

# Finding Feeding Bottlenecks on Dairy Farms

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**Finding Feeding Bottlenecks  
 on Dairy Farms**  
 Four State Dairy Conference  
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### Never Give Up Milk

- Ration dry matter is **10 to 12 cents per pound**
- One pound of dry matter should support **2 to 2.5 pounds more milk**
- If milk is \$0.16 cents a pound, 10 cents worth of dry matter yields **\$0.22 more profit / income**

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### Today's Program

- Four feeding pillars for 2016
- Using on-farm tools
- Focus on profitability

2016

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### Getting The Right Cows on the Bus—Mature Cows (Source: 2016 DRPG)

Level of milk	22,700 lb	28,300 lb
	-----Peak Milk (lb)-----	
1 <sup>st</sup> lactation	76.6	92.3
2 <sup>nd</sup> lactation	95.7	116.6
3 <sup>rd</sup> + lactation	103.0	125.4

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### Pillar #1

Never  
 give  
 up  
 milk

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### Pillar #2

Building  
 Your  
 Milk  
 Check

Never  
 give  
 up  
 milk

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## Milk Fat and Milk Protein Relationships (Hoards Dairyman, 2015)

	Fat %	Protein %	Protein vs Fat	Fat vs Protein
Ayrshire	3.87	3.16	82%	1.22
Brown Swiss	4.03	3.31	82%	1.21
Guernsey	4.53	3.31	73%	1.36
Holstein	3.73	3.02	81%	1.32
Jersey	4.83	3.64	75%	1.33

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## Additives For Lactating Cows

- Rumen buffers
- Yeast culture/yeast products
- Monensin (Rumensin)
- Silage inoculants
- Biotin
- Organic trace minerals

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## Value of Milk Components

(Prices for February, 2016)

- Holstein herd: 70 lb milk, 3.5% fat, and 2.9% true protein corrected to 3.7% fat and 3.0% true protein
- 70 lb x 0.2% point increase  
= 0.14lb of milk fat x \$2.38 / lb fat = **\$0.33**
- 70 lb x 0.1% point increase milk protein  
= .07 lb protein x \$1.78 / lb = **\$0.12**
- Profit potential: **\$0.45 / cow / day**

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## Additives For Close Up Dry Cows

- Yeast culture/yeast products
- Monensin (Rumensin)
- Silage inoculants
- Organic trace minerals + chromium
- Anionic product (if DCAD is > +20 meq/kg or 2 meq/100 gm)

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## Pounds of Protein and Fat (2015-DHIR)

Breed	Milk / Day	Fat	Protein	Total
Brown Swiss	22,509 / 61.6	2.46	2.04	4.5
Jersey	19,278 / 52.8	2.55	1.92	4.5
Holstein	25,476 / 70	2.61	2.24	<b>4.9</b>
	80	2.98	2.42	5.4
	90	3.36	2.72	<b>6.1</b>
	100	3.73	3.02	6.8

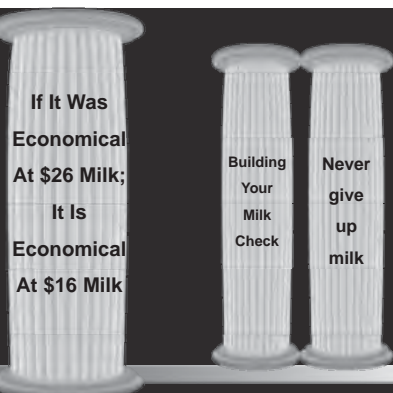
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## Additives For Fresh Cows

- Rumen buffers
- Yeast culture/yeast products
- Monensin (Rumensin)
- Calcium supplement (bolus/drench)
- Silage inoculants
- Biotin
- Organic trace minerals + chromium
- Rumen protected choline

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## Pillar #3



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## Pillar #4



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## Feeding Economics 2016

Feed costs per cow per day \$4.91  
 Feed cost per lb DM \$0.10

	Milk Production	
	80 lb	70 lb
Feed cost per cwt	\$ 6.14	\$ 7.01
Income over feed costs (\$16)	\$ 9.86	\$ 8.99
Feed efficiency (lb 3.5% FC milk/lb DM)	1.60	1.40

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## Using the Feed Efficiency



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## On-Farm Tools: Which Ones Are You Using?



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## Using Feed Efficiency as a Tool

- Evaluate the relationship between milk yield and dry matter intake
- Monitor changes with forage and ration shifts
- Compare group values on your farm

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## On-Farm Tools and Measurements

- Grain particle size
- Forage particle size
- Silage fermentation
- Feed efficiency
- Milk urea nitrogen
- Fecal washing
- Fecal scoring
- Fecal starch values
- Locomotion scoring
- Body condition scoring

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## Dairy Efficiency

Dairy Efficiency: Pounds of fat corrected milk divided by pounds of DM consumed

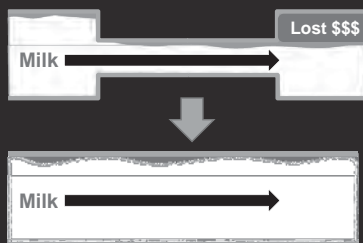
High group, mature cows	> 1.7
High group, 1 <sup>st</sup> lactation	> 1.6
Low group	> 1.3
One group TMR herds	> 1.5
Fresh cows	< 1.5
Concern (one group)	< 1.3

Example: 75 lb milk / 50 lb DMI = 1.5

3.5% FCM = (0.4324 x lb of milk) + (16.216 x lb of milk fat)

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## De-Bottlenecking



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## Economics of Feed Efficiency (70 lb milk, 10 cent lb DM)

Feed efficiency (lb milk/lb DM)	DMI (lb/day)	Difference (savings/day)
1.30	54	\$0.40
1.40	50	\$0.30
1.50	47	



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## Using the Forage NDFD



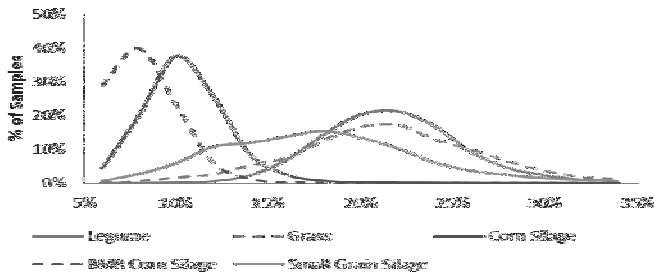
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## Shelled Corn Energy Values

	Mcal/lb DM
Cracked (2200 micron)	0.84
Ground (1500 micron)	0.89
High moisture (> 28%)	0.93
Steam flaked (26-28 bu)	0.93
High lysine (soft endo)	0.94
Finely ground (<800 mic)	0.96

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## uNDFom240 % DM



179,753 Samples – 2014 Crop Year

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## Particle Size Guidelines

Screen Size	#4	#8	#16	#30	Pan
H.M. Corn (>30%)	75	25	0	0	0
H.M. Corn (25-30)	25	50	25	0	0
H.M. Corn (<25%)	0	<10	30	50	<20
Dry corn	0	<10	30	50	<20
Sample Shakeout	1	20	29	44	6

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## Use of uNDF

- Determines rumen fill from forage sources
- Guideline is 6.0 to 6.2 pounds of uNDF-240 (Holstein) and 5.0 lb uNDF-30 (Jersey)

### Holstein Example:

$30\% \text{ ration NDF} \times 50 \text{ lb DM} \times 40\% \text{ uNDFD} = 6.0 \text{ lb uNDF}$   
 This herd should be able to consume this level of dry matter intake based on uNDF ration levels

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## Measuring On The Farm

- Send a sample to the forage lab and get a mean particle size and spread in particle size
- Dry corn: 500 to 800 micron depending on hybrid starch form
- Using a flour sifter, target 33% on the top and 67% as flour particle size

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## Corn Particle Measurements

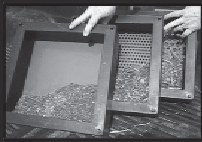


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## Forage Particle Measurements



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## Penn State Separator Guidelines

	Top	2 <sup>nd</sup>	3 <sup>rd</sup>	Bottom
	----- % (as fed) -----			
<b>TMR</b>	<b>10-15</b>	<b>&gt; 40</b>	<b>&lt; 30</b>	<b>&lt; 20</b>
<b>Haylage</b>	<b>&gt; 40</b>	<b>&gt; 40</b>	<b>&lt; 20</b>	<b>&lt; 5</b>
<b>Corn silage (3/4 TLC-Process)</b>	<b>5-15</b>	<b>&gt; 50</b>	<b>&lt; 30</b>	<b>&lt; 5</b>

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## CONSISTENCY

- **Score 1** Thin, fluff, arcs, green
  - Example: sick cow, off feed, cows on pasture
- **Score 2** Loose, splatters, little form
  - Example: fresh cow, cows on pasture
- **Score 3** Stacks up 1 to 1 1/2 inches, dimpled, 2 to 4 concentric rings, sticks to foot
  - Example: Recommended
- **Score 4** Stacks up 2 to 3 inches, dry
  - Example: Dry cow, low protein, high fiber
- **Score 5** Stacks up over 3 inches
  - Example: All forage, sick cow

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## Silage Fermentation Profile



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## Manure Scores (Hutjens Biases)

- **High pens**
  - < 10% score 1
  - < 25% score 2
- **Low pens**
  - < 0% score 1
  - < 10% score 2

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## Recommended Fermentation Profile for Ensiled Feeds

Measurement	Legume/grass	Corn Silage	H.M. Corn
Dry matter (%)	35 to 50	30 to 35	70 to 75
pH	4.3 to 4.7	3.8 to 4.2	4.0 to 4.5
Lactic acid (%)	4.0 to 6.0	5.0 to 10.0	1.0 to 2.0
Acetic acid (%)	0.5 to 2.5	1.0 to 3.0	<0.5
Propionic acid (%)	<0.25	<0.10	<0.10
Butyric acid (%)	<0.25	<0.10	<0.10
Ethanol (%DM)	≤1.0	≤3.0	≤2.0
Ammonia (%CP)	≤12.0	≤8.0	≤10.0
Lactic/Acetate	≥2.5	≥3.0	≥3.0
Lactic (% total)	≥70	≥70	≥70

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## Fecal Starch



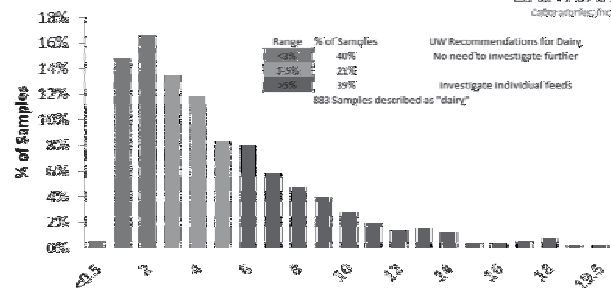
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## Manure Scoring



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## Dairy Fecal Starch %



## Milk response

- Fecal starch should be less than 4.5% represents total tract apparent digestibility of 90+ percent.
- If fecal starch can be reduced 1 unit (absolute decrease from 10% to 9%), milk production could increase 0.67 pound (dry matter intake remains constant).



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## Hutjens Guidelines/Checklist

uNDF	< 6.0 lb
• Fecal scoring	>80% @ 3.0
• Fecal starch values	< 4 %
• Locomotion scoring	< 10% @ 3
• Body condition scoring	2.75 to 3.25



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## Milk Urea Nitrogen (MUN)



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## MUN Values

- Old guidelines 12-16 mg/dl
- Old guidelines 10-14 mg/dl
- New guidelines 8 -12 mg/dl
- Reproductive concerns > 16 mg /dl
- Protein losses (10 to 15) 2+ lb sbm
- Environmental concerns



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## Hutjens Guidelines/Checklist

- Grain particle size 500 to 800 micron
- TMR particle size 10/45/45
- Silage fermentation 70% lactic/30% acetic
- Feed efficiency > 1.5 lb 3.5% FCM/lb DM
- Milk urea nitrogen 8 to 12 mg/dl



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# Using uNDF To Predict Dairy Cow Performance and Design Rations

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## Summary

- Our concept of fiber digestion has progressed from a 1-, to a 2-, and currently a 3-pool model.
- The major breakthrough in our understanding of fiber digestion was the recognition that some NDF is indigestible (iNDF) in the anaerobic ruminal environment.
- The measurements of undigested NDF (uNDF) at fermentation times up to 240 h (uNDF<sub>240</sub>) as estimates of iNDF resulted in the development of the 2- and 3-pool kinetic models that describe fiber digestion using first-order fractional rate constants.
- The uNDF of a feed is a better analytical indicator of nutritional availability than either NDF digestibility (NDFD) or lignin because both components of uNDF (NDF content and the proportion of NDF that is undegraded) are negatively associated with the total extent of fiber availability.
- The simple 2-pool model of digestion can be combined with a single-pool model of passage to develop a model of ruminal digestion and passage.
- The ruminal model provides insights about how fiber pools and flows change with 10% changes in dietary NDF concentrations, kinetic fractions of NDF, and rates of digestion (kp) and passage (kp).
- Assuming a constant dry matter intake, ruminal load of NDF is reduced, in order, by:
  - decreasing ration NDF concentration, then
  - increasing kp of NDF, then
  - reducing the proportion of iNDF and increasing the proportion of potentially digestible NDF (pdNDF), and then
  - increasing the kd of pdNDF.
- Assuming a constant dry matter intake, ruminal load of NDF is enlarged most by:
  - increasing the proportion of iNDF and decreasing the proportion of pdNDF.
- Using the rumen model to adjust intake so that the ruminal NDF pool was constant, dietary NDF concentration and iNDF had the greatest impacts on intake and milk production predicted by the simple ruminal model.

- Optimum dairy rations can be formulated by:
  - using NDF and physically effective NDF (peNDF) to defined the upper and lower limits of forage in rations,
  - managing forage harvest to minimize uNDF and maximize kd,
  - regulating forage particle size to optimize kp, and
  - allocating forages with lowest uNDF to cows with the largest milk production and energy demand.

## Introduction

Our concept of how neutral detergent fiber (NDF) affects the intake and digestion of dairy cows changed with the introduction of the concept of iNDF and the measurement of uNDF after extended periods of fermentation (> 72 h). The iNDF of a feed can never be measured because it requires an infinite time of fermentation; however, it can be estimated by mathematical models of digestion kinetics. The uNDF that we measure becomes closer to iNDF as fermentation times increase and the undigested NDF residue measured after 240 h of fermentation (uNDF<sub>240</sub>) is a practical estimate of the theoretical minimum iNDF. As with any measurement, uNDF can be affected by in vitro or in situ methodology (Mertens, 2016).

The chemical and physical nature of NDF has been used successfully to define the upper and lower limits of forage and coarse fiber intakes. At the upper limit, dairy cows can maximize their intake of forage while meeting their energy demands when the intake of total NDF is about 1.25% of their body weight per day. This upper limit assumes that the NDF of non-forage fiber sources (hulls, brans, etc.) are adjusted for their smaller particle size. The lower limit of fiber in dairy cow rations is limited by the physical properties of NDF that affect acceptable ruminal function. Ruminal characteristics that are acceptable for long-term health of the cow and milk component production are related to salivary buffering capacity, stratification of ruminal contents for selective retention of fiber, and VFA production. These characteristics are related to chewing activity and the concept of physically effective NDF (peNDF) was developed to define the physical and chemical attributes of feeds that influence chewing activity.

Given the roles of total NDF and peNDF in defining the feasible ranges of ration feed composition that optimize dairy cow production and health, what is the role of uNDF for improving dairy cow rations? The objectives of this presentation are to: (1) describe how the concept of iNDF affects our understanding of fiber digestion, (2) discuss the limitations of NDFD as a major characteristic of forages, (3) define the differences in uNDF among feeds and how it affects rumen conditions and our ability to allocate forages and formulate dairy rations.

### Central Role of uNDF in Fiber Digestion Kinetics

One of the important nutritional contributions due to the development of the NDF method (Van Soest, 1967) was its partitioning of feeds into neutral detergent solubles (NDS), which is an ideal nutritive entity with nearly complete digestion across most feeds (98% truly digestible), and NDF, which is not an ideal nutritive entity because its digestibility varies among feeds (original model, Figure 1). This analytical system allowed dry matter digestibility (DMD) to be calculated by a very simple summative equation

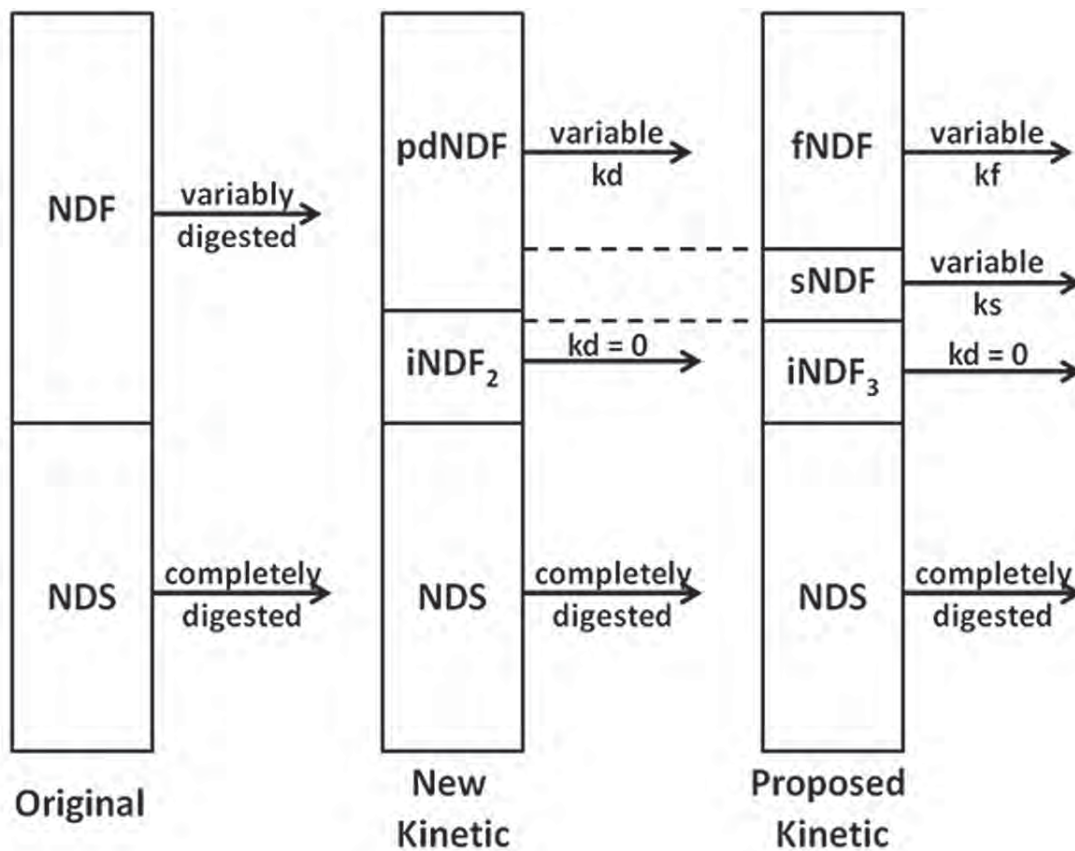
(Van Soest and Moore, 1965):

$$DMD = NDF * NDFD + 0.98 * NDS - 12.9.$$

Because  $NDS = (100 - NDF)$ , DMD is primarily a function of NDF and its digestibility (NDFD).

Waldo's (1969) hypothesis that a part of the cellulose in forages may not be digested after prolonged (6-day) fermentations changed our understanding of fiber digestion completely. The concept of iNDF, and its measured counterpart, uNDF, explains why NDF is not an ideal nutritive entity with uniform digestibility. The NDF in feeds is a combination of indigestible and potentially digestible fractions, each of which has homogeneous kinetic properties (new model, Figure 1). The iNDF pool has a  $k_d=0$  and the potentially digestible NDF (pdNDF) has a  $k_d$  that varies among feeds. The equation for the 2-pool model of NDF digestion is:

$uNDF_{(t)} = pdNDF * \exp(-k_d * [t - lag]) + iNDF_2$ ; where  $NDF_{(t)}$  is the undigested NDF remaining after any fermentation time = t, lag = the discrete lag time before digestion begins and  $iNDF_2$  is the indigestible NDF in a 2-pool model. For the 2-pool model,  $iNDF_2$  is reliably estimated by  $uNDF_{72}$ , which was measured after 72-h of fermentation (Smith et al., 1972).



**Figure 1.** Illustration of the changes in modeling feed digestibility based on NDF (NDS = neutral detergent solubles, pdNDF = potentially digestible NDF, iNDF = indigestible NDF, fNDF = fast-digestion NDF, sNDF = slow-digesting NDF and k = fractional rate for each pool).



Mertens (1977) observed that NDF continued to disappear after 72 h of fermentation, and when these endpoints were used to estimate iNDF, the plots of natural logarithm of pdNDF versus time (semi-log plots) were curvilinear. Curvilinear semi-log plots indicate that potentially digestible NDF may consist of fast- and slow-digesting pools, each of which has a homogeneous kd (proposed model, Figure 1). Raffrenato and Van Amburgh (2010) suggest that if uNDF<sub>240</sub> is used to estimate iNDF then a 3-pool model of NDF digestion is appropriate:

$$uNDF_{(t)} = fNDF * \exp(-kf*[t - lag]) + sNDF * \exp(-ks*[t - lag]) + iNDF_3$$

where fNDF is fast-digesting NDF with a fast digestion rate (kf), sNDF is slow-digesting NDF with a slow digestion rate (ks) and iNDF<sub>3</sub> is the indigestible NDF in a 3-pool model. Note that iNDF is a hypothetical pool defined by the model and that iNDF<sub>2</sub> and iNDF<sub>3</sub> are estimated by different uNDF (uNDF<sub>72</sub> and uNDF<sub>240</sub>, respectively).

Kinetic models of digestion more accurately predict DMD because a greater fraction of the feed is described as ideal nutritive entities (NDS and iNDF). After NDF and uNDF are measured for the 2-pool model of fiber digestion, the only remaining variable that affects DMD is the kd of the pdNDF fraction of the feed (Figure 1). This kd only applies to pdNDF, and iNDF (uNDF) has to be defined or measured before pdNDF and its kd can be estimated. To be clear, there is no kd that applies to total NDF because it is an heterogeneous nutritional entity. The kinetic model also makes clear that both iNDF and kd affect the extent of digestion in batch systems, such as in vitro and in situ. In general, iNDF, as a fraction of NDF, is higher in legumes, than in grasses or corn silage, but fractional rates of digestion for pdNDF are higher in legumes, than in grasses or corn silage (averaging about 0.12, 0.10 and 0.09/h, respectively) that are typically fed to dairy cows (Smith et al., 1972; Mertens, 1993). Assuming no lag time, these kinetic characteristics would predict NDFD<sub>24</sub> of 47, 64, and 66 % for legumes, grasses and corn silage, respectively

### Role of Lignin in Fiber Digestion

One of the benefits of kinetic models was to clarify the role of lignin in determining digestibility. In the original model (Figure 1), the variable digestibility of NDF was found to be related to logarithmic ratios of lignin to ADF or NDF (Goering and Van Soest, 1970). However, this correlative relationship did not provide insight into the mechanism by which lignin altered fiber digestibility. One of the earliest observations from kinetic models (Smith et al., 1972) was that uNDF<sub>72</sub>, which was used to estimate iNDF<sub>2</sub>, was highly correlated to lignin, but that kd was not. The relationship between lignin and uNDF has been confirmed by Traxler et al. (1998) for a wide variety

of forages, and Van Soest et al. (2005) argued that the factor (2.4 % lignin), which was derived from 60-d biodigester residues, could be used to estimate iNDF in the Cornell Net Carbohydrate-Protein System. Some reports suggest that the coefficient between uNDF and lignin is not constant among forage types; however, Mertens (2015) randomly selected 200 samples each of legumes, grasses and corn silages from a database provided by Dairyland Laboratories, Inc. (Arcadia, WI) and observed the regression:

$$uNDF_{240} = 2.86 \% \text{ lignin}; R^2 = 0.80$$

which appeared consistent among the three forages. This equation indicates that lignin binds about 1.86 times its mass of cellulose and hemicellulose in plant cell walls that is unavailable for microbial fermentation in the rumen.

Although there is a clear connection between lignin and indigestibility of NDF, this relationship is not perfect. Factors such as variation in the measurement of lignin and uNDF<sub>240</sub> or non-lignin characteristics of cell walls can affect NDF indigestibility. Mertens (2016) observed that the relationship between NDFD<sub>30</sub> and uNDFOM<sub>240</sub> (as a fraction of NDF) was better than that between NDFD<sub>30</sub> and lignin (as a fraction of NDF) when each forage was allowed to have an individual equation ( $R^2 = 0.70$  vs  $0.60$ ). This indicates that uNDF is a better analytical tool than lignin for providing information about digestibility.

### Utility of NDFD

Oba and Allen (1999) compiled data from seven experiments with 13 comparisons to quantify the effect of NDFD on lactating cow performance. They concluded that a .01 unit (or 1 %-unit) increase in forage NDFD, measured in situ or in vitro, resulted in a daily increases of 0.37 lb dry matter intake (DMI) and 0.55 lb 4% fat-corrected milk (FCM). Jung et al. (2004) selected trials that contained at least 40% corn silage and observed that each .01 increase in NDFD was associated with increases of 0.31 lb DMI and 0.26 lb of 3.5% FCM. Mertens (2006) added ten additional experiments to the database of Oba and Allen (1999) and used meta-regression to observe that each .01 unit of NDFD, measured in situ or in vitro at 48 h, resulted in daily increases of 0.21 lb DMI and 0.31 lb 4%FCM. Most of the studies were comparisons of lignin mutants (brown midrib) in corn and sorghum.

The results of Oba and Allen (1999), Jung et al. (2004) and Mertens (2006) were from trials in which the NDF of diets was equal or very similar, thus the only or primary variable among treatments was NDFD. However, this is not the circumstance when evaluating forages where both the NDF and NDFD can vary. If two forages had A = 0.45 and B = 0.55 NDFD<sub>48</sub>, the obvious choice would be forage B. But if A contained

50% and B contained 70% NDF, would the choice be the same? The effects of NDFD and NDF could be combined by calculating digested NDF in DM at 48 h ( $dNDF_{48} = NDF \% NDFD_{48}$ ), in which forage A = 22.5% and B = 38.5% of DM. Should forage B be selected? Does the positive effect of higher  $NDFD_{48}$  outweigh the negative effect of higher NDF? Mertens (2006) observed that the negative effects of increased NDF were about 3 times more detrimental than positive effects of NDFD. This conundrum of combining positive and negative effects can be solved by using  $uNDF_{48}$  because both components of  $uNDF$ , NDF undegraded (NDFU) and NDF, ( $uNDF_{48} = NDF \% NDFU_{48}$ ) have negative effects on intake and production. The  $uNDF_{48}$  of forage A = 27.5 and B = 31.5% of DM. Forage A has the least  $uNDF_{48}$  and would be the better selection for cows with high energy demand and limited space in the rumen or limited time needed to chew indigestible residue so that it can pass out of the rumen.

Forage NDFD can be used successfully as a diagnostic tool to evaluate forage quality when NDF concentrations are similar, but it cannot be used directly in rations formulation. Although, NDFD can be used indirectly to estimate energy value using TDN or DMD equations, it would be more accurate if dynamic estimate of digestibility could be developed to account for differences in intake and rate of passage, instead of single time measurements at 24, 30, or 48 h of fermentation.

### Rumen Models of Digestion

The kinetic models in Figure 1 describe fermentation of fiber in a batch system with no rate of passage. However, rate of passage can be combined with rates of digestion to develop rumen models that predict ruminal digestibilities over the full range of intakes and their corresponding rates of passage (Figure 2). At steady-state, the pools  $pdNDF$  and  $iNDF_2$  in Figure 2 are not changing. Thus, if we know (or assume) the flows into and out of each pool, we can calculate the pool sizes in the rumen using the following equations:

$$DMI/h \% (pdNDF \text{ in DM}) = pdNDF_{\text{pool}} \% kd + pdNDF_{\text{pool}} \% kp, \text{ solving for ruminal } pdNDF_{\text{pool}},$$

$$pdNDF_{\text{pool}} = [DMI/h \% (pdNDF \text{ in DM})] / (kd + kp) \text{ and}$$

$$DMI/h \% (iNDF_2 \text{ in DM}) = iNDF_{2\text{pool}} \% kp,$$

$$\text{solving for ruminal } iNDF_{2\text{pool}},$$

$$iNDF_{2\text{pool}} = [DMI/h \% (iNDF_2 \text{ in DM})] / kp.$$

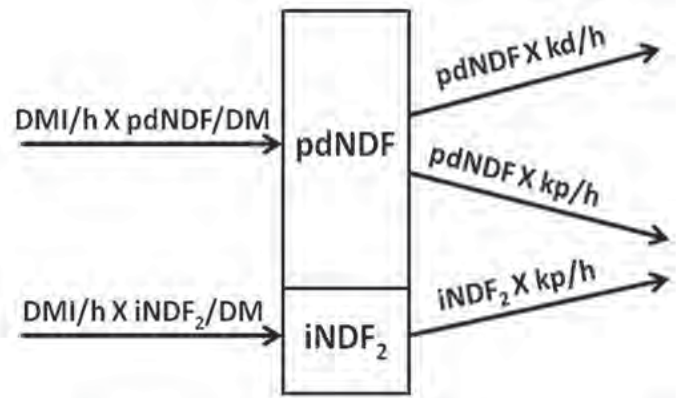
Alternatively, if we measure intakes of fiber fractions and measure pools by emptying rumens, we can rearrange the equations to solve for rates of diges-

tion and passage as demonstrated by Oba and Allen (2003) and others. We can also use the simple rumen model to calculate NDF digestibility (NDFD, as a decimal fraction) by the equation:

$$NDFD = [(pdNDF \text{ in DM}) / (NDF \text{ in DM})] \% [kd / (kd + kp)]$$

$$= [(NDF \text{ in DM}) - (iNDF_2 \text{ in DM})] / (NDF \text{ in DM}) \% [kd / (kd + kp)].$$

The importance of  $iNDF$  (or  $uNDF$ ) in DM is clear because it is the basis for estimating  $pdNDF$  (=  $NDF - iNDF$ ),  $fNDF$  or  $sNDF$  in DM. Without measuring  $uNDF$  or estimating  $iNDF$  in DM, it is impossible to determine  $pdNDF$  in DM and determine its rate of digestion.



### Simple Rumen Model of Fiber Digestion

**Figure 2.** Simple model of ruminal digestion of fiber assuming first-order fractional rate constants of digestion ( $kd$ ) and passage ( $kp$ ) for pools of  $pdNDF$  (potentially digestible NDF) and  $iNDF_2$  (indigestible NDF for a 2-pool model of digestion).

The utility of a simple model of digestion and passage is that we can use it to peek inside the ruminal “black-box” and begin to understand how rumen pools and flows change with changes in intake, rate of passage, fiber kinetic fractions and rates. To demonstrate the effects of changing kinetic rates and fractions (Table 1), a base ration was formulated using the NDF-Energy Intake System proposed by Mertens (summarized most recently by Mertens (2006) with adjustments for NDFD). Mertens’ system maximizes the proportions of forage and fiber in dairy rations that also meets target NEL requirements for maintenance, tissue balance and milk production. This system is based on the concept that the optimum intake of NDF is 1.15 to 1.25% of body weight per day for any target of dairy cow performance. For a 1430 lb cow in mid-lactation producing 99 lb of 3.5% fat-corrected milk and gaining 0.44 lb/d, Mertens’ system generated a base ration that contains 64.5% forage (mixture of 25% alfalfa and

75% corn silage - DM basis) and 33.5% concentrate (simple mixture of 78% corn, 18% soybean meal, and 4% minerals - DM basis).

The base ration contains 30% aNDF, 28.3% aNDFOM, and about 16% CP. The NDF-Energy Intake System predicts that the target cow will consume 57.2 lb/d of DMI, or 4.0% BW/d, to meet energy requirements and optimize NDF intake. The kinetic fractions and presumed rates of fiber digestion and passage of the base ration are described in Table 1. Rates of passage of the simple model were obtained from Oba and Allen (2003) and Grant (2015). Rates of digestion for forages were derived from data provided by Dairyland Laboratories (Arcadia, WI), and for concentrates, were obtained from Cumberland Valley Analytical Services (Hagerstown, MD). Milk production from intake of TDN was calculated as an independent check of the model using the total tract NDFOMD, NDFOM intake, and 0.98 % NDSOM intake with an endogenous loss of 12.0%. Total tract NDFOMD was determined assuming that pdNDF reaching the large intestine would digest for 8 h at the same fractional rate as the rumen. Starch was assumed to be fermented while ensiling and processed so that 98% would be digested.

