DM		
Feed	DM	NDF	CP
Corn silage	1.53	1.31	0.30
Alfalfa silage	2.10	0.57	0.18
Mixed silage	1.27	0.85	0.28
Grass hay	0.85	1.09	0.69
Mixed hay	0.81	2.24	0.33
Concentrate H	0.89	0.53	0.45
Concentrate D	0.32	1.33	1.25
Concentrate C	0.56	1.27	0.86
Whole cottonseed	0.49	4.21	2.20
SD from ingredients[2]			
TMR-H	1.27	0.92	0.32
TMR-D	1.51	1.17	0.59
TMR-C	1.24	1.77	0.89
SD from daily samples[3]			
TMR-H	1.31	0.67	0.061
TMR-D	0.91	0.87	0.31
TMR-C	1.08	1.06	0.36

[1] TMR-H was comprised of corn silage, mixed silage, and concentrate H. TMR-D was comprised for corn silage, alfalfa silage, mixed hay, and concentrate D, and TMR-C was comprised of corn silage, alfalfa silage, grass hay, whole cottonseed and concentrate C.

[2] SD were calculated as weighted average of SD for each ingredient (i.e., incorrect method)

[3] SD were calculated based on daily composition of the TMR. This is the correct method for calculating SD for TMR, however the values shown are just example values, they may not be correct for other situations.

Table 2. Example of how grouping cows to reduce within pen variation in milk yield can reduce feed costs. For the 1 group system, the diet would be formulated to contain adequate metabolizable protein to support 87 lbs. but with a 3 group system, the herd average diet would only need to contain adequate MP to support 81 lbs. of milk.

Grouping system	Average milk, lbs	SD[1], lbs	MP allowable milk, lbs
1 group	75	13	87
3 groups[2]			
Low cows	60	5	65
Medium cows	75	6	81
High cows	90	7	97
Average for herd	75	--	81

[1] SD for 1 group system was assumed to equal 16% of the average. For the 3 group system, SD was assumed to be reduced by 50% (i.e., 8% of mean)

[2]Group sizes were assumed to be equal.

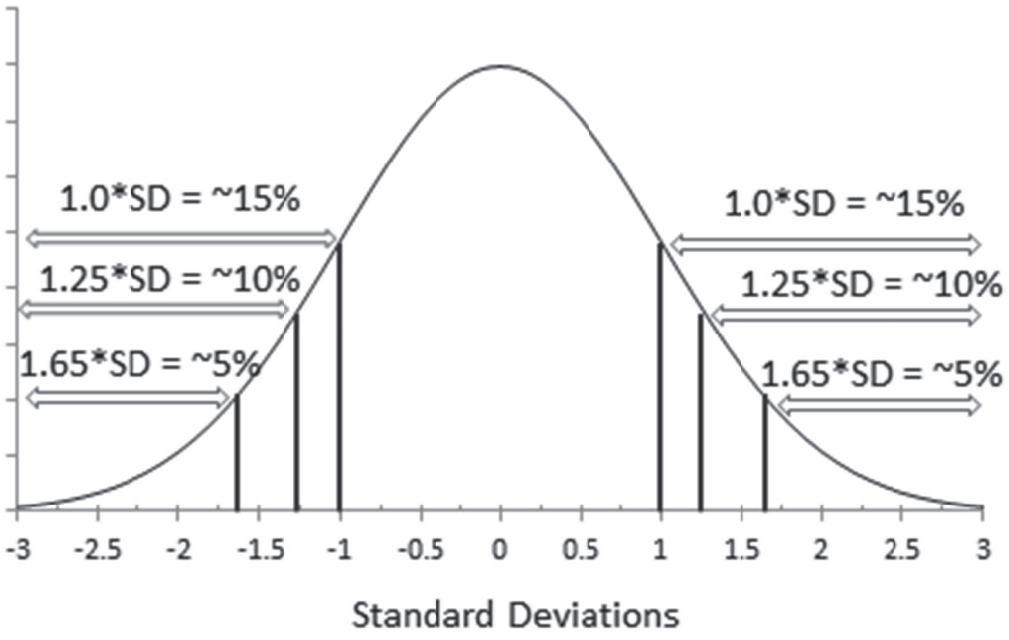

Figure 1. Normal distribution and approximate percentage of samples greater or less than specific distances (in standard deviation units) from the mean

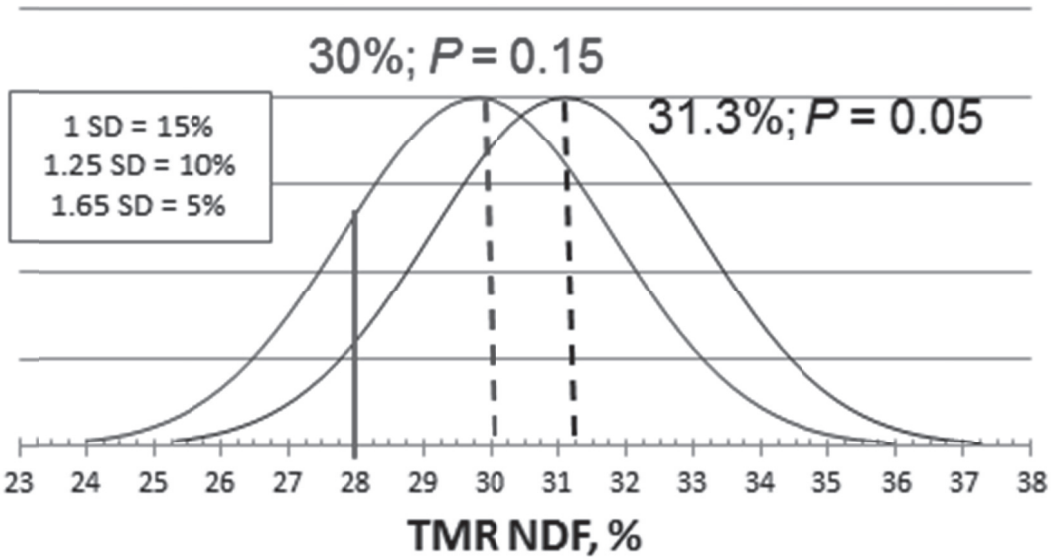

Figure 2. Example of using variation in TMR composition to formulate a diet. In this example, the nutritionist determined that she wanted to reduce the risk of feeding diets with <28% NDF and the SD for TMR NDF was 2.0. If the nutritionist was willing to accept a 15% risk (about once weekly) of feeding diets with <28% NDF, formulating for 30% NDF will be adequate. However, if she wanted to reduce the risk to 5% (1 day out of every 20), the diet should be formulated for 31.3% NDF. That was calculated as Lower limit (28% NDF) + SD (2.0) times risk factor (at 5% it is 1.65): 28 + (2 x 1.65) = 31.3.

Are All Clays Created Equal? Clay Utilization in Diets forDairy Cows

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TAKE HOME MESSAGE

- Clay's mode of action is commonly associated with its ion-exchanging capacity.
- Feeding clay can help to alleviate the effect of a grain challenge on the rumen environment and ultimately affected the performance of Holstein cows.
- Inclusion of clay products in the diet seem to linearly reduce aflatoxin transfer from the rumen and diet to the milk and feces of mid-lactation Holstein cows.

INTRODUCTION

Ruminant animals have evolved from their sole purpose in agriculture to a broad agricultural research role in areas of genetic engineering, biotechnology, clinical application, and more importantly for agricultural research itself (Underwood, et al. 2015). Agricultural research in the dairy industry has led to improvements in the performance of that animal i.e. reproduction, health, milk yield and components, and even medicinal purposes. Whatever the type of research, animals need to be healthy to be a candidate in order to conduct trials. All animals are subject to infection from bacteria, viruses, and fungi, but ruminant animals, specifically dairy cattle, can get diseases purely from what they eat (Underwood, et al. 2015). For example, the formulation of a diet can cause severe pH changes that can lead to acidosis, or their feed can be contaminated with fungi or bacteria that produce toxins.

Acidosis

Dietary ingredients in dairy cow diets affect animal efficiency and health. To produce milk at maximum efficiency, concentrates are required as a feed choice, but a high inclusion of concentrate in the TMR has gained popularity (Eastridge, 2006). Increasing concentrate to-forage ratios and more elaborate grain processing in lactating dairy cow diets have been associated with higher milk production (Khorasani and Kennelly, 2001; Yang et al., 2001). However, too much concentrate can challenge the cow's natural buffering capacity and leave the rumen susceptible to drastic drops in pH levels (Shaver et al., 2000). Knowing the diurnal rhythm of rumen pH is crucial to understanding when a cow confronts sub-acute ruminal acidosis (**SARA**) (Enemark, 2008). The minimum rumen pH fluctuates from 5.4 to 6.6, making it difficult to distinguish what is truly SARA (Duffield et al., 2004; Krause and Oetzel, 2006). Gozho et al. (2005) have defined SARA as when rumen pH is between 5.2 and 5.6 for at least 3 h/day (**d**). Cows facing SARA may experience symptoms such as decreased dry matter intake (**DMI**) and milk production, altered milk composition, diarrhea, and laminitis (Duffield, et al., 2004; Gozho et al., 2005; Krause and Oetzel, 2006; Plaizier et al., 2008). Even though SARA is difficult to diagnose, it is estimated to be prevalent in 19 to 26% of early- and mid-lactation dairy cattle (Enemark, 2008; Plaizier et al., 2008).

Toxins

Mycotoxins have constantly been a feed safety issue because of their harmful nature to ruminant animals when ingested (Campagnollo et al., 2016). There are a plethora of mycotoxins in the world, but most importantly, there has been a rising food safety concern with aflatoxins (**AF)** due to their capability of quickly being transferred into milk (Benkerroum 2016; Campagnollo et al., 2016; Zhu et al., 2016). There are no known treatments available to treat the toxic effects of aflatoxin, but in the United States the FDA has set regulations on the amount of contamination in feed to 20 µg/kg AFB1 and in milk to 0.5 µg/kg AFM1 (Peraica et al., 1999; Giovati et al., 2015). Aflatoxins are produced by many fungi species in the genus Aspergillus and are notorious for infecting 25% of crops in all stages of production, growth, harvest, and storage (FAO, 2004; Kabak et al., 2006; Campagnollo et al., 2016). There have been various technologies developed to diminish the impact of mycotoxins in the dairy industry. Some of these physical and chemical technologies such as UV-treatments or chemical reactions, are expensive and difficult to implement on farms (Kabak et al., 2006; Zhu et al., 2016). Overall, the addition of clay adsorbents, i.e. smectites, illites, and vermiculites seem to be a fairly easy and inexpensive way to mitigate the effects of mycotoxin on animal health and performance (Kabak et al., 2006; Zhu et al., 2016).

Aflatoxins create vast economic losses to the dairy industry. In terms of animal health, however, there are even more adverse effects ranging from depressed feed intake, lethargy, reproduction problems, to immune suppression (Whitlow and Hagler, 2005; Abrar et al., 2013; Zhu et al., 2016). Aflatoxins come in many forms, but the most toxic to ruminant animals is AFB1. Anywhere from 0.3% to 6.2% is biotransformed to AFM1, which is found in tissues or excreted in milk and other fluids (Campagnollo et al., 2016). This biotransformation has been detected in the serum 5 minutes after dosing and will stay in the cows system for 3-5 days after exposure (Mostrom and Jacobsen, 2011; Queiroz et al., 2012; Campagnollo et al., 2016). AFB1 can be metabolized by many pathways once ingested, but most importantly, it converts into a reactive epoxide (AFB1-8,9-) via cytochrome P450, which binds to DNA, RNA, and proteins to exert toxic effects on the animal (Abrar et al., 2013; Giovati et al., 2015; Campagnollo et al., 2016). Aflatoxins are lipophilic molecules, and because the liver is a predominantly lipophilic organ, they increased risks of hepatocellular carcinoma (Mostrom and Jacobsen, 2011; Di Gregorio et al., 2014; Campagnollo et al., 2016). In humans, aflatoxin has been known to negatively affect vitamin use and metabolism (Tang et al., 2009; Costanzo et al., 2015). Aflatoxins have been proven to impair gene regulation on inflammation processes in chickens (Yarru et al., 2009; Chen et al., 2014). For dairy cows, aflatoxins have been found to impair liver activity and suppress the immune responses (Bertoni et al., 2008; Queiroz et al., 2012). Aflatoxins are thought to suppress cell-mediated immune responses and can alter the proliferation and differentiation of cells (Corrier, 1991).

When toxins are introduced to the body the immune system first has to identify that a foreign body is present, which occurs via the innate immune system. In the case of mycotoxins, the focus will be placed on those pathways that link together the inflammation markers. To recall, the innate immune system works two ways. The first is to act as a first responder, sending signals for help. The adaptive immunity works to finish the job and keep records to know if or when the invader comes in again. When the innate immune system is working, cytokines are released as a signal to other cells in the body to know when they should perform their job. Cytokines like TNFα, IFNɣ, and IL-12 may reach all tissues and organs and stimulate a number of responses, but in the liver, they trigger the release of acute phase proteins such as haptoglobin and ceruloplasmin (Bertoni et al., 2008). Yarru et al., (2008) proved aflatoxins suppresses immune function by demonstrating that chicks fed a low dose of aflatoxin had downregulated the cytokine IL-6. Aflatoxin has also been shown to suppress innate immunity by suppressing activity of macrophages, T and B cells, and complement (Corrier, 1991). Mycotoxins fed to dairy cows also suppressed neutrophil phagocytosis in a study by Korosteleva et al. (2009).

CLAYS

Clay minerals widely come in contact with humans and animals on a daily basis. Clays can be found in a multitude of environments that involve soils and rocks, and even play an important role in research and development in many scientific fields (Meunier, A. 2005). Since the 16th century, clays have been discovered and researched and have accumulated a variety of definitions. According to the Clay Minerals Society, the term “clay” refers to a naturally occurring material composed primarily of fine-grained minerals, which is generally plastic at appropriate water contents and will harden when dried or fired (Guggenheim and Martin, 1995). However, the term “clays” can be used in three different ways; for size, for rock, and for minerals. For the purpose of clarification, clay minerals will be the focus of this article. Clay minerals are present in soil, sediments, and rock wastes, as well as in the matrix of the Earth’s crust (Mukherjee, S. 2013). Thus, it is vital to understand the structure and capacities of the various types of clays found in the environment.

There are two fundamental criteria to classify clay minerals, the type of layer structure in a ratio of 1:1, 2:1, or 2:1:1 and the type of octahedral sheet, di- or tri-. These structures can be seen in Figure 1. Each structure has sites where ions can bond to the structure and the number and positions of these bonds can determine its classification. For example, a 1:1 clay structure with dioctahedral orientation is Kaolinite (Rouquerol et al., 2014). These structures are tightly bound and cannot hold an interlayer space. The negative charges are located on the outer surfaces and bound by either Al or Si. The 2:1 layers are subdivided through an interlayer sheet that can undergo substitution with small atoms such as Mg, Fe, Li, Al, or Si in both the octahedral and tetrahedral layers (Meunier, 2005; Rouquerol et al., 2014). Smectites have many classifications according to the bound cations on the structure. They all have a charge of -0.2 to -0.6 but can be montmorillonite, beidellite, nontronite, saponite, stevensite, or hectorite. Vermiculites have charges of -0.6 to -0.9 but illites have charges or -0.9 to -0.75, the difference between the two being the crystalline features that are either hydrated or not hydrated, respectively (Meunier, 2005). Determining classification of various clay minerals can be done through many different techniques. X-ray techniques, such as X-ray diffraction (XRD), expose the target to a beam of electrons, with shorter wavelengths having

greater penetration power of the x-rays. Samples can be determined by analytical software and data files that are standard with an XRD machine (Mukherjee and Ghosh, 2013). For the purpose of this article, a focus will be placed on the clays with the highest swelling capacity, the 2:1 layer clays which its structure is represented in Figure 1.

An interesting fact about clays in the 2:1 layer category is their capability of "swelling". When these clays obtain a negative charge through ion substitutions, water and other molecules are able to penetrate the layers causing an increase in the layer spacing, leading to the cations attempting to retain their polar molecule "shell" (Meunier, 2005; Rouquerol et al., 2014). Clays that have the highest swelling capacity result from the nature of the interlayer cation that can form the most water or glycol layers and partial pressures of water or ethylene glycol (Meunier, 2005). This capacity for clay minerals has intrigued the scientific community for years and their use has been established in various household items. This specific property makes clays great kitty litter. In 1950, kitty litter was introduced to the world of clay adsorbents and has risen to account for 60% of litter products. Sodium bentonites are added for the characteristic clumping feature and added odor control (Yarnell, 2004; Murray, 2005). Almost all kinds of paints include clay additives to extend the life of the color and add specific features to paint such as gloss or matte finish (Murray, 2005; Jungang et al. 2012). Ceramic industries are conducting research with clays and different byproducts such as glycerin to make the same infrastructure that bricks have today (Martínez-Martínez et al., 2016). Other various items that include clay products are adhesives, cosmetics, floor absorbents, and pharmaceuticals. Medicines use clay products not only for suspension, capsules, and tablets, but also to treat gastro-intestinal disorders (Murray, 2005).

Geophagy, earlier termed pica, is the craving for substances not commonly regarded as food, i.e., clay, was first described in historical records as early as 10 BC. Danford (1982) described the recordings of geophagy throughout earth's history, each reason differing among cultures. Throughout the centuries, speculation on why pica occurred has ranged from mental illness, to help fetal development, and to treat mineral deficiencies, but mostly for gastrointestinal benefit (Danford, 1982; Mahaney et al., 2000). In areas and cultures where plants are barely tolerable to eat, such as Guatemala, clay eating is a common practice to mitigate gastrointestinal stress that results from ingestion and allows for broader diets to plants considered inedible otherwise (Johns, 1991). Humans are not the only species to ingest clays; animals have been hypothesized to practice geophagy long before humans have (Mahaney et al., 2000). Rats are ubiquitous in consuming clay when experiencing digestive disease or upset (Wiley and Katz, 1998). Slabach et al. (2015) observed mountain goats, known to be deficient in minerals, risking their visibility to predators in order to supplement their nutrients with provided mineral blocks. Eating earthen material such as clay has been thought to adsorb antinutrients and toxins like phenols, bacteria, and their metabolites (Johns and Duquette, 1991; Mahaney et al., 2000). Clays also are known to alleviate symptoms of gastrointestinal stress caused by changes in pH levels known as acidosis (Krishnamani and Mahaney, 2000; Slabach et al. 2015).

CLAY AS A BUFFER

Understanding how rumen, blood, and fecal pH are affected by clay after a grain challenge in Holstein cows and its effect on production parameters deserves attention. In an experiment, Sulzberger et al. (2016) used ten multiparous rumen-cannulated Holstein cows with 142 ± 130 (60 to 502) days in milk that were assigned to 1 of 5 treatments in a replicated 5 × 5 Latin square design balanced to measure carryover effects. Periods (21 d) were divided into an adaptation phase (d 1 to 18, with regular total mixed ration fed ad libitum) and a measurement phase (d 19 to 21). Feed was restricted on d 18 to 75% of the average of the total mixed ration fed from d 15 to 17 (dry matter basis), and on d 19 cows received a grain challenge. The challenge consisted of 20% finely ground wheat administered into the rumen via a rumen cannula, based on the average dry matter intake obtained on d 15 to 17. Treatments were POS (no clay plus a grain challenge), 3 different concentrations of clay (0.5, 1, or 2% of dietary dry matter intake), and control (C; no clay and no grain challenge).

Cows in the study encountered SARA when receiving a grain challenge (Gozho et al., 2005). Cows fed C had less area under the curve below rumen pH 5.6. These results were expected, because cows fed POS took longer to adjust their rumen environment to the normal pH range compared with cows fed C (Figure 2). Clays have been shown to work as alkalinizers and have great capacity for H+ exchange at different pH ranges (Yong et al., 1990). The authors reported that illite clay (a type of clay with high concentrations of magnesium and aluminum silicate) had the best buffer capacity in the pH range from 4.5 to 6, similar to the rumen pH range. Additionally, MgO when used as a buffer, may increase ruminal outflow, increasing the acetate:propionate ratio and improving milk fat tests (Davis, 1979). Earlier reports from Rindsig et al. (1969) concluded that cows fed clay at 5% (dietary

DMI) had increased acetate and decreased propionate in the rumen, leading to significant increases in milk fat percentage. In the Sulzberger et al. (2016) study, a positive linear effect of treatment on rumen pH indicated that clay at 2% was most efficient in buffering rumen pH and reducing the time spent below rumen pH 5.6 after a grain challenge. Greater concentrations of clay may have allowed for greater buffering capacity.

Clay's mode of action is commonly associated with its ion-exchanging capacity (Yong et al., 1990). For instance, clay materials are often used as backfill or buffer materials for radioactive waste disposal sites because of their ion-exchange properties, low permeability, and easy workability (Kumar and Jain, 2013). Hu and Murphy (2005) reported in a meta-analysis that buffers used in diets decreased molar proportions of propionate, which in turn increased the acetate:propionate ratio. Cruywagen et al. (2015) used buffered diets and reported a positive influence on milk fat as acetate was increased in the rumen. Interestingly, high-starch diets may increase the bioavailability of mycotoxins by a biochemical mechanism involving a lowered ruminal pH (Pantaya et al., 2016). The study demonstrated that such practice increased the bioavailability of AFB1 and ochratoxin A (OTA) and therefore exacerbate the toxic risk for animals.

CLAY AS AN ADSORBENT

Nones et al. (2016) studied the relationship between AF and stem cell damage in the presence of a bentonite adsorbent. They discovered that aflatoxin molecules occupy the interlayer space of the clay structures by forming complexes with the ions contained within the crystalline structure. The adsorbency of a clay mineral depends on the surfactant concentration and the polarity, the better the incorporation of surfactant in clay gives the higher the adsorbency power, and the more hydrophilic the clay, the higher adsorption with aflatoxin. There are many studies that have demonstrated the capability of clay minerals to adsorb aflatoxin and decrease AFM1 in milk and alleviate inflammatory suppression. Kutz el al. (2009) reported a 46% reduction in aflatoxin excretion and a 47% reduction in aflatoxin transfer from feed to milk by feeding a silicate clay mixture known as hydrated sodium calcium aluminosilicates (HCAS). A similar aluminosilicate product was used by Queiroz et al. (2012) and found a 45% reduction in milk AFM1 as well as a significant improvement to the immune challenge effect of aflatoxin on haptoglobin. Sodium bentonites have been found to decrease AFM1 concentrations by 60.4% (Kissell et al., 2012). Maki et al. (2016b) fed a calcium montmorillonite product that significantly reduced AFM1 excretion in milk.

In an experiment, Sulzberger et al. (2017) used ten multiparous rumen-cannulated Holstein cows (146 ± 69 days in milk), that were assigned to 1 of 5 treatments in a randomized replicated 5 × 5 Latin square design balanced to measure carryover effects. Periods (21 d) were divided in an adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). From d 15 to 17, cows received an AF challenge. The challenge consisted of 100 µg of aflatoxin B1 (AFB1)/kg of dietary DMI. The material was fitted into 10-mL gelatin capsules and administered into the rumen through a rumen-cannula based on the average DMI obtained on d 12 to 14. Treatments were no clay plus an AF challenge (POS); 3 different concentrations of clay (0.5, 1, or 2% of dietary DMI) plus an AF challenge; and a control consisting of no clay and no AF challenge (C).

Clay feed additives have been shown to decrease AF excretion and AF transfer from feed to milk (Kutz et al., 2009; Kissell et al., 2013; Barrientos-Velazquez et al., 2016; Maki et al., 2016a). Some studies have reported no changes in DMI or milk yield when feeding clay products during an AF challenge (Battacone et al., 2009; Queiroz et al., 2012; Maki et al., 2016a,b). However, in the Sulzberger et al. (2017) study, we detected a quadratic treatment effect for DMI and a negative linear treatment effect for milk yield. The small changes in these values tended to cause a difference for 3.5% fat-corrected milk (FCM) and differed for 3.5% FCM/DMI and milk/DMI. The differences seen in milk yield that reflect negatively on efficiency parameters could be the result of the cow's metabolism of AF. Kubena et al. (1998) reported a reduction in feed consumption that adversely affected feed conversion by broiler chickens exposed to AF.

As clay increased, AFM1 concentration in milk decreased and the highest reduction occurred in cows receiving 2% (Figure 3; Sulzberger et al., 2017). Queiroz et al. (2012) reported an increase in AF excretion in milk at low concentrations of dietary clay inclusion (0.2% of dietary DM) but when clay was increased to 1% of dietary DM, AF excretion decreased 16%. Maki et al. (2016a) used a clay feed additive at 0.5 and 1% of dietary DM and found that both percentages decreased AFM1 concentration in milk (51.3 and 69.7%, respectively). In the Sulzberger et al. (2017) study, we detected a significant decrease in AFM1 excretion (µg/d) that resulted in a reduction of 25% (0.5%), 18% (1%), and 41% (2%), which was seen as a decrease in the AF transfer percentage.

Even though clays have been reported to decrease AF, certain vitamins (A, D, and E) and minerals have been decreased in the presence of smectite clays

(Tang et al., 2009; Barrientos-Velazquez et al., 2016). In the Sulzberger et al. (2017) study, we detected no significant differences among treatment groups, suggesting that AF was not altering vitamin and mineral concentrations as previously reported in humans, swine, and chickens (Tang et al., 2009; Trckova et al., 2014; Fowler et al., 2015). In agreement with the results from the Sulzberger et al. (2017) study, Maki et al. (2016b) found no interference with serum vitamin A concentrations when montmorillonite clay was fed to bovine animals at 18 and 20 kg/d.

Ogunade et al. (2016) studied the effects of adding 3 mycotoxin-sequestering agents (SEQ) to diets contaminated with AFB1 (75 µg/kg of dietary DMI) on reducing milk aflatoxin M1 and immune status of dairy cows. Those authors reported that the greater mean fluorescent intensity of staining for CD62L and CD18 on neutrophils of cows fed SEQ1 (yeast cell culture) and SEQ3 (sodium bentonite) diets suggested that these agents altered the migration of neutrophils exposed to aflatoxin. Additionally, feeding the SEQ2 (yeast cell culture mixed with sodium bentonite) diet reduced the inflammatory response caused by the toxin diet (positive control), and the SEQ1 and SEQ3 diets tended to have a similar effect. Similarly, in our experiment, cows fed clay tended to have lower SOD plasma concentrations, possibly indicating less oxidative stress.

CONCLUSIONS

Feeding clay seems to help to alleviate the effect of a grain challenge on the rumen environment and ultimately affected the performance of Holstein cows. Cows fed 0.5, 1, or 2% clay tended to yield more milk and did yield more 3.5% FCM and ECM than cows not supplemented with clay. Production and physiological parameters (e.g.; rumen pH) suggest that clay may be an alternative buffer in diets for dairy cows. Additionally, the inclusion of clay products in the diet seem to linearly reduce aflatoxin transfer from the rumen (challenge) to the milk and feces of mid-lactation Holstein cows. Cows that were challenged with aflatoxin and not fed clay had poorer liver function and inflammatory response when compared with cows challenged and receiving clay.

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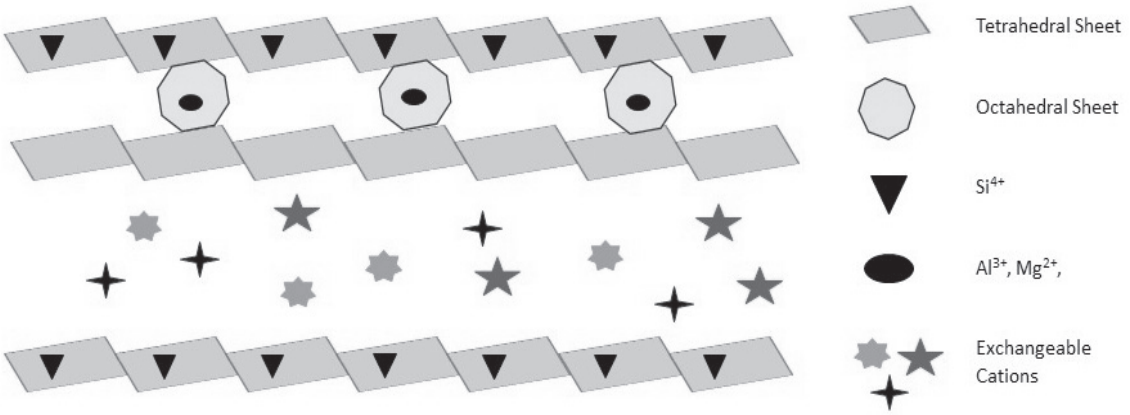

Figure 1. Clay Structure showing the ideal structure of a smectite clay in a 2:1 layer. Exchangeable ions represent the various ions that can interact with in the environment.

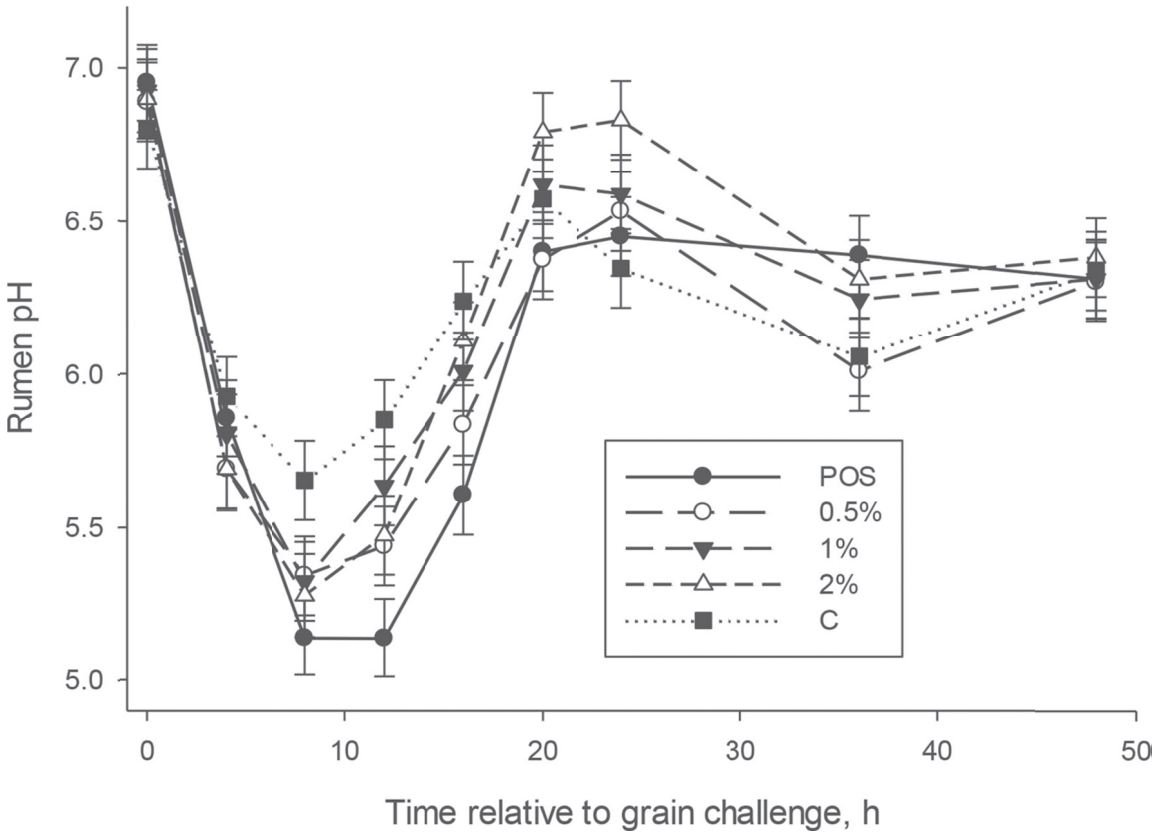

Figure 2: Least squares means ± SE for rumen pH response to a grain challenge (0 h) for cows in positive control with no clay (POS), 0.5, 1, or 2% clay, and negative control (C) treatments from 0 to 48 h (time points) relative to a grain challenge. Treatment: $P = 0.003$; time point: $P < 0.0001$; treatment × time point: $P = 0.01$.

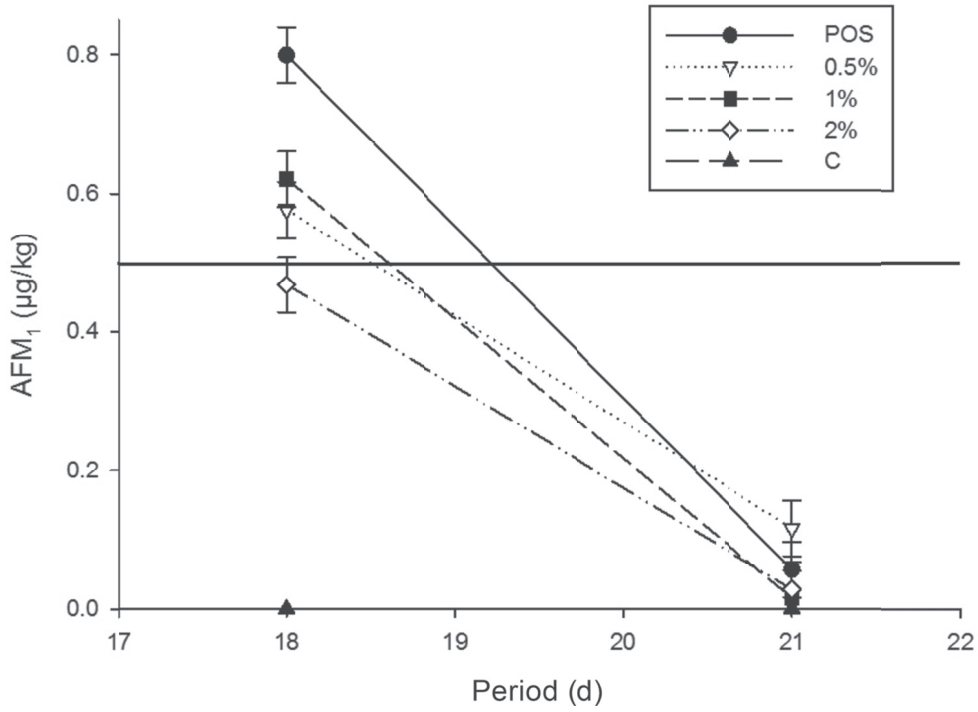

Figure 3. Least squares means ± SE for milk concentrations of aflatoxin M1 (AFM1) in response to an aflatoxin challenge (d15 to 17) for cows in 5 treatments: positive control with no clay (POS); 0.5, 1, or 2% clay; and a negative control with no clay (C) from d 18 to 21 of each period. On d 18, AFM1 concentrations in milk differed ($P < 0.0001$). Treatment × day: $P < 0.0001$. Horizontal solid line represents the Food and Drug Administration's allowable AFM1 concentration in milk (0.5 µg/kg).

Amino acids; Roles Beyond Being the Precursors for Protein Synthesis

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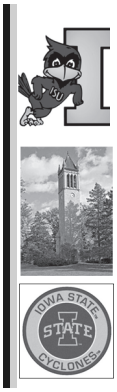
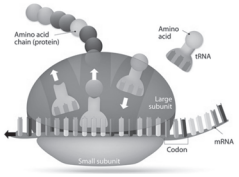


Amino acids; Roles Beyond Being the Precursors for Protein Synthesis



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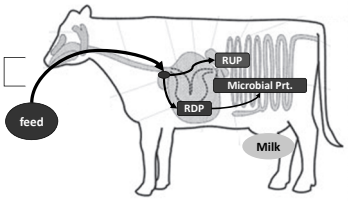
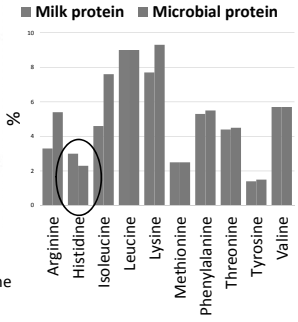
Feeding cows to Meet the AA Requirement of Milk Protein

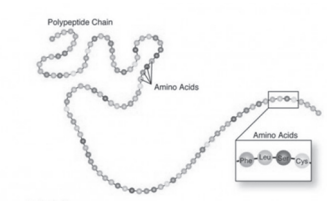




- Quite hard to determine AA supply
- A portion of dietary protein is remodeled to microbial protein before reaching the intestine

Every Building Block is Equally Important

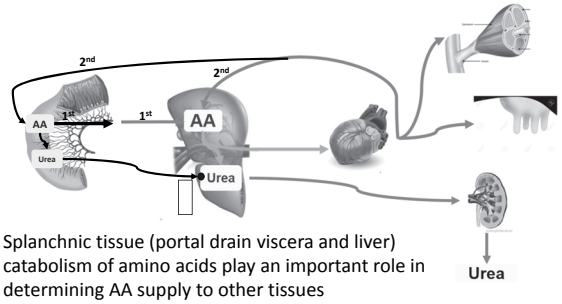
Amino acids sequence determines the biological function



Non-essential (NEAA)	Essential (EAA)
Alanine	Arganine
Asparagine	Histidine
Aspartate	Isoleucine
Cysteine	Leucine
Glutamine	Lysine
Glutamate	Methionine
Glycine	Phenylalanine
Serine	Threonine
Tyrosine	Tryptophan
Proline	Valine

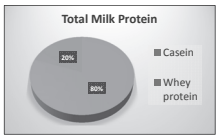
Essentiality: if body can synthesize enough or dietary supply is essential?

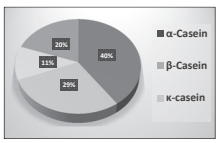
AA supply continues to be remodeled even after absorption

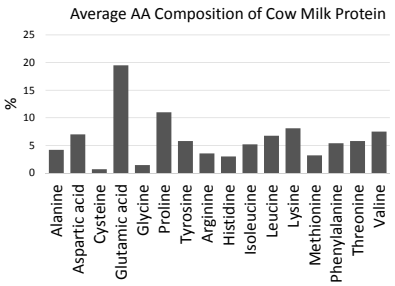


Splanchnic tissue (portal drain viscera and liver) catabolism of amino acids play an important role in determining AA supply to other tissues

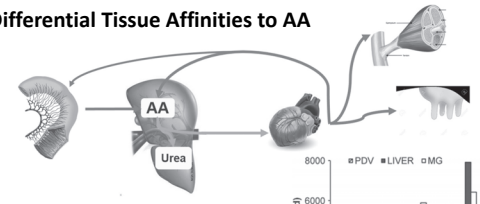
Milk Protein is Composed of both EAA and NEAA

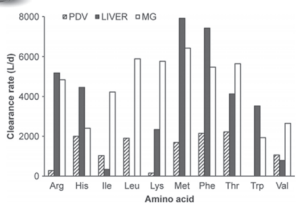






Differential Tissue Affinities to AA

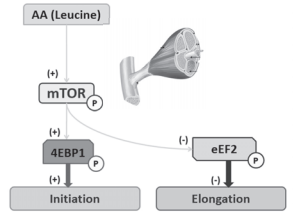


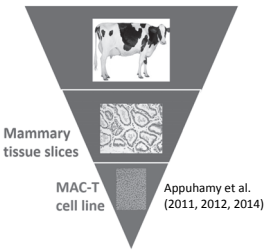


- Clearance of Met by both liver and the mammary glands is quite high
- Greater clearance of His and Thr by both gut and liver
- **Relatively low clearance of BCAA (Ile, Leu, & Val) by liver and viscera (gut)**

BCAA is an indicator for AA availability to extra-splanchnic tissues

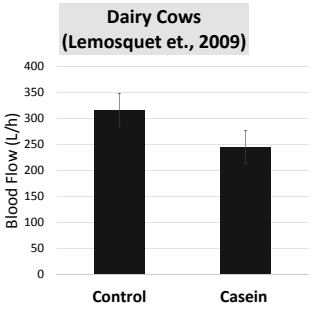
Early work on BCAA-induced cellular signals stimulating protein synthesis in muscle → Similar signaling effects in bovine mammary cells?

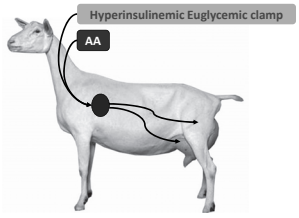




Yang et al., 2006; Wang et al., 2006; Kimball et al. 2006

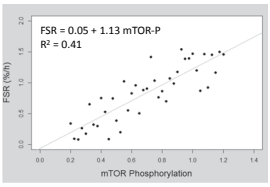
Surplus AA Supply Reduces Blood Flow to MG



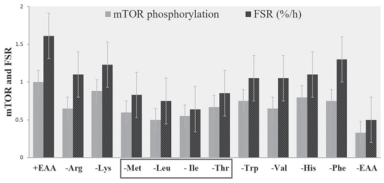


Infusion of AA increased blood flow to hind gut more than the blood flow to the udder (Bequette et al. 2001)

Fractional rate of casein synthesis (FSR) responds positively to BCAA signals mediated by mTOR

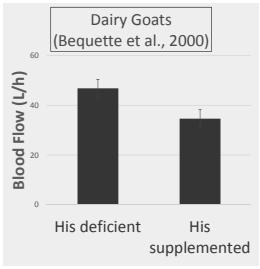


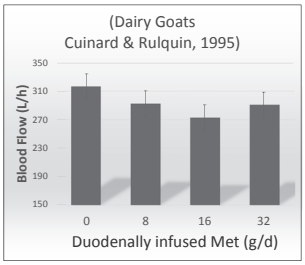
Casein synthesis rates were positively associated with mTOR signals in mammary tissues harvested from lactating Holstein cows (Appuhamy et al., 2012)



Both casein synthesis rates and mTOR signals significantly decreased, when extracellular Leu, Ile, Met, and Thr decreased in mammary tissues harvested from lactating Holstein cows (Appuhamy et al., 2012)

Mammary Blood Flow is Adjusted to Match with the Individual AA Requirements





No Apparent Effects of BCAA on Milk Protein *in vivo*

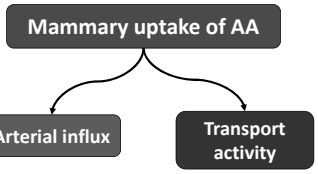
Variable	CTL	ML	ML+BCAA	SEM	*P*-value
Milk yield (kg/d)	51.7	52.9	53.8	2.65	0.420
Protein yield (kg/d)	1.39[a]	1.52[b]	1.51[b]	0.07	0.063
Protein (%)	2.71[a]	2.88[b]	2.83[b]	0.06	0.009
MUN yield (g/d)	6.43	6.29	5.85*	0.47	0.181
MUN content (mg/dL)	12.4*	11.8*	10.9[b]	0.70	0.006

- BCAA did not improve milk protein above the improvements caused by Met and Lys

AA	CTL	ML	ML+BCAA	SEM	*P*-value
Lys	69.2[a]	81.1[b]	72.6[ab]*	2.71	0.029
Met	23.3[a]	31.1[b]	26.7***	1.81	0.005
Ile	110[a]	91.6[b]	107[a]	8.33	0.034
Leu	182	179	205	10.9	0.214
Val	237	230	257	19.0	0.281

- Reduced MUN and the unchanged plasma BCAA concentrations indicate possible improvements in non-mammary protein synthesis

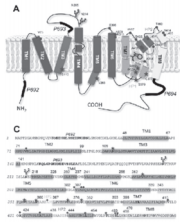
AA Transporters & Fractional Rate AA Uptake



Total amount of AA (mol) in the extracellular space

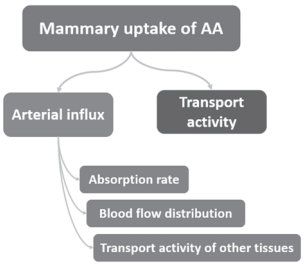
Fraction of available AA taken up by the cells

$$k_{AA} = \frac{AA\ uptaken}{Total\ AA}$$

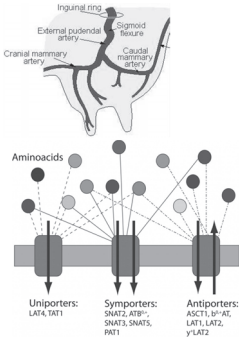


Rate of AA transporter expression and relocation to cell membrane significantly influence the fractional rate of AA uptake

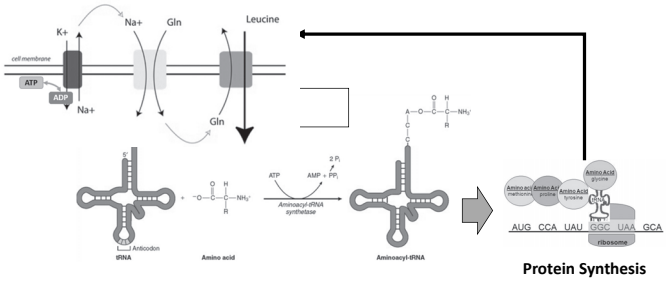
AA also regulate blood flow and tissue uptake of AA



Blood flow to the mammary glands (MG) is a key determinant of AA available for the MG



Coordination of mammary AA removal with cellular demand helps prevent wasteful energy (ATP) associated with AA transport and mRNA translation



Protein Synthesis

Mammary Uptake of AA (e.g., Met) complements the Protein Synthesis Rates

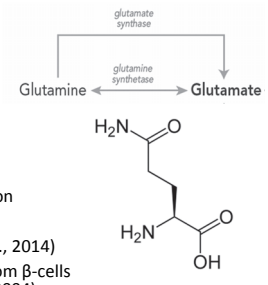
	DL-Met[1]				
	0 g/d	8 g/d	16 g/d	32 g/d	RMSE
Arterial concentrations, mg/100 ml	.33	.61	.72	1.26	.22
AV[3] Differences, mg/100 ml	.13	.15	.11	.14	.05
Extraction rates, %	58.3	34.2	14.8	10.7	10.8
Arterial flow, g/h	.83	1.31	1.45	2.55	.70
Mammary uptake,[4] g/h	.29	.30	.21	.30	.11
Mammary uptake:milk output[5]	.99	.99	.67	.90	.33

	DL-Met[2]					Effect[3]		
	0 g/d	8 g/d	16 g/d	32 g/d	RMSE	Linear	Quadratic	Cubic
True protein								
%	2.77	2.87	2.91	2.88	.06	.077	.068	.769
g	674	660	707	689	37	.433	.618	.198

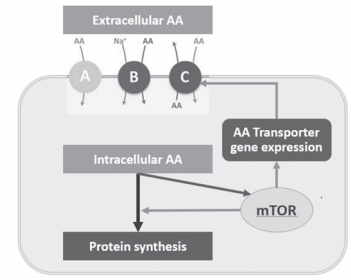
Guinard & Rulquin (1995)

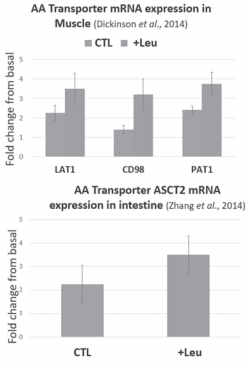
Functions of Glutamine (Gln)

- Protein synthesis
  - Milk protein: ~20% is Gln+Glu
  - Muscle protein: 90% of total body Gln
  - Production of Cytokines
- Synthesis of glutamate, a neurotransmitter
- Nitrogen donor in purine synthesis
  - cell proliferation (e.g., lymphocytes)
- NH_3 shuttle (non-toxic) in the blood circulation
- Cellular signal enhancing protein expression
  - e.g., tight junction protein (Wang et al., 2014)
- Cellular signal enhancing insulin secretion from β-cells (Greenfield et al. 2009; Li et al., 2004)

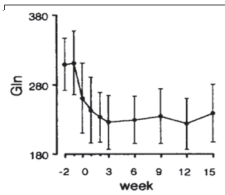


Leucine regulates AA transport gene expression via the mTOR pathway





Could Glutamine be Dietary Essential for Dairy Cows?