Using the base ration characteristics and fiber kinetics, based on NDFOM fractions instead of NDF the rumen model (Figure 2), the model predicted that the target cow's rumen will contain 9.50 lb of iNDFOM<sub>2</sub> and 3.47 lb of pdNDFOM, or 12.97 lb total NDFOM (Table 1). Recognize that these pools of fiber contain all particle sizes of each constituent in the rumen, and the digestion and passage rates are for the average size in each pool. Typically, the average size of particles in the rumen is quite small, especially for iNDFOM<sub>2</sub>. For comparison, Oba and Allen (2003) reported ruminal pools of 6.71, 4.57, and 11.28 lb for uNDF<sub>120</sub>, pdNDF, and total NDF, respectively, when averaged across all treatments. Taylor and Allen (2005) reported ruminal pools of 5.24, 6.81, and 12.09 lb for uNDF<sub>240</sub>, pdNDF, and total NDF, respectively, averaged across all treatments. Their diets were lower in NDF than the model base ration and obtained lower NDF intakes, which may explain the slightly smaller pools of total NDF than model predictions (Table 1). They also used uNDF<sub>120</sub> or uNDF<sub>240</sub> to estimate iNDF, which are smaller than the iNDF<sub>2</sub> of the 2-pool model (Figure 1, new kinetic model) that is estimated most appropriately by uNDF<sub>72</sub>. This would explain the smaller pools of uNDF and larger pools of pdNDF in the two trials compared to those generated by the simple

**Table 1.** Changes in inputs (bold font) and responses of a simple ruminal model to decreases in NDF organic matter (NDFOM) or increases in potentially digestible NDF (pdNDF), in fractional rates of passage (kp) and digestion (kd) of fiber, or in estimated indigestible NDF for a 2-pool model of fiber digestion (iNDF<sub>2</sub>). The base ration is defined in the text.

Model variables	Base Ration	Decr NDF	Incr pdNDF	Incr kp	Incr Kd	Incr iNDF <sub>2</sub>
<b>Model inputs</b>						
Ration aNDFOM (% DM)	28.32	25.49	28.32	28.32	28.32	28.32
Ration iNDFOM <sub>2</sub> (% DM)	11.16	10.04	10.04	11.16	11.16	12.88
Ration pdNDFOM (% DM)	17.16	15.44	18.28	17.16	17.16	15.44
Ration NDF kd (/h)	0.090	0.090	0.090	0.090	0.099	0.090
Ration NDF kp (/h)	0.028	0.028	0.028	0.031	0.028	0.028
<b>Model responses</b>						
Rumen Pool iNDFOM <sub>2</sub> (lb)	9.50	8.55	8.55	8.64	9.50	10.96
Rumen Pool pdNDFOM (lb)	3.47	3.12	3.69	3.39	3.22	3.12
Rumen Pool Total NDFOM (lb)	12.97	11.67	12.24	12.02	12.72	14.08
NDFOM passing out (lb/h)	0.363	0.327	0.343	0.370	0.356	0.394
NDFOM digesting (lb/h)	0.312	0.281	0.332	0.305	0.319	0.281
iNDFOM <sub>2</sub> total flow (%BW/d)	0.446	0.402	0.402	0.446	0.446	0.515
NDFOM total flow (%BW/d)	1.13	1.02	1.13	1.13	1.13	1.13
Ruminal NDFOMD (% of NDFOM)	46.2	46.2	49.2	45.1	47.2	41.6
3.5% fat-corrected milk (lb/d)	99.0	101.7	100.9	98.7	99.5	96.1

model. However, it appears that the pools generated by the model are reasonable. Another difference is that model predictions are based on NDFOM instead of NDF. This latter difference may create more difficulties than might be expected, if some of the ash from mineral supplements contaminates NDF residues from the rumen.

Using kd generated from commercial laboratory results, the model predicts ruminal NDFOMD from 42 (increase in iNDFOM2) to 49% (increase in pdNDFOM) and these values are within the range of published values. Reducing ration NDFOM by 10% did not change ruminal NDFOMD, but predicted higher 3.5% FCM (Table 1) due to increased TDN caused by shifting organic matter from NDFOM to NDSOM, which has greater digestibility. However, the largest impact of reducing ration NDFOM was a predicted increase in intake (6.4 lb/d, Figure 3), assuming cows can eat more when ruminal total NDFOM pool was increased from 11.67 lb to 12.97 lb. This increase in DMI seems large, but keep in mind that the base diet was designed to be fiber limiting and the cows would have to have milk production capability exceeding 99 lb/d. The practical adjustment for reduced NDF in the forages would be to reformulate the ration as shown in Table 2.

Holding NDFOM concentration constant by increasing pdNDF of the ration by 10% (with a corresponding decrease in iNDFOM2) decreased the total NDFOM pool in the rumen. This change had the greatest impact on reducing the iNDFOM2 pool and slightly increasing the pdNDFOM pool. With DMI held constant, this change would increase milk production from TDN, and if intake is adjusted to have a similar ruminal pool of total NDFOM to the base ration, the increase in intake (3.4 lb/d) and 3.5% FCM response would be substantial (Figure 3). The only practical method for increasing the fraction of pdNDFOM in forages would be by genetic selection/modification of forages to reduce lignin, or perhaps treatments (enzymatic, chemical or physical) that could convert some of the iNDF to pdNDF.

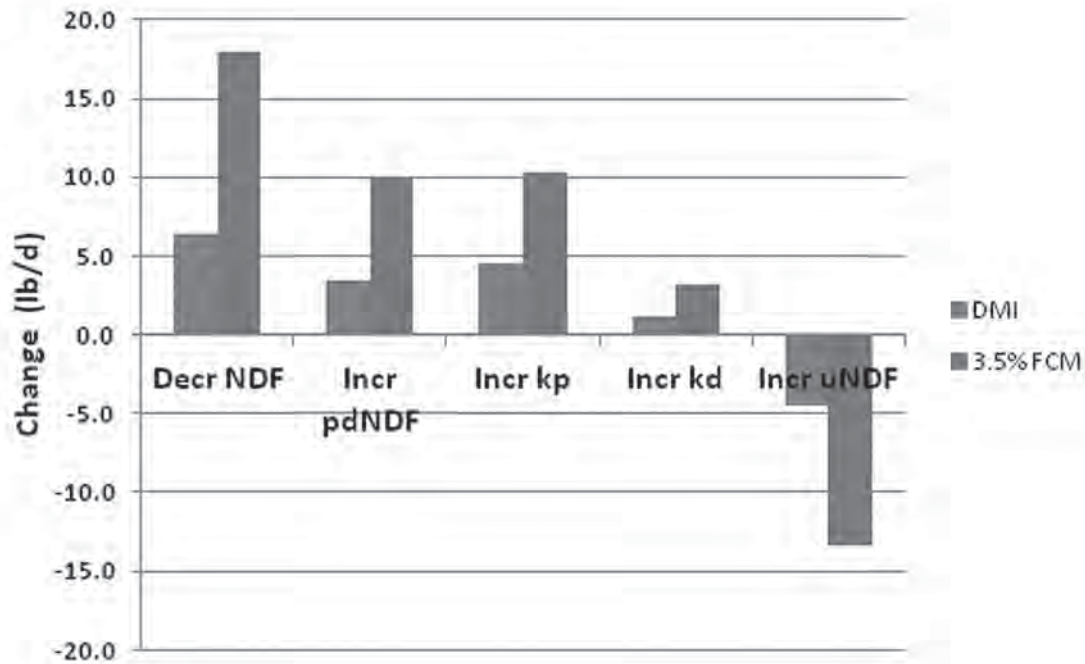
Increasing kp by 10% decreased the pools iNDFOM2 and pdNDFOM, and increasing the outflow of pdNDFOM decreased ruminal NDFOMD as expected (Table 1). However, the decrease in NDFOMD is relatively small and when intake is adjusted to have equal total NDFOM pool to the base ration, there is opportunity for substantial increases in intake and milk production (Figure 3). The only practical way of increasing kp is by reducing the particle size of forages. However, using longer (>3/4 inch) theoretical lengths of cut of corn silages to obtain higher peNDF may reduce kp and thus have a negative impact on the intake of high producing dairy cows.

Increasing kd had a small impact on the ruminal total NDFOM pool (Table 1), and on intake when adjusted to obtain the same NDFOM pool as the base ration. At this time, we do not know what affects kd other than environmental conditions (there usually is a year-affect in most studies of kd). Although kd may be manipulated by genetic selection/modification, it appears that the best practical recommendation for dealing with forages having slow kd, is to allocate forages with rapid kd to cows with the largest milk production and energy demand, and to add by-products that have rapid kd to increase the kd of the total ration.

The greatest negative impact of changing fiber kinetics was to increase iNDFOM2 and decrease pdNDFOM in the ration NDFOM (Table 2 and Figure 3). The model result certainly reinforces the concept that measuring uNDF is one of the most important analyses for nutritional evaluation of feeds, second only to aNDF. The only practical way of reducing uNDF is in genetic manipulation of plant cell walls by reducing lignin and other inhibitors or by harvesting more immature plants (difficult for corn silage). It may be advantageous to increase kp by reducing particle size so that iNDF can leave the rumen more quickly or by allocating forages so that those with the least iNDF are fed to the highest producing cows.

**Table 2.** Change in ration characteristics with changes in the NDF concentration of the forage mixture using the NDF-Energy Intake System (Mertens, 2006).

Corn silage NDF, % of DM	36	38	40	42	44
Optimum NDFI, % of BW/d	1.20	1.20	1.20	1.20	1.20
Forage, % of TMR DM	79.8	74.6	64.5	66.0	62.4
NDF, % of TMR	31.1	31.4	30.0	31.8	32.0
NDF from forage, % of TMR NDF	92.2	90.3	85.8	87.2	85.9
Expected intake, lb/d	57.8	57.4	57.1	56.7	56.4



**Figure 3.** Changes in intake and 3.5% FCM when intake is adjusted to obtain the same total ruminal load (NDFOM pool) as the model base diet.

It is worth noting that formulating rations based on iNDF may be more difficult than assumed. It would be nice if an upper limit of iNDF in the ration could be established, but it is likely that this can only be done within forage types. Mertens (2016) randomly selected forages from a database provided by Dairyland Laboratories, Inc. (Arcadia, WI) and observed that each forage type had different relationships between NDF and uNDF:

Legumes	$Y = 1.15 + .552 * NDF;$
Grasses	$Y = -3.00 + .401 * NDF;$ and
Corn silage	$y = 1.77 + .217 * NDF.$

Thus, if rations are balanced to have similar NDF, the proportion of iNDF in the ration will vary considerably among forage types, and will be highest for legumes, followed by grasses and corn silages. The model presented could be used to identify the iNDF that optimizes forage content in each type of ration and accounts for faster kp of legume compared to grass NDF. Because iNDF is unaffected by the particle size of the forage, peNDF is a better way to formulate minimum forage rations. However, it may be possible to fine-tune peNDF values for the effect of iNDF on particle size reduction and passage by cows (Grant, 2015).

Given the current interest in uNDF240, it can be argued that it should be used in model simulations. However, use of uNDF240 is only valid if a 3-pool model of fiber digestion is used. Using uNDF240 results in curved semi-log plots, which indicate that a

single fractional rate constant does not exist. It seems illogical to use a 3-pool model of fiber digestion with a single-pool model of passage that does not adequately represent the flow of large, medium and small particle reduction and escape. Mertens and Ely (1982) developed a ruminal model with 3 pools for both digestion and passage of fiber and Mertens (2011) showed that complex steady-state equations can be derived. But these models are limited by available data and it is unlikely that their simulations will refute any of the conclusions that can be generated by the simple model.

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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 homeostatic 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 depletion and increased oxidative and nitrosative stress (Hall et al., 2001). This contributes to tight junction dysfunction and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013). As a result, HS increases the passage of lu-

minal 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 (Cecilian 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., 2015c,d). 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

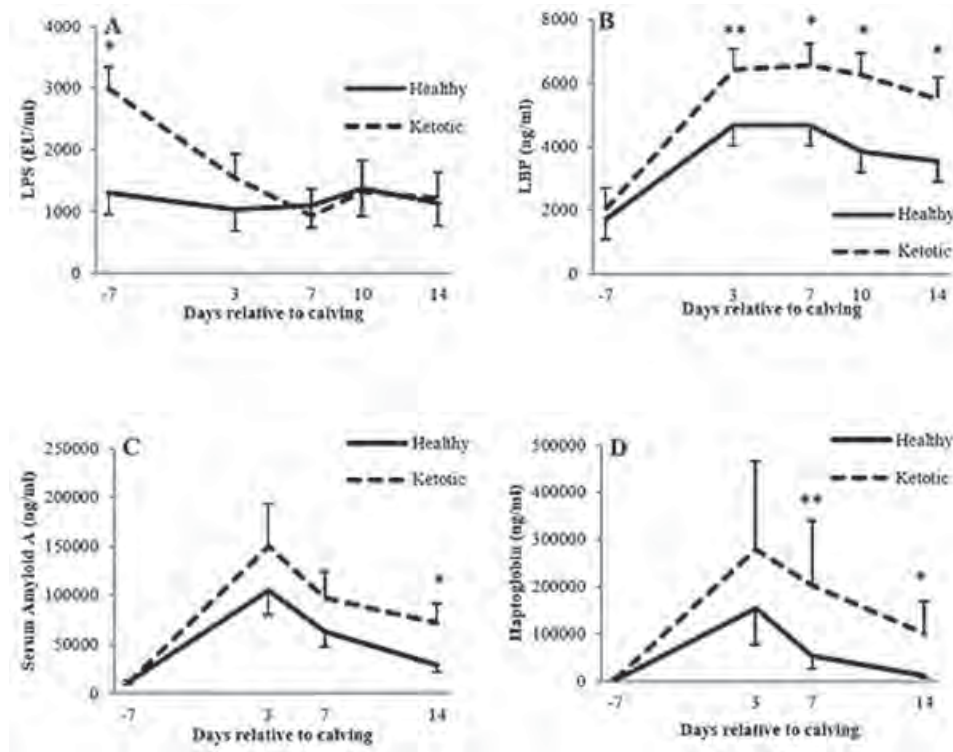
Recently, the concept that LPS impacts normal nutrient partitioning and potentially contributes to metabolic maladaptation to lactation has started to receive attention. Although LPS itself has not been the primary causative focus, general inflammation has been the topic of investigations. Increased 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 (Lor et al., 2005; Bertoni et al.,

2008), and this assumption was recently reinforced when TNF $\alpha$  infusion decreased productivity (albeit without overt changes in metabolism; Yuan et al., 2013; Martel et al., 2014). Additionally, in late-lactation cows, injecting TNF $\alpha$  increased (>100%) liver TAG content without a change in circulating NEFA (Bradford et al., 2009). Our recent data demonstrates increased inflammatory markers in cows diagnosed with ketosis only and no other health disorders. In comparison with healthy controls, ketotic cows had increased circulating LPS prior to calving and post-partum acute phase proteins such as LPS-binding protein, serum amyloid A, and haptoglobin were also increased (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 (Stoakes et al., 2014). 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.

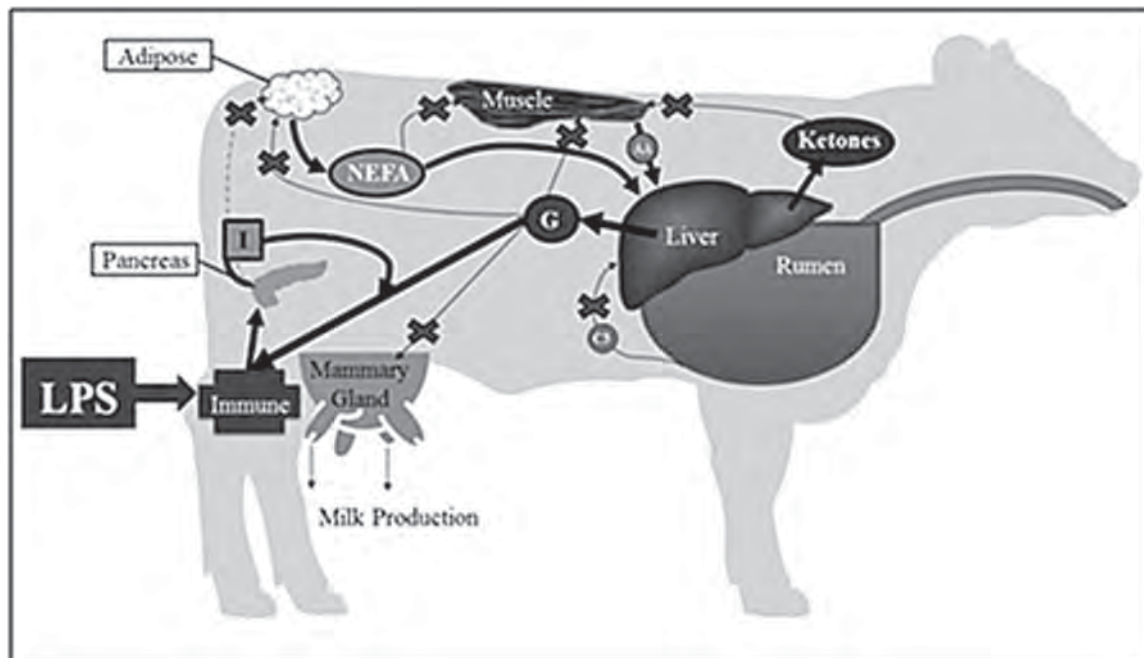


**Figure 1.** Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows.

## METABOLISM OF INFLAMMATION

LPS-induced inflammation has an energetic cost which redirects nutrients away from anabolic processes that support milk and muscle synthesis (see review by Johnson, 1997, 1998) and thus compromises productivity 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; Friesard 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 (Fig. 2) caused by immune system activation.



**Figure 2.** LPS induced alterations in glucose metabolism and insulin sensitivity.

### *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 BW<sup>0.75</sup>/h, respectively; Stoakes et al., 2015a,c,d). 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.

### **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.

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# Revisiting Starch for Lactating Dairy Cows

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## Introduction

The focal point carbohydrates in beef and dairy cattle nutrition research have been starch and fiber, respectively, likely in relationship to the feeding of high grain, energy diets to beef feedlot cattle and the Dairy NRC-established minimum fiber requirements to maintain normal milk fat content and rumen function in dairy cattle. Thus, the major concentration of starch-related research in beef cattle goes back nearly a half century, while in dairy cattle starch has been a relatively new hot research topic over the past decade.

Factors that have contributed to the rise in starch-related research in dairy cattle include: greater valuing of protein relative to fat as a milk component, focus on feed, energy and nitrogen efficiencies, interest in reducing methane production, establishment of corn silage as the predominant forage crop, and discussion of the hepatic oxidation theory of intake regulation. But, perhaps the most important factor contributing to the renewed or increased focus on starch is the two-fold or greater “new-normal” for the price of corn which largely establishes the cost of starch as a nutrient.

The intent of this paper is not to provide a review of the starch for ruminant’s topic, because the 28th ADSA Discover Conference – Starch for Ruminants was held late 2014 and the Committee for the new Dairy NRC (8th revised edition) is currently in the process of reviewing and establishing nutrient guidelines for dairy cattle diets. Rather the purpose of this paper is to present results from some of our lab’s recent experiments in the starch area.

## UW-Madison Dairy Science – Starch Research Update

### Corn Silage Processing Score and Kernel-Fraction Particle Size

It is now well-established that ensiling over extended storage times increases starch digestibility in whole-plant corn silage (**WPCS**; Ferraretto et al., 2015a,e) and high-moisture corn (**HMC**; Hoffman et al., 2011; Ferraretto et al., 2014), and that this likely occurs through the proteolysis of zein proteins cross-linked to starch granules in the starch-protein matrix (McAllister et al., 1993; Hoffman et al., 2011). This disruption of the starch-protein matrix may result in kernel particle size reduction during ensiling.

Across 2 experiments, we observed that corn silage processing score (**CSPS**; % of starch passing through a 4.75-mm sieve; Ferreira and Mertens, 2005) was increased by 7%- to 10%-units after ensiling in vacuum-sealed plastic bags for at least 30 d and up to 240 d (Figure 1; Ferraretto et al., 2015c). Furthermore, data summarized from 2 feeding trials suggest that silo baggers may significantly increase CSPS above what had been measured on fresh material coming from the forage harvester (L. F. Ferraretto, UW-Madison unpublished data). Together these observations suggest that CSPS determinations performed on fermented samples obtained from silos prior to feeding may be more accurate than those performed on samples obtained prior to ensiling. The determination CSPS on samples obtained directly from the harvester for processor set-up may be unreliable in some situations. More in-depth evaluation of this issue is warranted.

Results of survey samples obtained from commercial dairy farms suggests a weak, but positive, relationship between WPCS dry matter (**DM**) content and CSPS (Dias Junior et al., 2015). This could be a real effect of greater kernel fragmentation for drier WPCS kernels during processing, or possibly an analytical anomaly caused by fine starch from wetter WPCS kernels sticking to coarse fiber particles and thereby not passing through the 4.75-mm sieve during CSPS particle separation in the lab. The relationship between WPCS DM content and CSPS has been described by others (P. C. Hoffman, Vita Plus Corp., personal com-

munication), and should be investigated further with regard to the accuracy of CSPS measurements.

The foregoing discussion led us to explore a potential future alternative to CSPS (Dias Junior et al., 2016). Readers are referred to Dias Junior et al. (2016) for a complete listing of the experimental methods, but a brief summary is as follows: 80 WPCS samples were split into 2 subsamples, CSPS was performed on 1 subsample, the other subsample was dried and then subjected to a hydrodynamic separation procedure (Savoie et al., 2004) to separate the kernel and stover fractions, and the kernel fraction was then re-dried before dry sieving to determine its particle size parameters. Linear relationships between CSPS on WPCS and kernel fraction mean particle size (**MPS**), surface area, and proportion passing through a 4.75-mm sieve were poor ( $R^2 = 0.11, 0.06$  and  $0.34$ , respectively), thereby suggesting that hydrodynamic separation followed by dry sieving of the kernel fraction may provide a better determination of kernel breakage in WPCS than CSPS.

Simulations were performed using the Feed Grain V2.0 Evaluation System (Hoffman et al., 2012a,b,c) to predict the potential effect of MPS on extents of ruminal and total-tract starch digestibilities and ruminal rate of starch digestibility for dairy cows. Hydrodynamically separated WPCS kernel fraction MPS measurements from all samples were model inputs along with a constant ammonia-N concentration. Simulation results are in Figure 2, and suggest potential for enhanced modeling of starch digestibility in WPCS using results from the hydrodynamic separation of the kernel and stover fractions followed by dry sieving of the kernel fraction to determine its MPS.

More research is needed, however, to move forward with this approach. Neutral detergent fiber (**NDF**) dilution of the kernel fraction (11% NDF and 71% starch on average; DM basis) and starch loss to the stover fraction (57% NDF and 17% starch on average; DM basis) appeared to be relatively minor in our sample set, but more research is needed to better assess these potential procedural errors. Furthermore, potential loss of very fine starch particles in the water fraction during the hydrodynamic separation procedure was not determined by Dias Junior et al. (2016) and needs to be assessed as a potential source of error. Practical feasibility within the commercial lab setting would also need to be evaluated. Hydrodynamic separation of the kernel and stover fractions can be performed on undried fresh WPCS samples in the field to provide a subjective evaluation of kernel processing at the harvester for processor adjustments (Shinners and Holmes, 2013).

### Starch Digestibility in Earlage

We (Ferraretto et al., 2016) reported on an industry-university collaborative study of the effects of plant population, maturity, and ensiling time on silo fermentation parameters and starch digestibility in earlage samples (comprised of husks, kernels, and cob) from 4 hybrids. Plant populations tested were 26k, 32k, 38k and 44k plants per acre. Harvest maturities were ½ kernel milk line (**ML**) and black layer (**BL**) stages of kernel development. Ensiling was done in vacuum-sealed plastic bags for 30, 60, 120 and 240 d. Ruminal in vitro starch digestibility (**ivSD**) was determined with 7-h incubations on dried, 4-mm ground samples.

Plant population effects were minimal. The DM and starch concentrations were greater, lactate and total acid concentrations were lower, and thus pH was greater, for BL than ML earlage. Soluble-CP and ammonia-N concentrations and ivSD were reduced by 5.5, 1.0 and 8.3%-units, respectively, for BL compared to ML earlage. Gradual increases in soluble-CP and ammonia-N concentrations from 30 to 240 d of ensiling corresponded with ivSD of 58, 60, 68 and 70% of starch at 30, 60, 120 and 240 d of ensiling, respectively. Ammonia-N and soluble-CP were both good indicators of ivSD in earlage. Results coincide with previous work on HMC (Hoffman et al., 2011; Ferraretto et al., 2014) and WPCS (Ferraretto et al., 2015a,e).

### Sample Particle Size Effects on Ruminal In Vitro or In Situ Starch Digestibility Measurements

Feedstuff nutrient analysis and ruminal in vitro or in situ digestibility assays require the grinding of samples in the laboratory to homogenize feedstuffs and reduce sampling errors associated with the small assay sample sizes (0.5-1.0 grams) that are employed. The laboratory grind size for nutrient analysis and ruminal in vitro NDF digestibility (**ivNDFD**) measurements is typically about 1 mm. Therefore, ivNDFD measurements yield maximum potential rates and extents of ruminal digestion. For ivSD or ruminal in situ starch digestibility (**isSD**) measurements, however, fine grinding (i.e. 1-mm screen) in the lab to prepare the incubation samples could mask or eliminate differences among the test feedstuffs in particle size which is known to significantly affect starch digestibility (Ferraretto et al., 2013). In an attempt to allay this concern, ivSD or isSD incubation samples are typically prepared in the lab by grinding through a 4-mm or 6-mm screen.

We recently evaluated commercial dry ground corn samples for MPS by dry sieving as originally sent in

from feed mills and then after grinding in the lab through 4-mm or 6-mm screens as they would be prepared for ivSD or isSD assays (C. Willems, J. P. Goeser and R. D. Shaver unpublished data). Of the original samples sent in from feed mills, based on dry sieving 5 were categorized as “Fine” with a MPS of  $766 \pm 88$  microns (Range = 630 - 865 microns), 3 as “Medium” with a MPS of  $1,220 \pm 276$  microns (Range = 988 - 1,525 microns), and one “Cracked” corn sample had a MPS of 2,582 microns. Grinding through a 6-mm screen reduced the MPS of Fine, Medium and Cracked samples by 4%, 21% and 52%, respectively. Grinding through a 4-mm screen reduced the MPS of Fine, Medium and Cracked samples by 11%, 31% and 67%, respectively. It should be noted that a MPS of 1,200 – 2,500 microns is common for HMC (Tassoul et al., 2007; Hoffman et al., 2012) and the kernel fraction of WPCS (Dias Junior et al., 2016) samples.

Particle size is a major factor affecting starch digestibility (Ferraretto et al., 2013), and these results indicate a greater degree of particle size reduction by laboratory grinding for samples with a greater initial MPS. Therefore, ivSD or isSD results on field samples with varying initial MPS using 4-mm or 6-mm ground incubation samples must be interpreted with extreme caution. This may partially explain why Powell-Smith et al. (2015), in a field study of 32 high-producing commercial dairy herds in the Upper Midwest, reported that measurements of ivSD on TMR samples were unrelated ( $R^2 = 0.00$ ) to in vivo total tract starch digestibility calculated from dietary and fecal starch and 240-h undigested NDF or lignin concentrations. Also, a major flaw in ruminal ivSD and isSD measurements relative to in vivo digestibility is that post-ruminal starch digestion is ignored and the proportion of starch digested post-rationally can be very significant in dairy cattle (Ferraretto et al., 2013).

Another recent industry-university collaborative study (Goeser et al., 2016) evaluated particle size parameters and ruminal isSD performed on unground 3-gram lab incubation samples for commercial feed-mill samples of dry ground shelled corn ( $n = 38$ ). The corn MPS and surface area determined by dry sieving was  $715 \pm 233$  microns (Range = 405 to 1379 microns) and  $92.7 \pm 20.8$   $\text{cm}^2/\text{g}$  (Range = 50 to 139  $\text{cm}^2/\text{g}$ ), respectively. Clearly there is considerable variation in the field for particle size of dry ground shelled corn. Ruminal 7-h isSD (% of starch) determined on unground incubation samples was  $68.7\% \pm 10.6$ . Surface area was better related to isSD than MPS. Better characterization of actual particle size parameters of corns being fed on farms is warranted, as is further research on relationships between particle size parameters and starch digestibility.

### Corn Silage Endosperm Properties and Starch Digestibility

From a meta-analysis, Ferraretto and Shaver (2015) reported 7%-unit and 2%-unit reductions in vivo for ruminal (RSD) and total tract (TTSD) starch digestibility, respectively, in brown midrib (bm3) compared to near-isogenic or conventional WPCS hybrids. Compared to leafy hybrids, TTSD was 5%-units lower for bm3 WPCS hybrids. Reduced starch digestibility for bm3 WPCS hybrids could be due to greater kernel vitreousness (Fish, 2010; Glenn, 2013) and (or) faster passage rate through the digestive tract associated with increased DMI (Ferraretto et al., 2013). Additionally, Ferraretto et al. (2015d) reported 5%-units greater TTSD for lactating dairy cows fed an experimental floury-leafy WPCS hybrid compared to cows fed a bm3 WPCS hybrid that appeared related to reduced kernel vitreousness and greater WPCS ruminal ivSD and isSD for the floury-leafy hybrid.

Two other studies (Ferraretto et al., 2015a,e) were conducted to evaluate the interaction between hybrid types and ensiling time on starch digestibility of WPCS. Our hypothesis was that prolonged storage would attenuate, or perhaps overcome, the difference in starch digestibility between hybrid types. In the first experiment (Ferraretto et al., 2015e), another industry-university collaborative study, 8 WPCS hybrids (4 bm<sub>3</sub> and 4 leafy) were ensiled for 0, 30, 120 and 240 d. Although ivSD was similar between hybrids throughout the storage period, the N fraction response to time of fermentation varied with hybrid type suggesting greater effects on the breakdown of zein proteins in leafy than bm<sub>3</sub> hybrids. The second experiment (Ferraretto et al., 2015a) compared 3 hybrids (bm<sub>3</sub>, dual-purpose, and experimental floury-leafy) ensiled for 0, 30, 60, 120 and 240 d. Contrary to our hypothesis, however, extended ensiling time did not attenuate the negative effects of kernel vitreousness on ivSD. The results from these experiments emphasize the importance of further WPCS starch digestibility research with regard to potential interactions between hybrid, harvest maturity, kernel processing and ensiling. Furthermore, results suggest that the best opportunity for benefit from altering kernel endosperm properties for greater starch digestibility may reside within the bm<sub>3</sub> type hybrids.

### Rehydrated-Corn/HMC Experiments

A mini-silo study (Ferraretto et al., 2015b) was performed to evaluate the impact on ivSD for the following: 1) rehydration and ensiling of dry ground corn; 2) exogenous protease addition to rehydrated un-ensiled and ensiled corn; 3) exogenous protease addition or microbial inoculation in rehydrated ensiled corn; and 4) exogenous protease addition or



microbial inoculation in HMC. Rehydration increased ivSD of ground dry shelled corn only when ensiled. Exogenous protease addition increased ivSD in HMC and un-ensiled and ensiled rehydrated corn, but the benefits were greater when the corn was allowed to ferment. Microbial inoculation decreased pH and increased organic acid concentrations in rehydrated corn and HMC but did not affect ivSD.

An industry-university collaborative experiment (Ferraretto et al., 2014) using commercial laboratory data was performed to: 1) determine relationships between HMC DM and ivSD, and 2) evaluate the effect of ensiling time on ammonia-N, soluble CP and ivSD measurements in HMC. As fermentation progressed, soluble CP, ammonia-N and ivSD increased gradually. Furthermore, the ivSD decreased 1.6%-units per %-unit increase in DM content of HMC. Interestingly, DM content was negatively related to pH suggesting a reduction in the extent of fermentation for drier HMC. These results highlighted the importance of prolonged storage and maturity at harvest to optimize starch digestibility in HMC.

#### Dietary Starch Content and In Vivo NDF Digestibility

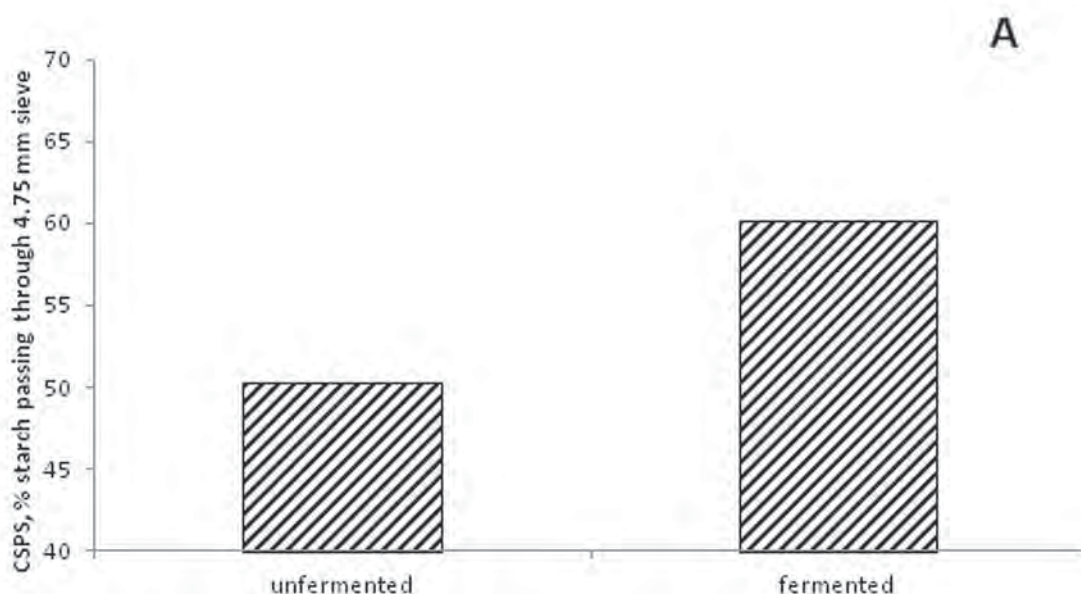
Presented in Figure 3 (meta-analysis by Ferraretto et al., 2013) is the effect of dietary starch concentration on in vivo NDF digestibility. Increased dietary starch concentrations reduced in vivo ruminal NDF digestibility ( $P = 0.01$ ) and in vivo total-tract NDFD (**TTNDFD**;  $P = 0.001$ ). The digestibility of dietary NDF decreased 0.61%-units ruminally and 0.48%-units total-tract per %-unit increase in dietary starch content. Decreased fiber digestibility may be partially explained by a decrease in rumen pH as a consequence of greater amounts of starch being digested in the rumen as starch intake increases. Low rumen pH is known to affect microbial growth and bacterial adherence and thereby fiber digestion. Also, the inherently high fiber digestibility of non-forage fibrous by-products used to partially replace corn grain in reduced-starch diets may be partly responsible.