- Arterial concentration of Gln can go down to as low as 185 µmol/L
- So, Gln uptake can go up to almost 100% of the arterial supply indicating a substantial demand by the mammary gland
- A massive amount of Glu is produced from Gln in the mammary gland because uptake ratio of Gln: Glu was 2:1 but the ratio in milk was 1:2 (Seymour et al., 1990)

Glutamine Is a Potentially Limiting Amino Acid for Milk Production in Dairy Cows: A Hypothesis

Gerwin A.L. Meijer, Jan van der Meulen, and Ad M. van Vuuren

Metabolism, Vol 42, No 3 (March), 1993: pp 358-364

AA as antioxidants

Methionine

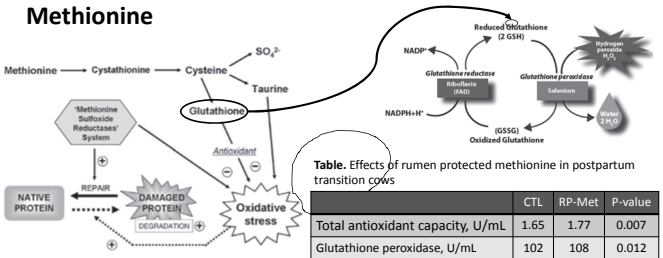


Table. Effects of rumen protected methionine in postpartum transition cows

	CTL	RP-Met	P-value
Total antioxidant capacity, U/mL	1.65	1.77	0.007
Glutathione peroxidase, U/mL	102	108	0.012
Fat corrected milk, kg/d	22.7	23.3	0.040
Milk protein, %	3.05	3.23	0.022

Sun et al., 2016

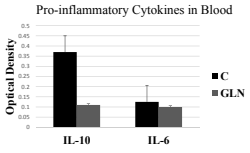
Could Glutamine be Conditionally Essential for Dairy Cows?

Dietary glutamine enhances immune responses of dairy cows under high ambient temperature

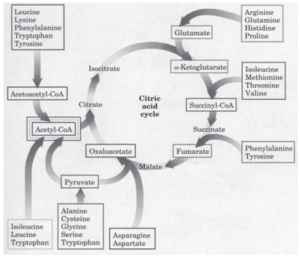
M. Caroprese,[1] M. Albenzio, R. Marino, A. Santillo, and A. Sevi

Journal of Dairy Science Vol. 96 No. 5, 2013

Item	C	GLN	SEM
Yield, kg/d			
Milk	19.1[c]	21.7[b]	0.8
Fat	0.668[c]	0.750[b]	0.022
Protein	0.603[c]	0.650[b]	0.021
Casein	0.472[c]	0.499[b]	0.002
Milk composition, %			
Fat	3.64	3.45	0.10
Protein	3.22	3.01	0.06
Casein	2.52[a]	2.31[b]	0.01
Lactose	4.81[a]	4.76[b]	0.01
Milk hygienic quality			
pH	6.63	6.63	0.01
SCC, $\log_{10}$ cells/mL	5.24[b]	4.90[c]	0.09

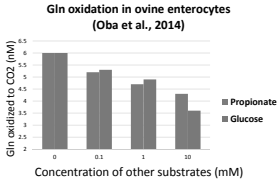


Glutamine, the most talked-about AA



All the amino acids are capable of generating ATP, even though they are not the most preferred energy substrates

- Intestine prefers amino acids particularly glutamine (Gln) as a major energy source



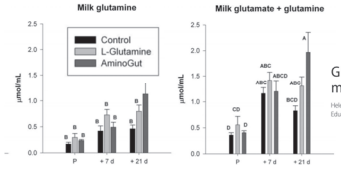
- All available data suggest that glutamine is not the predominant fuel for the intestine in ruminants.

Free Glutamine in Milk

Free Glutamine and Glutamic Acid Increase in Human Milk Through a Three-Month Lactation Period

	Colostrum	1st Month	2nd Month
Glutamine (µmol/L)	24	187	560
Total AA (µmol/L)	2204	2510	3175
Total EAA (µmol/L)	508	272	296
Protein (g/mL)	1.93	1.32	1.13

Agostoni et al., 2012

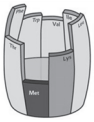


Glutamine and glutamate supplementation raise milk glutamine concentrations in lactating gilts

Helena Emilia CCC Manso[1], Helio C Manso Filho[1], Luiz E de Carvalho[2], Marianne Kutschenko[1], Eduardo T Nogueira[3] and Malcolm Watford[4]*

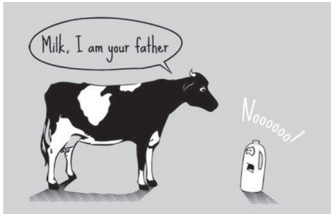
Summary

- Many other roles of AA than being the building blocks of proteins
  - signaling for protein synthesis and AA transport
  - immune functions



1. Limiting AA theory appears to be over-simplified
2. Other amino acids can be essential and limiting under special situations like illness and heat-stress
3. New benefits related to animal wellbeing & specialty food production

THANK YOU



Questions?

Feeding Strategies and Economic Returns in Robotic Milking Systems

Victor E. Cabrera[1] and Alex Bach[2,3]

[1]Department of Dairy Science, University of Wisconsin, Madison 53706, vcabrera@wisc.edu
[2]ICREA, Institució Catalana de Recerca i Estudis Avançats, Barcelona (Spain)
[3]Department of Ruminant Production, IRTA (Institut de Recerca i Tecnologia Agroalimentàries). Torre Marimon, Caldes de Montbui, 08140, Barcelona, Spain.

With exception of the economic case study, this paper is an excerpt from Bach and Cabrera (2017).

Introduction

Rationale

Cows in conventional milking parlors:
- Kept structured, consistent, and social milking and feeding routine
- Obtain all their nutrients from a TMR

Cows in automatic milking systems (AMS):
- Obtain a fraction of their nutrients during milking and through a partial mixed ration (PMR)
- Their milking frequency and time of milking vary across time

Overcome challenges and capture opportunities

- Behavioral Considerations
  - Nutritional Considerations
    - Economic Considerations

Bach & Cabrera, 2017

AMS

Challenges: milking frequency not only dependent on **concentrates at the AMS**, but
- the social structure of the herd,
- the farm layout design,
- the type of traffic imposed to cows,
- the type of flooring,
- the health condition of the cow

Opportunities
- manipulate the number of cows per AMS
- milking more frequently
- feeding more precisely

Behavioral Considerations

Maximum AMS return on investment ➡ full utilization of the AMS with little or no human intervention

Crucial ➡ maximizing milking frequency and minimizing fetching

Challenge ➡ consistent milking frequency throughout time

Behavioral Considerations

Common ➡ ~2.5 average milkings
Wagner-Storch et al., 2003; Bach et al. 2009; Deming et al., 2013

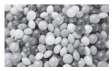
Variation ➡ can be high

Change frequency of milkings ➡ change in the AMS DMI

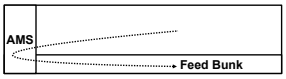
Behavioral Considerations

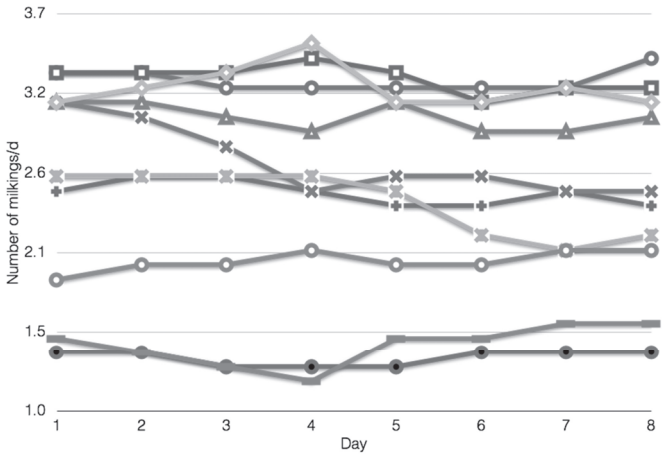
↑AMS visits & ↓variability ➡

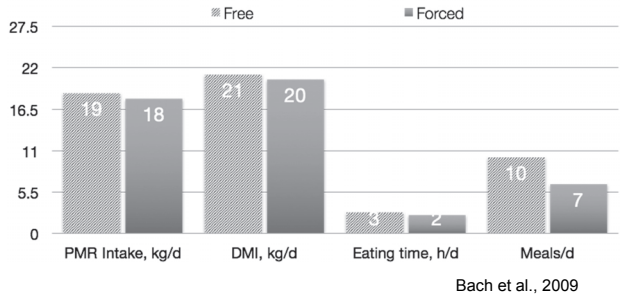
Palatable feed



Forced (guided) traffic

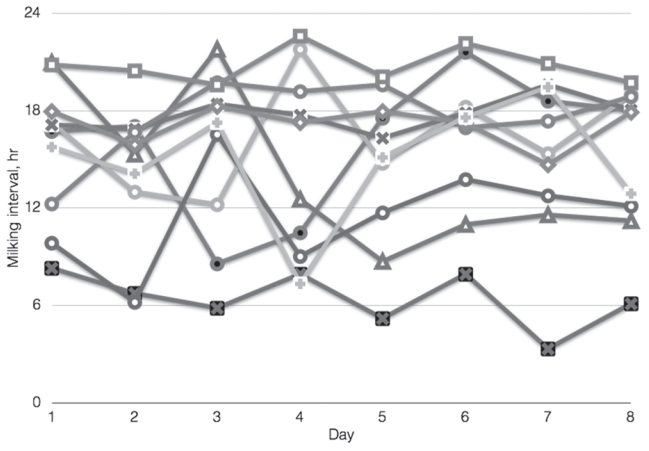


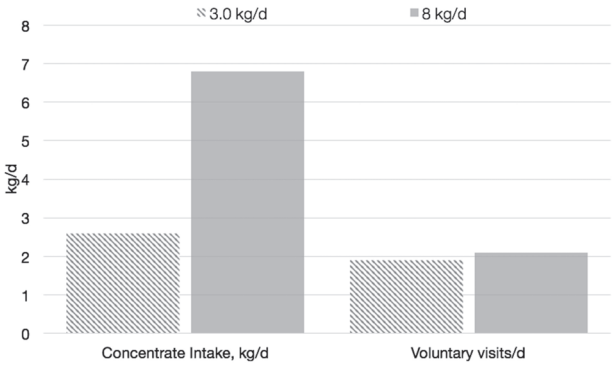




Bach et al., 2009

- Forced traffic reduces PMR intake Bach et al., 2009
- Forced traffic decreases milk yield Tremblay et al., 2016





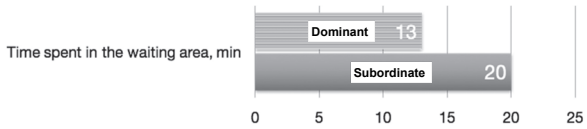
Bach et al., 2007

- ↑feed allowance does not ↑visits Bach et al., 2007
- 300 g/visit attracts grazing cows Scott et al., 2014

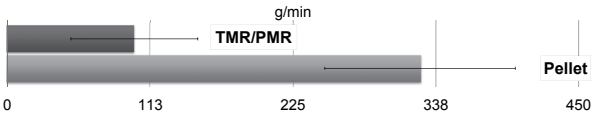
Behavioral Considerations

Cows are gregarious ➡
Sync behaviors
Benham, 1992

AMS force individualism
➡ unnatural



Dominant cows ➡ less time in waiting area
Halachmi, 2009



AMS time/milking ➡
7 min
Castro et al. 2012

A cow can consume ➡
< 2.8 kg/milking

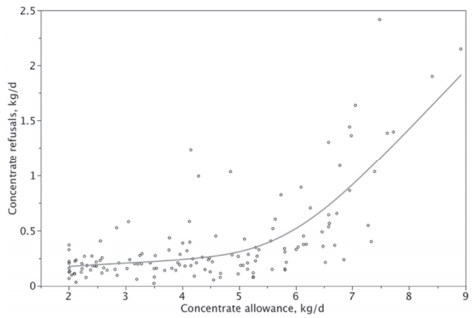
Theoretically, a cow can consume ➡ < 8.4 kg/3 milkings per d

To avoid variation
➡ better an allowance of 4 kg/d

Nutritional Considerations

AMS concentrate feeding ➡ main attraction to milking
Prescott et al, 1998

Cows do not consume all concentrate ➡ > 4 kg/d



Nutritional Considerations

AMS concentrate presentation➡ better pellet than mash

25 vs. 49% starch for 3 kg/d allowance➡ no change in milk yield, composition, or visits
Halachmi et al., 2006

Flavoring agents➡ in general no positive effects
Harper et al., 2016

Minerals and vitamins➡ normally not provided in AMS**➡ becomes an issue when cows rely more in concentrate**

Nutritional Considerations

Inconsistent nutrient supply➡ affects negatively milk yield
MacBeth et al., 2013

> AMS concentrate allowance ➡ < density PMR

Milk yield decreased➡
NEL variability in 22 herds
Sova et al., 2014

Milk yield decreased➡
> AMS concentrate allowance
Tremblay et al., 2016

Precision feeding opportunity

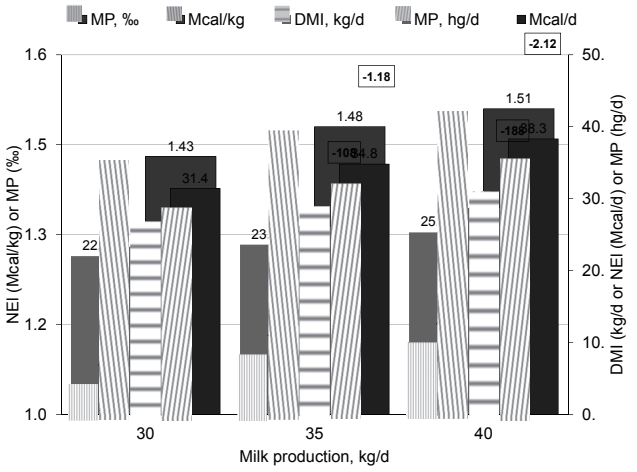
TMR or PMR inefficiencies➡ improved by AMS supplementation

Cows sort
Leonardi and Armentano, 2007

Composition changes
Kononoff and Heinrichs, 2003

Intake is variable➡ between cows and within cows

Balanced diet for a cow➡ unbalanced diet for another cow



Economic considerations

Maximizing milk production per AMS proposed as goal for economic efficiency
Sonck & Donkers, 1995

More cows per AMS -> milkings reduced and time AMS used by cows increased
Tremblay et al., 2016

Maximizing milking frequency -> should be the main goal of AMS

Precision feeding opportunity

Decrease imbalance➡ AMS concentrate

Most AMS only have single bin to deliver concentrates

Imbalance➡ will remain and progressively increase

How to overcome it➡ provide a custom-made cow-specific concentrate

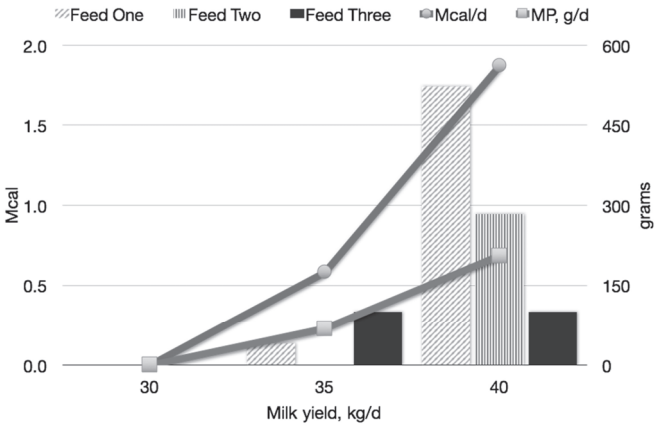
On the basis of milk, BW, state, components, etc.

Economic analyses

Data from a North Catalonian farm

AMS 1
- 64 cows
  - Primiparous (PMC)
- AMS concentrate
  - **3.84** kg/d
  - [0.98 - 7.42]
- Milk yield
  - **32.6** kg/d
  - [15.6 - 46.0]

AMS 2
- 70 cows
  - Multiparous (MPC)
- AMS concentrate
  - **4.70** kg/d
  - [1.60 - 9.04]
- Milk yield
  - **41.3** kg/d
  - [17.3 - 59.6]



Dataset

AMS concentrate
- 2.07 Mcal of NEl/kg
- 22.4% CP
- €274/MT

PMR feed
- 1.62 Mcal of NEl/kg
- 15.6% CP
- €92.5/MT

Cow consumption
- **DMI:** NRC (2001)
- **NEI & CP**: milk yield

Income over feed cost (IOFC)
- Milk price at **€0.32/kg**

1 - Change number of cows per AMS

70 to 65 MPC

Total milk harvested per AMS remained constant
Tremblay et al., 2016

2,892 kg milk AMS/d
- 70 MPC = 41.3 kg/cow.d
- 65 MPC = **44.5** kg/cow.d

Extra 3.2 kg/cow.d
- Required ~2.5 Mcal NEl/cow.d

Additional **PMR**
- Maintaining AMS concentrate allowance equal

2 - Limit amount of AMS concentrate

PMC
- 3.74 to **2 kg/cow.d**
- €7.9 to €8.1/cow.d
- ↑**€6,710/AMS.yr**

MPC
- 4.70 to **3 kg/cow.d**
- €10.0 to €10.3/cow.d
- ↑**€6,748/AMS.yr**

1 - Change number of cows per AMS

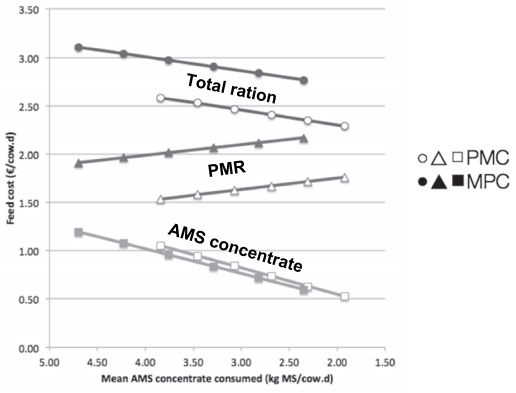
70 to 65 MPC

↑€**2,453**/AMS.yr
↑€**6.72**/cow.d

IOFC
- 70 MPC = €720.8/AMS.d
- 65 MPC = €727.5/AMS.d

65 MPC increased IOFC
- Less feed for maintenance

2 - Limit amount of AMS concentrate



2 - Limit amount of AMS concentrate

Less allowance of AMS concentrate
- minimize variability in concentrate consumption
- reducing feed costs
  - lower cost per unit of nutrient with PMR

PMC
- 3.74 to **2 kg/cow.d**

MPC
- 4.70 to **3 kg/cow.d**

3 - Precision feeding

AMS concentrate
- normally same density of nutrients for all animals
- ideally, it could be formulated individually

AMS concentrate
- 2 kg/cow.d PMC
- 3 kg/cow.d MPC

3 - Precision feeding

PMC IOFC
- ↑€1.30/cow.d

MPC IOFC
- ↑€1.56/cow.d

Whole farm
- ↑€192/d
- ↑€70,080/yr

Conclusions economic considerations

Reducing number of animals per AMS could **improve IOFC** if production does not decline

Restricting concentrate allowance to kg/cow.d **3** (PMC) and **4** (MPC) **improves IOFC** and minimizes variation nutrient intake

Precision feeding to meet cow-specific nutrient requirements may **greatly improve IOFC**

Keeping concentrate allowance low help to reduce digestion problems, feed costs, concentrate refusals, and milking regularity

Economic considerations

From an economic efficiency perspective, the main target is maximizing milk production per AMS (Sonck and Donkers, 1995). Milk harvested per cow and milking is related to the time elapsed since previous milking, with this relationship being more or less linear until 16 h and becomes constant thereafter (Delamaire and Guinard- Flament, 2006). Tremblay et al. (2016) showed that, as the number of cows per AMS increases, the number of milkings is reduced (i.e., milking interval increases) and the time that cows occupy the AMS increases. Despite the fact that both milking frequency and time spent in the AMS per milking increase milk production, these 2 aspects rarely increase simultaneously (Tremblay et al., 2016). It is commonly recommended that the number of animals per AMS should be around 60 to 70 cows. This number stems from the time required to clean the AMS, unit attachment failures, periods of nonattendance, and technical maintenance, which leaves around 20 to 22 h/d of available time for milking (Halachmi, 2004; Lyons et al., 2014), and because a single AMS has a limited capacity of around 8 milkings/h (Ketelaar- de Lauwere et al., 2000), leading to a theoretical total number of cows that can be milked 2.5 times every day between 60 and 70 cows. Results from the literature suggest that, to attain maximum milk harvesting capacity of an AMS, the goal should be maximizing milk yield per cow instead of increasing the number of cows. Typically, decreasing the number of cows per AMS decreases the time cows spend waiting in the pre-milking area, particularly for low socially ranked or less experienced cows (Halachmi, 2009); likewise, small reductions in cow numbers are commonly compensated by increases in milk production from the remaining cows because the number of milkings increase and time spent milking decreases, especially when cows are selected for high milking speed (Tremblay et al., 2016).

Case study

Data from a farm in North Catalonia (Spain) with 2 groups of cows milked in 2 AMS were used as a case study to evaluate the economic value of changing the number of cows per AMS under some general assumptions. One AMS was milking 64 primiparous cows that consumed 3.84 kg/d (min=0.98, max=7.42) of concentrate in the AMS and produced 32.6 kg of milk/d (min=15.6 max=46.0), whereas the other AMS milked 70 multiparous cows that consumed 4.70 kg/d (min=1.60, max=9.04) of concentrate in the AMS and produced 41.3 kg of milk/d (min=17.3, max=59.6).

1. Change number of cows per AMS. It could be safely assumed that the overall milk harvested per AMS would remain constant when the number of cows decreases and therefore milk production per cow would increase together with the cow's energy requirements (Tremblay et al., 2016). Furthermore, decreasing the number of cows per AMS could decrease the time cows spend waiting in the pre-milking area, particularly for low socially ranked or less experienced cows (Halachmi, 2009). Then, for an impartial analysis, cows were randomly selected out of the AMS system and the remaining cows' production was proportionally adjusted to reach the original AMS milk yield. For example, the 70 multiparous cows produced originally 2,892 kg of milk/d or 41.3 kg/cow.d. Then, after randomly removing 5 cows, milk production of the remaining cows was adjusted to increase to 44.5 kg/cow.d to make up the 2,892 kg of milk/d in the AMS. This difference of 3.2 kg of milk/cow.d required between additional 1.95 and 2.50 Mcal of NEl/cow.d,

which was compensated by additional consumption of PMR. Decreasing the number of animals from 70 to 65 and maintaining AMS production resulted in an income over feed cost (IOFC) of €727.5/AMS.d, compared with the original IOFC of €720.8/AMS.d; a difference of €6.72/AMS.d or €2,453/AMS.yr in favor of the 65 multiparous cows. A similar exercise with 60 multiparous cows resulted in a difference of €20.2/AMS.d or 7,366/AMS.yr in favor of milking 60 multiparous cows compared with the original 70 multiparous cows. With respect to the primiparous cows, a reduction from the original 64 cows that produced a total of 2,088 kg milk/AMS.d or 32.6 kg/cow.d to 60 cows, which then were assumed to produce 34.8 kg/cow.d, (additional 2.17 kg/cow.d) requiring between additional 1.41 and 1.61 Mcal of NEl/cow.d. Once again, 60 primiparous cows, instead of 64, resulted in an improved IOFC of €3.74/AMS.d (€524.6 vs. the original €520.8) or €1,365/AMS.yr. Therefore, the goal with an AMS would be to attain maximum milk harvesting capacity by maximizing milk yield per cow instead of increasing the number of cows.

2. Limit the amount of AMS concentrate. To support the maximum possible milk yield, however, the economic return from the feed needs to be accounted for. Feed represents 50 to 70% of all costs in dairy production (Bozic et al., 2012); therefore, increasing feed efficiency has a major effect on profitability. Furthermore, improving feed efficiency has positive consequences for the environment (Reed et al., 2015). Data from the Catalan farm described above with 2 groups of cows milked in 2 AMS were used to illustrate potential improvements in IOFC by implementing precision feeding approaches. The concentrate offered in the 2 AMS was the same and contained 2.07 Mcal of NEl/kg and 22.4% CP and had a cost of 274 €/MT (DM basis); whereas the PMR (which was also the same for both AMS) contained 1.62 Mcal of NEl /kg and 15.6% CP and had a cost of 92.5 €/MT. Then, the NRC (2001) model was used to estimate cow-specific DMI of PMR (given that concentrate intake was known) and consumption of NEl (Mcal) and CP (kg) based on individual milk yield and DMI was estimated. Lastly, individual and group IOFC were calculated using local current milk prices (€0.32/kg). The hypothesis was that a herd with an AMS could improve IOFC by providing a minimum amount of concentrate in the AMS and promoting maximum consumption of PMR. With this strategy, IOFC would be maximized by 1) minimizing variation in concentrate consumption, and 2) reducing feed costs due to the lower cost per unit of nutrient in the PMR compared with the concentrate. Assuming that the DM consumption per cow would adjust to remain iso-energetic at different target concentrate allowances at the AMS, PMR consumption was corrected to complete the energy required (i.e., cows would consume more PMR if a lower concentrate allowance was offered at the AMS). Target levels of concentrate varied proportionally to the known individual cow level of consumption (i.e., relative distribution of consumption of AMS concentrate remained among herd mates). For example, using an average concentrate allowance at the AMS for primiparous cows of 2 kg/cow.d (min=0.51, max=3.86) would result in an IOFC improvement from €7.85 to €8.14/cow.d, a gain of €105/cow.yr or €6,710 for 64 primiparous cows in 1 yr. For multiparous cows, using an average concentrate allowance at the AMS of 3 kg/cow.d (min=1.02, max=5.78) would generate an IOFC improvement from €10.03 to €10.30/cow.d, a gain of €96/cow.yr or €6,748 for 70 multiparous cows in 1 yr. Overall, in the whole farm, targeting the consumption of AMS concentrate to 52% for primiparous (2 kg/cow.d) and to 64% for multiparous (3 kg/cow.d) of what was actually being fed, would improve overall IOFC by €100.4/cow.yr or 13,449/herd.yr. Feed cost decreased as the amount of AMS concentrate consumed decreases because of the cost differential per unit of nutrient in the PMR. For instance, the cost for each energy unit is €0.06/Mcal of NEl for the PMR versus €0.13/Mcal of NEl for the AMS concentrate. On the other hand, in a scenario that would maintain the consumption of energy at the same level (iso-energetic intake), CP consumption would barely be affected: it would decrease from 3.44 kg/cow.d (min=1.66, max=4.48) to 3.40 (min=1.64, max=4.40) for primiparous and from 4.25 kg/cow.d (min=1.78, max=5.24) to 4.19 (min=1.74, max=5.17) for multiparous cows, which likely would not affect production performance. However, a more precise protein feeding would likely decrease N excretion.

3. Precision feeding. These economic returns could even be greater if a dynamic feeding approach was implemented, that is, combining 2 concentrates (energy and protein) at the AMS in different amounts and proportions at the AMS. Precision feeding provides, in theory, only the exact amount of nutrient required because the supplement changes in composition as needed, whereas conventional supplementation, because of a fixed profile of nutrients, provides some of those in excess without additional benefits and incurring in economic inefficiencies.

Following the previous case study, we assumed that cow productivity would remain constant and economic gains would result for nutrient savings: precision feeding provides only the exact amount of nutrient required, whereas conventional supplementation, because of a fixed profile of nutrients, provides some of those in excess without additional benefits. As previously, we calculated, according to NRC (2001), the cow-specific DMI, and NEl and CP requirements and then targeted the level of supplementation as before (2 and 3 kg in average for primiparous and multiparous cows, respectively). Next, we calculated individual and overall IOFC by either using conventional supplementation or precision supplementation. We found that using precision feeding would improve IOFC (€/cow.d) by 1.30 (min=1.02, min=1.56) and 1.56 (min=0.62, max=1.83) for primiparous and multiparous cows, respectively. Overall, in the whole farm, precision feeding would have a potential of improving IOFC by 192€/d or 70,080€/yr on the illustrated farm of 134 cows with 2 AMS.

Conclusions economic considerations

Reducing number of animals per AMS could **improve IOFC** if production does not decline

Restricting concentrate allowance to kg/cow.d **3** (PMC) and **4** (MPC) **improves IOFC** and minimizes variation nutrient intake

Precision feeding to meet cow-specific nutrient requirements may **greatly improve IOFC**

Keeping concentrate allowance low help to reduce digestion problems, feed costs, concentrate refusals, and milking regularity

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Bedding Choice, Udder Health & Productivity on Larger WI Dairy Farms

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Bedding Choice, Udder Health & Productivity on Larger WI Dairy Farms

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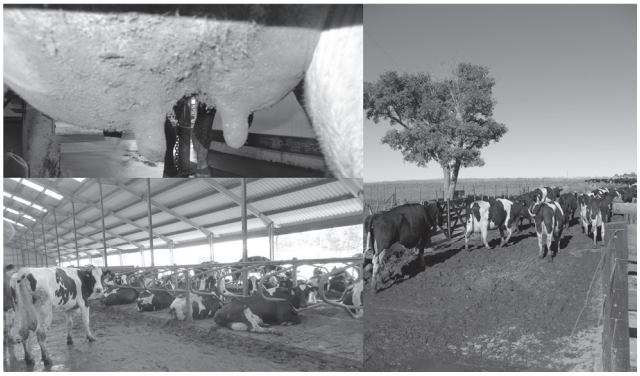
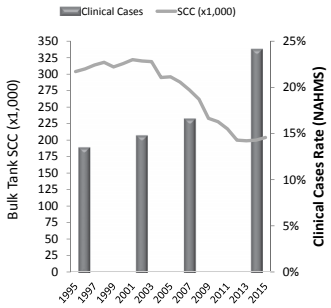
Most Pathogens are Opportunistic & Originate in the Environment



Trends in US Milk Quality & Production 1995 - 2015



- Reduced SCC
  - From 320,000 to 204,000 cells/mL
- Increased Clinical Case Rate
  - From 13 to 24%
- Milk yield increased
  - 11 lb/cow
  - Herd size increased
    - From 50 to 186 cows

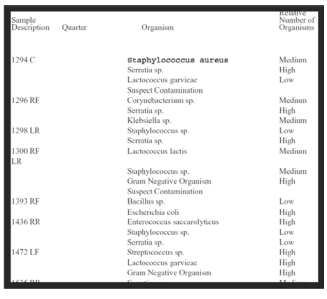
Opportunistic Bacteria

- Gram positive
  - Streptococcal organisms
  - Longer subclinical phase
  - Increase SCC
- Gram negative
  - Coliforms
  - Lipopolysaccharide in cell wall induced greater inflammation
  - Increase clinical case rate
- Reducing exposure
  - results in less mastitis
- Increasing exposure
  - results in more mastitis

Objective
Review factors influencing exposure and risk of infection

Evolution of Mastitis Pathogens

- Tremendous changes in prevalence of mastitis pathogens
  - Strep agalactiae is virtually eradicated
  - Staph aureus is highly controlled
- Other organisms are increasingly isolated
  - Changing Diagnostics
  - Changing herd structures



Sample Description	Quarter	Organism	Relative Number of Organisms
1294 C		Staphylococcus aureus	Medium
		Serratia sp.	High
		Lactococcus garvieae	Low
		Suspect Contamination	
1296 RF		Corynebacterium sp.	Medium
		Serratia sp.	High
		Klebsiella sp.	Medium
1298 LR		Staphylococcus sp.	Low
		Serratia sp.	High
1300 RF		Lactococcus lactis	Medium
LR			
		Staphylococcus sp.	Medium
		Gram Negative Organism	High
		Suspect Contamination	
1393 RF		Bacillus sp.	Low
		Escherichia coli	High
1436 RR		Enterococcus saccarolyticus	High
		Staphylococcus sp.	Low
		Serratia sp.	Low
1472 LF		Streptococcus sp.	High
		Lactococcus garvieae	High
		Gram Negative Organism	High

Bedding Types

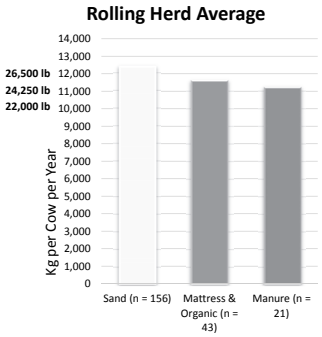
- Highly influenced by options for waste management
- Options are primarily
  - Sand
    - Clean or Recycled
  - Wood products
    - Mattresses or compost
  - Manure (biosolids)
    - Many forms

Survey of Bedding Practices of Larger WI Dairy Farms

- Selection criteria
  - Wisconsin dairy farms shipping at least ½ of a milk tanker per day
    - 25,000 lb/d
  - During period of May 2010 to April 2012
- Part of Dr. Rob Rowbotham's PhD

Herds Using Sand Had Higher RHA & Milk Income

- 2,542 lb greater RHA for herds using SAND
- **$393,000 greater milk sales per year for sand bedded herds**
- $18.52/cwt



Choice of Bedding Influences Milk Quality

- Studied 325 herds milking 255 to 8,100 cows
  - 282,235 lactating cows
- 80 lb/cow/day
- Bedding types
  - Sand (mostly clean)
    - n = 195 herds
  - Mattresses & org. bedding
    - n = 62
  - Recycled manure products
    - N = 29

Rowbotham & Ruegg, 2015 J Dairy Science

Management of Stalls Sand Bedding

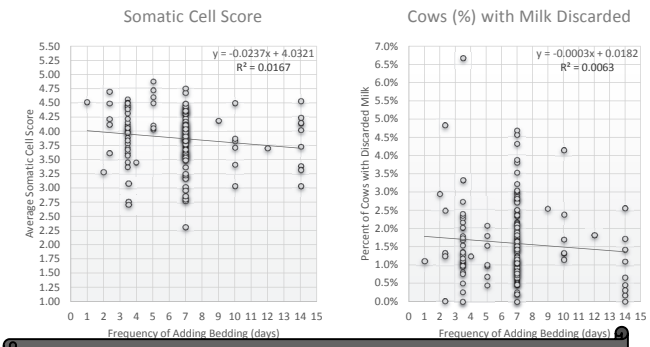
- About 80% fresh sand
- Stalls & adding bedding
  - Tires/traps = 14
    - 8.4 days
  - Mattresses = 13
    - 5.5 days
  - Deep bedded =129
    - 6.6 days
- 71% Never replace all bedding in stalls

Herds Using Sand Had Less Mastitis

Rowbotham & Ruegg, JDS 2015

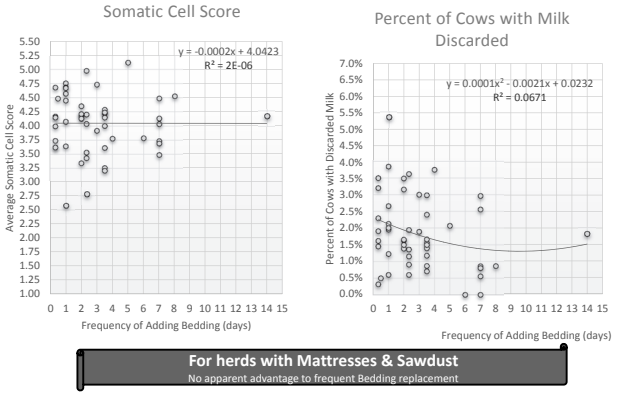
Outcome	Sand	Mattress & Bedding	Manure
Milk/cow/day (lb)	83 lb	76 lb	78 lb
Bulk milk SCC (cells/mL)	198,000	220,000	**248,000**
Cows with Milk not Sold (%)	1.6%	1.9%	**2.4%**
Cows milking <4 ¼ (%)	4.5%	4.8%	6.3%

Frequency of Adding or Replacing Sand Bedding For Deep Bedded Sand Herds



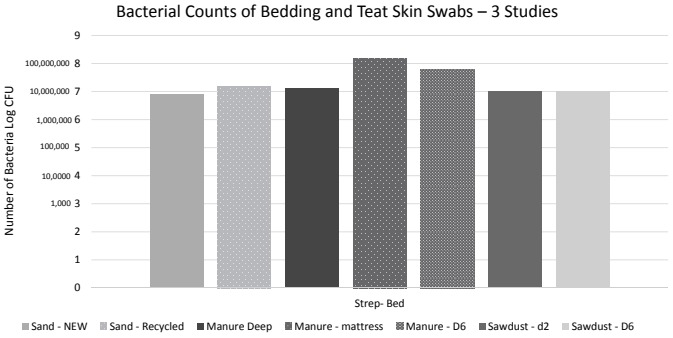
For deep bedded sand herds
No apparent advantage to adding Bedding more frequently than 1-2x per week

Frequency of Adding Bedding For Herds with Mattresses

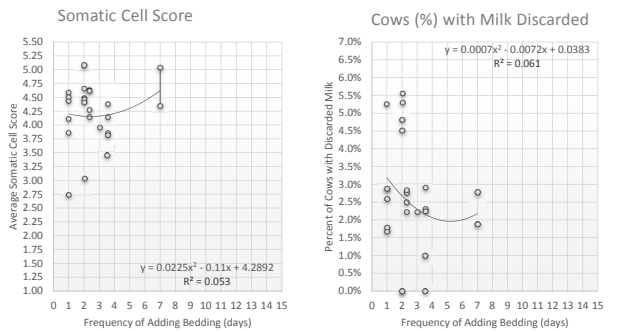


For herds with Mattresses & Sawdust
No apparent advantage to frequent Bedding replacement

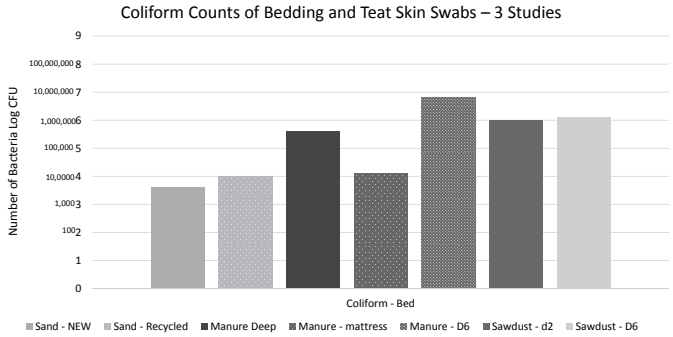
Exposure to Streptococci is High on all bedding types



Frequency of Adding Bedding for Herds using Manure as Bedding



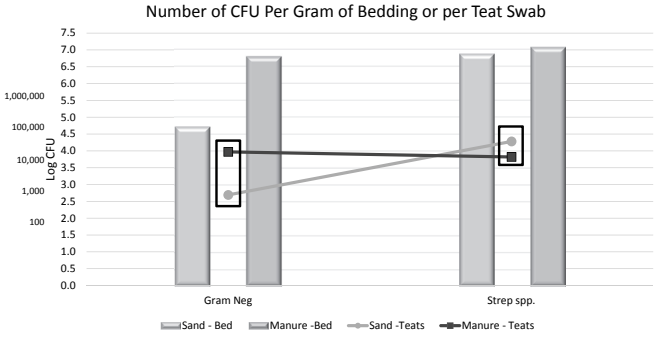
Exposure to Coliform Bacteria is >100 times Greater with Organic Bedding



What did we learn?
As compared to herds using Inorganic Bedding

- Herds with Manure or Other organic bedding
  - Produced considerably less milk
- Mastitis probably accounted for a portion of the loss
  - Greater BT SCC
  - Greater % milk discarded
  - Greater % cows with dry quarters

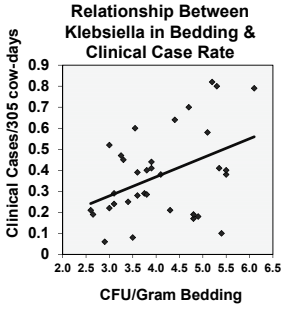
Teat Skin Has about 2 – 2.5 log less bacteria than bedding



Rowbotham and Ruegg, JDS 2016

Exposure Doesn't Always = Infection

- Linear relationship between bacteria in bedding, but…
  - Only 16% of variation was explained by bacterial count of bedding
- Mastitis is multifactorial
- What management & cows factors influence risk?



Hogan et al., 1989 JDS 72:250-258

Why Do Udders Become Dirty?