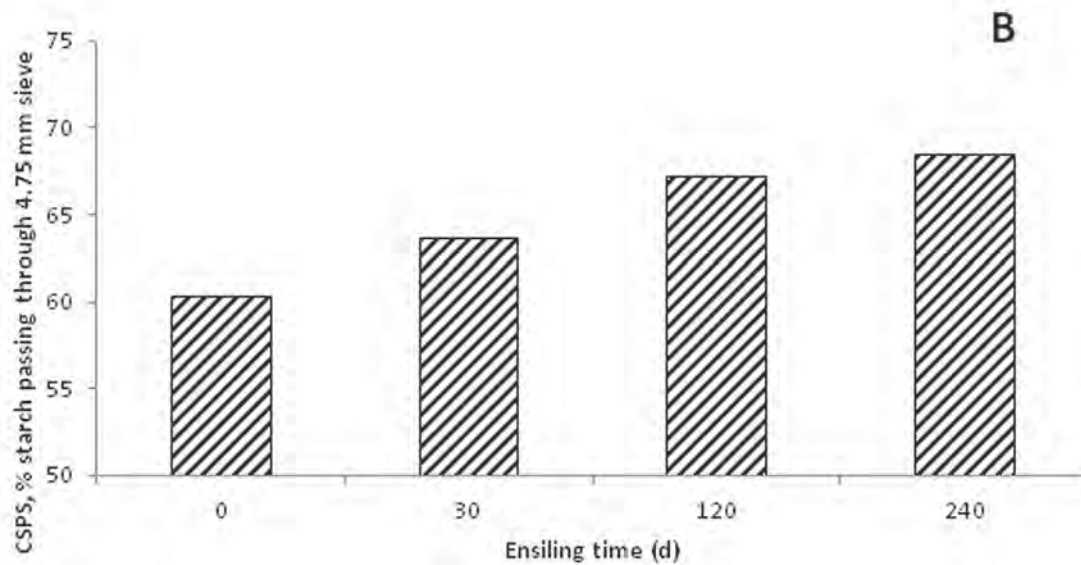
Weiss (2014; unpublished from 28<sup>th</sup> ADSA Discover Conference on Starch for Ruminants) used the slope of the Ferraretto et al. (2013) in Figure 3, or 0.5%-unit change in TTNDFD for each 1%-unit change in dietary starch content, to calculate effects on dietary energy values. In the Weiss example, a 5%-unit increase in dietary starch content (e.g. 30% vs. 25%) reduced TTNDFD 2.5%-units (46.5% to 44.0%) which resulted in a 5.3% increase in diet NEL content compared to a 6.5% increase had TTNDFD not been adversely affected by increased dietary starch content. Greater total tract starch digestibility (>90%) than TTNDFD (<50%) tempers the negative impact on diet NEL content of reduced TTNDFD with greater dietary starch concentrations.

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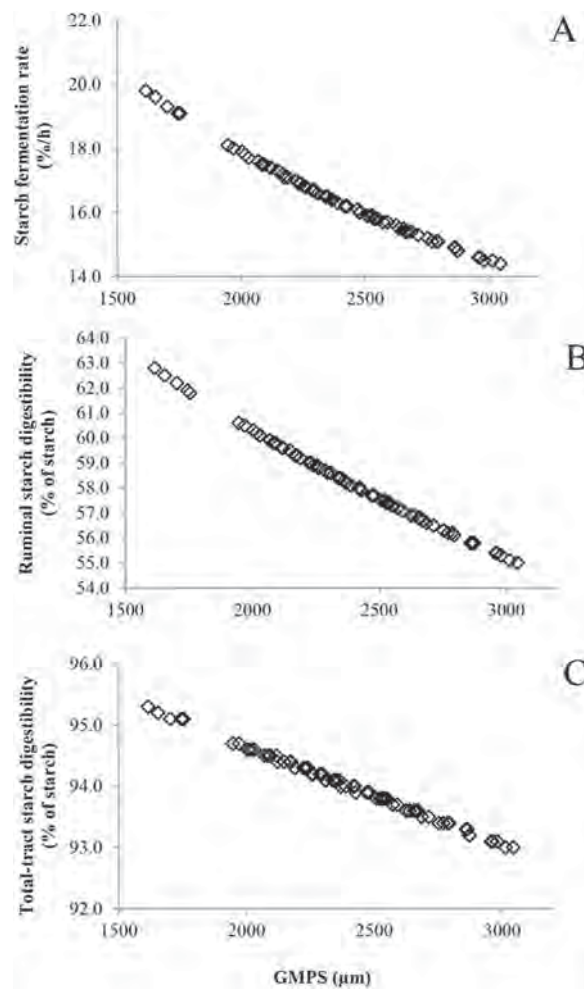
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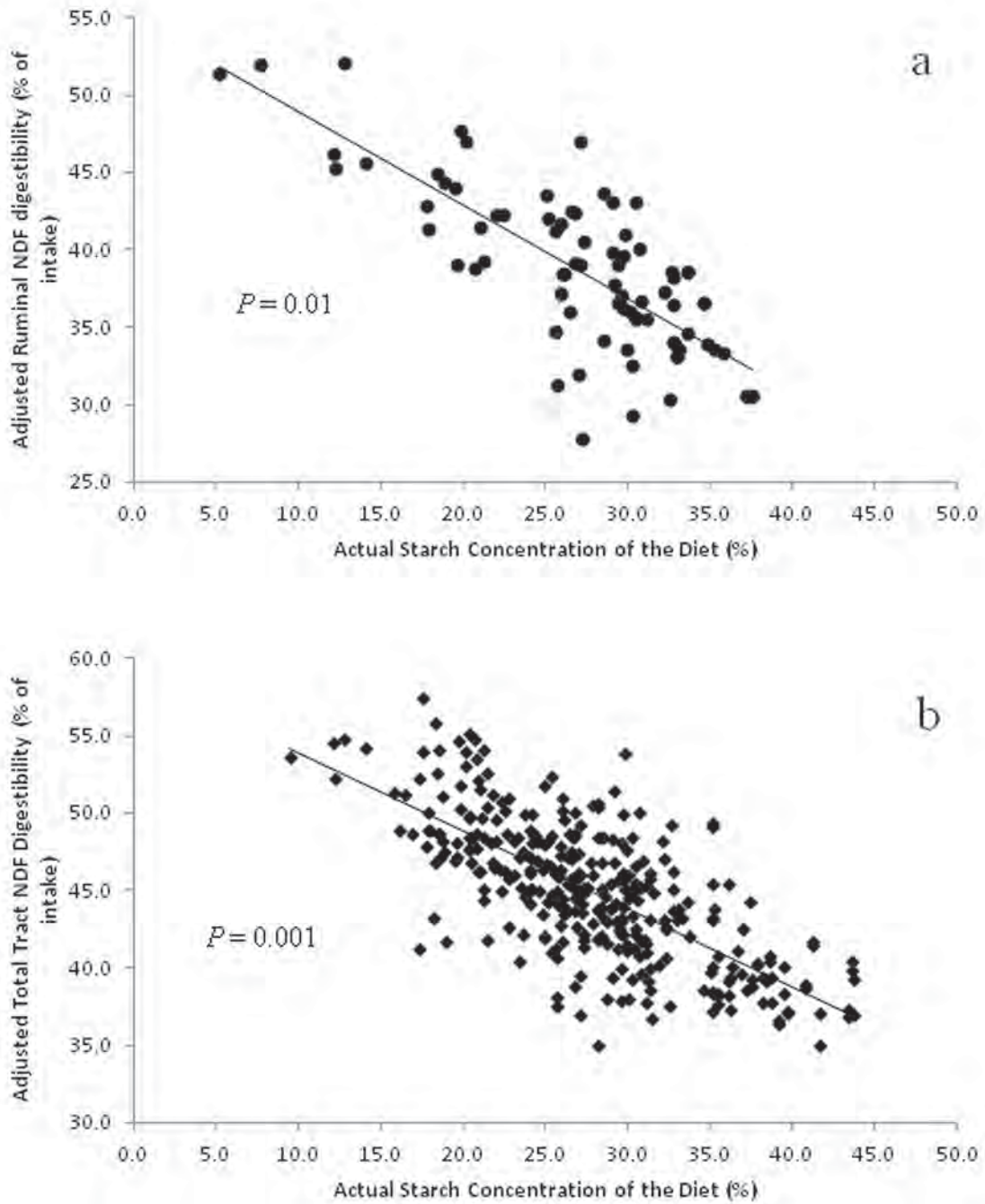




**Figure 1.** A. Effect of ensiling on corn silage processing score (CSPS) of whole-plant corn silage; n = 12, SEM = 3.1, P = 0.01. B. Effect of ensiling time on corn silage processing score (CSPS) of whole-plant corn silage; n = 3, SEM = 2.0, P = 0.08. Source: Ferraretto et al., 2015b.



**Figure 2.** Simulations (Dias Junior et al., 2016) of the effect of kernel fraction geometric mean particle size ( $\mu\text{m}$ ) on starch fermentation rate (%/h; A) and ruminal and total-tract starch digestibilities (% of starch; B and C, respectively) performed using the Feed Grain V2.0 Evaluation System (Hoffman et al., 2012a,b,c).



**Figure 3.** Effect of starch concentration of the diet on ruminal and total-tract digestibility of diet NDF adjusted for the random effect of trial. Ruminal digestibility data (Panel a) predicted from equation:  $y = 54.9746 + (-0.605 \cdot \text{starch concentration}) + (0.063 + 3.524)$ ;  $n = 70$ ,  $\text{RMSE} = 3.55$ . Total-tract digestibility diet (Panel b) predicted from equation:  $y = 58.2843 + (-0.4817 \cdot \text{starch concentration}) + (0.059 + 3.191)$ ;  $n = 320$ ,  $\text{RMSE} = 3.20$ . Source: Ferraretto et al., 2013.

# Review of Nutrient Partitioning

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## INTRODUCTION

Advances in animal productivity during the last century are remarkable, as modern dairy cows can produce more than ten times what their ancestors did just seven decades ago and the annual rate of milk yield increase does not appear to be diminishing (Collier et al., 2005). In addition to simply synthesizing more, the efficiency of producing milk has also markedly improved. Consequently, the inputs (feed, electricity, labor, barn space, etc.) necessary for making milk and the generated waste products per unit of milk produced have obviously decreased (Table 1; Bauman, 2000). This improved production efficiency is critical for sustaining farm economics, consciousness environmental stewardship and for satiating a growing global appetite for high quality protein.

**Table 1.** Performance and efficiency comparisons of Northeast American cows\*

Variable	Year		
	1930	1965	1999
Performance and Inputs			
Milk yield, kg/d	6.4	17.7	30.9
Milk yield/feed intake, kg/d	0.70	1.26	1.57
Use of netenergy intake, %			
Maintenance	70	45	32
Milk synthesis	30	55	68
Animal Waste Products			
Fecal output/milk yield, kg/kg	3.1	1.7	1.4
Urine output/milk yield, L/kg	3.1	1.1	0.6

\*Adapted from Bauman, 2000.

Despite incredible gains in the North American average milk production, there remain notable differences (i.e. > 5,000 kg) in average milk yield/cow between farms (even within farms from the same region and utilizing similar genetics and comparable feedstuffs) and this is likely in part due to farm management differences. However, within herds there is large variability between individual cows even though genetics, diet and management style do not differ. From an on-farm prospective, this is undoubtedly costly because low-producing cows are not as profitable. In addition, the unpredictability is also expensive because cows in a pen are fed based on an expected

(average) yield, therefore low and high producing cows are over-fed and under-fed, respectively. As a result, the low producing cows likely put on too much condition and yield in the high producing cows is probably limited by nutrient/energy availability.

The yield variation amongst cows begs the obvious questions: 1) what is the biological basis for differences in production efficiency? and 2) can these physiological systems be manipulated?

Sources of potential variation in production efficiency include nutrient digestion and absorption, efficiency of nutrient utilization, maintenance costs and nutrient partitioning. Although digestibility and nutrient absorption are heavily dependent upon dietary manipulation (Tyrrell and Moe, 1975), there appears to be little variability in the extent that which individual cows can digest and absorb a particular diet (Bauman et al., 1985). Likewise, although differences exist in the efficiency of utilizing metabolizable energy for a productive purpose between feedstuffs (i.e. dietary fat vs. fiber) there appears to be little inconsistency between individual cows (Bauman et al., 1985). There are obviously differences in maintenance costs in cows that differ in size and body composition, but the difference between maintenance requirements per unit of metabolic body size is very small and thus it does not appreciably contribute to the overall variation in production efficiency (Bauman et al., 1985; Collier et al., 2005).

The primary source of yield variation between cows (and the principal reason for the annual increase in milk yield/cow [and probably all productive indices since livestock domestication]) is nutrient partitioning. Nutrient partitioning was originally conceptualized by Hamman (1952) and can be broadly described as a change in tissue/system priority at a given plane of nutrition. For example (Table 2), how are metabolizable nutrients and tissue reserves “directed” towards the mammary gland in one animal, but in another animal on the same plane of nutrition those dietary derived nutrients are partitioned into tissue storage? It is the difference in how animals change the hierarchy of tissue/system priority that primarily explains why some cows give more milk, why some growing animals deposit protein at the expense of lipid and why high-producing cows de-emphasize the reproductive system in early lactation (Collier et al., 2005).

**Table 2.** Example of animal difference in nutrient partitioning

Variable <sup>a</sup>	Cow A	Cow B
Initial body weight (kg)	517	519
Diet Intake	Equal	
Live weight change (kg)	+39.1	-51.8
Milk yield (3.5% kg/d)	12.3	26.3

<sup>a</sup>For the first 67 DIM  
Adapted from Bauman et al., 1985

The mechanisms responsible for nutrient partitioning include both homeostatic and long-term homeorhetic adaptations that incorporate probably every tissue and physiological system in the body. Some of these homeorhetic changes are mediated by changes in circulating anabolic and catabolic hormones, hormone membrane receptors and intracellular signaling pathways. The coordinated change in how tissues and systems are re-prioritized includes a plethora of hormones (Table 3; and almost certainly ones that have not been discovered yet), but this brief review will primarily concentrate on insulin and somatotropin (growth hormone). For a more extensive description of nutrient partitioning see classic reviews authored by Bauman and Currie, 1980; Bauman et al., 1985; Bell and Bauman 1997; Chilliard et al., 2000; and Collier et al., 2005.

### Glucose-Sparing

Understanding the homeorhetic mechanisms responsible for physiological and metabolic adjustments lactating and growing animals initiate during periods of inadequate nutrition provides some insight as to how high producing animals prioritize valued tissues (mammary and muscle) compared to lower producing herd mates when on a high-plane of nutrition. These changes in post-absorptive nutrient partitioning occur to support a dominant physiological state (i.e. milk and skeletal muscle synthesis; Bauman and Currie, 1980) and one-well described homeorhetic strategy is the “glucose sparing” effect that both lactating and growing animals utilize when on a lowered-plane of nutrition.

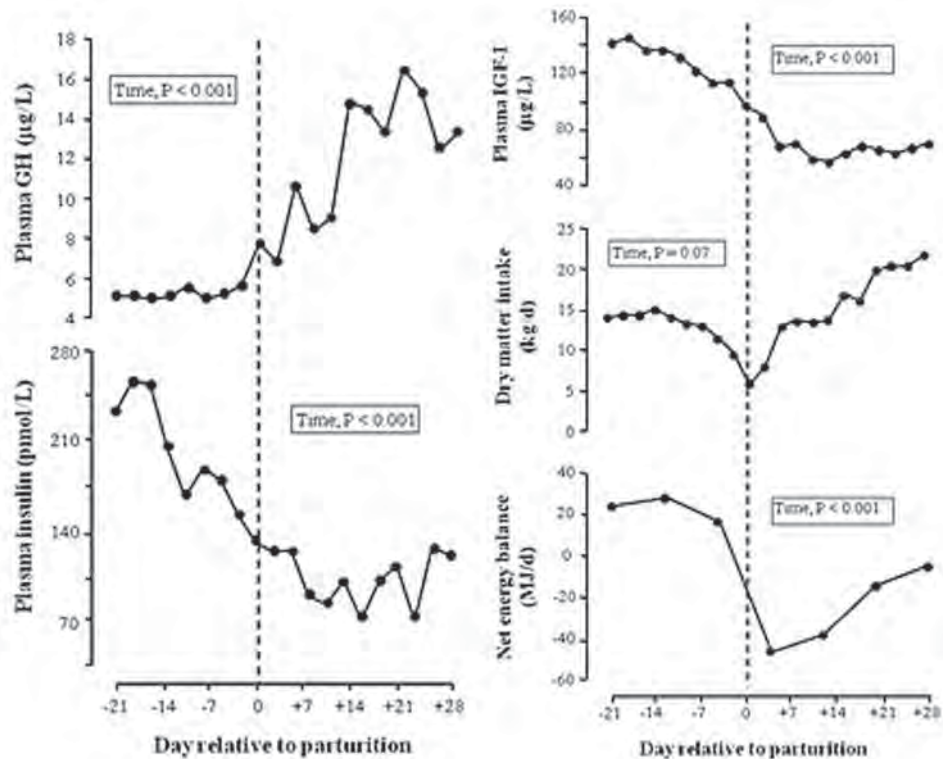
*Lactation:* Early lactation dairy cattle enter a unique physiological state during which they are unable to consume enough nutrients to meet maintenance and

**Table 3.** Partial list of physiological adaptations that occur in lactating dairy cows.

Process/Tissue	Response
Mammary Gland	Increased number of secretory cells Increased nutrient use Increased blood supply
Food Intake	Increased appetite
Digestive Tract	Increased size Increased absorptive capacity Increased rates of nutrient absorption
Liver	Increased size Increased rates of gluconeogenesis Increased glycogen mobilization Increased protein synthesis
Adipose Tissue	Decreased de novo fat synthesis Decreased preformed fatty acid uptake Decreased fatty acid reesterification Increased lipolysis and mobilization
Skeletal Muscle	Decreased glucose utilization Decreased protein synthesis Increased proteolysis Increased oxidation of NEFA
Bone	Increased Ca and P mobilization
Plasma Hormones	Decreased insulin Increased somatotropin Increased glucagon Increased prolactin Increased glucocorticoids Decreased thyroid hormones Decreased IGF-I

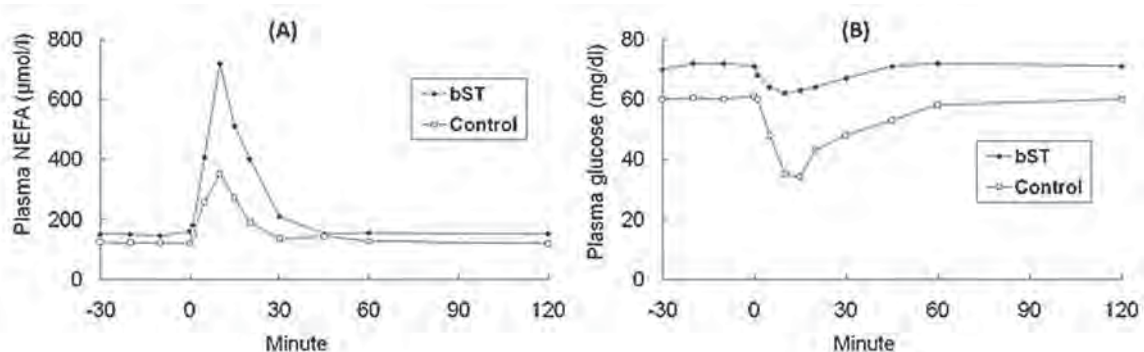
Adapted from Bauman and Currie, 1980; Vernon, 1989, 1998; Chilliard, 1999; Collier et al., 2005.

milk production costs and animals typically enter into negative energy balance (NEBAL; Figure 1; Drackley, 1999; Baumgard et al., 2006). Negative energy balance is associated with a variety of metabolic changes that are implemented to support the dominant physiological condition of lactation (Bauman and Currie, 1980). Marked alterations in both carbohydrate and lipid metabolism ensure partitioning of dietary and tissue derived nutrients towards the mammary gland, and not surprisingly many of these changes are mediated by endogenous somatotropin (Table 3) which naturally increases during periods of NEBAL (Figure 1; Bauman and Currie, 1980).

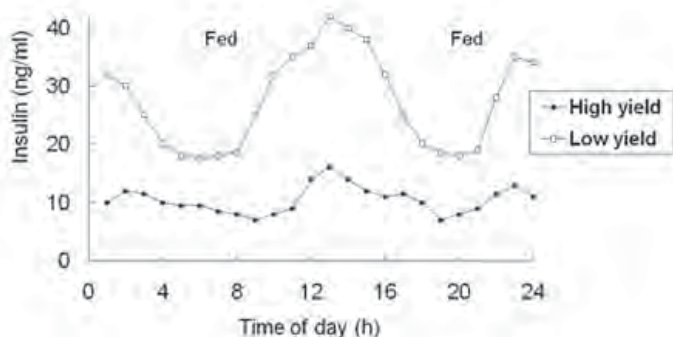


**Figure 1.** Temporal pattern of whole-animal energetics and key hormones responsible for nutrient partitioning in transitioning lactating Holstein cows.

During NEBAL, somatotropin promotes non-esterified fatty acids (NEFA) export from adipose tissue by accentuating the lipolytic response to  $\beta$ -adrenergic signals (Figure 2A) and by inhibiting insulin mediated lipogenesis and glucose utilization (Figure 2B; Bauman and Vernon, 1993). This reduction in systemic insulin sensitivity is coupled with a decrease in circulating blood insulin levels (Figure 1). The reduction in insulin action allows for adipose lipolysis and NEFA mobilization (Bauman and Currie, 1980). Not surprisingly, reduced circulating insulin is also a key mediating factor by which high producing cows partition nutrients away from storage and towards mammary utilization (Figure 3). Increased circulating NEFA are typical in “transitioning” and malnourished cows and represent (along with NEFA derived ketones) a significant source of energy (and precursors for milk fat synthesis) for cows in NEBAL. The severity of calculated NEBAL is positively associated with circulating NEFA levels (Bauman et al., 1988; Dunshea et al., 1990; Carriquiry et al., 2009) and it is generally thought that there is a linear relationship (concentration dependent process) between NEFA delivery, tissue NEFA uptake and NEFA oxidation (Armstrong et al., 1961). The magnitude of NEBAL and thus lipid mobilization, in large part explains why cows lose considerable amounts (> 50 kg) of body weight during early lactation.



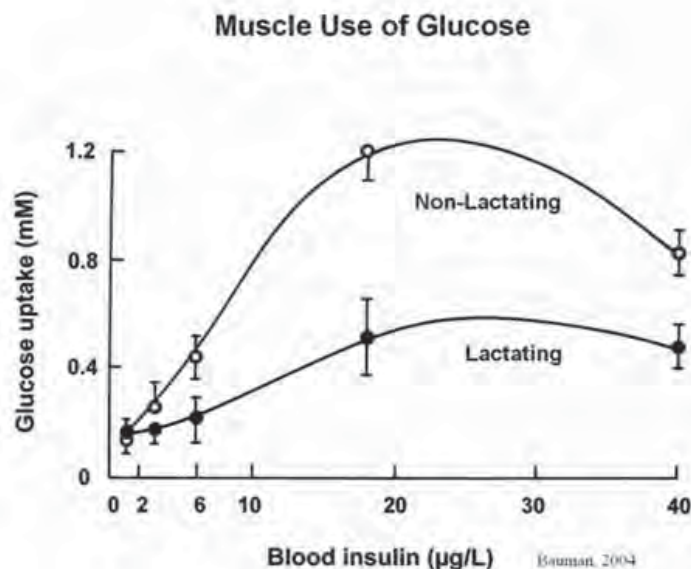
**Figure 2.** Effects of rbST on (A) the non-esterified fatty acid (NEFA) response to an epinephrine challenge and (B) the glucose response to an insulin tolerance test in lactating Holstein cows. Adapted from Sechen et al., 1990.



**Figure 3.** Plasma insulin levels in high and low yielding dairy cows. Adapted from Bines and Hart (1982).

Post-absorptive carbohydrate metabolism is also markedly altered by NEBAL and this is also, in large part, mediated by reduced insulin action. During either early lactation or inadequate nutrient intake, glucose is partitioned towards the mammary gland and glucose's contribution as a fuel source to extra-mammary tissues is decreased (Bell, 1995). This can be observed when comparing insulin's effectiveness at stimulating muscle glucose uptake in lactating and non-lactating animals (Figure 4). The early lactation or NEBAL induced hypoglycemia accentuates catecholamine's adipose lipolytic effectiveness (Clutter et al., 1980). This is a key "glucose sparing" mechanism because elevated NEFA levels decreases skeletal muscle glucose uptake and oxidation and this is referred to as the "Randle Effect" (Randle, 1998). The fact that insulin simultaneously orchestrates both carbohydrate and lipid metabolism explains why there is a reciprocal relationship between glucose and NEFA oxidation. Ultimately, these are homeorhetic adaptations to maximize milk synthesis at the expense of tissue accretion (Bauman and Curie, 1980). A cow in NEBAL could be considered "metabolically flexible" because she can depend upon alternative fuels (NEFA and ketones) to spare glucose, which can be utilized by the mammary gland to copiously produce milk.

**Growth:** Inadequate nutrient consumption is associated with a variety of metabolic changes implemented to support the synthesis of high priority tissues like skeletal muscle (Van Milgen and Noblet, 2003). Marked alterations in both carbohydrate and lipid metabolism ensure partitioning of dietary derived and tissue originating nutrients towards muscle, and many of these changes are mediated by altered concentrations of anabolic and catabolic signals. One characteristic response is a reduction in circulating insulin coupled with a decrease in adipose insulin sensitivity. Compared to a well-fed pig, the reduction in insulin action allows for adipose lipolysis and NEFA mobilization (Mersmann, 1987). Increased circulating NEFA are typical in restricted-fed animals and rep-



**Figure 4.** Effects of physiological state on insulin action in skeletal muscle. Adapted from Bauman, 2004.

resent a significant source of energy. The enhanced fatty acid oxidation during nutrient restriction is a classic strategy to "spare" glucose. Post-absorptive carbohydrate metabolism is also altered by reduced insulin action during feed restriction resulting in reduced glucose uptake by adipose tissue. In adipose tissue, the reduced nutrient uptake coupled with the prolonged net release of NEFA is a key homeorhetic mechanism implemented by malnourished pigs in order to maintain protein synthesis (Vernon, 1992).

## Summary

Much of the historical progress in animal productivity and a large part of the current production variability is due to changes in nutrient partitioning. The coordination of nutrient trafficking is an incredibly complex system, but somatotropin and insulin play critical roles in how tissues/systems are reprioritized or de-emphasized during different physiological states. This reprioritization can primarily be described by the enlistment of glucose sparing mechanisms and both insulin and somatotropin play key roles in this adaptation. As the role of other key regulators of nutrient partitioning become clearer, it is likely that those systems will be taken advantage of to accelerate the improvement rate of production efficiency.

\*Parts of this manuscript were first published in the proceedings of the 2010 Pacific Northwest Nutrition Conference



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# Keeping Post-Weaned Heifers Growing Great

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## Take-Home Messages

Proper nutrition of post-weaned heifers is necessary for the continued growth and development of heifers. At young ages, post-weaned heifers need readily available energy sources as their rumen continues to develop. Realizing that post-weaned heifers are still developing and are not yet ready to be fed like cows facilitates an understanding that specific feeding strategies need to be developed to allow for optimal growth and development of these heifers. Using feeding strategies specifically targeted for post-weaned dairy heifers allows them to continue to meet their growth potential while reducing costs per pound of gain and reducing the overall costs of raising dairy heifers.

## Introduction

Nutrition of dairy heifers is often discussed as a whole without referring to the growth stage of the heifer. Even though there is a lot of focus placed on feeding milk-fed calves, little research information is available regarding the best strategies for feeding post-weaned dairy heifers. Paying close attention to the diets of post-weaned heifers helps to make sure they are growing at a rate to make sure that they will be ready for breeding and that they are efficiently utilizing the diets they are fed. As feed costs are the greatest expense for raising dairy heifers, nutritional strategies to encourage growth and development while improving feed efficiency will be beneficial for both the animals and heifer raisers.

Dairy heifer nutrition should be based on the age and growth stage of the heifer. Similar to lactating cows in various stages of lactation, the nutrient requirements of dairy heifers vary substantially during their 2 years of development. Although milk-fed calves have obviously different feed requirements, the nutrient requirements of heifers continue to change, especially over the 4 to 5 months after weaning. It is important to keep in mind calves that were recently weaned have different nutrient requirements from year old heifers and, thus, need to be fed differently. Starter intake does help to promote the growth and development of the rumen in calves, but making the

assumption that weaned calves are fully functional ruminants is not correct. Therefore, continuing to pay close attention to how post-weaned heifers are fed will allow for the rumen to continue to develop and will maximize the growth and development of these heifers.

## Feed Delivery Methods for Post-Weaned Heifers

Dietary composition is an important aspect of feeding heifers, but the delivery method can also have an impact when feeding heifers. A study was conducted to evaluate the effects of feeding heifers a total mixed ration (TMR), feeding them concentrate and hay side-by-side in a feed bunk (SBS), or feeding grain in a bunk and hay in a feeder (HF) on growth and intake of post-weaned heifers (Table 1). In this study, heifers fed using HF were significantly heavier ( $P \leq 0.05$ ) than heifers fed using SBS from d 49 throughout the end of the study. Delivering feed using HF resulted in heifers that were, on average, 19.1 lbs and 14.5 lbs heavier than heifers fed using SBS and TMR, respectively, over the course of the study. Heifer weights at the conclusion of the grower period were 607, 572, and 576 lbs for HF, SBS, and TMR, respectively.

Average daily gains did vary depending on the time period of the study, as heifers fed using a TMR had lower ADG from d 7 to 14 ( $P = 0.05$ ) and d 14 to 21 ( $P = 0.07$ ) compared with HF and SBS, but higher ADG compared to SBS from d 21 to 28 ( $P = 0.03$ ). These results suggest that post-weaned heifers require more time to adjust to new diets when feeding a TMR compared with component-feeding.

During the grower period, heifers fed using HF averaged 0.8 lbs/d more DMI compared with SBS and TMR ( $P < 0.01$ ). The results of this study suggest that component-fed heifers receiving long-stemmed hay maintained intake and weight gains when transitioning to a new diet and throughout the grower period. From the responses observed in the current study, it appears that feeding growing dairy heifers dietary components separately may be a preferred feed management strategy early in the grower period compared to feeding a TMR.

**Table 1.** Body weight, intake, and skeletal measurements of prepubertal dairy heifers fed common diets using different feed delivery methods.

Item <sup>1</sup>	HF	SBS	TMR	SEM	P-value
Body weight, lb					
d 28 <sup>2</sup>	398.4	388.3	389.0	5.67	0.36
d 133	607.3 <sup>a</sup>	572.4 <sup>b</sup>	576.4 <sup>b</sup>	5.67	<0.01
Average daily gain, lb/d					
d 0 to 28	2.29	2.09	1.96	0.121	0.20
d 29 to 133	2.05 <sup>a</sup>	1.83 <sup>b</sup>	1.85 <sup>b</sup>	0.064	0.06
d 0 to 133	2.09 <sup>a</sup>	1.90 <sup>b</sup>	1.87 <sup>b</sup>	0.055	0.02
Dry matter intake, lb/d					
d 0 to 28	8.8	8.3	8.9	0.21	0.15
d 29 to 133	16.6 <sup>a</sup>	15.7 <sup>b</sup>	15.6 <sup>b</sup>	0.19	<0.01
d 0 to 133	14.9 <sup>a</sup>	14.0 <sup>b</sup>	14.1 <sup>b</sup>	0.16	<0.01
Feed efficiency <sup>3</sup>					
d 0 to 28	0.252 <sup>a</sup>	0.246 <sup>a</sup>	0.205 <sup>b</sup>	0.014	0.06
d 29 to 133	0.123	0.116	0.117	0.003	0.41
d 0 to 133	0.151 <sup>a</sup>	0.145 <sup>abx</sup>	0.137 <sup>by</sup>	0.003	0.03

<sup>1</sup>HF = hay feeder; SBS = side-by-side; TMR = total mixed ration; SEM = standard error of the mean.

<sup>2</sup>Day of study.

<sup>3</sup>Feed efficiency expressed as lb of ADG per lb of daily DMI.

<sup>ab</sup>Means differ at  $P < 0.05$  level.

<sup>xy</sup>Means tend to differ at  $0.10 \leq P < 0.05$  level.

## Feeding Hay or Ensiled Forages

Forages are an important component of heifer diets. However, little research has looked at how well post-weaned dairy heifers are able to utilize ensiled forages as compared to dry forages. A study was done to evaluate the performance of post-weaned dairy heifers that were fed either dry hay or baleage. In this study (Dennis et al., 2012), heifers fed a diet containing either 40% of their dietary DM as hay or baleage for a 28 d transition period had improved ADG, and the increase in ADG continued when heifers were fed the dry hay at 60% of the dietary DM for an additional 56 d grower period (Table 2). Interestingly, the DMI of the heifers during the transition period was not decreased; thus, the decreased gain was not a result of lesser intakes. During the grower period, the DMI was decreased for heifers fed baleage though there was still an overall tendency for improved feed efficiency for heifers fed dry hay.

**Table 2.** Body weight, intake, and feed efficiency of prepubertal dairy heifers fed either Hay or Baleage for 28 d Transition Period followed by a 56 d Grower Period (Dennis et al., 2012).

Item <sup>1</sup>	Hay	Baleage	SEM	P-value
Grower Period				
Initial body weight, lb	373.5	369.6	3.99	0.47
Final body weight, lb	482.2	467.5	4.37	0.02
Average daily gain, lb/d	1.94	1.75	0.04	0.04
Dry matter intake, lb/d	12.6	11.9	0.14	<0.01

<sup>1</sup>Hay or Baleage fed at 40% of diet DM in the Transition Period and 60% of diet DM in the Grower Period.

The results of this study indicate that feeding ensiled forages to post-weaned dairy heifers may result in decreased feed efficiency. In this study, the heifers fed hay were apparently able to better utilize the forage in their diet. Although measurements of rumen development were not determined in this study, it may be possible that the rumen of the post-weaned heifers was still undergoing development and the ensiled forage was not able to be fully utilized at that point in their development.

### Grain and Forage Ratios

In most dairy systems today, calves are fed ad libitum amounts of palatable grain-based starters within a few days of birth. As calves grow, they continue to increase their starter intake until they are to the point where they are able to consume enough nutrients from the starter to support their growth without consuming milk. Once calves are weaned, their starter intake continues to increase substantially to make up for the nutrients that are no longer being consumed through milk and to cover the increased nutrient needs of the calf as they continue to grow. The timing as to when calves should begin to receive

forage, the type of forage they should receive, and how much of that forage they should be given is still of some debate.

Research was conducted at Purdue University to look at different grain to forage ratios to help determine the best strategy for feeding post-weaned dairy heifers. Heifers began the study when they were approximately 330 lbs and 4.5 months of age and were assigned to diets containing either 80, 60, or 40% concentrate (on a DM basis) for 56 days before abruptly being switched to a common diet that was 40% concentrate.

In this study, increasing grain inclusion from 40 to 80% of the dietary DM resulted in a linear increase in BW and greater overall ADG (Table 3). Frame growth exhibited similar responses to those observed for BW and ADG. Hip heights, heart girth circumference, and body condition score linearly increased with increasing grain inclusion ( $P < 0.01$ ) during the treatment period, resulting in higher growth overall during the study for heifers fed 80% grain during the treatment period.

**Table 3.** Weight, skeletal measurements, and intake responses of prepubertal dairy heifers fed increasing levels of grain during the treatment period then switched to a common diet.

Item <sup>1</sup>	40:60	60:40	80:20	SEM	P-value
Body weight, lb					
d 57 <sup>2</sup>	369.2 <sup>c</sup>	398.6 <sup>b</sup>	428.8 <sup>a</sup>	6.01	< 0.01
d 112	476.1 <sup>c</sup>	504.7 <sup>b</sup>	524.9 <sup>a</sup>	6.03	< 0.01
Average daily gain, lb/d					
d 0 to 56	1.37 <sup>c</sup>	1.87 <sup>b</sup>	2.29 <sup>c</sup>	0.088	< 0.01
d 57 to 112	1.94 <sup>a</sup>	1.92 <sup>a</sup>	1.72 <sup>b</sup>	0.064	0.07
d 0 to 112	1.65 <sup>c</sup>	1.90 <sup>b</sup>	2.07 <sup>a</sup>	0.042	< 0.01
DM intake, lb/d					
d 0 to 56	9.3 <sup>c</sup>	10.7 <sup>b</sup>	12.7 <sup>a</sup>	0.198	< 0.01
d 57 to 112	14.3	14.1	13.7	0.291	0.31
d 0 to 112	11.8 <sup>c</sup>	12.4 <sup>b</sup>	13.2 <sup>a</sup>	0.165	< 0.01
Feed efficiency <sup>3</sup>					
d 0 to 56	0.147 <sup>c</sup>	0.178 <sup>b</sup>	0.196 <sup>a</sup>	0.008	< 0.01
d 57 to 112	0.136	0.139	0.128	0.005	0.31
d 0 to 112	0.142 <sup>b</sup>	0.158 <sup>a</sup>	0.161 <sup>a</sup>	0.004	0.02
Hip height, in					
d 56	43.7 <sup>c</sup>	44.4 <sup>b</sup>	45.1 <sup>a</sup>	0.13	< 0.01
d 112	45.8 <sup>c</sup>	46.8 <sup>b</sup>	47.2 <sup>a</sup>	0.13	< 0.01

<sup>1</sup>Grain:forage ratio.

<sup>2</sup>Day of study.

<sup>3</sup>Feed efficiency expressed as lb of ADG per lb of daily DM intake.

<sup>abc</sup>Means with differing superscripts are significantly different at  $P \leq 0.05$  level.

<sup>xy</sup>Means tend to differ at  $0.10 \geq P > 0.05$  level.

Feed costs per lb of DMI averaged \$0.11, \$0.12, and \$0.13 for heifers fed 40:60, 60:40, and 80:20, respectively, during the treatment period. Feed costs per lb of ADG were lowest for 60:40 heifers over the duration of the study compared to heifers fed 40:60, though they were statistically similar to the feed costs for the 80:20 heifers. When heifers were fed 60:40 or 80:20 during the treatment period, savings were \$0.24 and \$0.22 per lb of ADG compared to heifers fed 40:60.

This study demonstrated that feeding higher grain levels to post-weaned dairy heifers can improve growth and can actually decrease the cost of gain over higher forage diets. In addition, it reinforced that heifers fed high grain levels can be negatively impacted by abrupt changes to higher forages diets, with the heifers on the 80:20 treatment showing a definite decline in intake when they were switched to a 40:60 diet that took some time to recover from.

### **Non-Fiber Carbohydrates in Heifer Diets**

Even though previous research found that feeding higher concentrate diets improved gain and feed efficiency, the concentrate portion of the diet may be made up of a wide variety of different ingredients and nutrient compositions. Understanding the best strategies for designing the concentrate portion of the diet could further help to improve the gains and feed efficiency of dairy heifers.

In order to evaluate the effects of the composition of the concentrate portion of the diet on heifer growth, intake, and feed efficiency, studies were conducted to look at the effects of feeding concentrates that were formulated to provide either high or low levels of non-fiber carbohydrates (NFC). In the first study, heifers (averaging 320 lbs and 4.8 months of age at the start of the study) were fed a low NDF diet (LNFC), a high NFC diet (HNFC), and a low NFC diet with added fat (LNFC+) formulated to provide the same amount of Mcals of energy as the HNFC diet.

Heifers fed LNFC+ were heavier on d 56 and d 112 of the study compared to heifers fed LNFC. Heifers on the HNFC diet were intermediate and tended to be lighter on d 56 and d 112 compared to heifers fed LNFC+. Overall, heifers fed LNFC+ gained 19.4 lbs more BW than heifers fed LNFC during the study ( $P =$

0.05). Average daily gain in the first 56 d was 14.9% and 8.9% greater for heifers fed LNFC+ compared to heifers fed LNFC ( $P < 0.01$ ) or HNFC ( $P = 0.05$ ), respectively. During the first 56 d, treatment tended to affect feed efficiency (FE), as heifers fed LNFC+ were 12.7% more efficient than heifers fed LNFC and 9.3% more efficient than heifers fed HNFC, with a trend ( $P = 0.07$ ) towards improved feed efficiency for LFC+ from d 0 to d 112 as compared to HNFC.

During the NFC study, heifers fed LNFC maintained the lowest cost per heifer/d throughout the study as was expected due to the high inclusion rates of by-product feeds. However, feed costs per lb of ADG were lowest for heifers fed LNFC+ compared to HNFC, resulting in a cost savings of \$0.12 per lb of gain. However, feed costs per lb of ADG were similar among treatments overall. In our study, a larger proportion of the HNFC diet included corn and DDGS, resulting in greater costs per ton for the grain mix, especially due to higher corn prices from the 2012 crop year. Paired with increased DMI for heifers fed HNFC, our data suggests that alternative energy sources, such as supplemental fat, may be more cost-effective for feeding growing heifers.

A second study was conducted to evaluate the effect of NFC level in the diets of post-weaned heifers after being started on either a conventional (22:20) or higher plane of nutrition (28:20) milk replacer. One of the goals of this study was to determine if how a calf was raised pre-weaning affects subsequent heifer growth and performance. In this study, animal receiving the HNFC diet had greater weight gain during the growing period from 12 to 28 weeks. Interestingly, when the animals were started on a higher plane of nutrition during the milk feeding period and subsequently fed LNFC diets, their body weight gain was significantly decreased as compared to animals that were started with a convention milk replacer program (Table 4). This study indicates that when calves are started on diets with a higher level of nutrition, maintaining a greater level of nutrition into the growing period may be even more important than when calves are started on a conventional milk feeding program.

**Table 4.** Weight and skeletal growth responses of dairy heifers and steers at 28 wks of age fed a milk treatment (MILK) of either conventional milk replacer (CONV) or high nutrition plane milk replacer (HIGH) and fed a grower diet (GRWR) of high non-fiber carbohydrate (HNFC) or low NFC (LNFC) post-weaning grower diets from 12 to 28 wk of age.

Item	CONV		HIGH		SEM	P-value <sup>1</sup>		
	HNFC	LNFC	HNFC	LNFC		MILK	GRWR	MILK × GRWR
Body weight, lb								
28 wk <sup>2</sup>	516.4 <sup>a</sup>	503.0 <sup>ab</sup>	522.1 <sup>a</sup>	494.8 <sup>b</sup>	7.98	0.88	<0.01	0.04
Average daily gain, lb/d								
0 to 28 wk	2.12	2.03	2.14	1.98	0.053	0.95	0.01	0.49
Hip height, in								
28 wk	47.6	47.2	47.4	47.3	0.22	0.91	0.24	0.60
Hip width, in								
28 wk	13.9 <sup>ab</sup>	13.9 <sup>ab,x</sup>	14.1 <sup>a</sup>	13.7 <sup>b,y</sup>	0.10	0.85	0.15	0.08

<sup>1</sup>MILK = effect of pre-weaning milk treatment; GRWR = effect of post-weaning diet; MILK × GRWR = interaction of milk treatment vs. post-weaning diet effects.

<sup>2</sup>Weeks of age.

<sup>ab</sup>Means with differing superscripts significantly differ at  $P \leq 0.05$  level.

<sup>xy</sup>Means with differing superscripts tend to differ at  $0.10 \geq P > 0.05$  level.

## Conclusions

Using the best feeding strategies for post-weaned dairy heifers allows heifers to continue to meet their growth potential while reducing costs per lb of gain and reducing the overall costs of raising dairy heifers. Continuing to feed heifers high levels of grain post-weaning provides them with a digestible source of nutrients that facilitates growth and improves feed efficiency. At young ages, heifers appear to continue to need readily available energy sources as their rumen continues to develop. Realizing that post-weaned heifers are still developing and are not yet ready to be fed like cows facilitates an understanding that specific feeding strategies need to be developed to allow for optimal growth and development of these heifers.

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# Heifer Stocking Density and Performance

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## Introduction

Management programs for dairy replacement heifers prioritize rearing animals at a low economic and environmental cost, without compromising their performance as lactating cows (Hoffman et al., 2007). Generally, diets for replacement heifers are forage based, but oftentimes the forages available are too energy dense, resulting in over-conditioning. This is especially true if significant proportions of corn silage are included in the diet. While diets comprised of dairy-quality forages may exceed suggested energy-density targets for replacement dairy heifers, a concomitant problem is that these diets also may lack sufficient NDF to restrict DM intake by the gut-fill mechanism. Previous intensive evaluation of typical dairy-heifer diets in confined management systems has indicated that dairy heifers will consume approximately 1.0% of their bodyweight daily as NDF (Hoffman et al., 2008). As a result, heifers consuming diets containing inadequate NDF are susceptible to excessive DM intake, further compounding the risk of over-conditioning. Generally, two approaches have been developed to combat this problem: i) precision or limit feeding; and ii) dietary dilution with low-energy forages. Both strategies have advantages and disadvantages, and the effectiveness of both approaches can be affected by over-crowding. This summary will focus on recent research conducted at the University of Wisconsin Marshfield Agricultural Research Station that primarily addresses management questions associated with the dietary dilution approach to maintaining daily weight gains within reasonable proximity to often recommended targets for dairy heifers (~1.8 lbs/d).

## Effects of Dilution (Experiment 1)

Eastern gamagrass (EGG; *Tripsacum dactyloides* L.) is a perennial warm-season grass possessing the C4 photosynthetic pathway (Waller and Lewis, 1979), and is a distant relative of corn (Bates et al., 1981). Yields of DM ranging from 7.7 to 11.0 tons/acre can be obtained in Wisconsin using a 1-cut harvest system (Coblenz et al., 2010a), and the NDF concentration by mid-August is about 75 to 80% (Coblenz et

al., 2010b). Eastern gamagrass haylage was substituted primarily for corn silage at rates of 0, 9, 18, or 27% of DM within a base diet comprised of a 47% alfalfa haylage and 53% corn silage (Table 1; Coblenz et al., 2012). Diets were offered for 105 d to 120 Holstein heifers with an average initial bodyweight of 821 lbs. Heifers were housed in freestalls (8 heifers/pen), where each pen had 8 freestalls and 8 headlocking feed gates (no over-crowding; 100% of capacity). Substitution of EGG haylage for corn silage was effective at reducing energy intakes by two mechanisms: i) reducing the energy density of the diet; and ii) restricting voluntary intake. Furthermore, daily weight gains were reduced linearly with the serial addition of EGG haylage; however, it also was apparent that heifers did not exhibit any of the sorting behaviors commonly observed when chopped straw is added to blended diets.

## Sorting and Other Behaviors with Dietary Dilution (Experiment 2)

A follow-up trial (Coblenz et al., 2015) was conducted to evaluate heifer growth performance when heifers were over-crowded (133% of capacity) at the feedbunk, and offered diets similar to those in the first experiment, only the diluting agents (EGG haylage, chopped straw, or chopped corn fodder) varied with respect to sortability by heifers (Table 2). An alfalfa haylage/corn silage diet similar to that used in Experiment 1 also was included as a control. A total of 128 Holstein heifers (8 heifers/pen) with an average initial bodyweight of 1040 lbs were housed in the same facilities as described for Experiment 1; over-crowding was created by using plywood sheets to cover 2 of the 8 headlocking gates at the feed alley. Feedbunks were scored daily, and daily feed disbursements were adjusted to allow for ad-libitum intake, but with minimal orts (~2.5%). Heifers were not over-crowded with respect to available freestalls (100% of capacity). All diluting agents were effective in reducing nutrient intakes, as well as daily weight gains compared to the control diet; however, heifers receiving chopped straw achieved daily weight gains (1.74 lbs/d) closest to recommended targets. Serial

sampling of feedbunks indicated that the diet diluted with EGG haylage was much less sortable than those containing wheat straw or chopped corn fodder. However, the sortability of diets could not be related directly to daily weight gains. Although the diet containing chopped straw was sorted intermediately between those containing EGG haylage and corn fodder (Figure 1), daily weight gains were similar for EGG and corn fodder diets (2.17 vs. 2.14 lbs/d), but 0.41 lbs/d less for chopped straw. DeVries and von Keyserlingk (2009) concluded that competition for feed alters feeding patterns, reduces access to feed, and increases day-to-day feeding behaviors. In our study, the within-pen coefficient of variation (CV) for daily gain increased from 10.4 to 15.5% as the diet became more sortable; however, this variation was numerical only, and was not statistically significant. The feeding system within the research barn is managed to allow for ad-libitum intake, but with a very tight tolerance for orts (~2.5%). This system is consistent with recommendations for including straw within TMR diets (Shaver and Hoffman, 2010), and the results of Experiment 2 suggest that this management approach encourages (near) complete consumption of the TMR within a 24-hour period, and may partially decouple sorting behaviors from growth performance.

### **Over-crowding at the Feedbunk and in Freestalls (Experiment 3)**

A third experiment is being conducted currently with 240 Holstein heifers with a mean initial bodyweight of 903 lbs. Heifers were offered one of two alfalfa haylage/corn silage diets, both formulated identically, but with one diet containing well-processed straw (13.0% CP, 46.5% NDF, 60.5% TDN), and the other containing poorly processed straw (12.6% CP, 47.5% NDF, 59.5% TDN). In this trial, heifers were assigned to research pens at 100, 125, or 150% of capacity; therefore, over-crowding was established at both the feedbunk, as well as for freestall use. Data presented here represent two replications of the six interactive treatments (120 heifers), which is only 50% of the complete data set. Feeding management again was designed to allow for full ad-libitum intake, but with a minimal amount of orts. Descriptive performance and behavioral data appear in Table 3. Although the data for this trial are incomplete, preliminary evaluation suggests that over-stocking affected within-pen mean weight gains minimally, but some evidence of greater variability within pen was observed. To date, similar responses have been observed for hygiene scores of heifer flanks and legs (scale = 1 to 5; Cook, 2007), suggesting heifers in over-crowded pens were more likely to rest in the alleys instead of waiting for an available open stall. This was corroborated by pen counts; during night hours,

a greater percentage of heifers in over-crowded pens were observed resting in alleys or inactively standing (Figure 2).

### **Summary**

Although replacement dairy heifers are frequently offered forage-based diets, this management practice may still result in over-conditioning, especially if significant proportions of corn silage are included in the diet. Generally, two approaches are recommended to address this problem: i) precision or limit feeding; and ii) dietary dilution with low-energy forages. However, both strategies have advantages and disadvantages, and the effectiveness of both management approaches can be affected by over-crowding. The use of low-energy forages (dilution) acts to limit weight gains by two mechanisms: i) reducing the energy density of the diet; and ii) limiting voluntary intake via gut-fill, where heifers generally are limited to about 1% of their bodyweight for daily NDF intake. Although heifers will exhibit different sorting behaviors with various diluting agents, these behaviors could not be linked directly to growth performance in our studies. The variability of daily weight gains within each pen may trend greater with more sortable diets, but (to date) this variability has not been statistically significant in our trials. Feeding management in these trials was designed to maximize ad-libitum intake, but with minimal orts, thereby ensuring nearly 100% consumption of all feed components within a 24-hour period. This approach is consistent with current recommendations for including straw in TMR diets (Shaver and Hoffman, 2010), and may have restricted within-pen variability in growth performance. Over-stocking within the pen, such that heifers did not always have an available stall, resulted in increased (poorer) hygiene scores, as well as a greater percentage of heifers lying in alleys or inactively standing during night hours. Furthermore, within-pen variability of hygiene scores increased sharply with over-stocking.

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**Table 1.** Performance of 120 Holstein heifers offered diets containing eastern gamagrass (EGG) haylage substituted primarily for corn silage for 105 d without overcrowding at Marshfield, WI (Experiment 1; Coblentz et al., 2012).

Item	----- Blended Diet <sup>1</sup> -----				
	EGG0	EGG9	EGG18	EGG27	Limit-Fed
<b>Ingredients, % of DM</b>					
Alfalfa Haylage	46.5	45.8	44.6	43.4	46.5
Corn Silage	52.9	44.4	36.5	28.5	52.9
EGG Haylage	0	9.1	18.3	27.4	0
<b>Nutrients<sup>2</sup></b>					
DM, % (as fed)	40.1	39.9	40.5	40.6	40.1
CP	12.9	13.0	13.1	12.9	12.9
NDF	39.6	43.0	45.6	48.7	39.6
TDN	68.2	65.3	63.2	61.3	68.2
<b>Intake<sup>3</sup></b>					
DM	20.7	19.8	19.7	19.7	17.8
CP	2.67	2.56	2.58	2.54	2.29
NDF	8.20	8.49	8.97	9.59	7.03
NDF, % BW	0.88	0.92	0.97	1.04	0.77
TDN	14.1	12.9	12.4	12.1	12.1
<b>Performance</b>					
Gain, lbs	251	236	221	196	203
ADG, lbs/d	2.40	2.27	2.09	1.87	1.94
Feed:Gain, lbs/lbs	8.6	8.8	9.4	10.5	9.2

<sup>1</sup> Diets: EGG0 = alfalfa haylage/corn silage diet containing no EGG and offered for ad libitum intake; EGG9, EGG18, and EGG27 = alfalfa haylage/corn silage diet containing 9.1, 18.3, and 27.4% EGG haylage, respectively, and offered for ad libitum intake; and Limit-Fed = EGG0 diet offered at 85% of the daily intake of EGG0.

<sup>2</sup> Expressed as % of DM, unless otherwise indicated.

<sup>3</sup> Expressed as lbs/d, unless otherwise indicated.

**Table 2.** Performance of 128 Holstein heifers offered alfalfa haylage/corn silage diets with diluting agents differing in sortability for 118 d at Marshfield, WI. Heifers were overcrowded at 133% of capacity at the feedbunk, but not in the freestalls (Experiment 2; Coblenz et al., 2015).

Item	Diet <sup>1</sup>			
	Control	EGG	Wheat Straw	Com Fodder
<b>Ingredients, % of DM</b>				
Alfalfa Haylage	44.2	47.2	53.5	52.5
Corn Silage	55.8	26.7	25.2	32.6
EGG Haylage	0	26.2	0	0
Wheat Straw	0	0	21.3	0
Com Fodder	0	0	0	14.9
<b>Nutrients<sup>2</sup></b>				
DM, % (as fed)	32.6	34.9	39.2	36.1
CP	13.9	13.7	13.6	13.8
NDF	43.3	50.9	53.3	50.4
TDN	66.8	58.9	59.7	59.1
<b>Intake<sup>3</sup></b>				
DM	24.4	23.3	20.9	22.1
CP	3.40	3.20	2.84	3.06
NDF	10.6	11.8	11.1	11.2
NDF, % BW	0.89	1.02	0.97	0.96
TDN	16.3	13.7	12.5	13.2
<b>Performance</b>				
Gain, lbs	309	258	209	256
ADG, lbs/d	2.56	2.16	1.74	2.14
CV, % <sup>4</sup>	10.4	11.5	14.4	15.5
Feed:Gain, lbs/lbs	9.6	10.8	12.1	10.5

<sup>1</sup> Diets: Control = alfalfa haylage/corn silage diet containing no diluting agent and offered for ad libitum intake; EGG = alfalfa haylage/corn silage diet containing 26.2% eastern gamagrass haylage; Wheat Straw = alfalfa haylage/corn silage diet containing 21.3% wheat straw; and Corn Fodder = alfalfa haylage/corn silage diet containing 14.9% chopped corn fodder.

<sup>2</sup> Expressed as % of DM, unless otherwise indicated.

<sup>3</sup> Expressed as lbs/d, unless otherwise indicated.

<sup>4</sup> Coefficient of variation (%) for within-pen total gain or ADG.

**Table 3.** Performance, lying behavior, and hygiene scores for Holstein heifers offered alfalfa haylage/corn silage diets with well-processed or poorly processed wheat straw for 90 d at Marshfield, WI (Experiment 3; Coblenz et al., unpublished).

Item	Diets		Stocking Rate <sup>1</sup>		
	Well-Processed Straw	Poorly-Processed Straw	100%	125%	150%
<b>Performance</b>					
Gain, lbs	183	184	189	181	181
ADG, lbs/d	2.02	2.02	2.07	1.99	1.99
CV, % <sup>2</sup>	18.7	14.3	13.3	17.3	18.9
<b>Behavior</b>					
Lying Time, min/d	840	838	865	829	823
Standing Time, min/d	600	602	575	611	617
Bouts, #/d	11	11	11	11	11
Lying Time/Bout, min	80	83	82	83	80
Standing Time/Bout, min	58	62	56	62	63
<b>Hygiene Score<sup>3</sup></b>					
Flanks	1.9	2.1	1.7	2.0	2.2
CV <sup>2</sup> , %	32.5	26.2	18.0	30.6	39.5
Legs	2.2	2.5	2.1	2.4	2.5
CV <sup>2</sup> , %	13.6	15.0	9.4	13.9	19.7

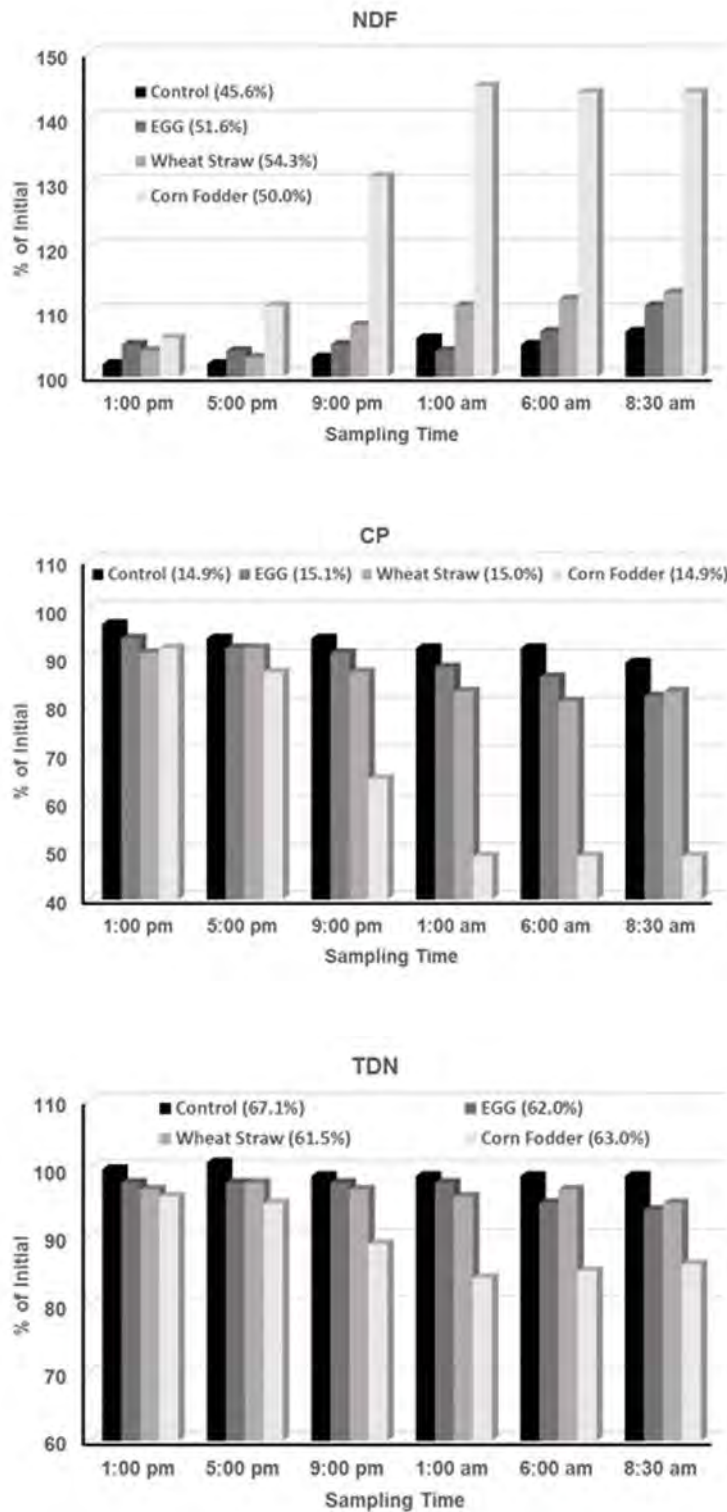
<sup>1</sup> Stocking Rate: 100%, 8 heifers/pen; 125%, 10 heifers/pen; and 150%, 12 heifers/pen. Each pen had 8 freestalls and 8 head-locking gates at the feedbunk.

<sup>2</sup> Coefficient of variation (%) for within-pen total gain or ADG, hygiene of flanks, and hygiene of legs.

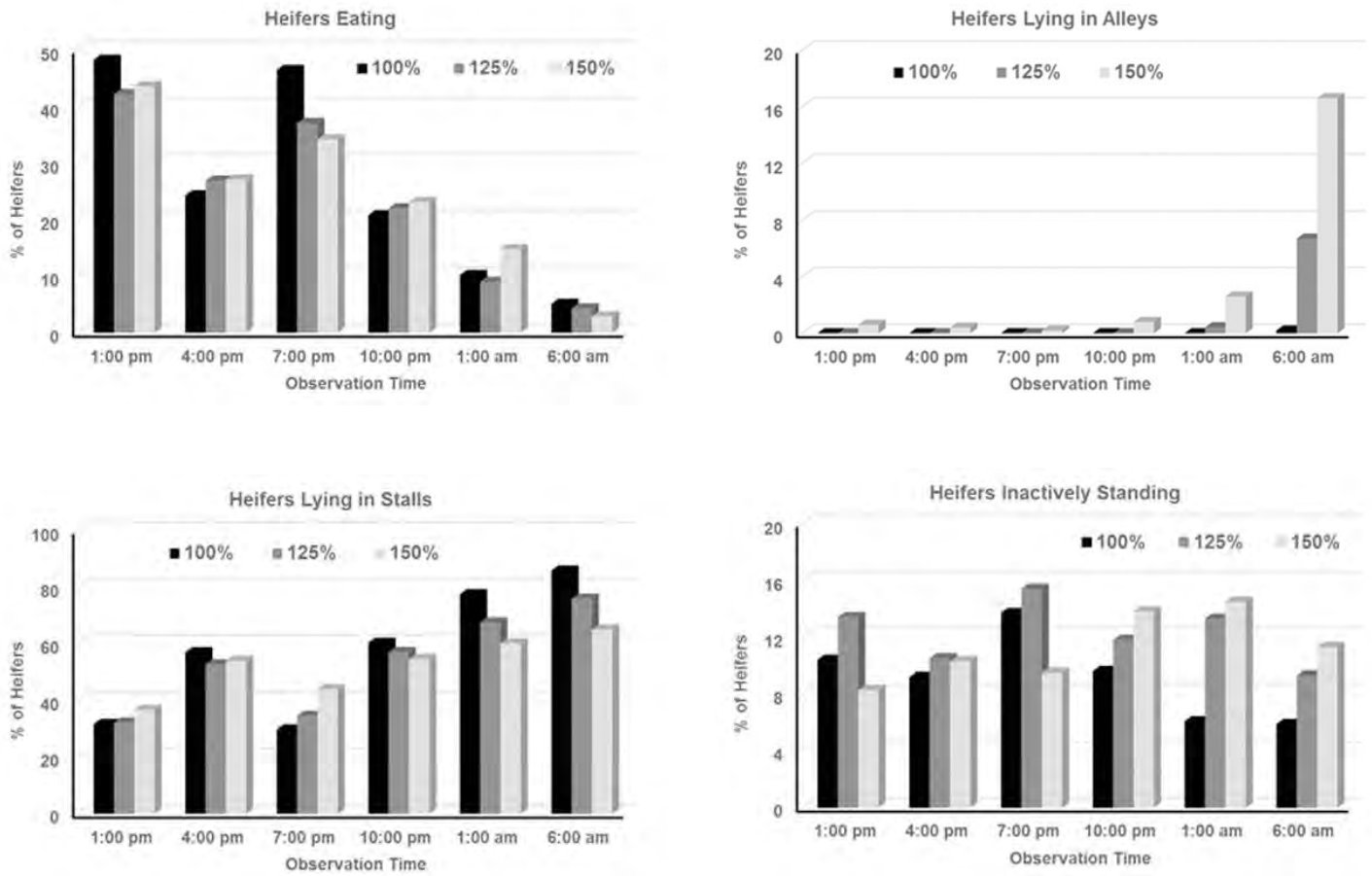
<sup>3</sup> Lying and standing behaviors determined by data logger (HOB0 Pendant® G Acceleration Data Logger; Onset Computer Corp., Bourne, MA), as calculated per Ledgerwood et al. (2010).

<sup>4</sup> Hygiene scores based on a scale of 1 (cleanest) to 5 (soiled) as described by Cook (2007).