- Studied risk factors for dirty udders on 79 WI Dairy farms
  - Different risk factors based on housing

- Dirtier udders occur when….
  - Tie stalls
    - When beds were dirty & cows had loose manure
  - Freestalls
    - Use of organic bedding, dirty beds
    - Access to outdoors
    - overstocking

Role of Teat Sanitation

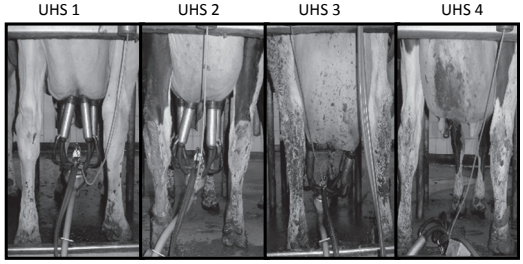
- Experimental studies show use of proven teat sanitizer reduces
  - Bacterial counts on teat skin by 2 – 5 logs
    - Enger et al., JDS 2015
  - Development of new intramammary infections by 50%
    - Pankey et al., 1987
    - Oliver et al., 1993
- On real farms, reduction is typically 2 log units

Parlor Factors Influencing Effective Teat Sanitation

- Operator training, compliance & distractions
- Parlor work routines
- Design of parlor stalls
- Compliance with teat dip storage & handling
- Willingness of cows to stand still during prep

Factors Influencing Effectiveness of Teat Sanitation

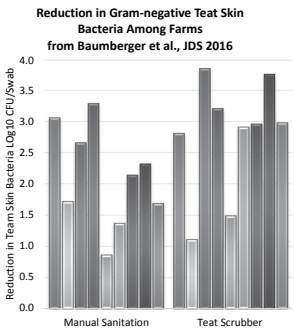
Dirtier udders = more bacteria on teat skin



Guarin et al., JDS 2017

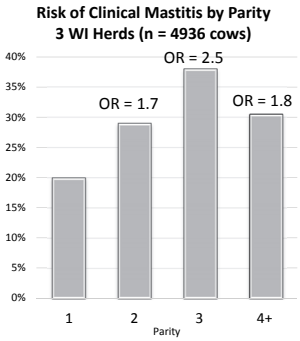
Variation Among Farms in Teat Skin Sanitation

- Performed same 2 pre-milking preps on 10 different farms
- Variation in reduction of teat skin bacteria was 1 – 3 logs
- Teat scrubber efficacy was strongly influenced by concentration of chlorine dioxide



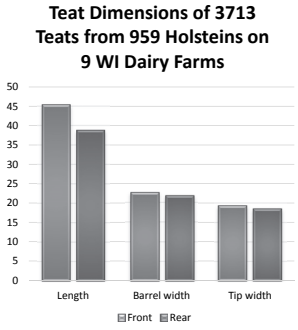
Not All Cows are at Equal Risk for Infection

- Older cows have 2X increased risk of clinical mastitis
  - Larger udder = greater exposure
- Cows with a history of clinical mastitis in previous lactation have 4x greater risk
  - Pantoja et al., JDS 2009



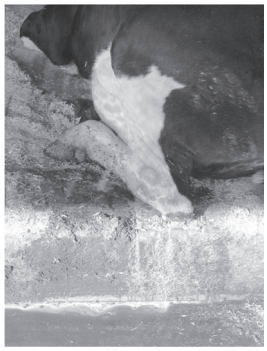
Location & Diameter of Teat

- Front teats are longer and have wider barrels
- Increased teat apex diameter is associated with increased
  - Risk of Clinical mastitis
    - Guarin & Ruegg, JDS 2016 99:8323-8329
  - Quarter SCC
    - Guarin et al., JDS 2017 100:643-652



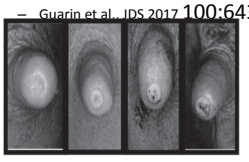
Leaking Milk Increases Risk

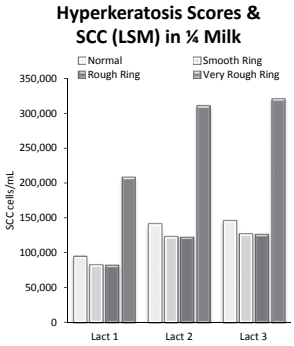
- Cows that leak milk have **greatly** increased risk of mastitis
  - Largest herd-level risk factor
    - Schukken, JDS 1990
- Immediate post-partum period is increased risk
  - First 7 days is high risk for clinical mastitis



Hyperkeratosis

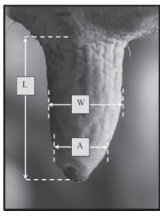
- Teats scored **VR** have increased SCC indicating increased subclinical mastitis
  - Guarin et al., JDS 2017 100:643-

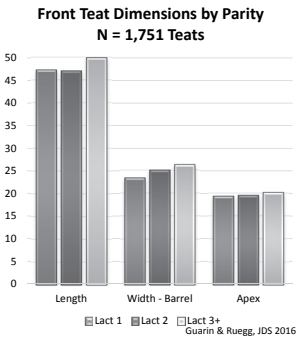




Teat Characteristics Influence Risk of Infection

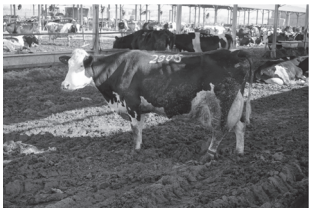
- Teats of older cows are longer and wider than younger cows





Maximum Risk

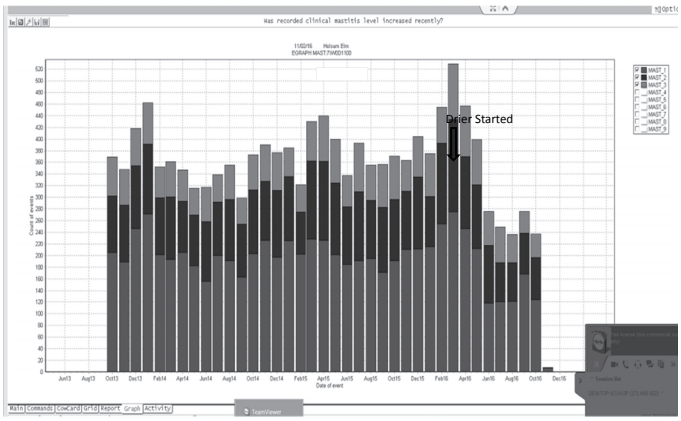
- Older (lactation 3+)
- History of previous clinical mastitis
- Leaking milk
- Early lactation
- Wide teat apex & VR
- Housed with high moisture, organic bedding



From Neave et al., 1969..

It is concluded from these results that the main reason for the failure to obtain a better control of new infection was the inability to keep teats free of the common pathogens all the time.

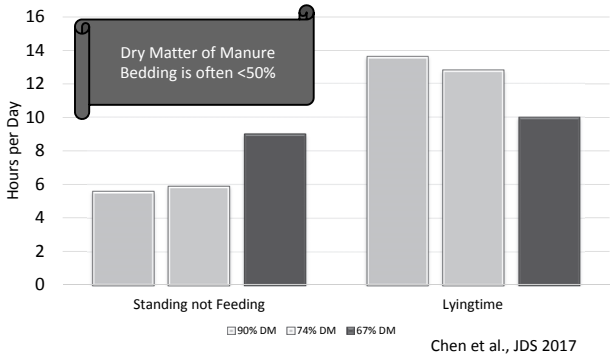
Anecdotal Data from a Large Dairy
Decline in CM After Installing Drier



Minimizing Risk – Reduce Exposure
Provide Sufficient Space

Minimum of 100 ft² (~~10m²~~)per cow in loose housing
<15% Overstocked in Freestalls

Reduce Exposure
Provide Dry Bedding

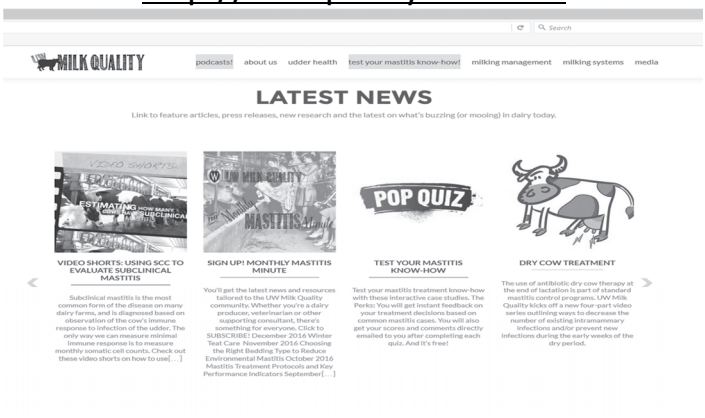


Chen et al., JDS 2017

Minimizing Risk
Identify Highest Risk Cows

- Manage high and low risk cows appropriately
- If dependent on using high risk bedding
  - Select cows with smaller udders and narrower teats
  - Milk younger animals
  - Cull cows that don't adapt
    - Leakers, recurrent cases
  - Maximize effectiveness of milking routine
  - Provide lower risk bedding to fresh cows

For more information:
http://milkquality.wisc.edu



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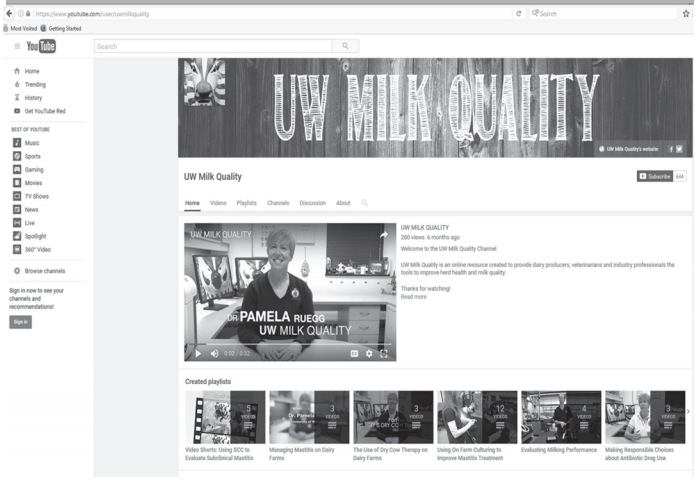

A Life Cycle, Lesion Oriented Approach to Lameness Control

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Introduction

We have a global lameness crisis in our dairy industry. The worldwide prevalence of lameness in dairy herds (defined as a cow walking with noticeable weight transfer and a 'limp') is approximately 24% across studies based in Austria, Canada, China, Finland, Germany, Italy, Netherlands, New Zealand, Norway, UK and the US (e.g., Amory et al., 2006; Barker et al., 2010; Chapinal et al., 2014; Cook, 2003; Cook et al., 2016; Dippel et al., 2009; Fabian et al., 2014; Kielland et al., 2009; Popescu et al., 2014; Sarjokari et al., 2013; von Keyserlingk et al., 2012), with a trend toward lower prevalence in grazing or mixed housing and grazing systems (e.g. 16.5% in Amory et al., 2006; 8.3% in Fabian et al., 2014; and 15% in Haskell et al., 2006), and a higher prevalence in confinement housed freestall herds (e.g. 31% in Chapinal et al., 2014; 54.8% in North East US dairy herds in von Keyserlingk et al., 2013).

Despite research and a significant improvement in our understanding of the causes of lameness over the last three decades, we appear to be fighting a losing battle, and the problem has been associated with increasing intensification of the dairy industry, higher milk production and confinement housing, with the obvious conclusion that lameness is an inevitable consequence of these decisions. Consumers carry an expectation that cows should graze and appear to place considerable value on cattle having access to the outdoors, where they have fresh air and freedom to roam (Cardoso et al., 2016). They emphasize the need for humane care of the animals (Cardoso et al., 2016), so the sustainability of the industry is threatened when the general public learns that production systems do not meet their expectations – and lameness is an obvious problem that has been and should continue to be a high priority for us to resolve.

What Causes Lameness in Dairy Cattle?

The aetiopathogenesis of a variety of hoof lesions has been researched and reviewed extensively (eg. Bicalho and Oikonomou, 2013; Cook and Nordlund, 2009; Cook, 2015; Evans et al., 2015), centering on genetic, nutritional, hormonal, mechanical, infectious and environmental factors.

Across numerous surveys in different production systems, three lesions emerge consistently as the most significant contributors to lameness – digital dermatitis (DD), white line disease (WLD) and sole ulcer (SU) (eg. Barker et al., 2009; Defrain et al., 2013). Our ability to impact lameness globally will depend on developing effective control strategies targeted at these three lesions. I will concede that some differences do exist between production systems and some countries. For example, DD has yet to become a dominant hoof lesion in New Zealand and Australia, likely due to the absence of environmental risk factors. However, the disease has spread in association with confinement housed dairy systems around the world, and now even in these locations DD is appearing at a low prevalence (Chesterton, 2015). In grazing systems, WLD appears to dominate, with sole bruising and axial wall fissures often reported. It is however important to note that a healthy sole is unlikely to 'bruise' unless the sole thickness is compromised, suggesting this as an underlying cause. We know thin soles emerge as a significant problem in larger confinement dairy systems in association with toe ulceration (Shearer et al., 2006), where cows are asked to walk long distances to and from the parlor for milking. It is therefore likely that hoof wear is the underlying issue in both, due to exposure to the track (grazing herds) or concrete alley (confinement herds).

I will contend that we now know more than ever what causes lameness, and while we still have more to learn, we know enough currently to solve the global lameness problem in our dairy industry.

A Life Cycle Approach

No matter what the causation of lameness, once the cow develops a lesion, they are at much greater risk for developing the same lesion again in the next lactation (Oikonomou et al., 2013), likely due to permanent anatomical changes to the structure and function of the claw – including the fat pad, the suspensory apparatus and the pedal bone itself (Table 1).

We are also aware that while claw horn disease is relatively uncommon in heifers, DD infection may impact 20-30% of heifers after breeding age in many rearing facilities, likely as a result of the same poor leg hygiene risk factors that have exacerbated the problem in mature cows. Laven and Logue (2007) and Holzhauer et al. (2012) have demonstrated the importance of the pre-partum period in affecting DD occurrence during the following lactation, and Gomez et al. (2015) were able to demonstrate increased risk for DD in primiparous cows when they suffer one or more episodes of DD during the rearing period, compared to heifers that are unaffected during the rearing period.

DD affects younger cows, with incidence peaking typically in the 1st or 2nd parity, while SU and WLD incidence increases with age to around the 4th lactation (Oikonomou et al., 2013).

These data therefore support an approach to lameness control that encompasses the life-cycle of the cow, starting during the heifer rearing period, with strategies that are lesion specific and age-specific, tailored to the type of lesions that are most prevalent on each farm. Understanding the motivation for farmers to implement change is critical for consultants (Leach et al., 2010). However, it would seem likely that with the growth and expansion of welfare audits globally, they will have little choice but to comply. Ultimately, producers that have succeeded in their control of lameness will become the best salesmen of prevention programs to the others that lag behind, and these producers will increasingly need an effective roadmap to expedite change.

Herd Risk Factor Oriented Strategies

Herd level risk factors for lameness have been studied in multiple countries and in a variety of production systems in recent years. A number of consistent findings have emerged from these studies. Factors which appear to be associated with lower lameness risk include; less time standing on concrete (Bell et al., 2009), deep bedded comfortable stalls (Chapinal et al., 2013; Cook, 2003; Dippel et al., 2009; Espejo et al., 2006; Rouha-Mulleder, et al., 2009; Solano et al., 2015), access to pasture or an outside exercise lot (Chapinal et al., 2013; Hernandez-Mendo et al., 2007; Popescu et al., 2013; Rouha-Mulleder, et al., 2009), prompt recognition and treatment of lameness (Barker at al., 2010), higher body condition score (Dippel et al., 2009; Espejo et al., 2006 Randall et al., 2015), use of manure removal systems other than automatic scrapers (Barker at al., 2010), use of non-slippery, non-traumatic flooring (Barker et al., 2010; Sarjokari et al., 2013; Solano et al., 2015a), use of a divided feed barrier (rather than a post and rail system), with a wider feed alley (Sarjokari et al., 2013; Westin et al., 2016).

It should be expected that routine professional hoof-trimming, access to a trim-chute for treatment and use of an effective footbath program would deliver improvements in lameness (eg. Pérez-Cabal and Alenda. 2014), but these effects are often confounded in associative observational studies (eg. Amory et al., 2006). It is also true that many poorly trained hoof-trimmers cause more harm than good, and many footbath routines are ineffective through poor design and management (Cook et al., 2012; Solano et al., 2015b). Similarly, several studies point to restrictive neck rail locations, high rear curb heights, and lunge obstructions as risk factors for lameness (eg. Chapinal et al., 2013; Dippel et al., 2009; Rouha-Mulleder, et al., 2009; Westin et al., 2016), however correct neck rail location and curb height is stall design specific and care should be taken in interpretation of these findings. Most recently, stall width has emerged as a significant factor impacting lameness (Westin et al., 2016)

High Production and Low Levels of Lameness

While we know that Holstein cows are perhaps more susceptible to lameness (eg. Sarjokari et al., 2013), and there appears to be a genetic component to the development of DD, SU and WLD, with a link to higher milk production (Oikonomou et al., 2013), I do not believe failure is inevitable.

We had the opportunity to visit 66 high performance Wisconsin herds that have been implementing strategies to prevent lameness for over a decade (Cook et al., 2016). These herds had a mean herd size of 851 cows, were confinement housed in freestalls and produced more than 40 kg energy corrected milk per cow per day on average. The prevalence of clinical lameness averaged 13.2% - which would rival the degree of lameness identified in grazing herds (e.g. 8.3% as reported by Fabian et al., 2014), and mixed housing and grazing or organic management systems elsewhere (e.g. 16.5% in Amory et al., 2006; 17.2% in Rutherford et al., 2008). Interestingly, it is lower than the prevalence found in similar herds in the Midwest a decade or more ago (e.g. 22.5% in Cook, 2003; 24.6% in Espejo et al., 2006), suggesting that the overall degree of lameness in the region may be improving. Severe lameness was also uncommon at a mean of 2.5%. This average is lower than that found in the majority of previous freestall surveys (e.g. 5.3% in Barker et al., 2010; 16% in Dippel et al., 2009; 4.8% in Husfeldt et al., 2012). Thus it would appear that high performance can be compatible with ac-

ceptable lameness levels, if we manage cows correctly. Table 2 highlights some of the management characteristics of these herds pertaining to lameness management.

When examining the management strategies with high levels of adoption in Table 1, there are consistencies with the herd level risk factors documented previously. These herds use deep loose bedded stalls, have 2-row pen layouts with headlocks, have solid flooring with strategic use of rubber floors, especially around the milking center. Notably, these herds were not using rubber flooring in their pens to control lameness. They clean manure from the alleys when the cows are outside the pen, and have aggressive hoof care, heat abatement and footbath programs. Two thirds of herds use rBST and milk three times daily, and perhaps surprisingly, 9% let their high producing cows outside the barn strategically – not to graze, but to spend time away from concrete floors inside the barn. In a multivariate model, deep bedded stalls, pasture access and fewer cows per FTE worker significantly reduced the risk for lameness overall.

Lameness management will continue to be refined, but these herds prove that we know enough right now to implement positive change in the dairy industry and achieve acceptably low levels of lameness, even in cattle which may be inherently more susceptible.

A Structured Approach to Lameness Prevention

When troubleshooting lameness problems, I use a structured approach starting with locomotion scoring, lesion analysis and assessment of the routine hoof-trimming and lame cow surveillance program. It is essential that the hoof-trimming is a component of prevention rather than a risk factor in itself. I then examine the risk factors for each of the key hoof lesions and finish with a review of feeding practices. From this examination, we can create a herd specific action plan designed to maximize impact on the key hoof lesions on the farm.

For DD prevention, we focus on the early identification of acute lesions (before the cattle are lame) and prompt effective treatment, starting around breeding age in replacement heifer pens and continuing throughout the life of the animal, coupled with an effective footbath program to control the chronic lesions and hold them in check. Trace mineral supplements have a significant role to play, particularly during the rearing period. For SU prevention we target risk factors that extend daily standing times – stall design and surface cushion, stocking density, milking times, heat abatement and lock up time for management tasks. We optimize the transition period to maximize rest and reduce BCS loss in early lactation. Finally, for WLD control, we examine areas of the farm where flooring puts the cow at risk of slipping, trauma and excessive hoof wear, and watch workers to ensure low stress handling – especially around the parlor operation.

The overall approach is summarized in Figure 2. Each assessment results in a problem list which can then be used to develop a targeted action plan for the herd.

Conclusion

In this article, I have made the case, that while we still have knowledge gaps to fill in our understanding of lameness, the global crisis that we face with 1 in 4 cows walking with a painful limp can be solved by implementing our current knowledge targeted at the key hoof lesions; DD, WLD and SU. The challenge we face is one of creating a simple roadmap targeted at an individual producers most significant problems and motivating that producer to implement the changes necessary. Dairy producers that have already achieved success in lameness prevention will serve an important role motivating others to follow in their foot-steps.

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Figure 1. Worldwide prevalence of lameness in dairy herds by location from the peer reviewed literature since 2003

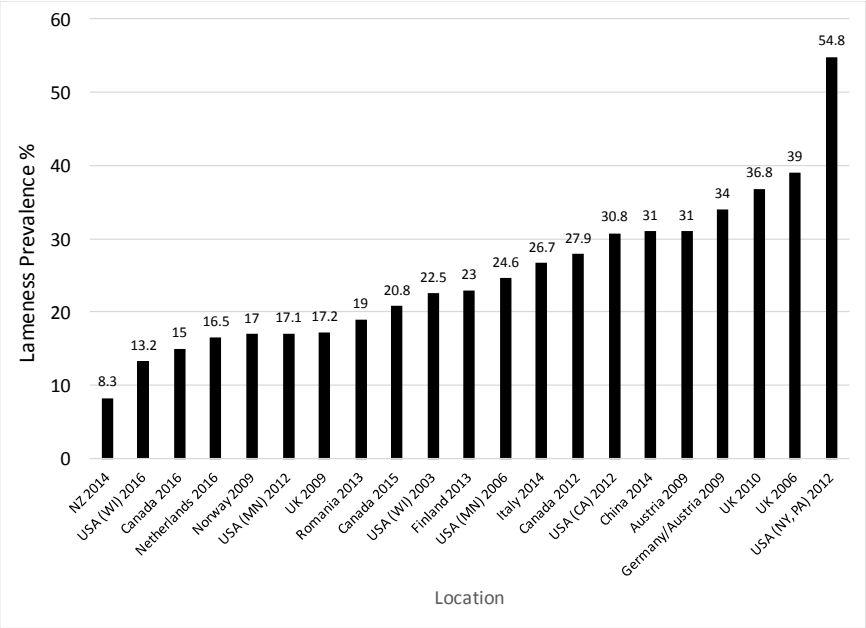

Figure 2. Herd lameness troubleshooting plan

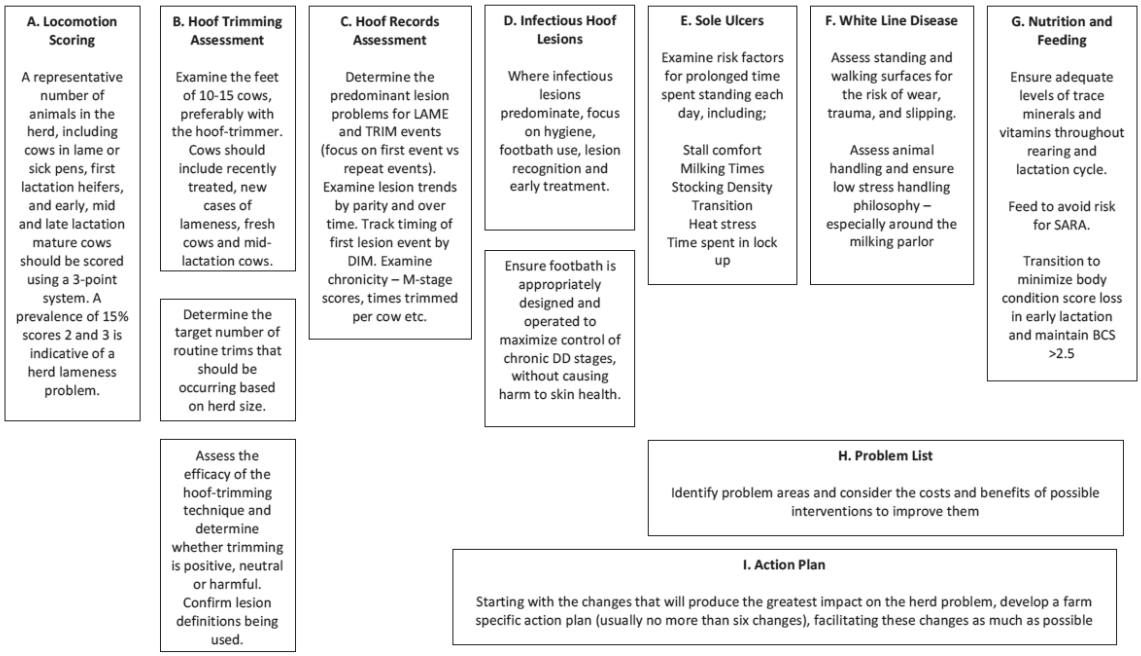

Table 1. Lactation adjusted incidence of lameness lesion (white line disease = WLD, sole ulcer = SU and digital dermatitis = DD) by lesion status (0 = no lesion, 1 = lesion) in the previous parity (1-3). (from Oikonomou et al., 2013)

Lesion	Parity	Lesion Status	Lactation Adjusted Incidence			P-value
			2	3	≥4	
WLD	1	0	6	11	15	<0.01
		1	20	21	24	
	2	0		9	13	<0.01
		1		20	18	
	3	0			10	<0.001
		1			21	
SU	1	0	12	20	26	<0.001
		1	44	32	23	
	2	0		15	24	<0.001
		1		40	30	
	3	0			18	<0.001
		1			41	
DD	1	0	7	7	8	<0.001
		1	32	15	10	
	2	0		5	7	<0.001
		1		19	12	
	3	0			5	<0.001
		1			14	

Table 2. Management characteristics of the high producing multiparous group cows in elite housed dairy herds in Wisconsin (from Cook et al., 2016).