**Figure 1.** Effects of sorting behaviors by Holstein dairy heifers on the composition of TMR remaining within the feedbunk (Experiment 2) at Marshfield, WI. The TMR was dispersed once daily at about 10:00 am, and orts were collected at approximately 8:30 am the following day. Mean initial concentrations of NDF, CP, and TDN during three sampling periods throughout the trial are shown parenthetically in the legend of each graph.



**Figure 2.** Eating and resting behaviors by 900-lb Holstein dairy heifers at 100, 125, and 150% of stocking capacity in freestall housing (Experiment 3).



# Automated Calf Feeders: What Makes Them work?

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Individual housing of preweaned calves reduces transmission of infectious diseases as a result of limited physical contact between calves. In addition, individually housed calves are easier to observe which can result in more effective disease treatment. However, individual calf housing results in lack of social contact among calves at an early age and limits their movement. Housing calves in groups allows them to interact with each other and have space to move around and play. In addition, dairy producers are housing calves in groups to facilitate improved labor efficiency and working conditions and to make it easier to deliver higher amounts of milk/milk replacer to young calves.

Feeding calves in groups allows calves to express some natural behaviors that cannot be expressed when they are housed individually, but offers some challenges in relation to maintaining good health, another important aspect of good animal welfare. Good health is achievable in group housed preweaned calves as long as appropriate management and maintenance of equipment are emphasized and implemented.

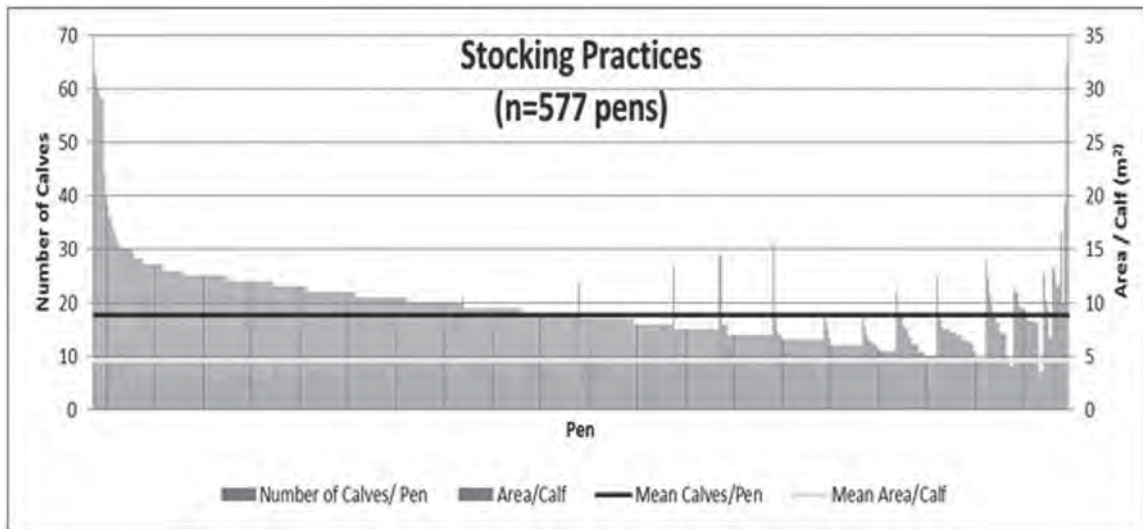
There has been consistent growth in the upper Midwest US on the number of farms installing automated computerized calf feeders. This paper summarizes some of the findings of a field study conducted recently at the University of Minnesota involving 38 farms with automated calf feeding systems. These types of longitudinal cross sectional studies can provide descriptive information on housing and management practices and by collecting many animal and facility measurements, we can identify factors that are associated with successful use of these systems. This methodology does not provide a direct 'cause and effect' connection, but we can identify guidelines and factors that are important and then further investigated by controlled research studies or experimented on the farm.

## Some management observations

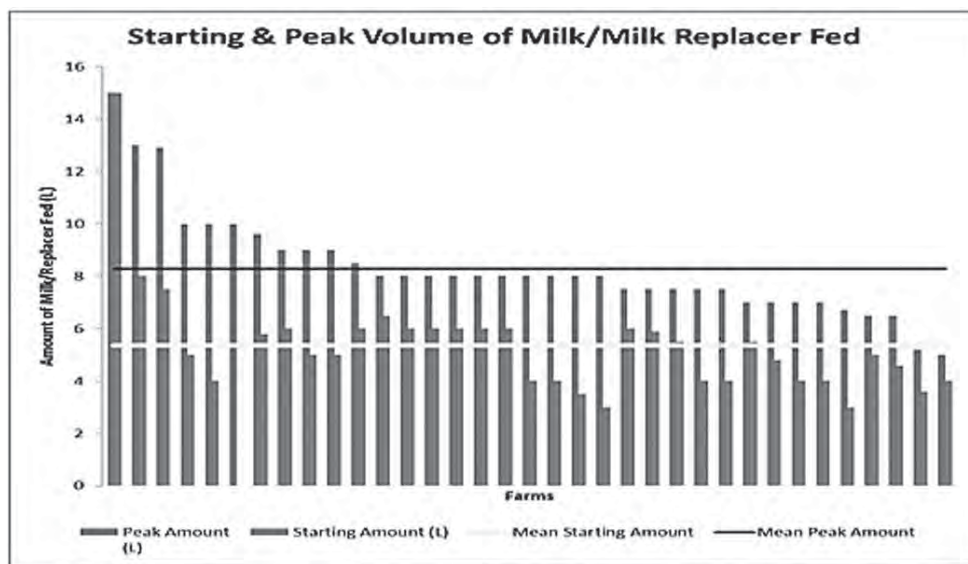
The following charts summarize some key practices used on the farms we visited. The average number of calves per pen (Figure 1) was approximately 17.6, which is less than the maximum suggested by the dealers (up to 30), and the space per calf was 4.6 square meters (~49 square feet). Average peak milk was 8.3 liters per day and start milk 5.4 liters per day (Figure 2). Calves were placed on the feeder at 5.2 days of age (range of 0 to 14 days; Figure 3); 10 farms placed calves in the group at 0 to 1 day of age. Most of the farms (87%) used positive pressure tubes to improve ventilation in the barn.

## Calf health

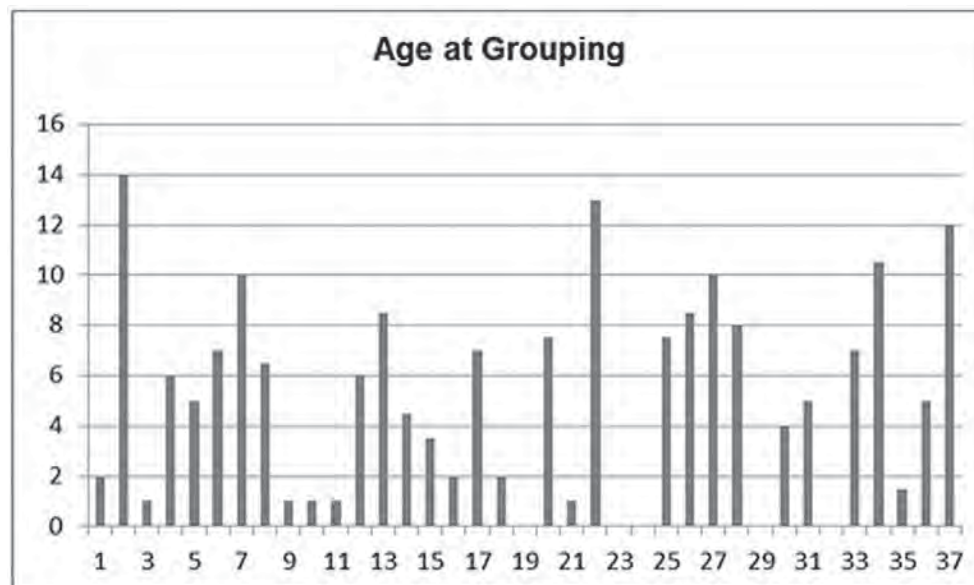
At each visit, the same trained observer scored calves for health in the youngest and oldest (plus a middle one in larger dairies) pens including attitude, eyes, ears, nose, cleanliness and body condition (n= 10,185 calves). Blood samples were collected from calves younger than 5 days of age to test for serum protein concentration as an indicator of passive immune transfer (n = 985 calves). Body temperature was measured if a calf had an abnormal health score. During five visits in different seasons, milk samples were collected from the mixer and the feeder tube to test for standard plate count (SPC) and coliform count. Figure 4 summarizes the calf health scores for the top 10th and the bottom 10th percentile farms. There was considerable variation among farms, indicating that housing and management factors can definitely influence the success of using these feeding systems. Table 1 summarizes the SPC and coliform counts for the top and bottom farms. Again, there is a lot of variation and some very extreme numbers were detected. The milk/milk replacer fed to preweaned calves should have a standard plate count of less than 100,000 CFU/ml.



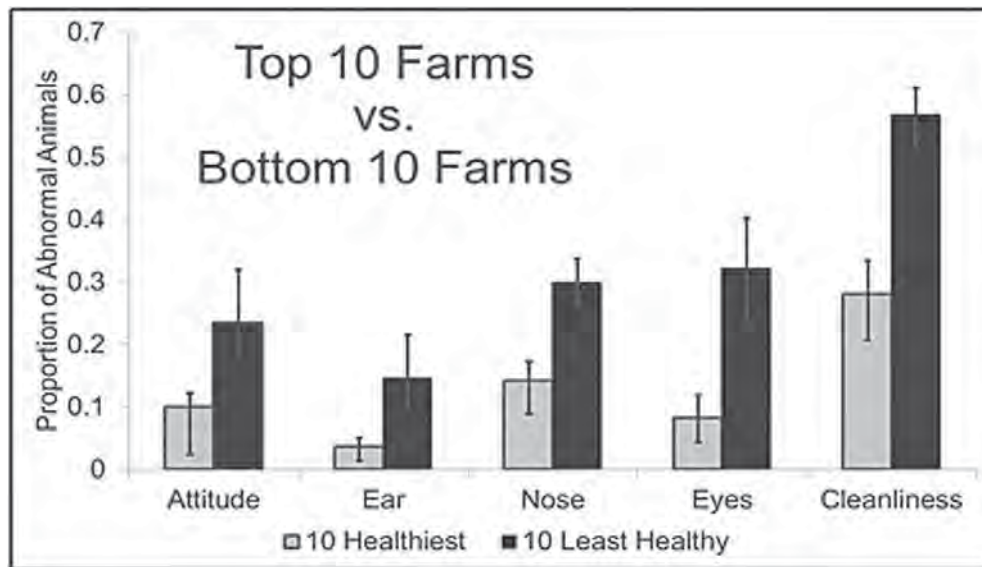
**Figure 1.** Stocking density as number of calves per pen and area per calf



**Figure 2.** Starting and peak amounts of milk/milk replacer fed



**Figure 3.** Age calves are introduced to group feeding



**Figure 4.** Average proportion of abnormal scores (indicating potential disease presence)

**Table 1.** Farm average bacterial counts (cfu/ml) across visits for top and bottom 10 farms

Item	Tube Coliform	Mixer Coliform	Tube SPC	Mixer SPC
Median of Top 10 (Q1-Q3)	887 (206-1,211)	12 (3-15)	87,590 (32,603-134,940)	9,006 (2,308-9,392)
Median of Bottom 10 (Q1-Q3)	5,659,567 (1,198,059-14,344,063)	522,263 (64,564-20,001,213)	21,140,625 (18,644,538-71,642,610)	10,209,920 (3,204,500-43,673,293)

### Risk factors for abnormal health scores

Our statistical analysis indicated that the following factors are positively associated with abnormal health scores:

- Number of calves per group – the greater the number, the more sick calves
- Space per calf – less space per calf associated with higher number of abnormal scores
- Time to reach peak milk allowance – sooner was better
- SPC on tube samples >100,000 cells/ml – higher counts were associated with higher number of abnormal health scores. Cleanliness is a key for success!

A preliminary analysis of factors associated with mortality rate showed significant relationships with serum total protein concentration (an indicator of passive immune transfer), use of drinking speed provided by the software as an alarm that a calf might be sick, performing navel and between group disinfection, age difference in calf groups and bacteria count in milk/milk replacer.

It was interesting to learn that some producers were not very clear about the need for cleaning the equipment on a routine basis, which resulted in a wide distribution for the quality of the milk/milk replacer fed to the calves across farms. It is extremely important to run circuit and mixer cleaning as recommended by the manufacturer (or more), replace hoses and nipples regularly (biweekly and daily, respectively), use the recommended cleaner to remove biofilms from the surfaces, keep the area around the feeder clean, provide clean and dry bedding to the calves, provide high quality milk, calibrate the equipment to deliver appropriate concentration of nutrients and temperature for the milk, etc.

Dietrich et al (2015) collected milk samples daily for four weeks before and after aut feeder circuit cleaning in 10 herds and showed that circuit cleaning reduced bacteria in milk. However, machines with more circuit cleanings per week had greater counts possibly because circuit cleaning may be loosening bacterial cells from biofilms. Authors recommended a combination of three times per day mixer/heat exchanger cleaning before major feeding times along



with once a day circuit cleaning after major feeding times to reduce bacterial counts in milk. Circuit cleaning involves hand cleaning of the nipple and machine cleaning of the lines and internal workings of the feeder which must be instituted by the operator. The mixer/heat exchange cleaning is automated and involved cleaning of the element used for heating milk if used and the mixer.

Suggestions for making automated calf feeders work Although more research and on farm observations are still needed, here are some general recommendations for using automated calf feeder systems:

- Excellent colostrum management programs are essential!
- Clean, dry, comfortable bedding and minimum of 40-45 square feet per calf.
- Milk/milk replacer with low bacterial count (less than 100,000 cells/ml).
- Adequate training of calves to use the feeders by gently leading them to the nipple when they are moved into the group housing.
- Stocking rates of no more than 12-15 calves per group, although research has shown that 7 to 8 calves per group is best for good health outcomes. A balance between health outcomes and economics needs to be considered. Larger group sizes are more successful when the age range among calves is narrow.
- Milk allowances range from 1.5 to 3.7 lb of milk solids per calf per day. On a volume basis this amounts to 5.5 to 12 L of liquid per day. Most farms offer 8 L per calf per day as peak amount and start with 4 to 6 L per day. Calves will easily drink 10 L per day.
- Meal sizes of 1.8 to 2.5 L each. Meal size recommendations for younger calves tend to be lower and increase to upper limits by 2 to 3 weeks of age. Calves typically consume their daily allocation in 4 to 6 meals per day.
- When milk replacer is used, powder is diluted with water to approximately 13 to 15% solids. It is important that the feeder is calibrated routinely and all parts kept clean so that powder flows properly and dilution is consistent.
- Cleaning of the equipment and its various components is one of the most important keys to making these systems work successfully. Change/clean nipples daily; change feeder hoses/tubes weekly as minimum.

## Conclusions

Automated calf feeders for raising young calves in groups are growing in popularity as producers want more flexible labor management and consumers want animals to have a more natural life. Feeding calves in groups allows calves to express some natural behaviors that cannot be expressed when housed individually, but offers some challenges in relation to maintaining good health, another important aspect of good animal welfare. Good health is achievable when using automated calf feeders to raise pre-weaned calves as long as appropriate management and maintenance of equipment are emphasized and implemented.

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## Reference

Dietrich, A. M., W. A. Knauer, S. A. Godden, C. S. Petersson-Wolfe and R. E. James. 2015. Factors associated with aerobic plate count, coliform count and log reduction of bacteria in automated calf feeders. *J. Dairy Sci.* 98, E-Suppl.2, 214.

# Forage Quality of Two Different Pasture Systems Incorporating Warm and Cool Season Forages for Grazing Organic Dairy Cattle

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Pasture is the primary source of forage for grazing dairies, and for organic dairies, the National Organic Program livestock production regulations require a minimum of 120 days grazing per animal. In the northern United States, this requirement is typically met by a May to October grazing season, and profitability depends on pastures that provide a uniform, season-long supply of high quality forage. However, in the northern United States, seasonal variation in temperature and precipitation creates a challenge, as the predominant forage plants, which include perennial grasses such as Kentucky bluegrass and smooth brome grass, and legumes such as white clover, undergo a “summer slump” in production. Most pastures in the upper Midwest consist of perennial cool season species. These grasses and legumes grow well in Midwestern soils and climate and are considered high quality forage options that provide adequate nutrition for grazing dairy cows. The decreased feed availability in pastures because of slower growth of these forages may lead to decreased milk production. In addition, farmers may have to feed stored forages, which can increase their feed costs. Incorporating warm season annual grasses into pasture systems has been suggested as a solution, as these grasses will experience their fastest growth rates at the time that cool season perennials may have delayed growth. Some farmers may be hesitant to implement this solution as it is generally believed that warm season annuals have lower forage quality than cool season perennials. To create a more uniform and extended forage supply, research studies have recommended diversifying pasture systems to include warm season species in the summer.

An approach to increasing diversity in a farm’s forage base is to combine annual and perennial crops in separate fields. An example for the northern United States, would be to use cool season grasses and legumes for forage in spring and early fall, and warm season annuals like teff and sudangrass for forage in summer. Grazing systems using these different approaches to achieve diversity require biological, environmental and economic analysis.

It is important for organic dairy farmers to establish good pasture management to be able to follow the pasture rule for organic cattle. Organic cattle must graze pasture for at least 120 days of the year and 30% of their dry matter intake must come from pasture forage. Milk production is directly related to dry matter intake, which is directly related to amount of available dry matter in pasture. For cattle grazing pasture to be productive, there must also be productive pastures that provide adequate forage quality and biomass to feed cattle.

## **Plan your forage supply for summer grazing.**

There are a lot of disagreements regarding the ideal number of species to include in pasture mixtures. Most agronomic guidelines recommend the use of a small number of species in grazed mixtures. Past research in the Northeast United States found that six to nine grass species were more productive than a white clover-orchardgrass mixture.

When selecting pasture grass species, producers should consider yield potential, palatability, survival of grasses. Producer should select species that are winter hardy, have good seasonal yield distribution, and are rust resistant. Quite possibly, variety is as important as or more important than specie choice.

At the University of Minnesota West Central Research and Outreach Center, in Morris, we are measuring the performance of dairy cows grazing two unique pasture systems designed to maximize seasonal forage yield and quality and extend the grazing season. System 1 will increase within-field species diversity targeting perennial cool season, polyculture pastures to enhance multi-seasonal productivity (spring, summer and fall). System 2 will increase across-landscape diversity achieved by adding a combination of perennial polycultures and annual warm season grasses fertilized with livestock manures. Regional differences in soil fertility and rainfall may favor different pasture species in other locations.

Our current perennial pasture species mixtures and seeding rates are as follows:

1. Perennial ryegrass (4 lb), White clover (2 lb), Red clover (3 lb), and Chicory (2 lb);
2. Orchardgrass (3 lb), Meadow Fescue (6 lb), Chicory (1 lb), Alfalfa (10 lb); and
3. Perennial ryegrass (3 lb), Meadow Fescue (8 lb), White clover (4 lb), Red clover (2 lb), and Chicory (1 lb)

### **Warm-Season Summer Annual Grasses**

Why should summer annuals be considered by livestock producers? They are very drought tolerant and can fill a gap in feed when other species experience the “summer slump”. They are great emergency forages during dry weather and are multipurpose, so you can use them for grazing, silage, or for baling.

### **Sorghum-Sudangrass and Teff Grass**

During the summer for three grazing systems (2013 to 2015), we planted two summer annuals for grazing at the University of Minnesota WCROC dairy in Morris. BMR Sorghum-Sudangrass and Teff grass were planted to extend our forage supply. These grasses were seeded with a drill the third week of May each year.

BMR Sorghum-Sudangrass has increased in popularity due to the BMR gene and increased NDF digestibility (5-10% higher than regular sorghum-sudangrass). The plants have thick stems and are very leafy. Sorghum-sudangrass has moderate regrowth potential, but you should not graze or cut for forage until the plants are at least 18 inches tall to reduce prussic acid concentration. The ideal height for forage is 18 to 36 inches tall. When grazing sorghum-sudangrass animals should be moved so they leave 6 to 8 inches of stubble, but they might waste 20-30% of the forage through grazing. Lastly, sorghums and sudangrass are consumers of potassium, so they should not be used for dry cow forages. For seeding rate, we seeded our fields and pastures at 20 lbs/acre.

BMR sorghum sudangrass has been fed as silage to dairy cattle. Nutrition studies have been conducted in dairy cattle comparing sorghum sudangrass silage to corn silage, showing similar production. It is typically not grazed in a pasture system, so very little is known about sorghum sudangrass as pasture forage, and how it may affect grazing dairy cattle.

Teff grass is native to Northern Africa. Teff is drought tolerant and can be seeded into many different soil types. With this grass, you will have high yield with

competitive forage quality, and will have rapid growth for 9 to 12 weeks. The seed is very, very small, and we seeded our pastures at 8 lbs/acre. Both of these annuals should be planted at 60 to 65-degree soil temperature and planted 1 to 1.5 inches deep. Perhaps, manure should be added as a fertilizer before planting because they have nitrogen requirements that are similar to corn.

Teff grass originated in Ethiopia and is extremely drought and heat tolerant. It has occasionally been used by some rangeland cattle producers as emergency forage but is usually fed as hay. Very little is known about the forage quality of teff grass, especially in a grazing system.

### **University of Minnesota Grazing Study**

The University of Minnesota chose to study BMR sorghum sudangrass and teff grass, as organic dairy farmers in Minnesota are beginning to incorporate these grasses in their grazing programs and are interested in learning more about them. We wanted to determine how the forage quality of annual warm season grasses compare to perennial cool season pasture mixtures, as well as how they influence milk production and health parameters in grazing organic dairy cows.

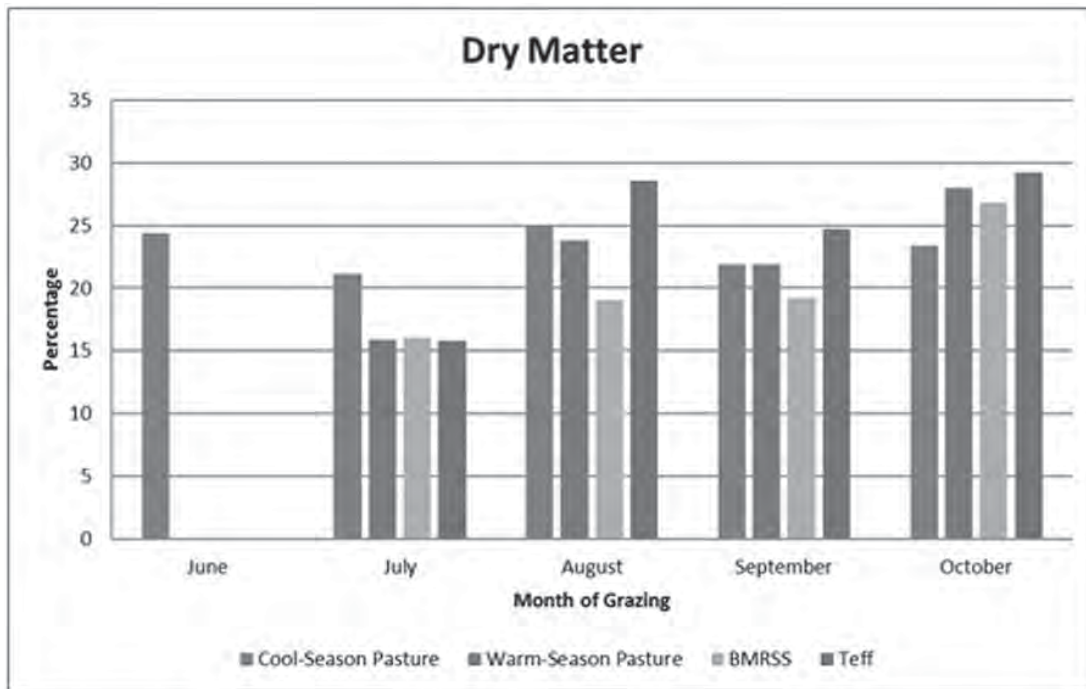
For our study, ninety organic dairy cows were used in a study to compare two different pasture systems at the West Central Research and Outreach Center in Morris, MN. The first system (cool system) included a diverse mix of cool season perennial grasses and legumes such as perennial ryegrass, white clover, red clover, chicory, meadow bromegrass, orchardgrass, meadow fescue, and alfalfa. The second pasture system (warm system) was a combination of the cool season perennial mixtures and warm season annuals BMR sorghum sudangrass and teff grass. Perennial pastures were established in 2012. Warm season annuals BMR sorghum sudangrass and teff grass were planted in individual paddocks during the third week of May of each year. Forage samples were collected daily throughout the grazing seasons of 2013-2015. Dry matter was analyzed immediately after sample collection. Forage samples were tested at Rock River Labs in Watertown, WI for the forage quality characteristics neutral detergent fiber (NDF), total tract NDF digestibility (TTNDFD), crude protein (CP), and mineral content.

Holstein and crossbred dairy cows were blocked by breed, parity, days in milk, and randomly assigned to one of two systems. Cows were moved to a new paddock every two days, were supplemented 5 lb. of corn per day, and provided with free-choice mineral

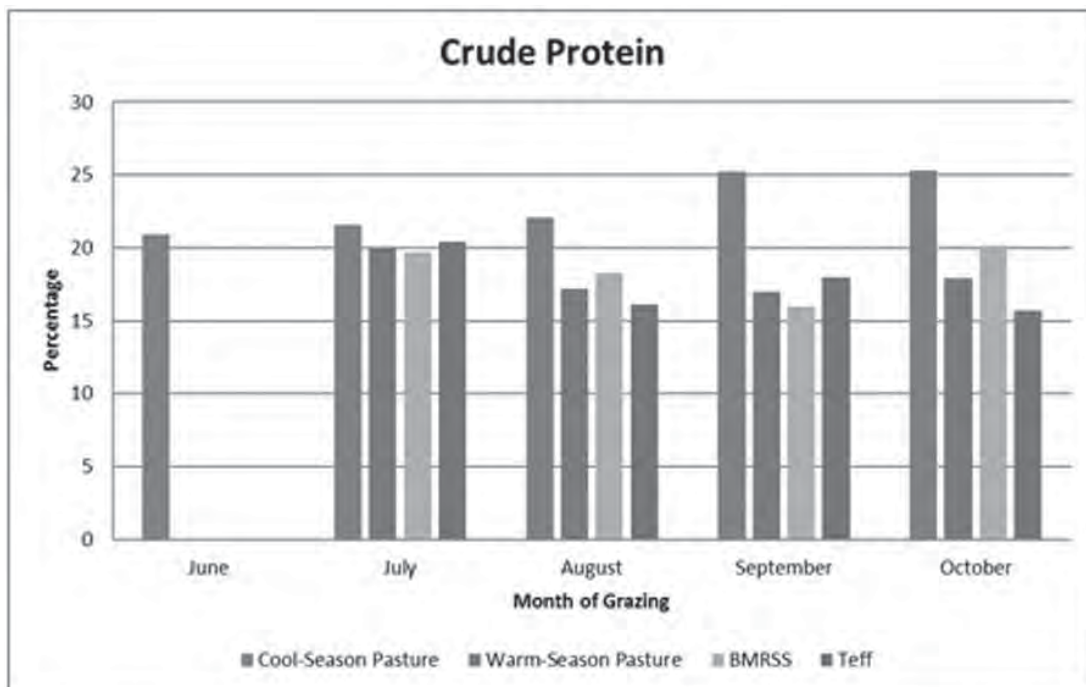
in pasture. Milk production data was collected daily. Fat, protein, MUN, and SCC were from monthly DHI testing. Body weight was recorded on cows using a digital scale as cows exited the milking parlor approximately once every 2 weeks during lactations, and BCS was measured at the same time as BW on a 1 to 5 scale in increments of 0.25, with 1 = excessively thin, and 5 = excessively fat. Cows were also fitted with SCR Heattime HR-LD Tags to monitor daily rumination and activity across the grazing season.

Across the grazing season, spring pasture dry matter fluctuated across the grazing season and was higher during August and October compared to the early part of the grazing season (June and July; Figure 1). Seasonal average crude protein concentrations were greater for the perennial pastures in the fall; however, the warm season grasses were greater for crude protein during July at the time of first grazing (Figure 2).

**Figure 1.** Dry matter of pasture grass species across the grazing season



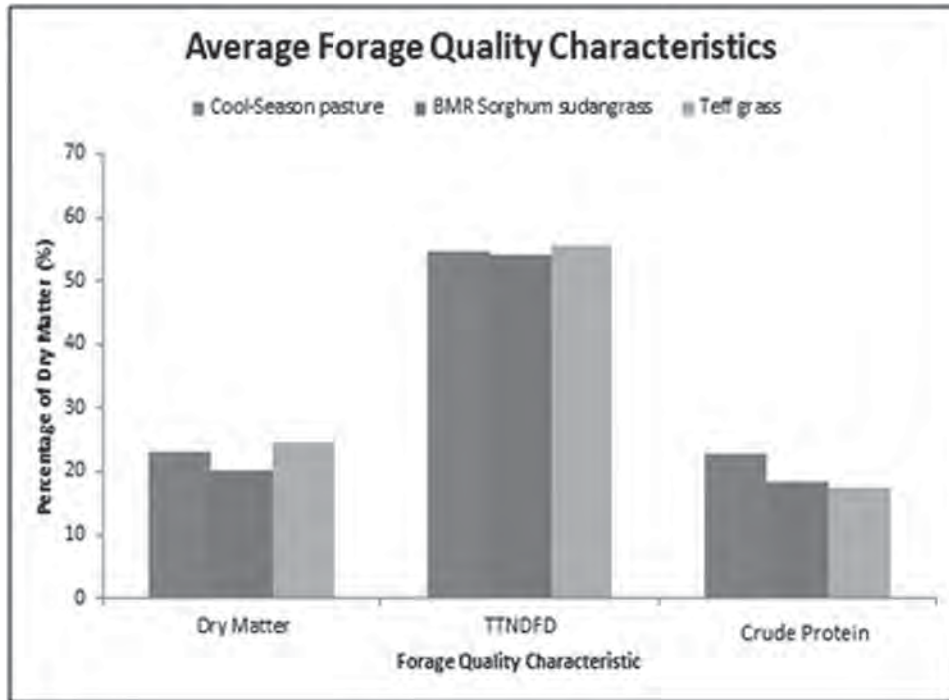
**Figure 2.** Crude protein of pasture grass species across the grazing season



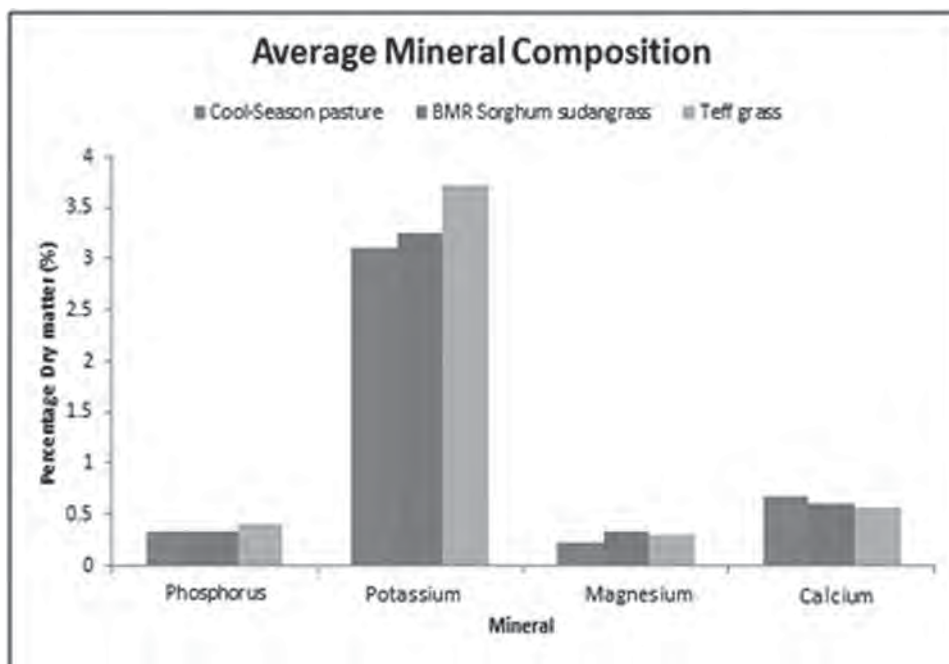
Forage quality was similar between cool season perennial pasture grasses and the warm season species evaluated in this study (Figure 3). Cool season pasture had higher average crude protein (23.0%) than the warm season grasses, but BMR sorghum sudangrass and teff grass still had adequate levels of protein for lactating cow diets (18.5 and 17.5%, respectively). Dry matter was higher in cool season pasture

(23%) and teff grass (24%) than BMR sorghum sudangrass (20%). TTNDFD was similar between all types of forage. The mineral composition varied between the different grasses (Figure 4).

**Figure 3.** Forage quality of pasture grass species



**Figure 4.** Mineral composition of pasture grass species



There were no differences in milk production, components or quality between cows grazing only cool season pastures and cows in a system that incorporated warm season annuals. Average milk production was 32.3 lb for the cool system and 32.5 lb for the warm system. There was also no difference in body condition score, body weight, or activity between systems. Cows on cool season grasses did have higher daily rumination than cows in the warm season system. Cows in both systems follow similar trends in production including decreased production during times of high temperature and humidity. In 2015, cows in the warm system achieved higher production than cows in the cool system during July and August.

**Figure 5:** Milk production of cows in cool system and warm system across 2014 and 2015 grazing seasons



In the first year of the study, cows in the cool season system needed to be supplemented with stored feed in a TMR due to a shortage of forage biomass in pasture, while cows in the system incorporating warm season grasses were still able to graze. The following year there were no difference between pasture systems. Therefore, warm season annuals in grazing systems for dairy cattle may be beneficial in certain years to compensate for weather that affects pasture production.

Warm season grasses like BMR sorghum sudangrass and teff grass may be incorporated into a pasture system for grazing organic dairy cattle without sacrificing forage quality. Milk quality and production can also be maintained when warm season grasses are incorporated in a grazing system for organic dairy cattle. This study will be repeated for a third year to evaluate the economics of including warm season annuals in a pasture system compared to a system that uses only cool season perennials for organic dairy grazing operations. A continuation of this study is currently being conducted using a dual flow continuous culture fermenter, and results will include digestibility of the grasses used in this study.

## Conclusions

Grazing systems using these different approaches to achieve diversity require biological, environmental and economic analysis. Pasture management and forage species selection within a farm can influence the forage quality of pasture forage for grazing dairy animals.

BMR sorghum-sudangrass and teff grass can be used in rotational grazing systems in the Midwest without sacrificing forage quality or milk production. Remember, sorghum-sudangrass and teff grass are not replacements for cool-season forages, but they should be added to a forage program to complement the cool-season grasses.

## Acknowledgments

The authors express gratitude to Darin Huot and co-workers at the Morris (Minnesota) organic dairy facility for their assistance in data collection and care of animals. This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2012-51300-20015, "Strategies to Improve Profitability of Organic Dairy Herds in the Upper Midwest".

# Impact of Feeding Amino Acids on Reproduction

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## TAKE HOME MESSAGE

- Rumen-protected methionine (RPM) added to the diet of Holstein cows improves the survival rate of preimplantation embryos.
- Cows fed methionine have more lipid droplets inside the preimplantation embryo, which could be used as energy by the embryos.
- Embryonic death has been shown to drop from 19 percent to 6 percent in cows fed methionine.

## INTRODUCTION

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

Strategies have been used to improve the reproductive performance of dairy cows through alteration of nutritional status (Santos et al., 2008a; Santos et al., 2001). In other species, dietary supplementation with specific AAs (e.g., arginine, glutamine, leucine, glycine, and methionine) had beneficial effects on embryonic and fetal survival and growth through regulation of key signaling and metabolic pathways (Del Curto et al., 2013; Wang et al., 2012). Methionine is the most limiting AA in lactating cows (NRC, 2001), but supplementation of diets with crystalline methionine has been excluded because free methionine is quickly and almost totally degraded by the

microorganisms in the rumen (NRC, 2001). In contrast, supplementing rumen-protected methionine (RPM) has a positive effect on milk protein synthesis in dairy cows (Pisulewski et al., 1996; Ordway, 2009; Osorio et al., 2013). Although the role of methionine in bovine embryonic development is unknown, there is evidence that methionine availability alters the transcriptome of bovine preimplantation embryos in vivo (Penagaricano et al., 2013) and its contents (Acosta et al., 2016).

The DNA methylation in promoters is an important mechanism for regulation of gene expression and gene silencing. However, DNA methylation in other regions may have a more complex role in regulation of transcription (Bird and Wolfe, 1999; Van de Veyver, 2002; Suzuki and Bird, 2008). Methylation of the DNA depends on the availability of methyl donors supplied by AAs such as methionine and by compounds of one-carbon metabolic pathways such as choline (Van de Veyver, 2002). Increased methionine bioavailability is likely to increase the entry of methionine into the one-carbon metabolism cycle where it is initially converted into S-adenosylmethionine, the major biological methyl donor (Martinov et al., 2010). Nonruminants fed diets deficient in methyl donors (e.g., choline and methionine) have hypomethylated DNA (Locker et al., 1986; Tsujiuchi et al., 1999). These changes occur not only in global methylation (Wilson et al., 1984) but also in the methylation of specific genes (Bhave et al., 1988). However, effects of methionine in preimplantation embryos are still controversial. Bonilla et al. (2010) suggested that extracellular methionine is not required for DNA methylation in the cultured blastocyst. Nevertheless, gene expression changes caused by alteration of DNA methylation (i.e., absence of the methylase genes) can result in embryo death or developmental defects in preimplantation embryos (Reik et al., 2001).

## REPRODUCTION AND NUTRITION

Nutrient demands for milk synthesis are increased in early lactation, and if no compensatory intake of nutrients is achieved to cope with milk production requirements, reproductive functions (i.e., synthesis and secretion of hormones, follicle ovulation, and embryo development) may be depressed. The inci-

dence of diseases and disorders can be high during the periparturient period and have a negative impact on reproductive performance. The risk of pregnancy was reduced if cows lost more than one body condition score (BCS) unit (Butler, 2003; Butler 2005; Santos et al., 2008b). Milk production increases faster than energy intake in the first 4 to 6 weeks after calving. High yielding cows will experience negative energy balance (NEB) and blood concentrations of non-esterified fatty acids (NEFA) increase, and concentrations of insulin-like growth factor-I (IGF-I), glucose, and insulin are low. If extreme, these changes in blood metabolites and hormones may compromise ovarian function and fertility (Butler, 2005).

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

## THE IMPORTANCE OF AMINO ACIDS

Some AA are limiting for optimal milk production as evidenced by an increase in milk yield, percentage of milk protein, and milk protein yield after supplementation with specific, rumen-protected amino acids. The first three limiting amino acids for milk production are considered to be Methionine, Lysine (NRC, 2001), and Histidine (Hutannen, 2002). In addition, many amino acids can have positive effects on physiological processes that are independent of their effects on synthesis of proteins (Wu, 2013). Fertilization and the first few days of embryo development occur in the oviduct. By about 5 days after estrus the embryo arrives in the uterine horn. The embryo reaches the blastocyst stage by 6 to 7 days after estrus. The embryo hatches from the zona pellucida by about Day 9 after estrus and then elongates on Days 14-19. The elongating embryo secretes the protein interferon-tau that is essential for rescue of the corpus luteum and continuation of the pregnancy. By Day 25-28 the embryo attaches to the caruncles of the uterus and begins to establish a vascular relationship with the dam through the placenta. During all the time prior to embryo attachment, the embryo is free-floating and is dependent upon uterine secretions for energy and the building blocks for development, including amino acids. Thus, it is critical to understand the changes in amino acid concentrations in the uterus that accompany these different stages of embryo development.

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

Hugentobler et al. (2010) summarized the concentrations of amino acids in plasma (average of days 0, 2, 3, 4 and 6 of estrous cycle), in the oviduct of cross-bred beef heifers, and in the uterus (average days 6, 8, and 14 of estrous cycle). There was no effect of day of the cycle on oviductal concentrations of amino acids. Nine of the 20 amino acids were present at significantly greater concentrations in the oviduct than plasma indicating that mechanisms are present in the cells of the oviduct that allow concentration of amino acids. The uterus also had greater concentrations of many amino acids than found in plasma from cows on the same days of the estrous cycle. The amino acids that were most elevated in uterus, Asp, Asn, Glu, were mostly similar to the oviduct.

In addition to the mechanisms that concentrate amino acids in the uterus in non-pregnant ruminants, there are additional mechanisms that result in further increases in concentrations of amino acids in the uterine lumen in pregnant ruminants near the time of embryo elongation (day 14-18). Three studies have provided amino acid concentrations near the time of embryo elongation; two in sheep (Gao et al., 2009) and one in cattle (Groebner et al., 2011). Although there seems to be very little change in amino acid concentrations between Day 10 and 16 in non-pregnant sheep, there are large increases from 3 to 23-fold in specific amino acids in the uterine lumen of pregnant sheep (Gao et al., 2009). In order to provide



some idea of changes in uterine amino acids during early pregnancy, Wiltbank et al. (2014) combined the results from these 3 studies into a fold increase in amino acids during the time of embryo elongation. There is an increase in almost all amino acids at the time of embryo elongation. Of particular interest for dairy cattle, the three amino acids that are considered limiting for milk production, Met, His, and Lys, are the amino acids with the greatest increase in concentrations in the uterine lumen during embryo elongation (> 10-fold increase on average from these three studies). Disturbances in the temporal relationship between uterine blood flow, induction of uterine amino acid transport, uterine amino acid concentrations, embryonic growth, embryonic interferon-tau production, and rescue/regression of the corpus luteum may reduce fertility and increase pregnancy losses.

### **EFFECT OF METHIONINE ON EMBRYO DEVELOPMENT.**

One particularly interesting study (Coelho et al., 1989) used serum from lactating dairy cows in the media to grow head-fold stage rat embryos (day 9.5 after breeding). Complete development of these embryos requires serum and development is normal in rat serum. When embryos are grown in serum from dairy cows embryonic development is abnormal when measured as total embryo protein, somite pairs, or percentage of the embryos that are abnormal (no neural tube closure, abnormal shape, no development of eyes and branchial arches). Supplementation of bovine serum with amino acids and vitamins produced normal development. Amino acid supplementation alone but not vitamin supplementation produced normal development. Use of serum from cows that were supplemented with rumen-protected methionine also produced normal embryo development. Thus, bovine serum has such low methionine concentrations that normal development of rat embryos is retarded.

The requirements for complete development of bovine embryos have not yet been determined. Current culture conditions allow development of bovine embryos to the blastocyst stage (day 7-8) and even allow hatching of a percentage of embryos (day 9), however conditions have not been developed in vitro that allow elongation of embryos. The methionine requirements for cultured pre-implantation bovine embryos (day 7-8) was determined in studies from University of Florida (Bonilla et al., 2010). There was a surprisingly low methionine requirement (7  $\mu\text{M}$ ) for development of embryos to the blastocyst stage by Day 7, however development to the advanced blastocyst stage by day 7 appeared to be optimized at around 21  $\mu\text{M}$  (Bonilla et al., 2010). Thus, the results

of these studies indicated that development of morphologically normal bovine embryos did not require elevated methionine concentrations (>21  $\mu\text{M}$ ), at least during the first week after fertilization.

Ikeda et al. (2012) evaluated whether methionine metabolism was required for normal development of bovine embryos. The researchers added ethionine or additional methionine to cultures of bovine embryos. Ethionine blocks metabolism of methionine into the one-carbon pathway (termed antimetabolite of methionine). Ethionine did not block development to the morula stage but blocked development to the blastocyst stage (Control = 38.5%; Ethionine = 1.5%). Development to the blastocyst stage in the presence of ethionine was partially restored by adding S-adenosylmethionine (SAM) which would restore the methylation pathway but not restore protein synthesis. Thus, methionine has an essential role in the development of the bovine embryo from morula to blastocyst that is probably partially mediated by hypomethylation in the absence of sufficient methionine.

Souza et al. (2012a,b) evaluated the effect of supplementation with rumen-protected methionine on early embryo development in super-ovulated cows. Super ovulation increased the number of embryos available and thus the statistical power to test the in vivo effects of methionine supplementation on early embryo development in lactating dairy cows. In this experiment, animals were blocked by parity and calving date and randomly assigned to two treatments differing in level of dietary methionine supplementation: 1) Methionine (MET); diet composed of (%DM) corn silage (39.7), alfalfa silage (21.8), HMSC (17.2), roasted soybeans (8.6), grass hay (4.6), canola meal (4.0), mineral-vitamin mix (2.7) and ProVAAL Ultra (w/Smartamine<sup>®</sup>, 1.4), formulated to deliver 2875 g MP with 6.8 Lys %MP and 2.43 Met %MP; 2) Control (CON); cows fed the same basal diet but replacing ProVAAL Ultra by ProVAAL Advantage (no added Smartamine<sup>®</sup>), formulated to deliver 2875 gr MP with 6.8 Lys %MP and 1.89 Met %MP. There was an increase in both kg of milk protein produced and percentage of protein in the milk (Souza et al., 2012b). Thus, from a milk protein synthesis standpoint, methionine was concluded to be the first limiting amino acid. A large significant effect of feeding the rumen-protected methionine on circulating methionine concentrations (Control = 16.8  $\mu\text{M}$  vs. Met-supplemented = 22.9  $\mu\text{M}$ ) was observed.

Even though methionine supplementation during the later stages of follicle development and early embryo development may not have produced morphological changes in the early embryo, it is well known that methionine during this time can have effects on the

epigenome of the embryo (Sinclair et al., 2007). This means that the genes can be changed in such a way that they are not expressed in the same way due to addition of groups, generally methyl groups to the DNA of the cells. To test this hypothesis, Penagari-cano et al. (2013), evaluated whether the embryos that were recovered from cows that had been sup-plemented or not supplemented with methionine had differences in gene expression. The objective was to evaluate the effect of maternal methionine supplementation on the transcriptome of bovine pre-implantation embryos. Only high quality embryos from individual cows were pooled and then analyzed by a powerful technique that allows evaluation of all genes that are expressed in these embryos, called RNA sequencing. Remarkably, the small difference in circulating methionine produced a substantial difference in expression of genes in the embryo. Methionine supplementation seemed to change gene expression in a way that may lead to improved pregnancy outcomes and improved physiology of the offspring.

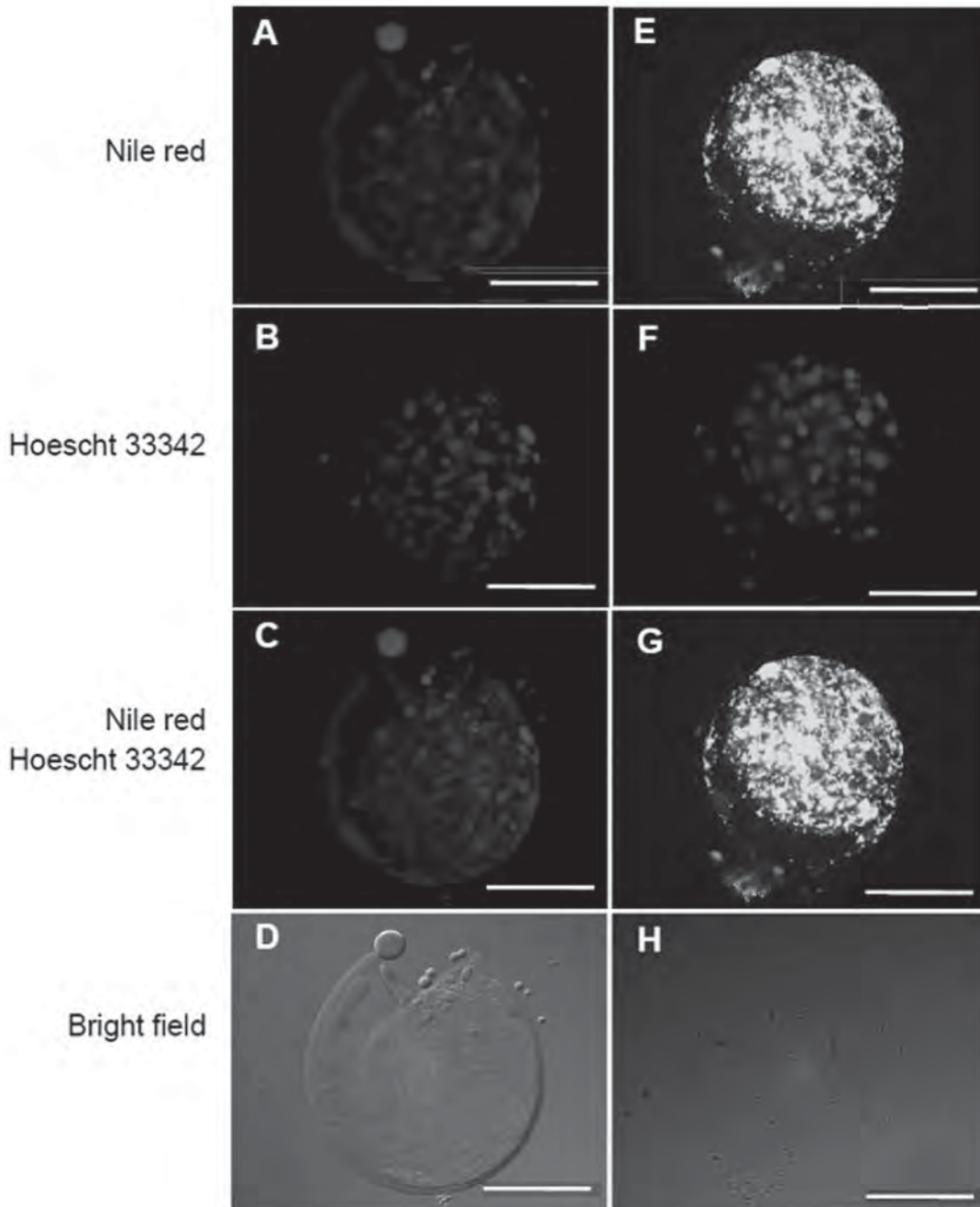
Researchers from the same laboratory at the Univ. of Wisconsin conducted a trial with a total of 309 cows (138 primiparous and 171 multiparous) that were blocked by parity and randomly assigned to two treatments; 1) CON: Cows fed a ration formulated to deliver 2500 g of MP with 6.9% Lys (% MP) and 1.9 Met (% MP) and 2) RPM: Cows fed a ration formulat-ed to deliver 2500 g of MP with 6.9% Lys % MP) and 2.3 & Met (% MP). Cows were randomly assigned to three pens with head-locks and fed a single basal TMR twice daily. From 28 to 128 DIM, after the AM milking, cows were head-locked for 30 minutes and the TMR of CON and RPM cows were individually top dressed with 50 g of DDG or 50 g of a mix of DDG (29 g) and Smartamine M(21 g) respectively. Following a double ovsynch protocol, cows were inseminated and pregnancy checked at 28 (plasma Pregnancy Spe-cific Protein-B concentration), and at 32, 47 and 61 d (ultrasound). Individual milk samples were taken once a month and analyzed for composition. There were no statistical differences in milk production, but RPM cows had a higher milk protein concentra-tion. Cows fed the methionine enriched diet had a lower pregnancy loss from 21 to 61 after AI (16.7 % RPM cows vs. 10.0% from CON cows). Pregnancy losses between days 28 and 61 were not different in the primiparous cows (12/8% CON and 14.6% RPM), however, pregnancy losses between treatments were significant for the multiparous cows (19.6% CON vs. 6.1% RPM; Figure 2; Toledo et al., 2015).

## CONCLUSIONS

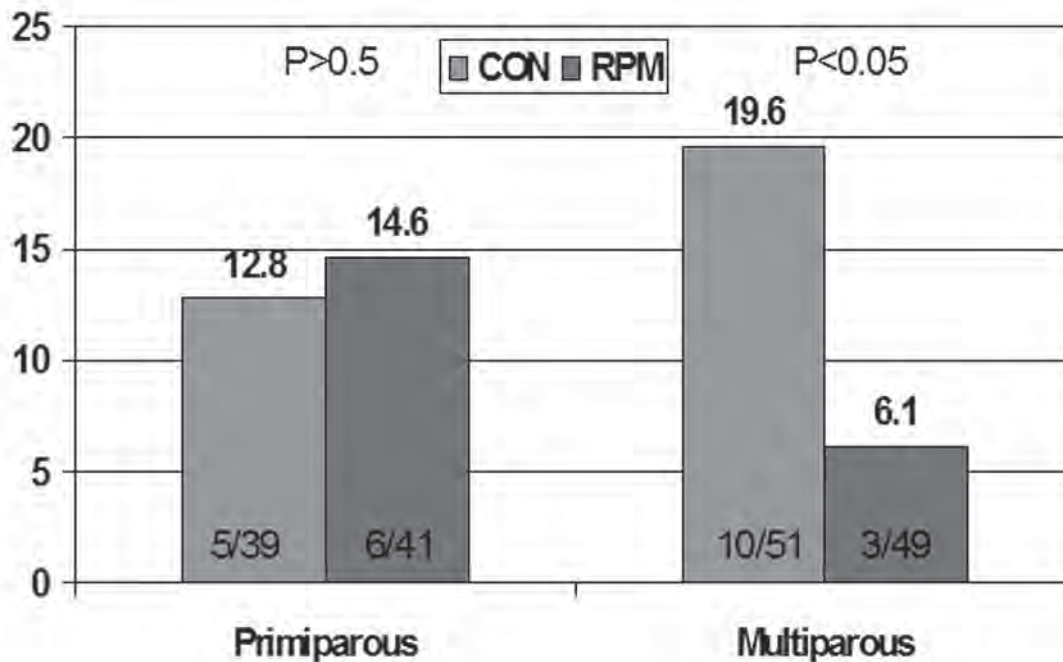
The elevated concentration of the amino acids, Met, His, and Lys, in the uterine fluid of pregnant cows near the time of embryo elongation suggests that elevated amounts of these amino acids may be criti-cal for this important stage of embryo development. Supplementation of cows with methionine during the final stages of follicular development and early embryo development, until Day 7 after breeding, lead to lipid accumulation changes in the embryos and resulted in differences in gene expression in the em-bryo. Methionine supplementation seems to impact the preimplantation embryo in a way that enhances its capacity for survival because there is strong evidence that endogenous lipid reserves serve as an energy substrate. The lower pregnancy losses from cows fed a methionine enriched diets suggest that methionine favors the embryo survival, at least in multiparous cows. Further studies are needed to cor-roborate whether supplementation with methionine would have a beneficial impact on embryo survival and if these changes in the early embryo translate into changes in pregnancy outcomes or physiology of the resulting calf.

## FIGURES

**Figure 1.** Nile red labeling for analysis of lipid content in embryos produced in vivo from cows fed methionine (SMT, fed the basal diet plus methionine; E–H) or a control diet (CNT, fed a basal diet) after 30 days in milk (A–D; magnification:  $\times 40$ ; scale bars = 100  $\mu\text{m}$ ). Note that the labeling intensity in (A) is higher than (E). (A) and (E), Nile red labeling; (B) and (F), Hoescht 33342 labeling (nuclear stain); (C) and (G), merged image of Nile red and nuclear labeling; (D) and (H), bright field image



**Figure 2:** Pregnancy losses between days 21 and 61 after timed AI of primiparous and multiparous cows fed a control diet (CON) or a methionine enriched diet (RPM)



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# Fertility Programs to Achieve High 21-d Pregnancy Rates in High-Producing Dairy Herds

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## Introduction

Hormonal synchronization protocols have been incorporated widely into reproductive management programs by dairy farmers (Caraviello et al., 2006; Norman et al., 2009). The initial impact of TAI protocols on 21-day pregnancy rates in U.S. dairy herds has been to increase the AI service rate (Norman et al., 2009); however, a deeper understanding of the physiology underlying the Ovsynch protocol has allowed for a dramatic increase in fertility to timed artificial insemination (TAI). As the title of this paper suggests, perhaps it is now more appropriate to refer to the latest iteration of hormonal synchronization protocols as fertility programs for lactating dairy cows.

Progesterone (**P4**) is the most biologically active progestogen in cattle and is primarily produced and secreted into circulation by the corpus luteum (**CL**) during the estrous cycle and the placenta during pregnancy. Much of the recent research published in the scientific literature has focused on the role of P4 during an Ovsynch protocol (Figure 1) or at various time points during an Ovsynch protocol on fertility as measured by pregnancies per artificial insemination (**P/AI**) 32 days after TAI. For the purposes of this review, the initial GnRH treatment of an Ovsynch protocol to which TAI occurs will be referred to as **G1** and the final GnRH treatment of an Ovsynch protocol immediately preceding TAI will be referred to as **G2** (Figure 1).



**Figure 1.** Schematic diagram of an Ovsynch protocol. G1 = first GnRH treatment; PGF = prostaglandin F<sub>2α</sub> treatment; G2 = last GnRH treatment; TAI = timed artificial insemination.

## Effect of Progesterone at G1 and PGF on Fertility to Timed AI

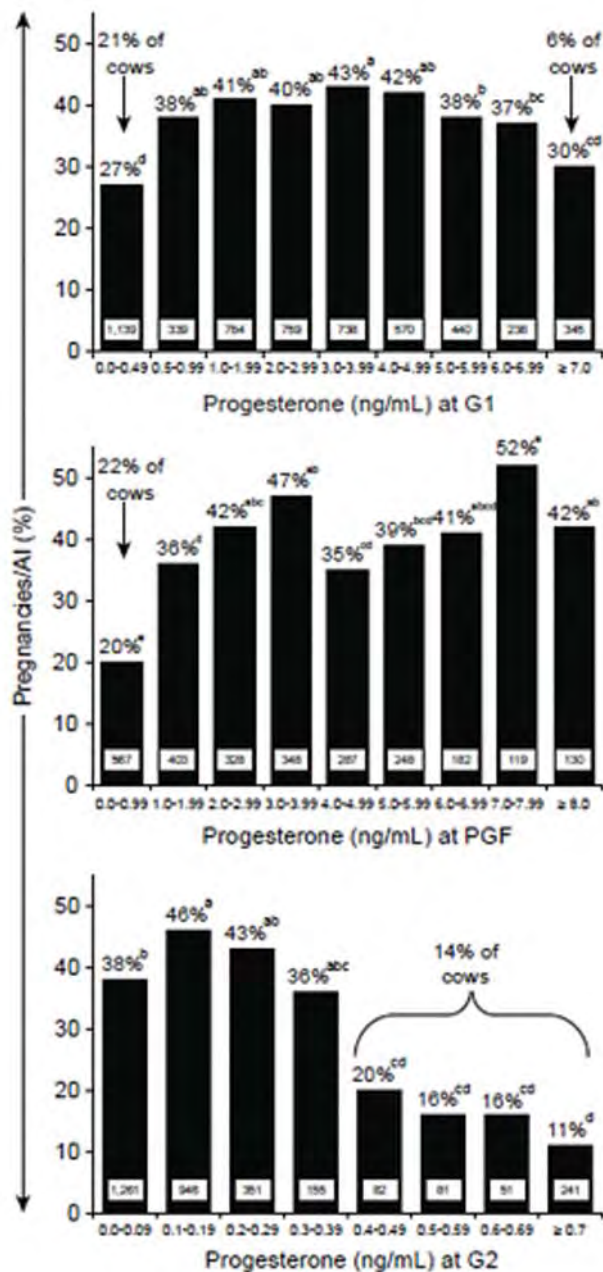
To assess the association between P4 concentrations at each treatment of an Ovsynch protocol and P/AI to TAI in lactating Holstein cows, we analyzed data from 7,792 cows from 14 experiments in which P4 was measured at the three hormonal treatments during an Ovsynch protocol (Figure 2; Carvalho et al., 2015b). The association between P4 during the Ovsynch protocol and P/AI to TAI was analyzed independently because P4 was not measured for all cows at all hormonal treatments during the Ovsynch protocol in all experiments.

At G1, cows (n = 6,144) were stratified into 9 P4 categories from 0 to ≥ 7 ng/mL using 0.5 ng/mL increments (Figure 2, upper panel). Overall, P/AI differed (P < 0.01) among P4 categories at G1 with fewer P/AI for cows with P4 < 0.5 ng/mL or P4 > 7.0 ng/mL than for cows with intermediate P4. At the PGF<sub>2α</sub> treatment, cows (n = 3,383) were stratified into 9 P4 categories from 0 to ≥ 8 ng/mL using 1.0 ng/mL increments (Figure 2, middle panel). Overall, P/AI differed (P < 0.01) among P4 categories at PGF<sub>2α</sub> with a 51% relative decrease in P/AI for cows with P4 < 1.0 ng/mL than for cows with P4 > 1.0 ng/mL. Based on this large dataset, suboptimal P4 concentrations could be identified at G1 in 26% of cows (26% lower P/AI) and at the PGF<sub>2α</sub> treatment in 21% of cows (51% lower P/AI).

Presynchronization strategies before initiation of an Ovsynch protocol at first TAI or Resynch TAI can optimize P4 at G1 and PGF<sub>2α</sub> in most cows resulting in more P/AI than for cows submitted to an Ovsynch protocol with no presynchronization. Presynchronization strategies tested thus far have used one PGF<sub>2α</sub> treatment administered 10 days (Cartmill et al., 2001) or 14 days (Silva et al., 2007; Bruno et al., 2013) before initiation of an Ovsynch protocol two PGF<sub>2α</sub> treatments administered 14 days apart with the second treatment administered 10 to 14 days before initiation of an Ovsynch protocol (i.e., Presynch

Ovsynch; Moreira et al., 2001; El-Zarkouny et al., 2004; Navanukraw et al., 2004; Galvão et al., 2007), a single GnRH treatment 7 days before Ovsynch (i.e., GGPG; Giordano et al., 2012b; Lopes Jr et al., 2013; Bruno et al., 2014; Carvalho et al., 2014a), a combination of GnRH and PGF<sub>2α</sub> 6 to 7 days before initiation of an Ovsynch protocol (i.e., G6G, Double-Ovsynch, and PG-3-G; Bello et al., 2006; Souza et al., 2008; Stevenson and Pulley, 2012). Independent of the presynchronization strategy tested, there was an increase in P/AI when P4 concentrations were increased at the time of the PGF<sub>2α</sub> treatment of the Ovsynch protocol (Bello et al., 2006; Bisinotto et al., 2010; Denicol et al., 2012; Stevenson et al., 2012; Martins et al., 2011).

Figure 2



**Figure 2.** Effect of progesterone at each treatment of an Ovsynch protocol on pregnancies per AI in lactating Holstein cows. At G1, concentrations of progesterone in 6,144 cows were stratified into nine P4 categories from 0 to  $\geq 7$  ng/mL using 0.5 ng/mL increments (upper panel). At the PGF<sub>2α</sub> treatment, concentrations of progesterone in 3,383 cows were stratified into nine P4 categories from 0 to  $\geq 8$  ng/mL using 1.0 ng/mL increments (middle panel). At G2, concentrations of progesterone in 3,148 cows were stratified into eight P4 categories from 0 to  $\geq 0.7$  ng/mL using 0.1 ng/mL increments (lower panel). Numbers within bars denote number of cows in each progesterone category. Adapted from Carvalho et al. (2015b).

### Effect of Progesterone at G2 on Fertility to Timed AI

Based on our analysis of cows from 14 different studies in which P4 was measured at the various treatments during an Ovsynch protocol (Figure 2; Carvalho et al., 2015b), a critical factor associated with P/AI to TAI is P4 at G2. At G2, cows ( $n = 3,148$ ) were stratified into 8 P4 categories from 0 to  $\geq 0.7$  ng/mL using 0.1 ng/mL increments (Figure 2, lower panel). Overall, P/AI differed ( $P < 0.01$ ) among P4 categories at G2 with a 66% relative decrease in P/AI for cows with P4  $> 0.4$  ng/mL than for cows with P4  $< 0.4$  ng/mL. Based on these data, a major problem with current TAI protocols is that a subset of cows fails to fully regress their CL resulting in P4 levels at G2 that limit fertility. The underlying physiology by which slightly increased P4 levels at G2 cause this decreased fertility to TAI is not clear. Some possibilities include a negative association between P4 during the estrous cycle and oviducal and uterine motility thereby decreasing gamete transport and fertilization rate (Bennett et al., 1988) or decreased uterine thickness at TAI associated with decreased fertility to TAI in cows (Souza et al., 2011).

### Addition of a Second PGF<sub>2α</sub> Treatment Increases Fertility to Timed AI

Based on the analysis of the large dataset of P4 profiles during an Ovsynch protocol (Carvalho et al., 2015b), suboptimal P4 concentrations were identified at G1 in 26% of cows (26% lower P/AI), at PGF in 21% of cows (51% lower P/AI), and at G2 in 14% of cows (66% lower P/AI). Our conclusion based on this analysis was that achieving optimal P4 during an Ovsynch protocol may allow for a dramatic increase in fertility in lactating dairy cows. Incomplete luteal regression measured as P4  $\geq 0.4$  ng/mL at G2 has been associated with decreased P/AI at first and Resynch TAI. Decreased P/AI associated with incomplete luteal regression is particularly manifested in cows in which an Ovsynch protocol is initiated in a low-P4 environment (Giordano et al., 2012c; Carvalho et al., 2015a; Santos et al. 2015). This is likely because cows with one young CL ( $\sim 6$ d) at the PGF<sub>2α</sub> treatment during an Ovsynch protocol fail to fully regress to a single PGF<sub>2α</sub> treatment because some cows have young CL



that have not fully acquired luteolytic capacity (Nascimento et al., 2014).

Based on an analysis of data from an experiment in which cows were resynchronized using a Double Ovsynch protocol (Giordano et al., 2012c), we classified cows based on the age and number of CL present at the PGF<sub>2α</sub> treatment of an Ovsynch protocol and assessed the rate of complete luteal regression (Table 1). Cows with a single CL ~13 days of age had a 97% luteal regression rate, and cows with a CL ~13 days of age and a CL ~6 days of age had a 92% luteal regression rate. By contrast, cows with a single CL ~6 days of age had only a 64% luteal regression rate. Cows that initiate an Ovsynch protocol in a low P4 environment (whether anovular or cyclic and lacking a CL) have a high ovulatory response to G1 resulting in a single CL ~6 days of age present at the PGF<sub>2α</sub> treatment of the Ovsynch protocol. Approximately one-third of these cows fail to fully regress this young CL resulting in slightly elevated P4 levels at G2 which dramatically decrease P/AI.