Management Characteristic	***% Herds or Mean***
% Sand bedded stalls (deep loose bedding including manure solids)	62 (70)
% 2-row stall layout pens (vs 3-row)	61
% Use of headlocks at the feedbunk	83
Milking Frequency (% 3 times a day)	67
% Use of rBST	67
% Solid floor (vs slatted)	100
% Rubber floors in freestall alleys	5
% Rubber floors in transfer lanes	15
% Rubber floors in holding areas	41
% Rubber floors in parlors	68
% Manual manure cleaning from the alleys	73
% Use of fans over the resting area	96
% Use of water soakers in the pens	79
% Allow access to the outside to roam	9
% Trimming at least once per lactation	88
% Trim cows at least twice per lactation	65
% Trim heifers before calving	49
Mean footbath frequency (milkings per week)	4.5
Mean cows per full time equivalent (FTE) worker	62

The Fundamentals For Good Hoof Health

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The Fundamentals For Good Hoof Health

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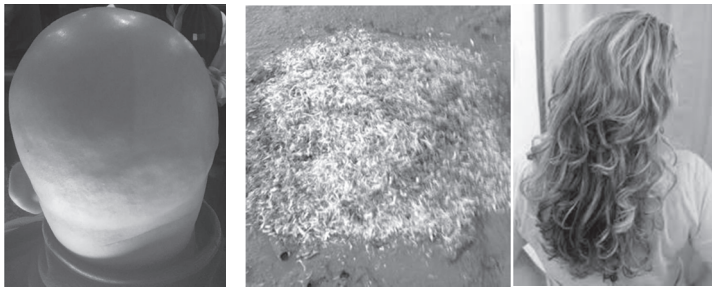
The Roadmap to target three hoof lesions

- Hoof trimming accountability
- Functional and therapeutic hoof trimming
- Hoof trimming schedule
- Lameness treated within 24hrs
- Integrated approach to managing digital dermatitis
- Making the hoof bath work

Considerations for improving hoof health

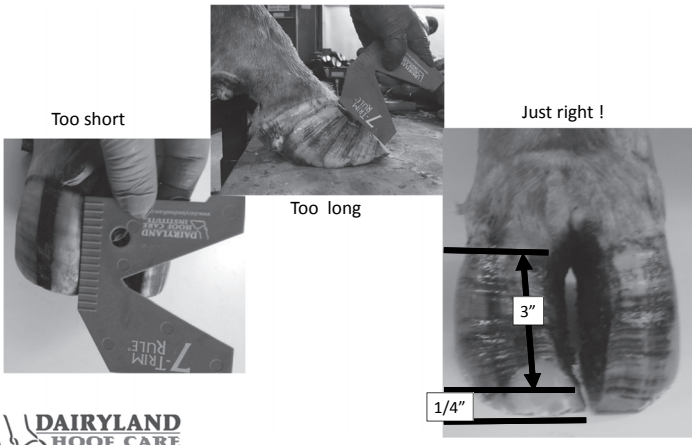
- Using best practice management tools and action plans.
- Animal welfare = "No Lameness Tolerance" policy
- A scientific approach
- Improving the bottom line

What is a producer paying for?



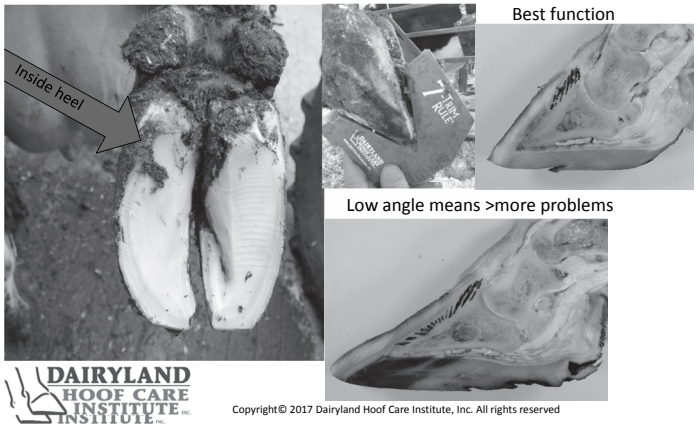
The hoof chips on the floor?

1. Trimming toes too short

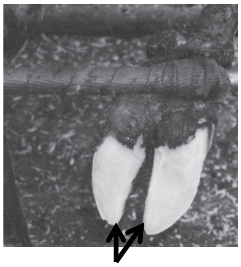


2. Excessive trimming

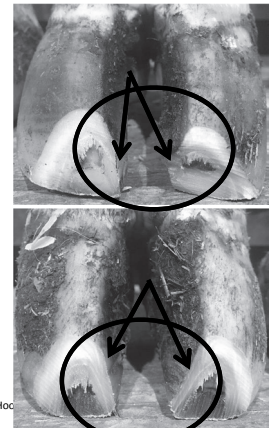
Heel of the inside claw. White soles means = over-trimming



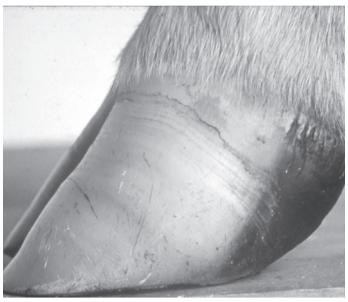
3. Removal of the axial or inside wall of the toe



Trimming between the toes with the grinder

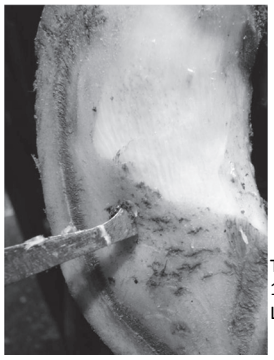


4. Excessive removal of the abaxial or outside wall

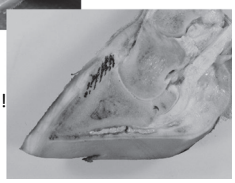


The wall is the supporting edge of the claw!
It should never be removed except when lame!

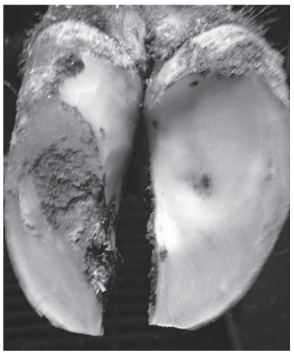
5. Trimming the soles too thin



The corium requires
1/4 of sole to stay healthy.
Less sole = more lameness!



Industry measuring stick



“How many cows can you trim in a day”?

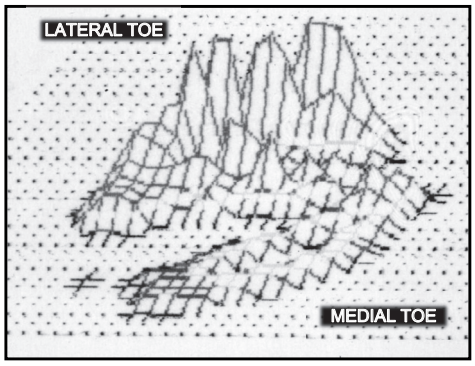
What is the correct question?

Do we prevent or cause claw horn lesions with hoof trimming?

Industry measuring stick

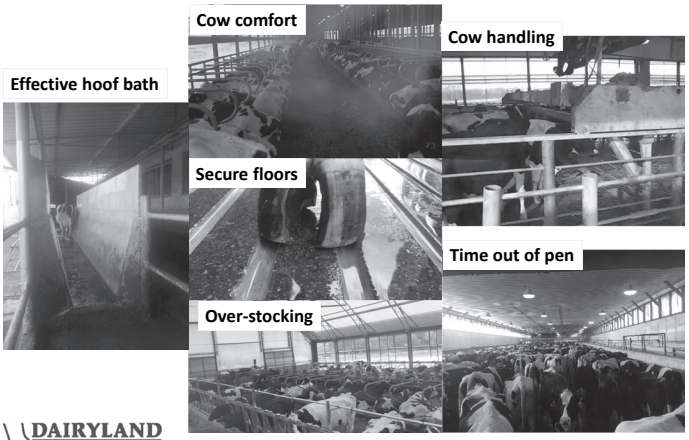
- Is hoof trimming preventing lameness or causing lameness?
- Are lame cows recovering following therapeutic hoof trimming?
- Do cows become lame and stay lame?
- Low lameness = good hoof trimming!

95% Lameness = rear outside claws

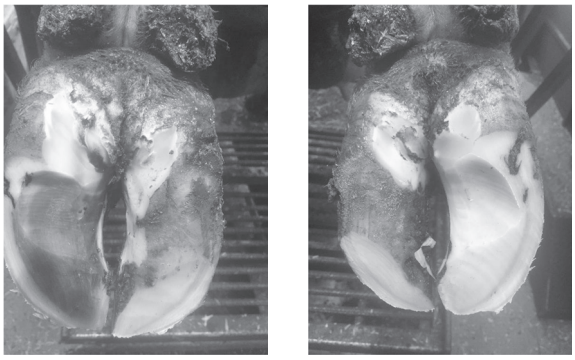


Left rear hoof pressure plate results!

Is hoof trimming the problem?

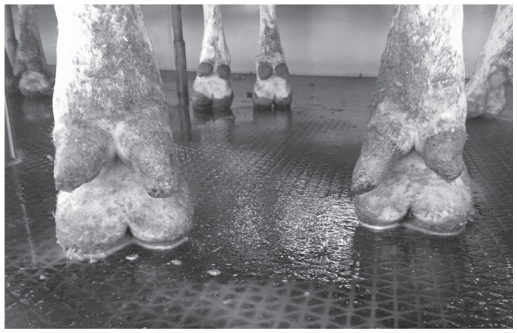


Functional hoof trimming



Re-establishes healthy claw function

95% claw lesions = rear outside claws



The real story

Timed trimming schedule

- Every dry cow, every springing heifer is assessed and functionally trimmed 8 to 3 weeks prior to calving
- Perform one or two more lactation assessments and trims depending on:
  - Cow housing, environment and management
  - Age of cow
  - High maintenance cows

Timed trimming schedule

- First lactation cows next trim at 125 days
- Second lactation and over at 80 days for mattress barns, 125 days sand barns
- All cows every 120 - 150 days thereafter
- SOP for chronic lame cows (check rear feet 3 to 6 times extra per year) Flag in management software!

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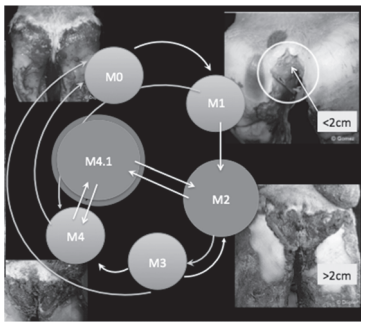
Digital dermatitis (hairy warts)



- Primary cause: breakdown in immune system
- Compromised skin integrity
- Opportunity for bacteria to enter
- Also need low oxygen environment
- **NOTE: Placing bacteria that cause digital dermatitis on healthy skin *will not* result in digital dermatitis**

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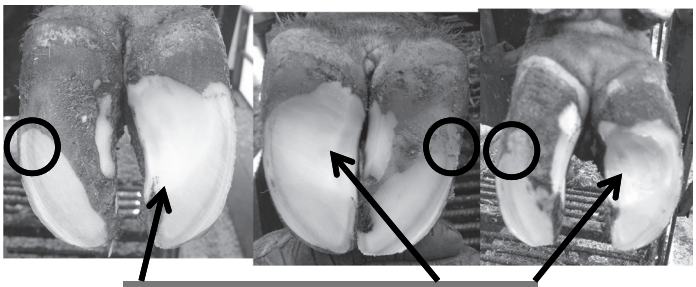
Trim springing (pre-calving) heifers



More modeling of the outside hind claw significantly reduced onset of digital dermatitis in 1st lactation animals

Sparing the heel of the inside rear claw is good for DD prevention

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< 20 mm

Regular foot bathing will prevent disease progress or disease outbreak.

M 1
Subclinical stage

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The typical sign of digital dermatitis

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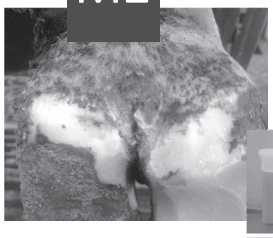
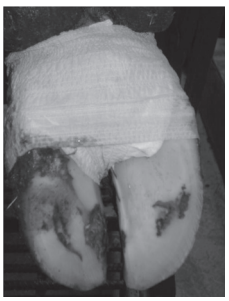
Early identification and prompt treatment can *interrupt* this disease!

M2



Less is successful and safe ! 2 grams!

2 g of Tet 324 activated with Quick Hit Gel or vinegar



Remove bikini wrap after 24 hrs !!!

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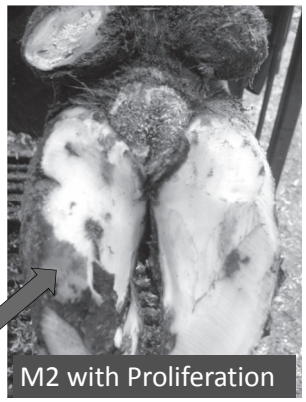
Delayed treatment results



Progression of disease. Bacteria have begun to migrate deeper into the epidermis and encyst!

Hygiene influences DD occurrence

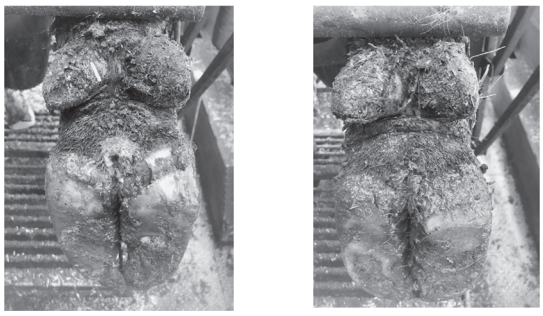
Leg hygiene score in animals with no DD is better than in animals with DD

This is a permanent DD lesion

Lesion present for life

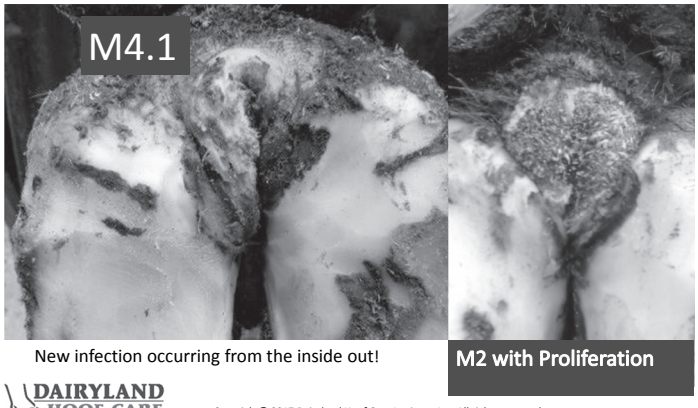
- Spirochetes migrated deep into the epidermis = are encysted
- Hyperkeratosis present
- Encysted bacteria have colonized/organized and will surface again
- New infections must be prevented with regular hoof baths and good hygiene

Hygiene influences DD occurrence



DD increases with higher leg hygiene scores.
Animals with DD have higher leg hygiene score!

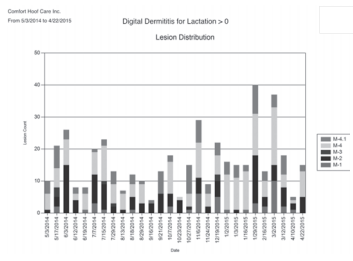
New infection = disease shedder



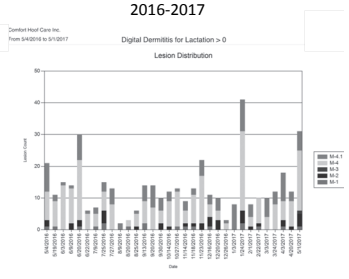
New infection occurring from the inside out!

Improving hygiene and its effects on DD

2014-2015



2016-2017



June 2015 - Introduced Hypochlorite NaClO as a cleaning product twice per week!

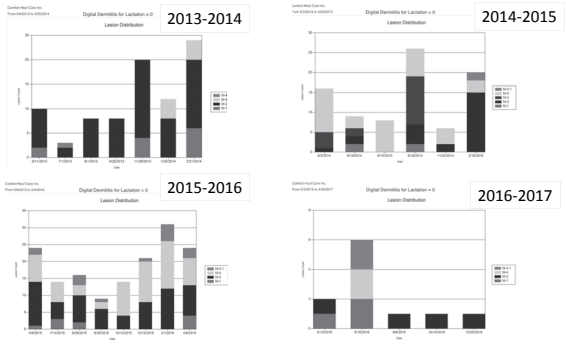
Integrated approach for controlling DD

- Close observation of heifers >10 month
- Prompt treatment of early lesion
- Must use topical antibiotic the first time!
- Excellent hygiene and low stress environment
- Footbath to control M4 lesions and prevent M1 lesions
- If the hoof trimmer is treating all DD the approach is not integrated

The Role of the Hoof Bath

- Improve hygiene condition of hooves
- Disinfect hooves for prevention and control of hygiene influenced hoof diseases
- Prevent foot rot infections
- Control and treat early DD (M1) infections
- Control chronic DD (M4) from re-infecting

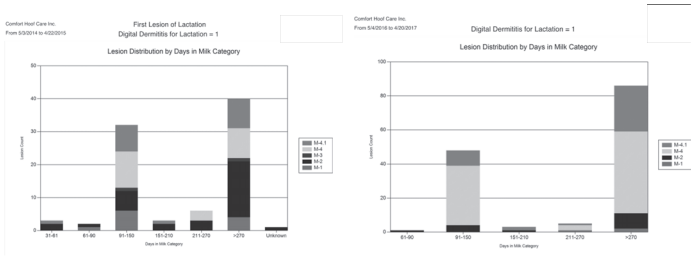
Heifer DD program with Availa Plus® and early antibiotic treatment



Efficacy of hoof bath solution

- "The best solution in the hoof bath is 12 feet or 4 meters in length"
- Dr. Dopfer at UW Veterinary school will run a test to determine solution efficacy
- Change solution after "x" amount of cows walk through
- Defecation into the bath
- Leg hygiene score determines hoof bath frequency

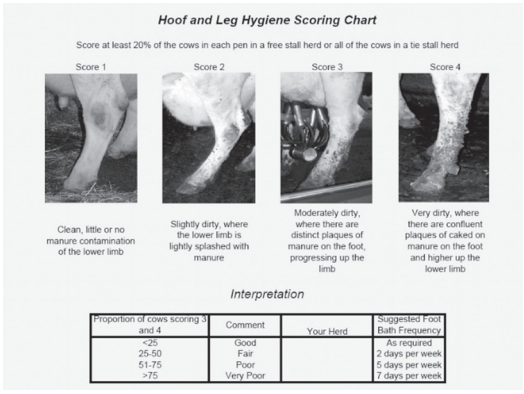
DD 1st in Lactation change in 3 years



2014-2015 2016-2017

Constant inoculation = challenging

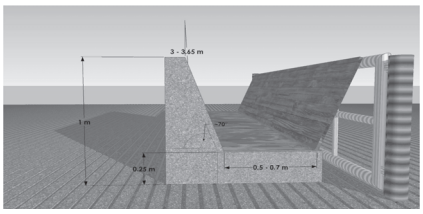
Hoof and leg hygiene



Footbath with sidewalls or a race

Cows will pass through the bath without defecating!

The ideal hoof bath? H



Hoot bath with sidewalls or a race

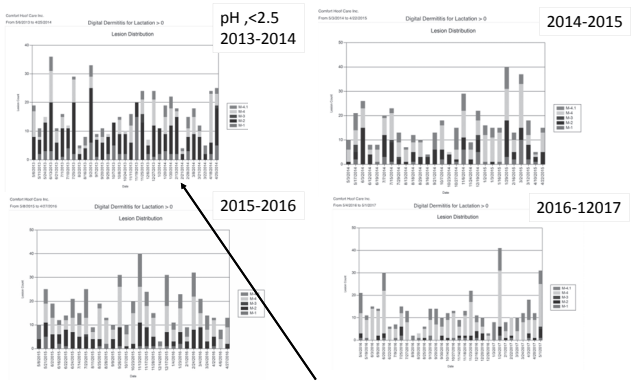
Effective hoof bath dimensions

- 12 feet (4m) long
- 20 inches (50cm) wide
- 36 inch (75cm) sides
- 6 foot (1.80m) side panels
- 12 inch (25cm) entrance and exit curb
- 3 ½ inch (10cm) solution

Hoof bath chemicals

- Manure contamination dependent (200 – 600 cow passes?)
- At what point does the chemical cease to kill Treponeme spp ?
- ***Acid based hoof bath*** keep pH between 3.0 and 5.0 for best results
- How much does the chemical promote skin hyperkeratosis !!!? (low pH, strong concentration, etc.)

Low pH chemical caused more DD



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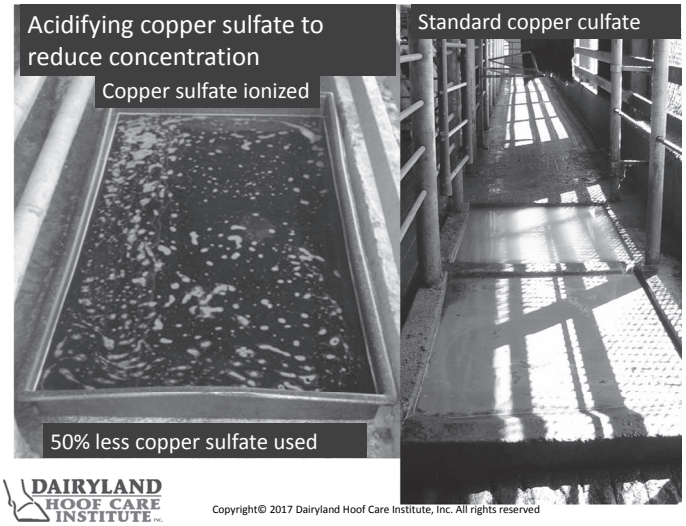

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Common hoof bath solutions - cleaning

- Mild soap/ bleach 1 quart soap/4 quarts bleach 50 gal water
- Mild soap/ bleach/ salt 1 quart soap/4 quarts bleach 5 lbs salt/50 gal water
- Hypochlorite NaClO 2 1/2 gallon 50 gal water

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Footbath frequency

- Farm Dependent!
- Adapt the footbath frequency based on DD prevalence (M4) and foot rot prevalence
- Use records to predict changes in stocking density or determine high risk periods
- Careful with environmental accumulation of chemicals and costs

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Common hoof bath solutions - disinfecting

- Copper Sulfate 2.5% 12 lbs (5kg) CU +
  Sodium Bisulfate 6oz(100g)/50gal water
  (NaHS04) (.0.5g/l)
  (<u>monitor pH regularly, 3.0 – 5.0</u>)
- Use hot water for initial mix of CU and NaHSO4
- Hoof Zink (Follow manufactures recommendation)
- Formalin 1.5 - 2% 2-3 quarts/50gal water
- **Integrate other commercial products only if effective!**

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Take Home…

- Hoof baths are used to keep chronic or subclinical DD from going into active DD
- The hoof bath design matters!
- Hoof bathing protocol is farm dependent

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Conclusion for achieving good hoof health

- Evaluate functional and therapeutic hoof trimming
- Evaluate lame cow recovery
- Every cow is assessed 1 to 3 times per year
- Identify DD early and treat first lesion with topical antibiotic
- Use a well managed hoof bath
- Ensure hoof bath chemical proves efficacy

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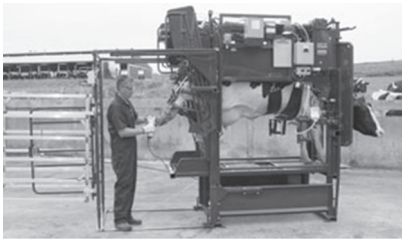
Success in the Details

Thank You!



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Mycotoxins in Dairy Cattle: Who, What and Why

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Introduction

Feeding high-producing dairy cows involves a combination of forages, grains and supplements to meet nutrient requirements and support rumen function. In addition to impacting yield, weather and growing conditions in the field have additional effects that can impact forage and grain quality. Stress factors such as water availability and temperature may promote growth of fungi or molds on the developing plants. In addition, molds can also develop during storage and processing of crops and grains. The presence of molds on feeds poses an increased risk for health and productivity problems due to the presence and ingestion of mycotoxins. These secondary metabolites are produced by different species of molds and can cause toxicosis, in animals and humans, if ingested in large quantities or for prolonged periods. Molds in the Aspergillus and Fusarium are commonly found in feeds for livestock, these molds and their toxins will be the focus of this paper. Each mycotoxin varies in chemical structure which dictates the nature of its toxic effects. Some effects of mycotoxicosis include anorexia, immunosuppression, reproductive failure, and cancer.

Mold Growth and Production of Mycotoxins

Mold spores are naturally present in the soil and in crop residue on the field, therefore it is almost impossible to prevent presence of mold spores on crops. Environmental conditions conducive to further development of spores into active mold growth include extremes in weather and insect damage. Water and temperature are determinant factors for mold growth; while irrigation may be an option for some producers to reduce draught stress on crops, is virtually impossible to do anything to manage ambient temperature. Having methods to control insects and fungi on the growing plants is a practical approach to reduce the risk of pre-harvest mycosis; such practices include growing insect resistant crops and applying foliar fungicide.

Post-harvest mycosis is a latent risk during associated with poor harvest and storage practices. One of the most critical factors that modulate mold growth is moisture availability, determined as water activity (aw). For this reason, post-harvest prevention of mold on dry feedstuffs focuses on maintaining aw below 0.7, from a practical stand point this translates to a moisture content below 15% for grains and hays. For high-moisture feeds such as fermented forages and some industrial y-products, aw is inherently favorable for mold growth which indicates that other conditions and management practices must be addressed to prevent fungal growth. Although some species are tolerant to acidic conditions, most molds grow in pH from 4 to 8. For most fermented forages, the final pH is acidic at the final stage of fermentation but mold could grow shortly after harvest and during initial fermentation when pH is still high. Failure to maintain an anaerobic environment in fermented forages may also lead to fungal growth. Promoting an effective anaerobic fermentation of forages has two-fold advantages by preserving feed quality and increasing food safety for animals and humans alike.

Types of Mold and Their Toxins

Aspergillus

Aspergillus flavus and A. parasiticus produced a mycotoxin known as aflatoxin. This mycotoxin is particularly important in the dairy industry because it is known to be mutagenic and carcinogenic. Approximately 1.5% of aflatoxin intake is transferred to milk as aflatoxin metabolite M1. Collectively, this mycotoxin is a serious health threat for animals and human and the FDA has set limits on the concentration of aflatoxin in feeds and milk, the maximum aflatoxin level in milk is set at 0.5 ppb. This mycotoxin is rapidly absorbed, some reports have indicated its presence in blood of cows upon hours of exposure to contaminated feed, the authors indicate that this observation likely indicates that it can be absorbed through mucosa and clearance rate in controlled has been achieve within 72 to 96 after last ingestion with no deleterious effects on performance.

Ingredients utilized in Midwest rations that may have aflatoxin more frequently include corn and cottonseed. The degree of damage or diseased caused by aflatoxicosis depends on the length of exposure and level of contamination. Although controlled studies rarely show negative effects on animal performance,

it is important to highlight that most experiments are short term exposure to purified aflatoxin. In contrast, field conditions generally involve low level of exposure during sustained periods, in addition, the toxicity of naturally occurring aflatoxin has been shown to be more potent than purified forms. Therefore, it is important to consider that consumption of naturally contaminated feeds can cause negative effects not observed in controlled studies. Some of the external symptoms of toxicosis by aflatoxin include anorexia and low milk production, and increased susceptibility to diseases due to immuno-supression; internal damage is commonly seen in enlarged liver.

Fusarium

Fumonisin is one of the several mycotoxins produced by Fusarium molds. It has been widely reported to cause liver and kidney toxicity. Exposure to fumonisin is hard to detect via milk analysis because there is little to no transfer. Due to its hepatotoxic effects, concentration of liver enzymes in serum could be used as an indicator of liver damage. In addition to organ damage, its toxic effects also extend to tissues that contain sphingolipids, for example nervous tissue, because its chemical structure is similar to sphingosine, a component of sphingolipids.

Another fusarium-produced mycotoxin is zearalenone; this mycotoxin is very relevant in the dairy industry because it can have negative implications on reproductive performance. Zearalenone has a chemical structure that resembles estrogen, hence the potential for impaired reproductive function. This mycotoxin has been associated with abortion, irregular estrous cycles and undersized corpora lutea.

Deoxynivalenol (DON), also known as vomitoxin because it is commonly reported to cause vomiting bouts in swine. Unfortunately, controlled experiments documenting effects of DON on dairy cattle are rather scarce and there are still conflicting reports. Like many mycotoxins, appetite suppression and low milk production have been reported upon consumption of contaminated feed with ≥ 2.5 ppm of DON. Even though the body of research of DON on dairy cattle is limited, there is some agreement in the fact that consumption of DON-contaminated wheat altered rumen protein metabolism resulting in protein degradation and ammonia concentration. In addition, flow of microbial protein to the duodenum was reduced when cows consumed a contaminated diet.

T-2 toxin is another fusarium-produced mycotoxin. This mycotoxin has been reported with greater frequency in corn grown under draught conditions. Epithelial damage of intestinal mucosa, as inflammation and hemorrhage, lead to the external symptoms of toxicosis by T-2 including bloody feces and death.

Take Home Messages

- Most mycotoxins are an animal and human health risk
- Drought-stressed crops are more susceptible to fungal infection
- Field conditions are hard to control, but harvesting and storage conditions can be managed to reduce risk of fungal infection in grains and forages
- Aflatoxin is a public health concern, limit set by FDA is 0.5 ppb
- Negative impacts on dry matter intake and milk production upon chronic exposure
- Co-occurrence is very likely, combinations of mycotoxins exacerbate negative effects
- Beware of no or mild negative effects reported in the literature, purified mycotoxins are less potent than the naturally occurring toxins

Potential for Sorghum Forages for Dairy Heifers in the Midwest

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Marshfield, WI

Introduction

Dairy heifers require moderate quality diets (60-65% TDN) to maintain adequate body weight gains (1.8 to 2.0 lb average daily gain). Diets for heifers are typically forage based but can still have excess energy, especially when corn silage is used a significant part of the diet. These diets often do not have enough fiber to control feed and energy intake causing excess gains and body condition in heifers compared to lower energy diets that include high fiber forages like straw, fodder or perennial warm season grasses (Coblentz et al, 2015). This can especially be a problem for pregnant heifers with excess bodyweight and condition leading to calving difficulties and metabolic issues after calving that lower milk production and profitability. When fed high forage diets, heifers will typically eat close to 1% of BW as NDF each day (Hoffman et al., 2008) so feeding a higher NDF diet can help control intakes. Production of lower energy, higher fiber forages that have similar yield to corn silage and at potentially lower costs would be useful for feeding dairy heifers. Sorghum and sorghum-sudangrass forages have a moderate nutritive quality (higher fiber, lower starch). Also, sorghums have 75% of the nitrogen and water requirements compared to corn (Bean and McCollum, 2006). This makes sorghum a potentially useful crop to control heifer growth, and reduce input costs for nitrogen and where irrigation is necessary for crop production. New types of sorghum (photoperiod sensitive) are available, however limited work has been done with these in the Midwest. Photoperiod sensitive (PS) varieties stay vegetative until total daylight reaches 12 hours and 20 minutes (mid-September), allowing the plant to accumulate greater forage mass. This summary will provide yield and quality information from recent plot research focusing on the agronomic management (planting date, harvest strategy, nitrogen fertilization, and irrigation) of sorghum forages in Central Wisconsin.

Effects of Planting Date and Harvest Strategy

Planting date can impact forage yield with later dates generally lowering forage yield. In addition, the strategy to harvest sorghum forage (single cut or multiple cut) can impact yield and quality. In 2015 and 2016, seven sorghum forages (see Table 1 for varieties) and 1 corn silage hybrid were evaluated at 2 locations (Marshfield Agricultural Research Station and Hancock Agricultural Research Station) in a plot study. Marshfield and Hancock were chosen due to having very different growing conditions. Soils in Marshfield have good fertility but have poor drainage which slows warming, while Hancock has very sandy soils that warm quickly in spring but require irrigation and multiple fertilization events to prevent nutrient leaching. Sorghums evaluated included 1 PS forage sorghum, 1 PS sorghum-sudangrass, 1 conventional forage sorghum, 1 BMR forage sorghum, 1 conventional sorghum-sudangrass, 1 BMR sorghum-sudangrass, and 1 PS BMR sudangrass. Plots were seeded in the first week or third week of June (approximately 2 weeks apart) with seeding rates of 32,000 seeds/acre for corn, 100,000 seeds/acre for forage sorghum, 20 lb/acre for sorghum-sudangrass, and 15 lb/acre for sudangrass. Sorghums were planted at 15" rows with a no-till drill and corn planted at 30" rows. Plots were harvested either using a single or multiple (2 harvests) cut strategy with 4 plots for each variety.

Forage Yield

Single cut plots had 2-3 times greater yields than using multiple cuts, however using a 2-cut system at Marshfield was more similar to a 1-cut system than at Hancock. Forage yields were 3-4 tons DM/acre greater for single cut plots at Hancock than at Marshfield likely due to faster emergence and better soils conditions for sorghum production. As expected, the early June planting resulted in greater yields than when planting in mid-June, but when emergence was delayed at Marshfield in 2015 the difference was smaller. The single harvest PS varieties and non-PS

sorghum-sudangrass had greater yields than BMR varieties, with corn and forage sorghum being intermediate. Also, sorghum-sudangrass and sudangrass had more similar yields using either 1 or 2 harvests than other varieties due to greater tiller production than forage sorghums. Compared to corn, sorghum forages generally produced similar or greater yields of moderate quality forage using a single cut system.

Forage Quality

The single harvest strategy decreased NDF levels especially for varieties that were reproductive (corn silage, non-PS forage sorghums, and the conventional sorghum-sudangrass) due to the accumulation of starch in the corn kernels or seed-head which diluted NDF (Table 2). The decreased NDF in the PS sorghum varieties and BMR sorghum-sudangrass may be due to increases in sugars accumulating in the stalk due to cool temperatures in the fall. It was anticipated that these forages would have higher NDF content with increased growth. Crude protein was lower when using a single harvest. Protein levels were lower at Hancock than at Marshfield possibly due to differences in soil properties and available N. Protein levels for forages at Hancock would not be sufficient to meet pregnant heifer needs and need to be fed with a higher quality forage or protein supplement. At Marshfield, the protein content of the multiple harvest sorghums (except conventional sorghum-sudangrass) would meet the needs of pregnant dairy heifers. Fiber digestibility was highest for the BMR varieties at Hancock when harvested twice with minimal differences at Marshfield. Fiber digestibility was generally high using multiple harvests ranging from 62% up to 75%. Harvesting in fall with increased maturity caused lower fiber digestibility at both sites especially for sorghum varieties with higher yields. The same result was found for total digestible nutrients (TDN) with the higher yielding sorghums having lower energy values. Corn had the highest energy level using a single cut system at Hancock but was similar to other sorghum varieties at Marshfield except PS sorghum-sudangrass, conventional forage sorghum, and conventional sorghum-sudangrass which were lower. Overall, the energy levels of single cut sorghum forages would better meet the needs of pregnant dairy heifers compared to corn silage.

Nitrates

Nitrate levels are a major concern for producers growing sorghums with nitrates accumulating during periods of slow growth (drought or after rains following a drought; low temperatures or after a frost) which reduced N conversion to amino acids causing nitrates to accumulate. The site location affected nitrate levels with Hancock having very low levels due to the sandy soil not holding N well and having low N levels (Table 3). At Marshfield, when harvested as a single cut sorghums had lower nitrate-N levels below the threshold of 1000 ppm nitrate-N for potential toxicity. However, using a multi-cut strategy increased nitrate-N levels above 1000 ppm for several varieties. Ensiling can reduce nitrate levels but testing is advised when using sorghums to monitor this risk.

Effects of Irrigation Rate and Nitrogen Fertilization

In 2016, another set of experiments with the same sorghum cultivars were done to evaluate variable rates of irrigation and nitrogen fertilization. The effect of irrigation was evaluated at Hancock ARS with 5 rates of irrigation relative to needs of corn (0, 25, 50, 75, and 100%). The irrigation rates were applied using a linear irrigation system with each rate applied as one strip with rates attained using different flow nozzles. Within each irrigation rate strip, the forage cultivars were randomly assigned with 3 replicate plots.

The effect of nitrogen fertilizer was evaluated at Marshfield ARS with 4 rates of nitrogen fertilization (0, 50, 100, and 150 lb N/acre). Nitrogen was applied at planting (15 lb N/acre) in a starter fertilizer and then the remaining N applied at 3-5 leaf stages. The 0 lb N/acre rate did not receive any fertilizer. The study had 3 replicate blocks, with N rates randomized in each block, and the cultivars assigned within each N rate. For both studies, forage was harvested using a single cut system based on the maturity (1/3 to 1/2 milk-line for corn and soft to hard dough for sorghums) or after a frost. Planting was in the first week of June with the same seeding rates as the previously described study.

Precipitation as rainfall at Hancock ARS totaled 24.5” from planting to harvest. Additional water as irrigation totaled 2.9”, 5.9”, 8.8”, and 11.75” for 25, 50, 75, and 100% rates. The 2016 season had very consistent rainfall events without extended dry periods.

Irrigation Rate

Forage Yield:

Forage yield was improved with additional irrigation and depended on forage cultivar. All of the cultivars responded positively with linear increases except for BMR sorghum-sudangrass and PS BMR sudangrass. The PS cultivars and BMR forage sorghum were especially responsive to irrigation when additional irrigation was used (Figure 1). Compared to corn, all the sorghum cultivars had similar or greater yields across all irrigation rates. The yields of forage sorghum, BMR forage sorghum, PS forage sorghum, PS

sorghum-sudangrass, sorghum-sudangrass, and PS BMR sudangrass at 50% or less irrigation were similar or greater than when corn irrigated was at 100%. This would allow producers to use significantly less irrigation water to produce forage for either heifer or lactating cows.

Nutrient Content:
Irrigation had only minor effects on forage quality with no definite trends across the irrigation rates. Most of the cultivars were unaffected by irrigation, however all the forage sorghum varieties (PS, BMR, and conventional) were affected by irrigation but in different ways. Conventional forage sorghum had decreased TDN with additional irrigation, while both PS and BMR forage sorghum had increased TDN with irrigation above 0%. The conventional and PS sorghum-sudangrass had TDN levels that would work well to use in pregnant heifer diets, while all other sorghums had energy levels suitable for pre-breeding heifers.

Nitrogen Application Rate
Forage Yield:
Nitrogen application rate had generally positive effect on forage yield, however there was variation and inconsistent increases. All the PS cultivars had significant linear effects of nitrogen on yield. All other cultivars did not have significant linear effects, even though most had a positive response to nitrogen. It appears that all cultivars had a diminishing response as additional nitrogen was applied with a maximum between 50 and 100 lb N/acre and smaller increases in yield at 150 lb N.

Nutrient Content:
Unexpectedly, nitrogen application rate had minimal effects on forage quality with no significant effects on CP, NDF, in vitro digestibility or TDN. There were small decreases in NDF and in vitro digestibility and small increases in CP. Corn silage and BMR forage sorghum were the only forages that had linear increases in CP content with additional nitrogen fertilizer. NDF digestibility was reduced with use of nitrogen fertilization. This may be due to the 0 lb N rate being severely deficient and delaying development. This would result in greater NDF content but less developed lignin structure and greater digestibility.

Similar to the irrigation rate study, more variation occurred between the forage cultivars for all quality parameters, with corn silage having the highest quality (lowest NDF and highest TDN) compared to all other forages. The BMR forages had moderate energy with higher NDF and NDF digestibility than corn silage resulting in these having a range of 61-66% TDN. The conventional and PS forage sorghum and sorghum-sudangrasses had the lowest energy contents with similar NDF content and TDN contents (57-58% TDN). Protein content of all forages was low (3.6-6.2% averages across N rates). The PS cultivars and conventional sorghum-sudangrass had minimal or no increase in CP content with additional nitrogen fertilization and may be due to the large response in forage yield for these cultivars to the additional nitrogen.

Sorghums in the Dairy Forage System

Sorghum forages would fit well in the dairy forage system for producers that use cereal grain forages. Typically, these forages will be harvested in mid to late May depending on the weather, forage species, and desired quality. After harvest, sorghum forages could then be established in late May or early June with soil needing to be above 60° F for fast germination. This system would provide heifer quality forage from both cereal forages and sorghum forages and take advantage of available growing days.

Summary

Dairy heifers require lower dietary energy needs (63-65% TDN for 6-12 month old heifers; 58-60% TDN for >12 month old heifers) with high forage diets containing significant amounts of corn silage often exceeding the needs of pregnant heifers. Use of low energy forages to decrease energy and increase NDF content has been successful to control intake and growth of pregnant heifers. Based upon the studies summarized, sorghum forages would fit well in these diets with higher NDF (50-65% NDF) and lower TDN (57-65% TDN) with the PS and conventional varieties having the lowest TDN values. The higher quality BMR sorghum forages would fit well into pre-breeding heifer or lactating cow rations. A multi-cut system would provide higher quality forage if needing forage for lactating cows. Yields from the 3 studies show that sorghum forages can have similar or greater yields to corn silage when planted in early to mid-June with sorghums being more responsive to lower irrigation and nitrogen applications than corn. Most sorghums had yields at 50% or lower irrigation or 50-100 lb N/acre that were similar or greater than yield of corn at the highest irrigation and nitrogen rates. Costs of heifer forage production may be decreased by using sorghum forages due to lower seed costs and nutrient needs compared to corn while maintaining similar yield production. Overall, sorghum forages are high yielding with lower energy content that is well-suited for dairy heifers.

Recognition of Support

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References

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Hoffman, P. C., K. A. Weigel, and R. M. Wernberg. 2008. Evaluation of equations to predict dry matter intake of dairy heifers. J. Dairy. Sci. 91:3699-3709.