**Table 1.** Effect of age and number of CL at the final PGF<sub>2α</sub> treatment during a Double Ovsynch protocol on the proportion of Holstein dairy cows undergoing complete luteal regression by G2 (P4 < 0.4 ng/mL)<sup>1</sup>.

Age and number of CL at PGF <sub>2α</sub> treatment	Proportion of cows with complete luteolysis, % (n)
Day 6 CL	64 (59)
Day 6 and Day 13 CL	92 (74)
Day 13 CL	97 (166)

<sup>1</sup>Adapted from Giordano et al., 2012c

Several experiments have assessed the effect of adding a second PGF<sub>2α</sub> treatment during an Ovsynch protocol to decrease P4 at G2 on fertility to TAI at first TAI as well as at Resynch TAI.

**First TAI.** Lactating Holstein cows were randomly assigned to a Double Ovsynch protocol (control) or a Double Ovsynch protocol that included a second PGF<sub>2α</sub> treatment 24 hours after the first (Brusveen et al., 2009). Cows receiving 2 PGF<sub>2α</sub> treatments during the Ovsynch protocol had a greater incidence of luteal regression than cows receiving 1 PGF<sub>2α</sub> treatment (98% vs. 86%); however, P/AI to first TAI did not differ between cows receiving 2 vs. 1 PGF<sub>2α</sub> treatments (53% vs. 47%, respectively). The 6 percentage point difference in P/AI would be expected based on the 12 percentage point increase in luteal regression combined with a 50% conception rate to TAI in this experiment. Further, the physiological impact of adding a second PGF<sub>2α</sub> treatment during a Double Ovsynch protocol may be limited because a Double Ovsynch protocol results in most cows having a CL

~13 days of age, or a CL ~13 days of age and a CL ~6 days of age at the PGF<sub>2α</sub> treatment and avoids setting up cows with a young CL ~6 days of age at the PGF<sub>2α</sub> treatment that fail to fully regress (Table 3).

**Resynch TAI.** Whereas resynchronization strategies have yielded significant increases in P/AI to first TAI, many herds struggle with poor fertility to an Ovsynch protocol used for Resynch TAI. In several studies, 16%, 22%, and 35% of cows diagnosed not pregnant 32 days after TAI and that did not receive a GnRH treatment 7 days before pregnancy diagnosis lacked a CL (Fricke et al., 2003; Sterry et al., 2006; Giordano et al., 2015). When cows were synchronized for first TAI and P4 profiles and CL diameter was measured until a pregnancy diagnosis 32 days later, 19% of cows diagnosed not pregnant lacked a CL > 10 mm in diameter (Ricci et al., 2014). Thus, up to one-third of nonpregnant cows initiate a Resynch protocol in a low P4 environment which leads to a lack of luteal regression and low fertility to Resynch TAI. We conducted an experiment to determine the effect of adding a second PGF<sub>2α</sub> treatment 24 hours after the first within an Ovsynch protocol would increase P/AI to TAI after a Resynch protocol (Carvalho et al., 2015a). A greater (P < 0.01) proportion of cows receiving 1 PGF<sub>2α</sub> treatment had incomplete luteal regression (≥ 0.4 ng/mL) than cows receiving 2 PGF<sub>2α</sub> treatments regardless of P4 concentrations at G1 (Table 4). For cows with P4 concentrations < 1.0 ng/mL at G1, cows receiving 2 PGF<sub>2α</sub> treatments had more (P = 0.03) P/AI than cows receiving 1 PGF<sub>2α</sub> treatment, whereas for cows with P4 concentrations ≥ 1.0 ng/mL at G1, P/AI did not differ (P = 0.46) between cows receiving 1 vs. 2 PGF<sub>2α</sub> treatments (Table 2).

Table 2. Effect of 1 vs. 2 PGF<sub>2α</sub> treatments during an Ovsynch protocol on luteal regression and pregnancies per AI (P/AI) for Holstein dairy cows with low vs. high progesterone (P4) concentrations at the first GnRH treatment of an Ovsynch protocol (G1)<sup>1</sup>.

Item	Treatment	
	1 PGF <sub>2α</sub>	2 PGF <sub>2α</sub>
	----- % (n) -----	
Cows undergoing complete luteal regression		
Low P4 (<1.0 ng/mL) at G1	70 <sup>a</sup> (76)	96 <sup>b</sup> (74)
High P4 (>1.0 ng/mL) at G1	89 <sup>a</sup> (236)	98 <sup>b</sup> (214)
Overall	83 <sup>a</sup> (312)	98 <sup>b</sup> (288)
P/AI 32 days after TAI		
Low P4 (<1.0 ng/mL) at G1	33 <sup>c</sup> (107)	46 <sup>d</sup> (110)
High P4 (>1.0 ng/mL) at G1	33 (312)	37 (289)
Overall	33 <sup>c</sup> (419)	39 <sup>d</sup> (399)

<sup>1</sup>Adapted from Carvalho et al., 2015a.

<sup>a,b</sup>Proportions differ (P < 0.01).

<sup>c,d</sup>Proportions differ (P < 0.05).

## Achieving High Fertility in High-Producing Dairy Herds

### Reproductive Management

All cows are submitted for first TAI between 77 to 83 DIM after a Double-Ovsynch protocol as described by Souza et al. (2008; Figure 8, lower panel). The second Ovsynch of the Double-Ovsynch protocol is conducted as an Ovsynch-56 protocol as described by Brusveen et al. (2008) with the addition of a second PGF<sub>2α</sub> treatment 24 h after the first PGF<sub>2α</sub> treatment (Wiltbank et al., 2015). For second and subsequent TAI, all cows are treated with GnRH 25 d after TAI, and few cows are detected in estrus to receive AI after first TAI. Pregnancy diagnosis is conducted using transrectal ultrasonography 32 d after TAI, and cows diagnosed not pregnant are classified as having or lacking a CL > 10 mm in diameter. Nonpregnant cows with a CL continue an Ovsynch-56 protocol by receiving a PGF<sub>2α</sub> treatment 32 d after TAI with the addition a second PGF<sub>2α</sub> treatment 24 h after the first PGF<sub>2α</sub> treatment. Nonpregnant cows lacking a CL restart an Ovsynch-56 protocol that includes a second PGF<sub>2α</sub> treatment 24 h after the first as described by Carvalho et al. (2015b). Intravaginal P4 inserts (i.e., CIDR inserts) are included within the Ovsynch protocol for cows lacking a CL. This strategy was designed based on studies in which exogenous P4 increased fertility for cows lacking a CL at initiation of an Ovsynch protocol (Bilby et al., 2013; Bisinotto et al., 2015).

### Reproductive Performance

During a one-year period (January 2015 to January 2016), The non-adjusted 21-day pregnancy rate (based on a 50-day VWP) was 25%, whereas the adjusted 21-day pregnancy rate (based on a 76 day VWP) was 33%. The 21-day service rate averaged 68%, and overall fertility for all TAI averaged 52% (n = 1,093). Overall, fertility to first TAI averaged 56% (n = 563), fertility to second TAI averaged 50% (n = 264), and fertility to third TAI averaged 45% (n = 129). The first three TAI occur from 77 to 180 DIM (i.e., a 100-d period), and 90% of cows became pregnant after the first three TAI. Over 95% of the inseminations in the herd are based on TAI. Although not conducted in this herd, detection of estrus after first TAI for cows that return to estrus after failing to conceive to TAI could further drive the 21-d pregnancy rate but would also require AI to occur every day of the week rather than on a prescheduled day of the week.

The intensive reproductive management protocol based on the concepts presented in this chapter integrates the latest information on technologies for synchronization of ovulation and TAI and pregnancy diagnosis and results in reproductive performance that is heretofore unprecedented for a herd of high-producing Holstein cows. Although use of an aggressive fertility program is important for achieving a high 21-day pregnancy rate, cows must be healthy to achieve high fertility. Many cow health factors have been reported to decrease fertility to TAI includ-

ing the incidence of mastitis between TAI and the first pregnancy diagnosis (Fuenzalida et al., 2015), a decrease in body condition score during the first 21 days after calving (Carvalho et al., 2014b), and poor uterine health (Lima et al., 2013).

## Conclusion

This intensive reproductive management protocol based on the concepts presented in this review has resulted in reproductive performance that is unprecedented for a herd of high-producing Holstein dairy cows. Although use of an ideal fertility program is important for achieving a high 21-day pregnancy rate, cows must be healthy to achieve high fertility. Many cow health factors have been reported to decrease P/AI to TAI including the incidence of mastitis between TAI and the first pregnancy diagnosis (Fuenzalida et al., 2015), a decrease in body condition score during the first 21 days after calving (Carvalho et al., 2014a), and poor uterine health (Lima et al., 2013).

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# Dairy Reproduction

## How to Turn the Research Into a Breeding Program

Dr. Don Niles, DVM  
Partner Dairy Dreams

### Dairy Reproduction

How to turn the research into a breeding program

### Example: Ketosis

- All Fresh cows are tested 2x weekly
- Moved out of fresh pens after 2 normal tests
- Any BHBA > 1.2 is ketosis
  - All cases are entered in DC and assigned a protocol
- Treatments continue as defined in protocol

### 30 is the New 20 What changed?

- Nutrition
- TAI Programs
- Genetic strategies

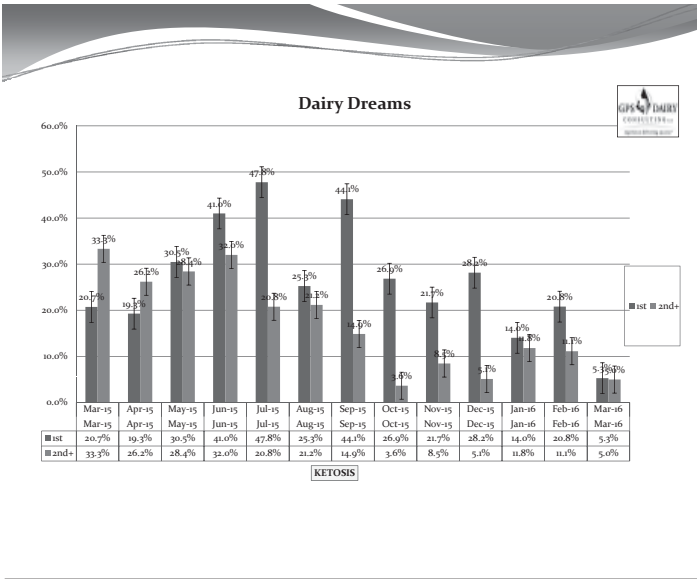


### Nutritional Effects

Dr Marty Faldet GPS

- Good Transition
  - Clean, healthy cows delivered to the breeding team
  - Good energy control/ketosis





### NKETO > 0

Date	Br Elig	Bred	Pct	Pg Elig	Preg	Pct Aborts
10/07/14	145	105	72	138	46	33 11
10/28/14	133	78	59	127	31	24 4
11/18/14	115	79	69	115	30	26 3
12/09/14	97	52	54	96	24	25 5
12/30/14	84	57	68	81	26	32 1
1/20/15	77	47	61	72	16	22 7
2/10/15	93	68	73 *	84	34	40 4
3/03/15	72	41	57	70	11	16 3
3/24/15	93	62	67	86	34	40 4
4/14/15	64	41	64 *	57	13	23 0
5/05/15	75	50	67	74	17	23 0
5/26/15	81	48	59	79	23	29 1
6/16/15	88	66	75	86	30	35 3
7/07/15	85	48	56	84	18	21 1
7/28/15	104	80	77	102	27	26 0
8/18/15	110	67	61	108	29	27 0
9/08/15	104	70	67	0	0	0 ????
9/29/15	85	66	78	0	0	0 ????
<b>Total</b>	<b>1516</b>	<b>989</b>	<b>65</b>	<b>1459</b>	<b>409</b>	<b>28 47</b>

Wait Period 67

- ## Results – Pen 10 – Fresh Cows
- 66 cows in fresh pen
  - 28 tested for ketosis
  - 1 positive and treated
  - All results entered on hand held
  - 16 minutes elapsed
  - Interesting observation- Virtually all positive heifers have metritis

- ## Nutritional Effects
- Good Transition
    - Clean, healthy cows delivered to the breeding team
    - Good energy control/ketosis
  - Body Condition
    - Good nutrition and good reproduction work together
  - Products
    - Megalac R, Choline, By-Pass Fats, Glucoboost ....

### NKETO = 0

Date	Br Elig	Bred	Pct	Pg Elig	Preg	Pct Aborts
10/07/14	444	331	75	417	134	32 27
10/28/14	436	268	61	421	111	26 16
11/18/14	495	377	76	479	194	41 31
12/09/14	414	250	60	396	129	33 16
12/30/14	425	339	80	407	148	36 26
1/20/15	366	206	56	352	93	26 23
2/10/15	412	307	75	394	142	36 25
3/03/15	428	257	60	410	108	26 20
3/24/15	406	272	67	392	106	27 11
4/14/15	386	239	62	376	102	27 5
5/05/15	408	290	71	394	126	32 13
5/26/15	384	234	61	376	98	26 12
6/16/15	435	331	76	424	144	34 14
7/07/15	418	228	55	406	90	22 7
7/28/15	459	320	70	431	122	28 6
8/18/15	409	263	64	399	94	24 1
9/08/15	467	351	75	0	0	0 ????
9/29/15	318	229	72	0	0	0 ????
<b>Total</b>	<b>6725</b>	<b>4512</b>	<b>67</b>	<b>6474</b>	<b>1941</b>	<b>30 253</b>

Wait Period 67

- ## TAI Program
- Dr Paul Fricke
- Combination TAI and Tail Chalk
  - All first breedings are Dbl OvSynch
    - All OvSynch protocols employ 2x PG
  - ReSynch sets up all Preg checks
    - G-G-PG
  - Open cows at Preg check are checked for a CL
    - CL+ receive PG
    - CL- receive CIDR

## TAI Program

- All treatments and exams are dictated in DC305
- No “thinking” cowside
- Culture for success – No Cow Left Behind
- VWP is now 74 DIM
- Constantly updated DNB list
  - Flag=D

Date	Br Elig	Bred	Pct	Pg Elig	Preg	Pct	Aborts
10/15/14	558	310	56	536	132	25	27
11/05/14	626	482	77	601	221	37	34
11/26/14	539	320	59	520	142	27	20
12/17/14	563	423	75	541	212	39	26
1/07/15	473	281	59	458	124	27	22
1/28/15	488	364	75	464	155	33	37
2/18/15	477	277	58	456	131	29	19
3/11/15	530	396	75	505	162	32	29
4/01/15	476	266	56	456	102	22	6
4/22/15	492	354	72	471	152	32	12
5/13/15	467	266	57	458	115	25	8
6/03/15	522	397	76	509	187	37	21
6/24/15	494	308	62	481	122	25	14
7/15/15	541	363	67	517	154	30	12
8/05/15	518	295	57	504	102	20	7
8/26/15	580	443	76	564	175	31	1
9/16/15	551	337	61	0	0	0	0
10/07/15	434	359	83	0	0	0	0
Total	8344	5545	66	8041	2388	30	295

Wait Period 67

## TAI Program

- Focused DNB program
  - DD uses ECM cutoffs
    - Lact=1 80#
    - Lact=2 90#
    - Lact>2 100#
  - Use a Flag switch
- High value fat cull vs low value fresh cull (difference?)

Date	Br Elig	Bred	Pct	Pg Elig	Preg	Pct	Aborts
4/21/2015	775	447	58	755	205	27	21
5/12/2015	907	702	77	877	345	39	26
6/12/2015	770	449	58	743	209	28	20
6/23/2015	901	728	81	867	325	37	37
7/14/2015	818	475	58	798	187	23	14
8/4/2015	939	708	75	899	317	35	32
8/25/2015	833	498	60	816	210	26	21
9/15/2015	929	671	72	900	309	34	31
10/16/2015	816	447	55	791	195	25	20
10/27/2015	914	694	76	894	345	39	19
11/17/2015	886	593	67	864	294	34	25
12/18/2015	884	654	74	857	303	35	14
12/29/2015	910	632	69	876	313	36	21
1/19/2016	872	623	71	834	316	38	13
2/9/2016	862	627	73	830	316	38	4
3/1/2016	826	572	69	789	270	34	0
3/22/2016	830	580	70	0	0	0	0
4/12/2016	623	468	75	0	0	0	0
Total	13842	9520	69	13390	4459	33	318

## By Breeding Code from 12/22/14 through 12/22/15

Breeding Code	95% CI	%Conc	#Preg	#Open	Other	Abort	Total	%Tot	SPC
Embryo Transfe	36-49	42	95	131	3	13	229	9	2.4
Ovsynch First	56-63	60	526	358	10	37	894	36	1.7
Standing Heat	35-45	40	162	245	24	8	431	17	2.5
MULTI-NO-CL	26-57	41	15	22	1	2	38	2	2.5
OVSYNCH	40-46	43	355	469	66	35	890	35	2.3
Cystic-CIDR	-	100	1	0	0	0	1	0	1.0
WAIT1 WEEK-CL	6-34	15	4	22	1	1	27	1	6.5
TOTALS	46-50	48	1158	1247	105	96	2510	100	2.1

## Genetic Strategies

Dr Nate Zwald

- New advances in genetics have dramatically increased the speed and precision of genetic progress
- DD program is based upon parent average estimates
  - “Poor man’s genomics”
- A single value genetic index can be created to match any herd’s goals
  - DDINX- composite of DPR, PL, #Prot, #Fat
- This requires very accurate sire ID’s



## Are Lact=1 cows performing according to DPR genetic predictions?

By DPR	Pct	Count	AvMEPRO	AvMEFAT	Av	DPR	Av	PTAP	PR
0.6	25	263	920	1128	0.7	11.7	26		
1.5	25	267	913	1122	1.5	8.8	32		
2.2	25	262	921	1131	2.2	8.9	36		
3.3	26	276	939	1108	3.3	7.2	40		
=====									
<b>Total</b>	<b>100</b>	<b>1068</b>	<b>924</b>	<b>1122</b>	<b>2.0</b>	<b>9.0</b>			

## 2016 Dairy Dreams Genetic Plan

- All animals still assigned parent average derived genetic score (DDINX)
  - Poor man's Genomics - requires accurate sire ID
- The top 50% of heifer herd is bred up to 2x using sexed semen
- The bottom 50% of heifer herd is implanted with surrogate embryos up to 2x
- First lact animals of high genetic score are bred 1x with sexed semen
- First lact animals with the lowest score may be implanted with embryos

## Are Lact=1 cows performing according to DDINX?

By DDINX	Pct	Count	AvMEPRO	AvMEFAT	Av	DPR	Av	PTAP	PR
0		32	894	1106	0	0	30		
143	25	266	901	1101	1.3	3.3	25		
227	24	259	914	1113	1.8	7.1	33		
279	26	275	935	1127	2.1	10.1	35		
368	25	268	944	1145	2.5	14.6	38		
=====									
<b>Total</b>	<b>100</b>	<b>1068</b>	<b>924</b>	<b>1122</b>	<b>2.0</b>	<b>9.0</b>	<b>301</b>		

## 2016 Dairy Dreams Genetic Plan (cont)

- Until recently the lactating recipients were 1<sup>st</sup> breeding
- Currently using the following criteria:
  - 1<sup>st</sup> and 2<sup>nd</sup> lact found open on herd check
  - Leukosis neg
  - Due to weekly herd check and biweekly implant date:
    - Preg check at either 32 or 39 days
    - Immediate CIDR synch
- 10 calves selected for genomic sire confirmation monthly - variety of breeding types

## 2014 Dairy Dreams' Genetic Plan

- All animals assigned a genetic score (DDINX) at birth, based on pedigree
  - DDINX composite of DPR (50%), prot# (40%), fat# (10%)
- Based on DDINX top 10% of calves are genomically tested.
  - Those that remain in top are bred with sexed semen 1x.
  - The top 4-5 in each test period are flushed

## SUM DDINX BY LACT

By LACT	Pct	Count	AvDDINX
0	48	2817	423
1	22	1278	313
2	16	963	262
3	9	535	186
4	4	232	126
5	1	65	141
6	0	13	91
7	0	3	-1
8	0	1	-20
=====			
<b>Total</b>	<b>100</b>	<b>5907</b>	<b>330</b>

# Including Ethanol Co-Products in Dairy Rations: A Moving Target

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## Introduction

The corn ethanol industry is a source of various feed co-products that are incorporated in rations for ruminants primarily as a source of protein and energy. The fermentation for ethanol only utilizes the starch portion of the corn kernel and the remaining nutrients that are left become concentrated approximately three-fold. The final nutrient profile varies depending upon the downstream processing of the co-products. The two main streams of co-products are distillers solubles and distillers grains which represent the liquid and solid fraction of the fermentation process, respectively. These two are often blended and dried to produce dried distillers grains with solubles (DDGS). The technology for processing corn kernels for ethanol production has evolved to improve fermentation efficiency with concomitant modifications of downstream processing and variation in the nutrient content of co-products. Therefore it is important to outline the various grain processing practices and their respective downstream effects on nutrient content of feed co-products. This paper presents information regarding nutrient content of ethanol co-products and their effects on cow performance.

## Distillers grains

The term “distillers grains” is used widely but it should be noted that there have been many products developed under this umbrella term and it is very important to consider the specifics of each one:

- Distiller grains.- This portion represents the solid material left over after the fermentation and distillation process.
- Distillers grains with solubles.- This co-product is the combination of distillers grains (solid stream) and the fluid fraction of the fermentation slurry, called solubles. It can be fed wet (WDGS) or dried (DDGS).
- High protein distillers grains (HP-DDGS).- Removal of the germ and bran prior to fermentation results in a co-product with high protein content.

- Reduced-fat distillers grains with solubles (RF-DDGS).- This co-product is obtained by separating the oil, mainly by centrifugation. The process reduces fat content by 45 to 50% compared with conventional distillers grains with solubles.

## Energy content

The energy provided by DDGS comes from two main sources: fermentable fiber and lipids. Corn bran is the fibrous portion of the feed and it is fermented in the rumen; on the other hand, the lipid portion comes primarily from the germ and in some part from free corn oil, and it is digested in the small intestine. Birkelo et al. (2004) reported that WDGS contain 3.36 Mcal/kg DM and 2.27Mcal/kg DM of metabolizable energy and net energy for lactation. These values are 10 to 15% greater than those reported in the 2001 Dairy NRC. This observation highlights the importance of accurate feed analyses for proper formulation. A recent modification in the co-products stream involves removal of oil to produce RF-DDGS. Removal of oil in RF-DDGS decreases the lipid-derived energy, which is a concern because of possible energy shortages when formulating rations for high producing dairy cows. Recent data by Foth et al. (2015) indicate that the metabolizable and net energy content of RF-DDGS is 3.41 and 2.03 Mcal/kg, respectively. Inclusion of rumen-inert fat is an option to compensate for the removal of oil. Such strategy was used by Mjoun et al. (2010) and Castillo-Lopez et al. (2014) with diets containing 0, 10, 20 or 30% RF-DDGS and the results showed that cows performed similarly to the control treatments at all levels of inclusion. Surprisingly, supplementation with rumen inert fat may not be necessary in all cases, Ramirez Ramirez et al. (2016) reported that addition of rumen-inert fat to diets with RF-DDGS had no effect on cow performance when compared to conventional DDGS. This seems to indicate that improved fermentation may compensate for the reduced supply of lipid-derived energy. In vitro fermentation data by Williams et al. (2010) indicated that defatted DDGS resulted in shorter lag time and increased proportions of fibrolytic and proteolytic bacteria.

## Protein content

Corn grain has approximately 9 to 10% crude protein (DM basis), by removing the starch through fermentation the content of crude protein of most DDGS rises to 28-30% (DM basis). However, up-front fractionation of the corn kernel allows for separation of the germ and bran. This process results in high protein distillers grains with approximately 45% CP, which makes very similar to soybean meal (SBM). Kelzer et al. (2009) reported that a diet containing 15% HPDDGS with no soybean meal and bypass protein resulted in similar results to a diet containing DDGS, SBM and bypass protein. An important aspect of the protein content of distillers grains is the proportion of rumen undegradable protein (RUP). Most reports on the RUP content of DDGS indicate that it is around 50 to 55% of CP but ranges from 33 to 63% (NRC, 2001; Janicek et al., 2008; Kelzer et al., 2010; Castillo-Lopez et al., 2013). This may be the reason for the occasional positive response in milk protein as it may be related to increased supply of RUP to the dairy cow to support milk protein synthesis by DDGS and RFDDGS. Because of this, it is important to balance rations that meet the protein requirements of the rumen microbes and the cow. For example, Kleinschmit et al. (2007) reported that a combination of 15% DDGS and alfalfa hay tended to increase milk protein yield compared to a diet that included corn silage instead of alfalfa hay. In addition to protein solubility, Hollmann et al. (2011) underscored that origin of protein is another factor to consider when including ethanol co-products in dairy rations. This concept considers corn-protein and non-corn protein, this becomes relevant when including high concentration of corn based products as they supply of lysine may become limiting.

## Diet characteristics to consider

### *Fermentability*

When considering including ethanol co-products in dairy rations, several dietary factors need to be considered in order to obtain the desired performance. For example, a diet that is highly fermentable may cause altered rumen environment which leads to altered biohydrogenation pathways associated with milk fat depression (MFD). Such situations may arise when combining high moisture corn (Owens, et al., 2009) compared to dry ground corn or high inclusion of corn silage with DDGS (Ramirez-Ramirez, 2012). One alternative to counteract this effect is to use fermentable fiber as a replacement for starch to reduce the rate of ruminal fermentation. Ranathunga et al. (2010) reported that a combination of DDGS and soy hulls to lower the starch content to 20% dietary DM

resulted in similar response compared to other diets containing 23, 26 or 29 % starch DM. Another alternative is to lower the content of fat or its availability so that biohydrogenation issues are reduced. The fat contained in DDGS seems to be divided in two pools, free oil and germ-bound oil; Aldelqader et al. (2009) evaluated diets with similar fat content but from different origin for lactating cows, the results showed MFD when feeding corn oil and DDGS compared to germ. With the current advancements in oil removal, it is likely that formulating dairy rations with high inclusion of ethanol co-products will be less challenging.

## Particle size and rumen kinetics

There is limited information on the combined effects of rate of passage, biohydrogenation and feeding DDGS to dairy cows. It has been reported that inclusion of DDGs increases intestinal flow of polyunsaturated fatty acids in steers. In dairy cows this situation may lead to MFD as more CLA isomers leave the rumen and reach the intestine. An experiment conducted at the University of Nebraska-Lincoln evaluated the effect of feeding a high corn oil diet with short and long forage particle size and high inclusion of RFDDGS. Feeding short particles and high oil resulted in MFD, this effect was less severe when cows consumed long particles. The mechanism for this may be related to more thorough biohydrogenation due to slower passage rate thus reducing the outflow of isomers of conjugated linoleic acid that may cause MFD.

## Conclusion

The corn ethanol industry has been an important source of feed co-products for dairy cows. The nutrient profile of first-generation co-products is markedly different from the ones currently available because of improved fermentation and distillation processes, kernel fractionation and separation of oil in the downstream processing of co-products. The main aspects these co-products involve their energy and protein content; within these nutrients, it is important to consider the profile of fatty acids and balance between rumen degradable and undegradable protein. Research has shown that feeding ethanol co-products to dairy cows, most reports agree that 20% dietary DM is a safe inclusion level as long as other nutrients and diet characteristics are taken into account. Some of these characteristics include fermentability, particle size and rate of passage. As the ethanol industry and its co-products continue to evolve it will be even more relevant to use actual laboratory analysis to fine-tune dairy formulations to according to the nutrient profile of the next-generation feedstuffs.

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# Ruminating on Cow Behavior Monitors: A “Real Time” Look!

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4 State Dairy Nutrition 2016

## RUMINATING ON COW BEHAVIOR MONITORS:



A “Real Time” Look

<http://www.extension.iastate.edu/dairyteam/>

IOWA STATE UNIVERSITY  
Extension and Outreach  
Healthy People, Environments, Economies

**Drs. Leo Timms & Ryan Breuer**  
Extension Dairy Specialists

### BEHAVIOR IS NOT ALWAYS PRECISE!!



**Movement!!** → **Behavior!**  
**Direction!**  
**Speed!**  
**Force!**

**Active!**  
**Non-Active**  
**Ruminating**  
**Eating**

IOWA STATE UNIVERSITY Dairy Extension Team

### Monitoring

\$500 - \$15,000 Readers / software, \$80 - \$150 tags



**Animal ID**  
**Activity / Non-Activity!**  
**Eating ? RUMINATING!!**



**Microphone**

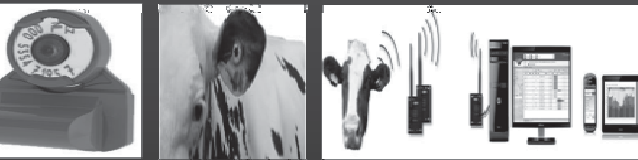


**Accelerometers**

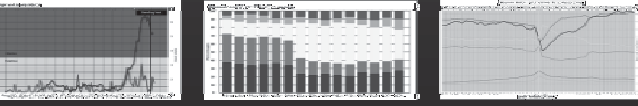



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### COW MANAGER





**Fertility** **Health** **Sickness** **Rumination** **Eating**




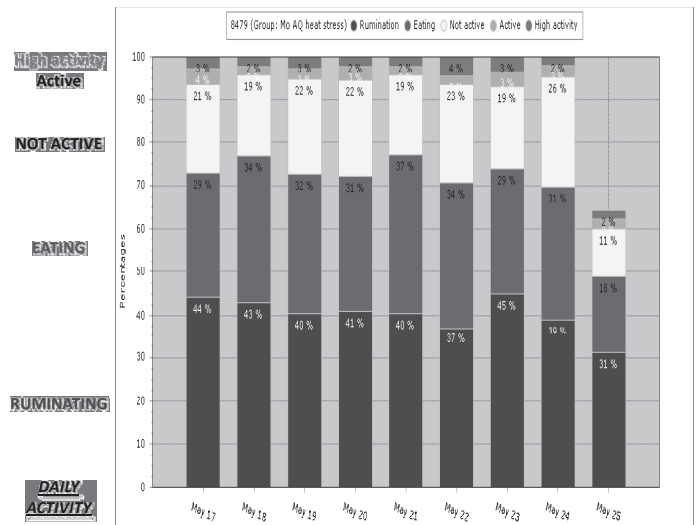


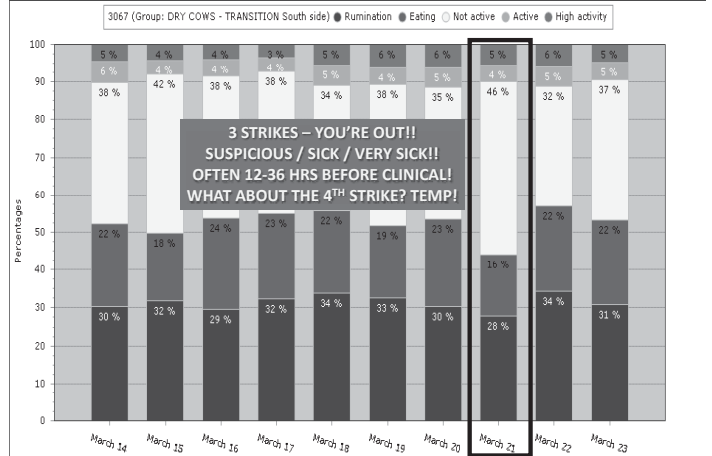
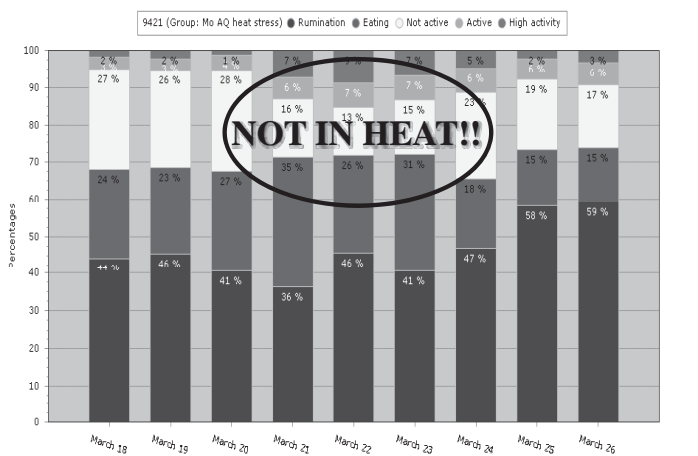
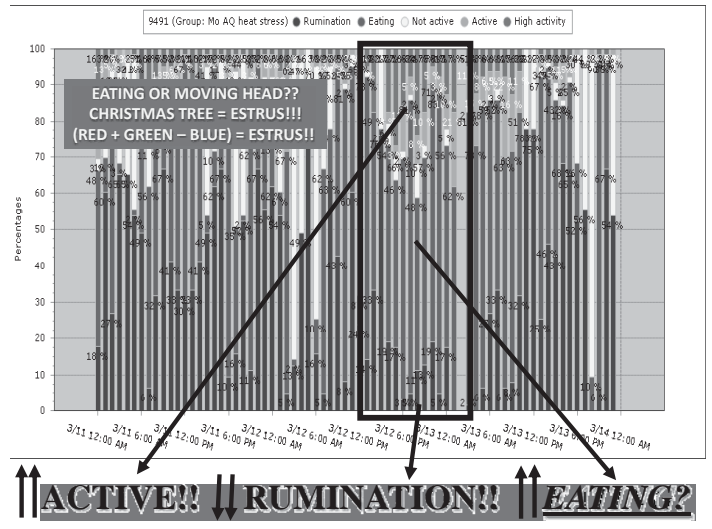
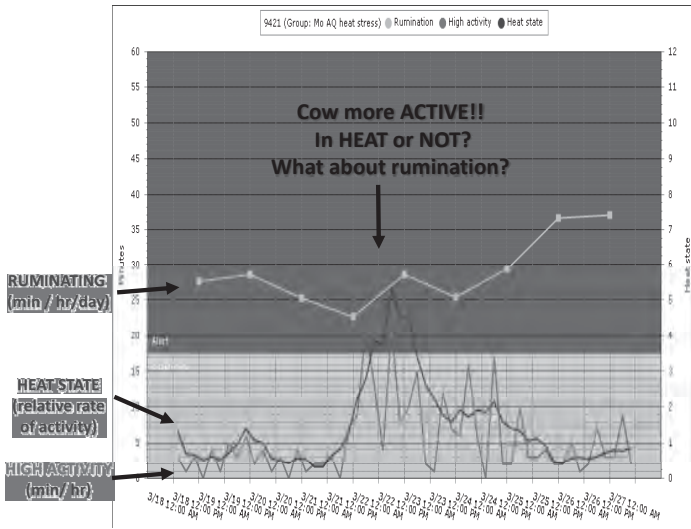
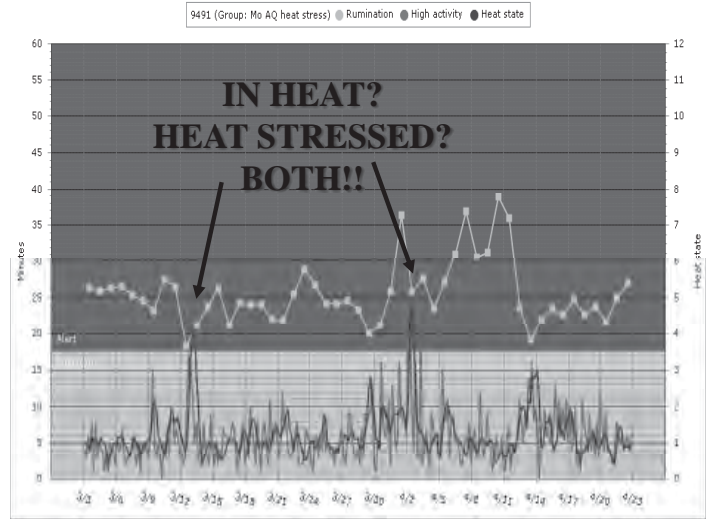
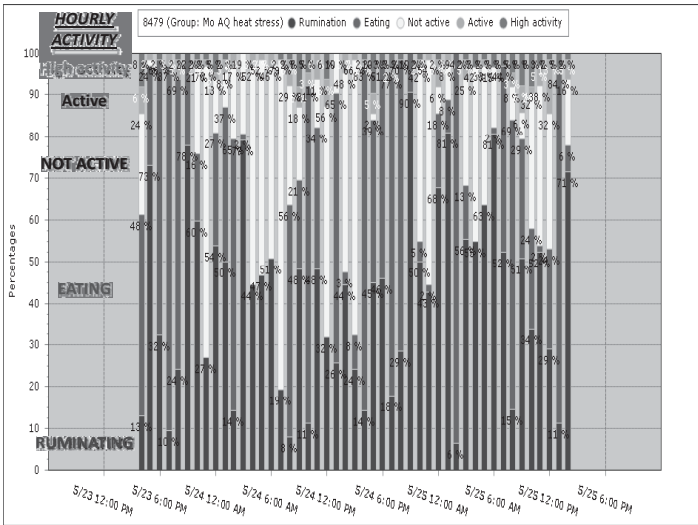
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### 3-D accelerometer sensor

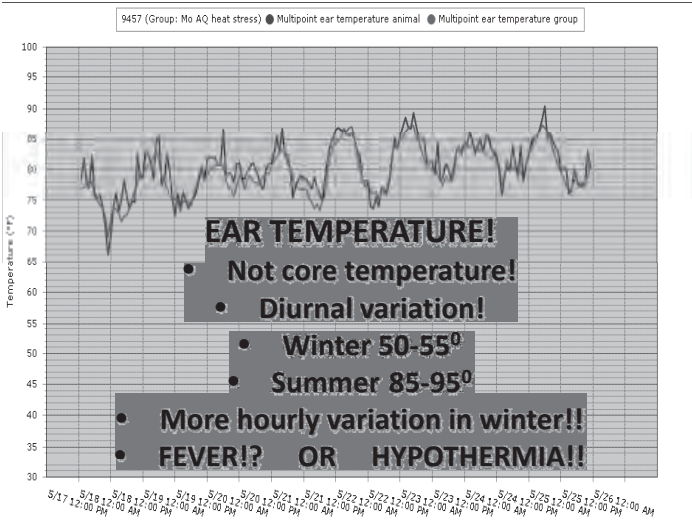
- Measure both static and dynamic forces
- First patent- 12/06/1963 Isemi Igarachi, Takecio Chiku
- Common types: piezoelectric, capacitance
- Ubiquitous in modern devices-consumer, medical, robotics.



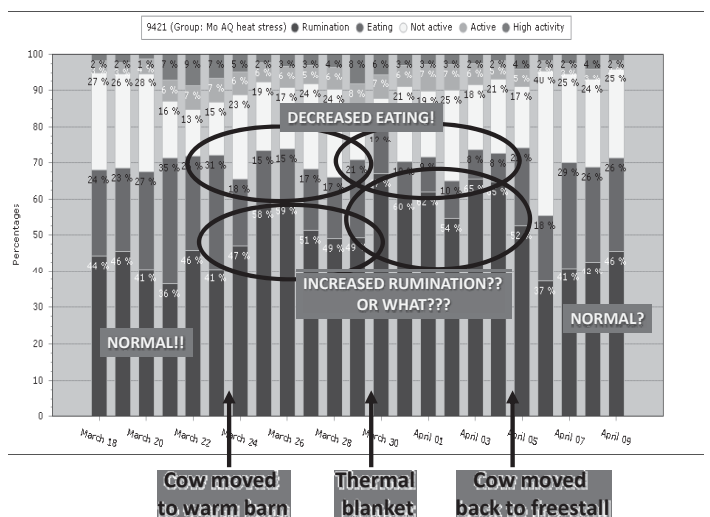
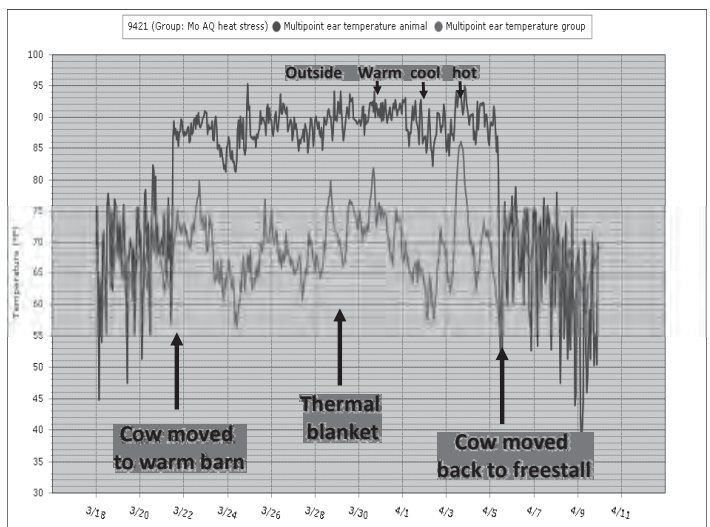
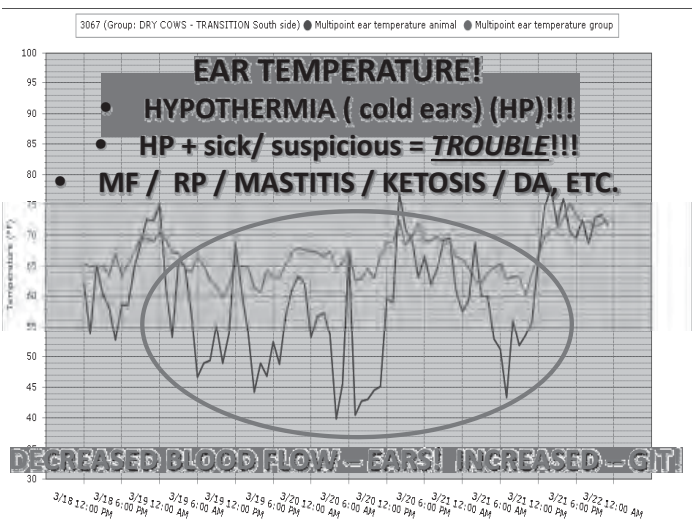
**ACTIVE!! NO CHANGE - RUMIN/EAT!!**

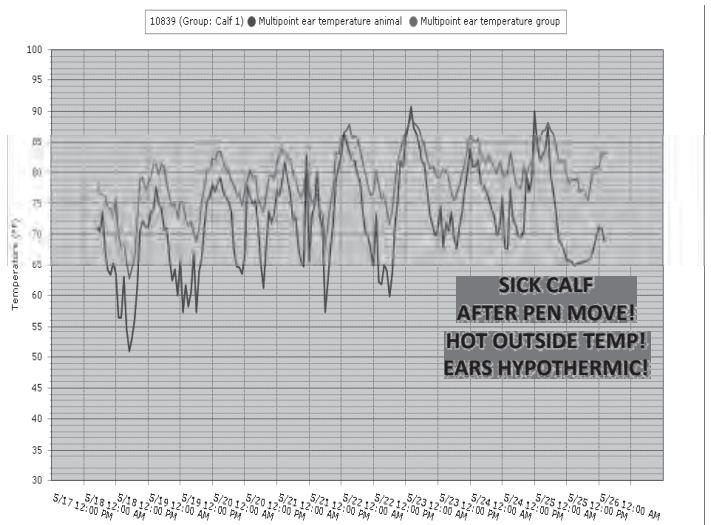
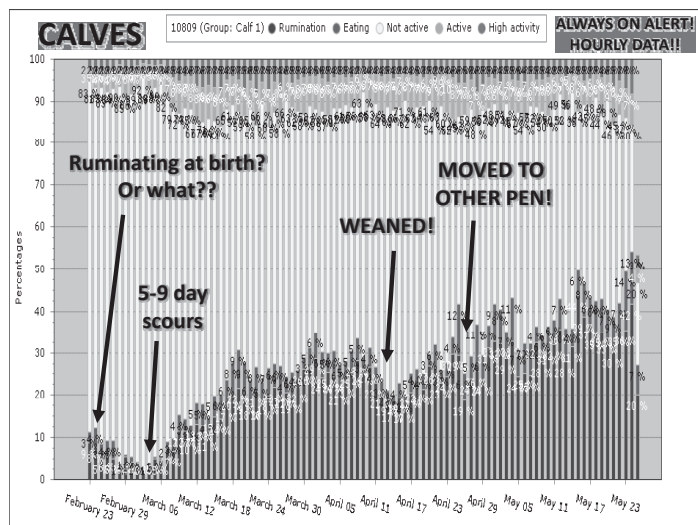
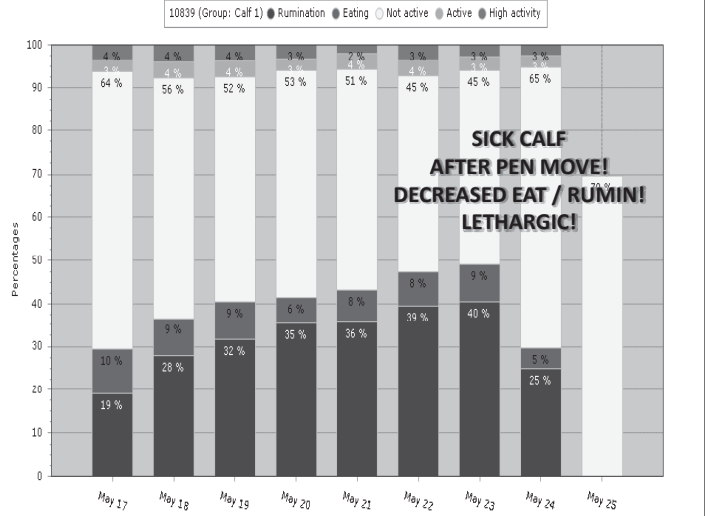
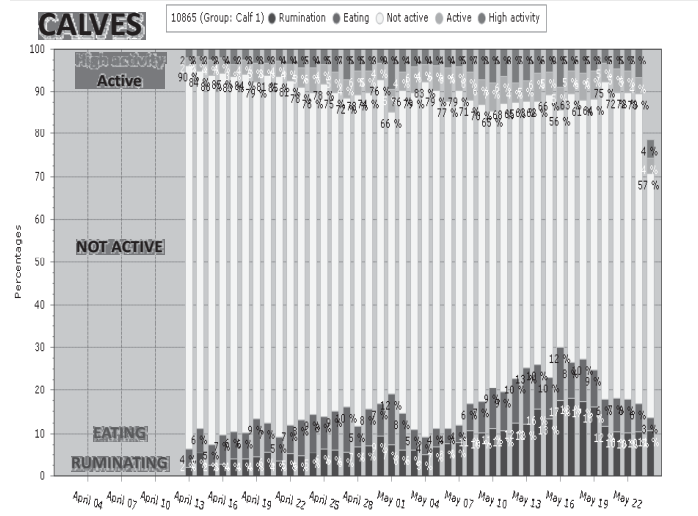
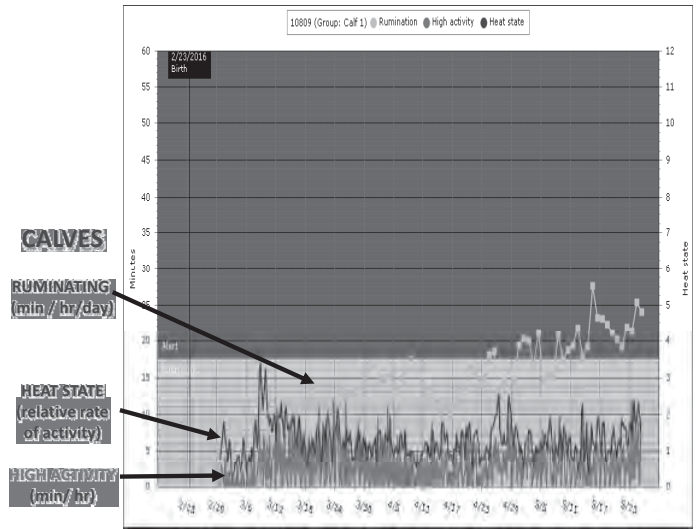
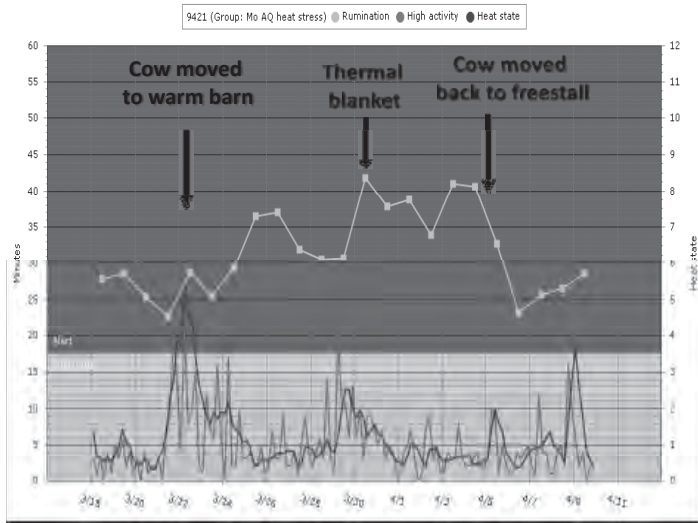
**NON-ACTIVE!! RUMINATION!! EATING!!**



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Dairy Extension Team







# Forage Value of Cover Crops

Jim Paulson, UM Dairy Extension Educator  
Work funded by a MFA Grant  
University of Minnesota Extension

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MAKING A DIFFERENCE IN MINNESOTA: ENVIRONMENT + FOOD & AGRICULTURE + COMMUNITIES + FAMILIES + YOUTH

## Forage Value of Cover Crops



JIM PAULSON, UM DAIRY EXTENSION EDUCATOR  
WORK FUNDED BY A MFA GRANT

1

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## Function of Cover Crops

- Erosion control
- Water infiltration
- Soil health
- Build organic matter
- Nitrogen uptake
- Nitrogen production
- Mineral movement

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## COVER CROPS - DEFINED

- A non-cash crop grown between two cash crops?



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## Function of Cover Crops

- Soil health – being healthy allows us to do what we are supposed to be able to do.
- Enhancing the soil biome so it can do the functions of soil



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5

## Roots of Cover Crops

- Variation in root depth
- Keeping plants doing something in the soil – life, organisms – we are measuring
- Build organic matter and soil carbon through plant and root growth

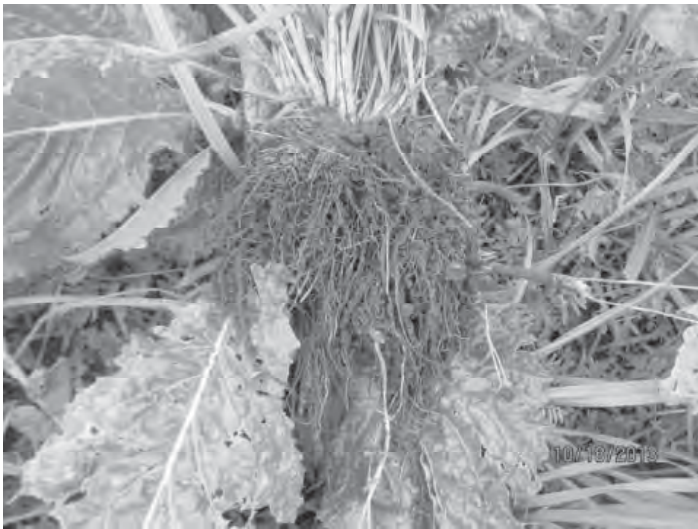


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3

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6



## SARE SURVEY

- Large increase in adoption of cover crops
  - Growers
  - Acres

Prevent plant – 48%

No-till farms – 42%

Conventional tillage – 23%



## SARE SURVEY – CONCERNS

**Cost**

**Termination of cover crop**

**Reduced yields of the next crop**

**Limited information**

**No financial incentive**

**Attitude**

**Time to get it done, early enough to get fall growth**

## Cover Crop Guidelines

- Diversity is a goal
  - Root depth, type
  - Plant type (grasses, legumes, annuals, broadleaves, pollinators)

Be specific for your farm and fields

Time of year for growth

How much diversity?

3 or 5 or 10 or 20?

Plant populations?

Carbon : Nitrogen

## SARE SURVEY – BENEFITS

**Increase organic matter**

**Reduce erosion**

**Reduce compaction**

**Control weeds**

**N management – produce and/or scavenge**

**Increase yields**

**Root growth/effects**

## SARE SURVEY – CROPS USED

- Winter cereals – 73% (cereal rye, triticale)
- Legumes - 54% (clovers)
- Brassicas – 54% (turnips, kale)
- Annual grasses – 53% (annual ryegrass, So/Su)
- Multi-species – 33%
- Two species – 26%
- Annual broadleaf – 20%

## WHEN DO WE PLANT?



- After winter wheat is very common
- At last cultivation
- After corn silage is harvested

## SARE SURVEY – WHEN USED

- After small grain – 33%
- After specialty crops
- Before or after corn / soybeans – 50%
- Prevent plant

## WHEN DO WE PLANT?



- At last cultivation



## SARE SURVEY – HOW PLANTED

- Drilled
- Broadcast with incorporation
- Arial
- Broadcast with no incorporation
- With liquid slurry

## Strategies for Cover Crops

- Corn Silage → Winter Rye or Winter Triticale
- Alfalfa (3<sup>rd</sup> yr: 3<sup>rd</sup> Crop) → Fall Oats, Winter Rye/Triticale
- Alfalfa (3<sup>rd</sup> yr: 1<sup>st</sup> Crop) → Corn Silage
- Winter Rye/Triticale → Sorghum Sudan

## > August 15- Fall Oats

- Planted August 15 + or -
- Grows Backwards in Decreasing Day Length
- Low Lignin Static NDF
- Can Have Very High Sugar Levels
- Late Cold Weather Silage Harvest
- Versatile with High TDN Potential



## BUILDING A FORAGE CHAIN

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Cool Season Perennials												
Warm Season perennials												
Cool Season annuals												
Warm season annuals												

*Every day we can graze is a day we don't have to feed!*

## Common Cover Crops

- Cool season
  - Grasses - ryegrass
  - Legumes- peas, clovers, vetches
  - Cereal grains- oats, triticale, rye
  - Brassicas- turnips, radishes
- Warm Season
  - Sorghum and Sudan as well as crosses.
  - Annual grasses- millets, Teff,



## EXAMPLE COVER CROPS



- Common vetch
- Berseem clover
- Crimson clover



## Stockpiling Forage

- Any forage can be stockpiled, but quality of most declines sharply with duration of stockpiling time
- Some species retain quality better into the winter
- Tall fescue
- All brassicas, but especially rape and kale



## EXAMPLE COVER CROPS



- Common vetch
- Buckwheat
- Austrian winter pea



## Yield and forage analysis

	DM kg/acre	Ton/acre	CP	NDF	LIGNIN	T.D.N.
<b>Crimson Clover</b>	<b>1371</b>	<b>1.51</b>	<b>20.44%</b>	<b>38.14%</b>	<b>3.88%</b>	<b>63.61%</b>
<b>Berseem Clover</b>	<b>1013</b>	<b>1.11</b>	<b>22.36%</b>	<b>38.51%</b>	<b>6.62%</b>	<b>60.89%</b>

## EXAMPLE COVER CROPS



## Yield and forage analysis

	DM kg/acre	Ton/acre	CP	NDF	LIGNIN	T.D.N.
<b>Pearl Millet</b>	<b>3066</b>	<b>3.37</b>	<b>15.92%</b>	<b>54.83%</b>	<b>2.60%</b>	<b>60.60%</b>
<b>Buckwheat</b>	<b>1507</b>	<b>1.65</b>	<b>13.57%</b>	<b>42.36%</b>	<b>7.32%</b>	<b>58.01%</b>

## Yield and forage analysis

	DM kg/acre	Ton/acre	CP	NDF	LIGNIN	T.D.N.
<b>Kale</b>	<b>1239</b>	<b>1.36</b>	<b>23.21%</b>	<b>39.00%</b>	<b>4.54%</b>	<b>65.15%</b>
<b>Turnip</b>	<b>1600</b>	<b>1.76</b>	<b>17.23%</b>	<b>28.64%</b>	<b>2.36%</b>	<b>67.77%</b>

## Yield and forage analysis

	DM kg/acre	Ton/acre	CP	NDF	LIGNIN	T.D.N.
<b>Sugarbeet</b>	<b>2845</b>	<b>3.13</b>	<b>21.68%</b>	<b>29.33%</b>	<b>3.32%</b>	<b>68.59%</b>
<b>FodderBeet</b>	<b>1266</b>	<b>1.39</b>	<b>24.01%</b>	<b>33.42%</b>	<b>3.72%</b>	<b>66.69%</b>

## Yield and forage analysis

	DM kg/acre	Ton/acre	CP	NDF	LIGNIN	T.D.N.
Forage Peas	2909	3.2	13.52%	41.08%	7.22%	45.52%
Phacelia	404	0.44	21.40%	34.16%	4.22%	63.66%
Forage Oats	1436	1.58	16.61%	50.99%	3.66%	62.23%
Annual Ryegrass	2183	2.40	21.72%	37.91%	5.40%	60.61%

## Your Goals for Cover Crops

- What root depth do you want?
- Warm season or cool season?
- Grazing?
- No till, minimum till
- Before manure or after?
- Cost of seed?
  - \$10-\$40/ acre
  - Other consideration
    - Drill, Brillion
    - Two boxes needed
    - Apply with manure slurry: 3 - 5 thousand gals/acre with minimum tillage

## Yield and forage analysis

	DM kg/acre	Ton/ acre	CP	NDF	LIGNIN	T.D.N.
Teff	3059	3.36	17.68%	59.02%	4.01%	60.23%
BMR sorgh	4045	4.45	14.34%	53.65%	2.84%	62.18%
sorg/sud	6580	7.23	10.90%	56.10%	3.32%	58.37%
graze corn	5797	6.38	13.37%	32.70%	3.34%	48.38%
Rox Cane	9130	10	12.69%	51.25%	3.02%	63.18%

Thank You

Questions?

[jcp@umn.edu](mailto:jcp@umn.edu)



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## SARE SURVEY – COSTS

Establishment costs -  
median cost \$12  
Seed costs – median cost  
\$25



# Options to Improve Forage Quality

Tim Meister  
 Division Marketing Manager  
 John Deere Ottumwa Works



**Options to Improve Forage Quality**

Tim Meister  
 Division Marketing Manager  
 John Deere Ottumwa Works

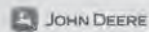


## Factors in Alfalfa Silage quality

JOHN DEERE  
 CONFIDENTIAL



4




**Forage Quality**

## Factors in Corn Silage quality

JOHN DEERE  
 CONFIDENTIAL



5



## Quality Silage

JOHN DEERE  
 CONFIDENTIAL


### Challenges

- Kernel processing score
- Proper packing
- High bunk density
- Consistency
- Proper chop length
- Proper rate of inoculant
- Cob destruction
- Effective fiber
- Low ash content
- Nutrient value and documentation

- Effective/consistent fiber/cost
- Rule of 800, cost, effective fiber
- Consistent length of cut
- Kernel processing score/ moisture
- Cost and availability of fiber
- Solved via yield and moisture data
- Effective/consistent fiber
- Speed/mechanization/kernel proc.
- Speed of harvest
- Technology and cost

Overall goal, increase production, lower cost

3



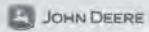
## Real time moisture measurement

### HarvestLab

- NIR Sensor mounted in the spout (17 readings/sec.)
- The same sensor can be removed and used on the desktop for ration balance
- Developed in conjunction with Carl Zeiss and Dairyland Labs



6



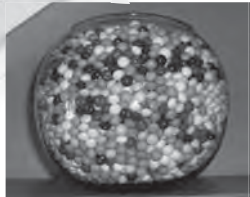
### Real Time Moisture Readings - HarvestLab



30,000 lbs of Forage in a truck load (2:12)

← 2,244 res

**Get Rid of Sampling Error!**



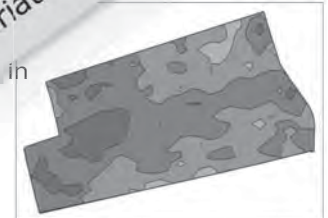
200 grams of Forage in a Koster Tec  
200 grams of Forage in a Koster Tec

68,000 Skittles

### Drymatter Variation Within One Field Corn

Moisture varied from 59 to 75 % moisture

- Difference absolute: 16%
- Average: 69,5 %
- Standard variation: 6%
- 22 points of difference in California!



38 Acres

**There is dry matter variation in corn fields**

### Real time moisture measurement

#### HarvestLab

#### • Harvest Choices

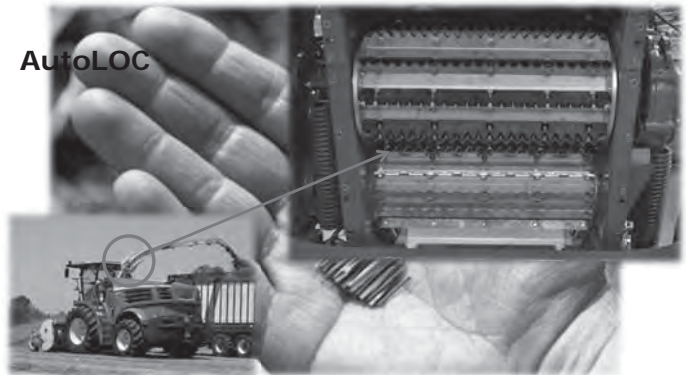
- One 15 ton alfalfa truck = \$2,250
- Alfalfa \$1,000/minute
  - \$3,000/minute
  - Higher Quality Silage

#### • Yield Monitoring



### Field Variability And LOC

#### AutoLOC



### Real Time Yield/Moisture Measurement

#### HarvestLab

#### • Harvest Choices

- One 15 ton alfalfa truck = \$2,250
- Alfalfa \$1,000/minute
  - \$3,000/minute
  - Higher Quality Silage

#### • Yield Monitoring

- Know what you're harvesting
- Make decisions on the spot
- Decide what to do next year

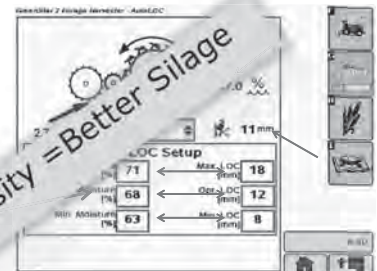
**You Only Get One Chance to Make Good Silage!**



### Field Variability And LOC

#### AutoLOC

- 3 dry matter/3 LOC
  - Operator Selected
- 17%+ better bunk density in grass
- Added Productivity
- Better kernel processing



**Better Bunk Density = Better Silage**

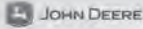


**Real time moisture measurement**



Photos via Connormareting.wordpress.com, Truthordairy.Blogspot.com, thesimplecountrylife.com

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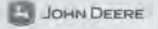


**Variable rate/Twin Line Inoculant dosing**

How Much Inoculant Do I Need to Use?

**ALL OF IT!**

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**Real time moisture measurement**

**HarvestLab**

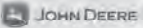
- Use at feedout for Ration Balance Dry Matter
- 1.5 min
- Real-time moisture measurement
- Reduces risk of acidosis



Desktop Unit

*No Need to Wait For it To Happen*

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**Variable rate/Twin Line Inoculant dosing**



– High volume 95 Gallon (360L) rear tank with easy filling

– Low volume 8 gallon (30L) concentrate tank

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**Real Time Constituent Sensing**

**HarvestLab**

- Constituent Sensing
- Make Agronomic Decision
- Tied to management
- Know when you have changed cuttings
- Change in Cut Height?
- Super Silage
- Value at feed out
- It is about change



*Know immediately when something has changed!*

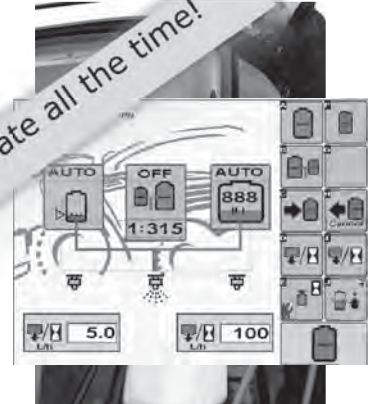
ADFV... in/Starch

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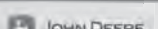
**Variable rate/Twin Line Inoculant dosing**

- Inoculant costs are \$5/minute
- Fully integrated solution on board
- Control via CommandARM™ display for on the go adjustments and ease of use
- Combine the (rate) for a ratio of (rate) to water
- Variable (rate) according to HarvestLab readings based on real time (rate) measured by Harvestlab and the feedrolls

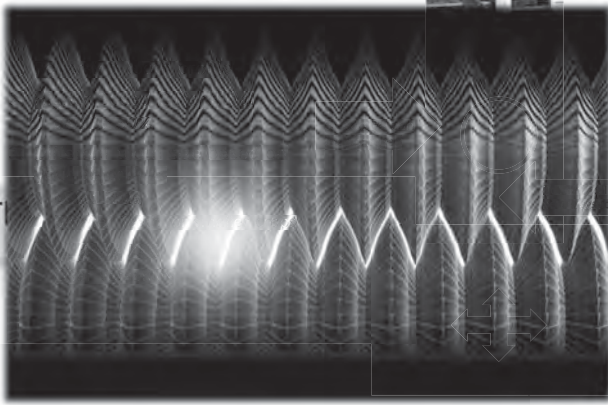


*Right Application rate all the time!*

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## High KP Scores and High Throughput



T

can

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JOHN DEERE

## Factors affecting KP Scores

1. Reversing tooth profile
- 2. Higher Speed Differential**
  1. 24% to 50%
3. Different tooth size based on your objectives
  1. Mixed Rolls
  2. Smaller Teeth for smaller
- 4. Disk Style KPs**
  1. Offer Versatility



5. Settling

6. You Can't Fix Processing in February!

Nutritionist?

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## Summary



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