Table 1. Sorghum variety information

Forage cultivar	Variety	Company
Forage sorghum	AF8301	Alta Seeds
Sorghum-sudangrass	AS5201	Alta Seeds
PS forage sorghum[1]	4-Ever Green	Walter Moss Seeds
PS sorghum-sudangrass	Mega Green	Walter Moss Seeds
BMR forage sorghum[2]	BMR 3411	Croplan®
BMR sorghum-sudangrass (male sterile)	Greentreat® 1731	Croplan®
PS BMR sorghum-sudangrass	Greentreat® Rocket	Croplan®

[1] PS = photoperiod sensitive; [2] BMR = brown mid-rib

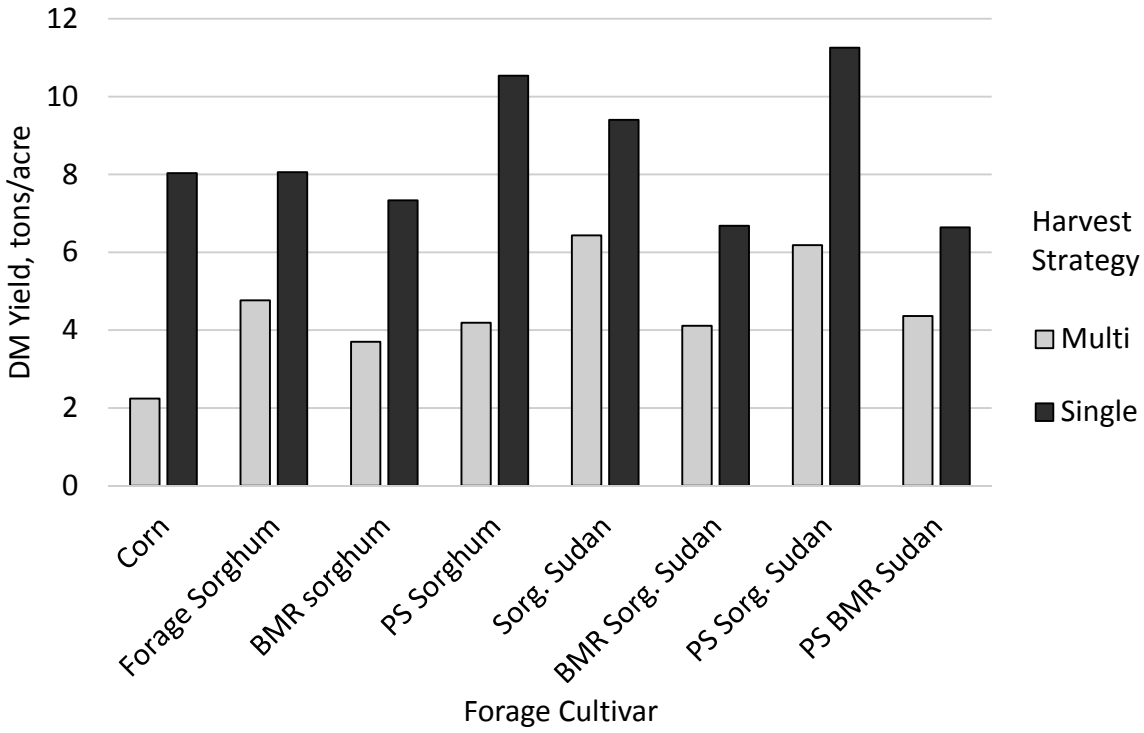

Figure 1. Forage DM yield of various sorghum forages harvested using a multiple or single cut strategy. Corn was only harvested once for the multiple harvest strategy due to no regrowth.

Table 2. Forage quality (DM basis) of sorghums and corn silage sampled using a single or multiple cut harvest strategy in 2015

		CP		NDF		TDN	
Forage:	Harvest:	Single	Multi	Single	Multi	Single	Multi
Corn silage		6.3	8.75	50.8	65.5	65.9	65.9
PS forage sorghum		5.8	11.0	60.8	64.3	59.8	63.8
PS sorghum-sudan		5.7	10.1	60.5	65.6	56.0	62.5
Forage sorghum		7.2	10.3	54.7	64.3	60.8	65.6
Sorghum-sudan		7.1	9.2	54.0	65.0	57.3	63.5
BMR forage sorghum		7.5	10.5	51.3	63.0	62.5	67.4
BMR sorghum-sudan		7.7	10.7	59.1	63.5	62.7	66.3
PS BMR sudangrass		7.3	11.5	56.0	62.5	62.8	66.4

Table 3. Nitrate-N levels (ppm) for sorghums and corn silage using a single or multiple cut harvest strategy at Hancock and Marshfield Agricultural Research Stations

	Location:	Hancock		Marshfield	
Forage:	Harvest:	Single	Multi	Single	Multi
Corn silage		11.4	18.3	44.9	83.1
PS forage sorghum		166.6	82.0	423.2	1952.0
PS sorghum-sudan		280.3	168.4	476.3	1441.9
Forage sorghum		63.5	72.0	568.0	1037.9
Sorghum-sudan		65.1	109.6	391.1	871.3
BMR forage sorghum		99.8	111.7	404.3	820.9
BMR sorghum-sudan		209.5	91.2	334.7	988.7
PS BMR sudangrass		160.4	108.3	615.8	2180.3

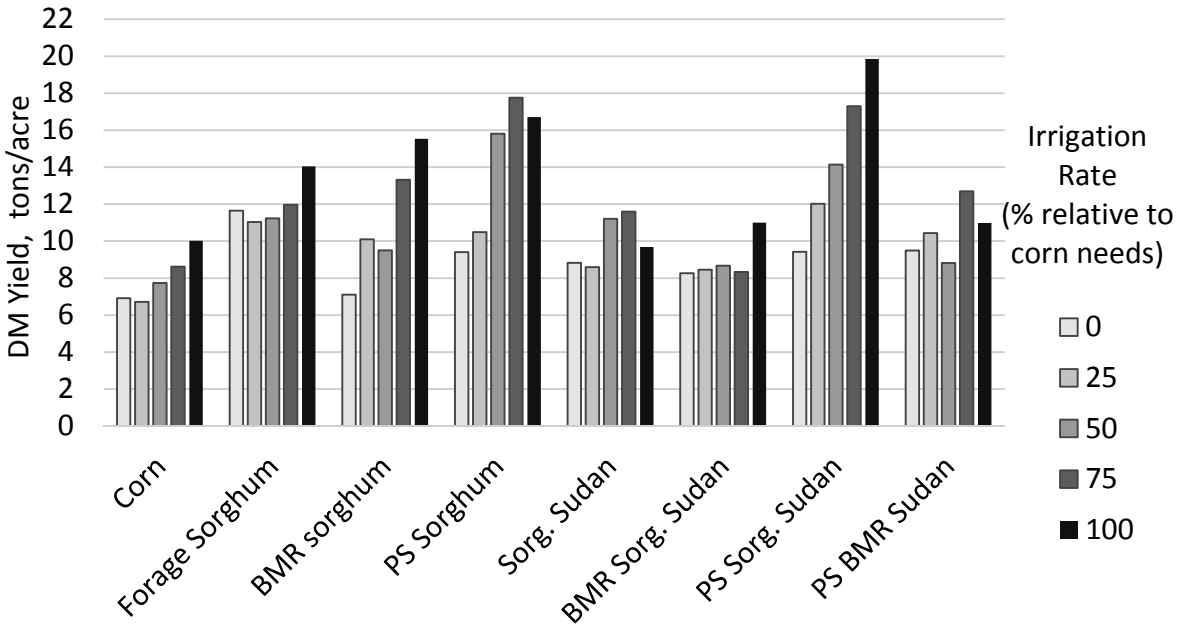

Figure 2. Forage yield (tons DM/acre) of various sorghum forages and corn at different irrigation levels at Hancock ARS. PS = photoperiod-sensitive; BMR = brown mid-rib

Table 4. Forage nutrient values (DM basis) of sorghums and corn silage with different irrigation rates.

Irrigation Rate (% of corn needs)	NDF	CP	NDFD	TDN
0	53.1	6.6	49.9	63.5
25	52.2	7.5	53.6	65.9
50	54.1	6.9	51.0	63.6
75	51.9	6.7	49.0	63.6
100	53.2	6.9	50.1	63.6
Forage Cultivar				
Corn silage	39.0	6.9	51.6	71.9
PS forage sorghum[1]	59.0	5.8	54.8	63.1
PS sorghum-sudan	60.8	5.6	47.0	57.9
Forage sorghum	48.9	8.0	44.1	63.2
Sorghum-sudan	56.4	6.9	43.5	58.5
BMR forage sorghum[2]	47.5	8.2	52.0	67.2
BMR sorghum-sudan	55.5	7.0	56.0	66.0
PS BMR sudangrass	56.3	7.1	56.6	64.5

[1] PS = Photoperiod sensitive; [2] BMR = Brown mid-rib

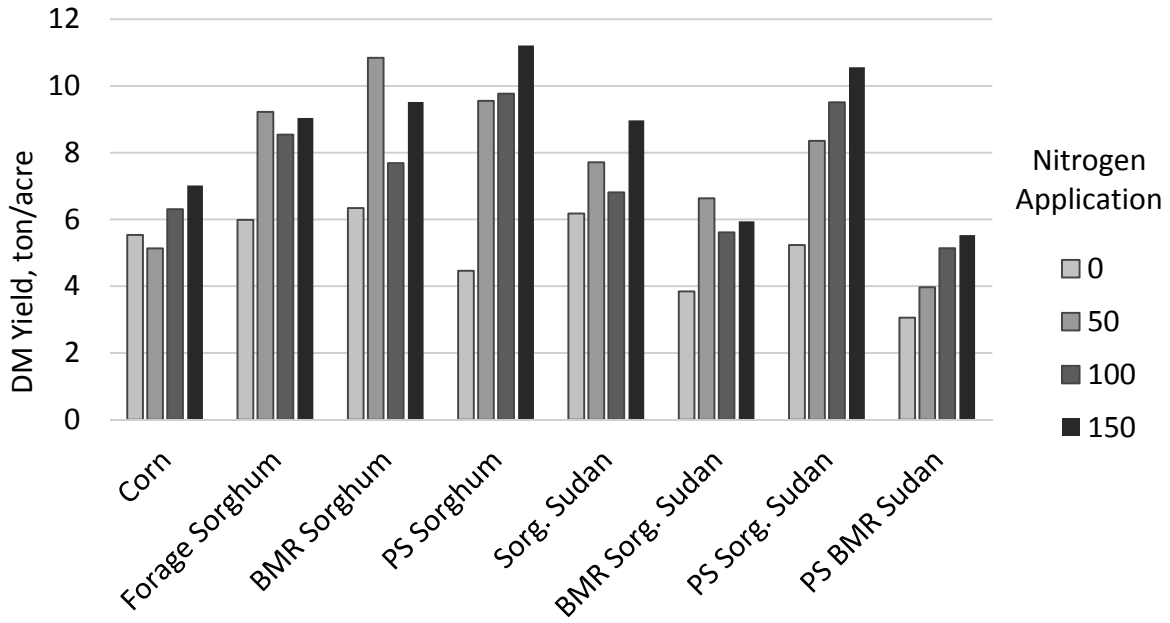

Figure 3. Forage yield (tons DM/acre) of various sorghum forages and corn at different nitrogen application levels (lb N/acre) at Marshfield ARS. PS = photoperiod sensitive; BMR = brown mid-rib

Table 5. Forage nutrient values (DM basis) of sorghums and corn silage with different nitrogen application rates

Nitrogen Rate (lb N/acre)	NDF	CP	NDFD	TDN
0	58.9	4.4	57.9	62.4
50	57.7	4.9	53.5	61.1
100	56.8	5.0	52.4	60.8
150	56.9	5.3	52.7	61.1
Forage Cultivar				
Corn silage	42.1	5.8	52.1	69.3
PS forage sorghum[1]	62.5	4.2	54.8	58.2
PS sorghum-sudan	62.9	3.6	51.8	57.4
Forage sorghum	59.8	5.3	49.1	57.8
Sorghum-sudan	59.5	3.6	49.7	58.4
BMR forage sorghum[2]	52.4	5.6	58.2	65.8
BMR sorghum-sudan	59.2	6.2	57.4	63.0
PS BMR sudangrass	61.2	4.9	60.0	61.0

[1] PS = Photoperiod sensitive; [2] BMR = Brown mid-rib

Producing More Milk Using More High Quality Forages

Randy Shaver, Ph.D. PAS, ACAN
Dairy Science Department
University of Wisconsin

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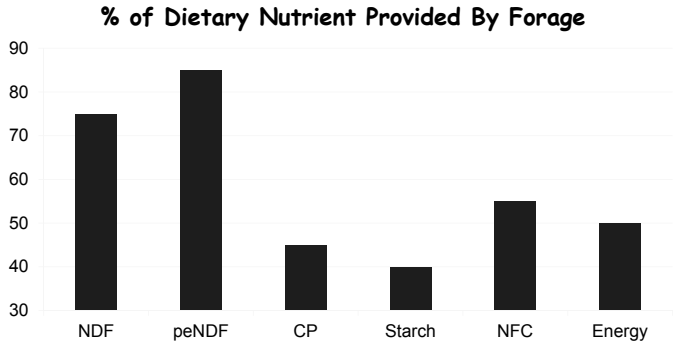
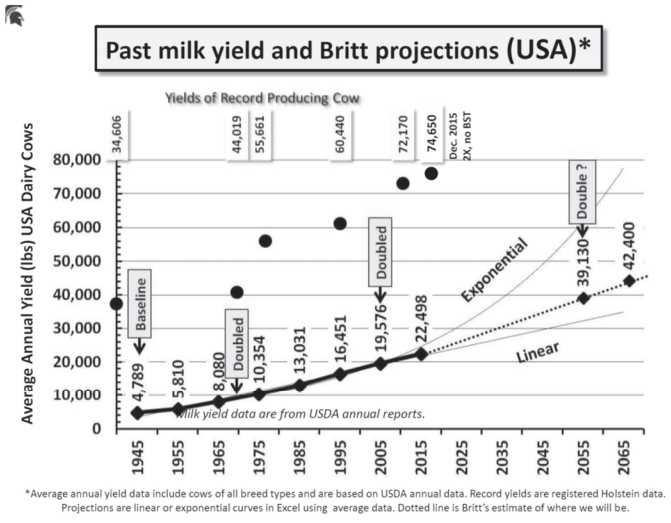
Calculated from Survey Summaries





*Average annual yield data include cows of all breed types and are based on USDA annual data. Record yields are registered Holstein data. Projections are linear or exponential curves in Excel using average data. Dotted line is Britt's estimate of where we will be.

Calculated from Survey Summaries

Maintenance & BWG energy requirements apportioned to forage or concentrate according to diet F:C ratio

Corn Silage Quality Indicators for High-Producing Dairy Herds

Parameter	Indicates Better Quality	Primary Reason
NDF	↓	↓ Rumen Fill Limitation of DMI Potential for production response or feeding of higher-forage diets
Lignin	↓	
$uNDF_{240}$	↓	
$NDFD_{30}$	↑	
TTNDFD	↑	
Starch	↑	↑ Energy Density Potential for production response or feeding less corn grain
Milk per ton	↑	Quality Index for Ranking

Corn Silage Quality Indicators for High-Producing Dairy Herds

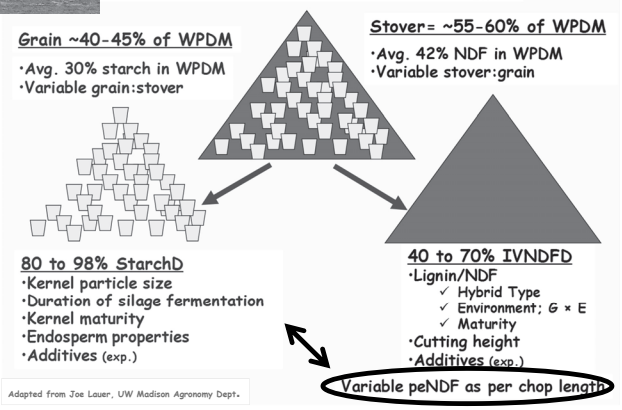
Parameter	Indicates Better Quality	n	Average ± 1 STDEV
NDF (% DM)	↓	384,715	41 - 36
Lignin (% DM)	↓	344,134	3.3 - 2.6
$uNDF_{240}$ (% NDF)	↓	81,418	27 - 24
$NDFD_{30}$ (% NDF)	↑	170,634	54 - 60
TTNDFD (% NDF)	↑	27,954	41 - 46
Starch (% DM)	↑	347,759	32 - 39
Milk per ton	↑	136,056	3320 - 3683

Summary of combined multi-year, multi-lab (CVAS, DairyOne, RRL, DLL) data, except TTNDFD only from RRL

Haycrop Silage Quality Indicators for High-Producing Dairy Herds

Parameter	Indicates Better Quality	Primary Reason
NDF	↓	↓ Rumen Fill Limitation of DMI Potential for production response or feeding of higher-forage diets
Lignin	↓	
$uNDF_{240}$	↓	
$NDFD_{30}$	↑	
TTNDFD	↑	
NFC (includes soluble fiber)	↑	↑ Energy Density Potential for production response or feeding less corn grain
CP	↑	↓ Supplemental Protein
Ash	Minimal Soil Contamination	↑ Energy Density
RFV; RFQ	↑	Quality Index for Ranking

Whole-Plant Corn Silage

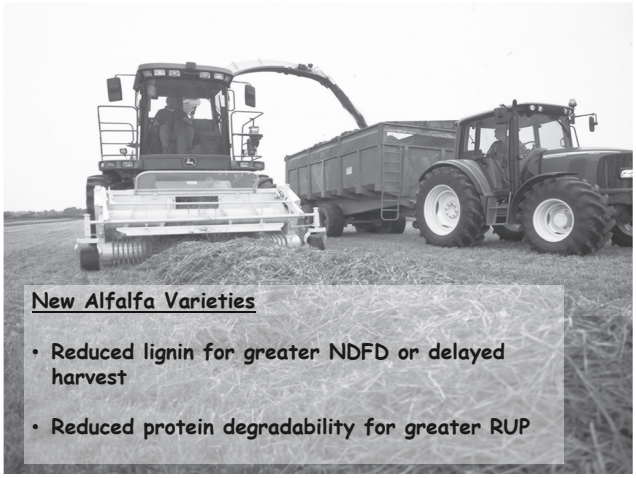


Adapted from Joe Lauer, UW Madison Agronomy Dept.

Legume Silage Quality Indicators for High-Producing Dairy Herds

Parameter	Indicates Better Quality	n	Average ± 1 STDEV
NDF (% DM)	↓	111,310	42 - 37
Lignin (% DM)	↓	100,029	7 - 5
$uNDF_{240}$ (% NDF)	↓	25,541	45 - 36
$NDFD_{30}$ (% NDF)	↑	61,568	46 - 57
TTNDFD (% NDF)	↑	24,498	44 - 51
NFC (% DM)	↑	94,337	26 - 30
CP (% DM)	↑	112,423	21 - 24
Ash (% DM)	Minimal Soil	100,888	<13
RFV	↑	100,831	141 - 167
RFQ	↑	51,453	155 - 179

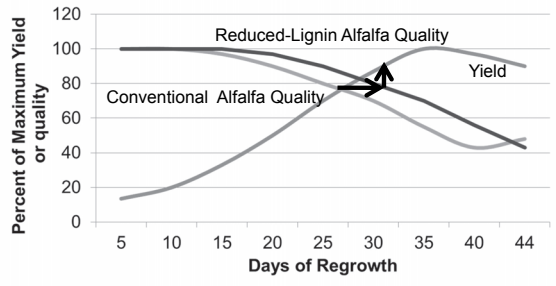
Summary of combined multi-year, multi-lab (CVAS, DairyOne, RRL, DLL) data, except for TTNDFD from RRL



New Alfalfa Varieties

- Reduced lignin for greater NDFD or delayed harvest
- Reduced protein degradability for greater RUP

Yield and Quality Curve of Alfalfa

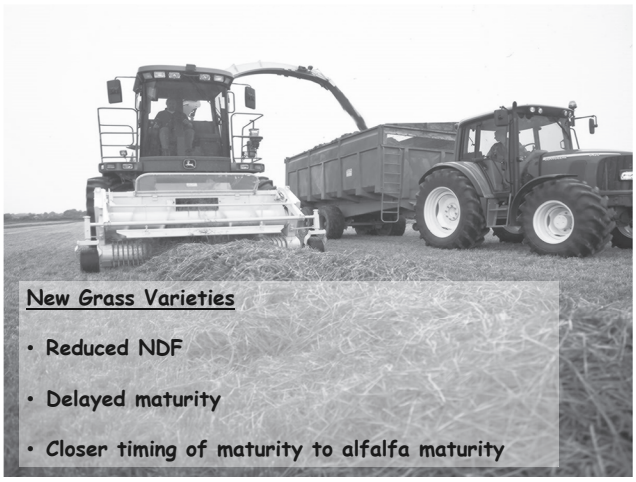


Slide courtesy of Dave Combs, UW Madison

Grass/MMG Silage Quality Indicators for High-Producing Dairy Herds

Parameter	Indicates Better Quality	n	Average ± 1 STDEV
NDF (% DM)	↓	85,213	55 - 48
Lignin (% DM)	↓	76,222	6 - 4
$uNDF_{240}$ (% NDF)	↓	15,972	33 - 24
$NDFD_{30}$ (% NDF)	↑	34,833	54 - 62
TTNDFD (% NDF)	↑	9,000	47 - 56
NFC (% DM)	↑	80,008	20 - 25
CP (% DM)	↑	85,889	15 - 18
Ash (% DM)	Minimal Soil	76,530	<10
RFV	↑	79,702	112 - 136
RFQ	↑	24,541	135 - 167

Summary of combined multi-year, multi-lab (CVAS, DairyOne, RRL, DLL) data, except for TTNDFD from RRL



Practical forage-NDF range in high-group TMR

24% forage-NDF

- High Quality Forages
- Large Forage Supply
- Forages Favorably Priced

i.e. 60% Forage @ 40% NDF

16% forage-NDF

- Limited Forage Supply
- Use of High-Fiber Byproducts
- Forages Expensive
- Moderate/Low Quality Forages

i.e. 35% Forage @ 46% NDF

Nutritional Constraints

24% forage-NDF

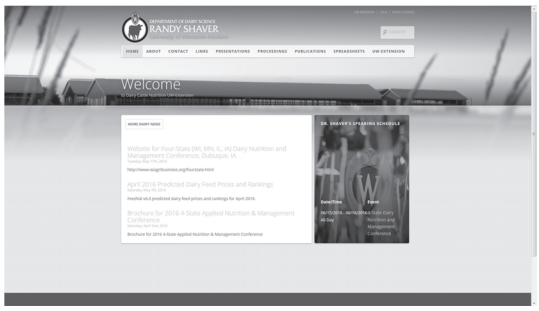
- NDF, ivNDFD
- Fill Limitation of DMI
- Reduced Milk Yield

16% forage-NDF

- peNDF
- Milk Fat Depression
- Cow Health

Visit UW Extension Dairy Cattle Nutrition Website

http://www.shaverlab.dysci.wisc.edu/



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