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Pre-Conference Symposium *Sponsored by Diamond V*

4-State Dairy Nutrition and Management Conference

Nutrition to Improve Performance and Cow Health

Breakfast ***Sponsored by DuPont Pioneer***

General Session

Nutrition to Improve Reproductive Performance

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Upcoming Conference Dates

June 14-15, 2017
June 13-14, 2018

Diamond V Welcomes You
to the 4-State Pre-Conference

New Perspectives on the Control of Salmonella and Related Enteric Pathogens

Steve A. Carlson, DVM PhD
Associate Professor of Molecular Pharmacology
Iowa State University College of Veterinary Medicine

New Perspectives on the Control of *Salmonella* and Related Enteric Pathogens

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Steve A. Carlson, DVM PhD
Associate Professor of Molecular Pharmacology
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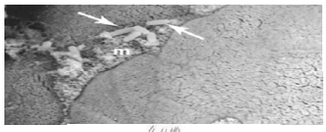
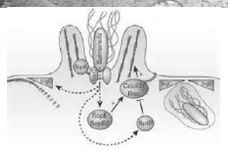
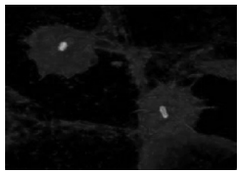
(I) Importance of *Salmonella* in Cattle



- ✓ **Three important facets:**
- ✓ **(A) Attachment to the gut epithelium**



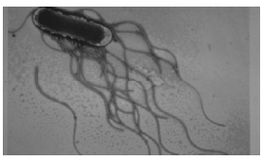
- ✓ **(B) Invasion of cells lining the gut epithelium**



- ✓ **(C) Survival within bovine macrophages**

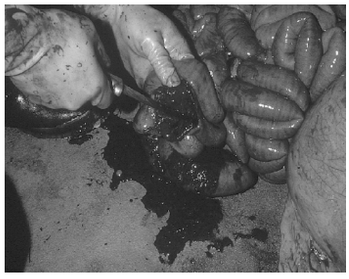
Overview of the Presentation

- **(I)** **The importance of *Salmonella* in cattle**
- **(II)** **Strategies to mitigate *Salmonella* in cattle**
- **(III)** **New approaches that use the anti-*Salmonella* strategies**
- **(IV)** **Cost effectiveness of these approaches**
- **(V)** **Conclusions**



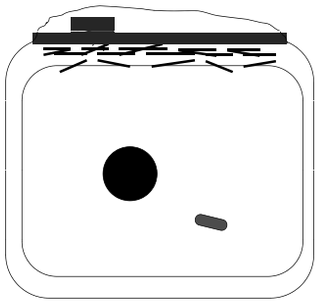
(I) Importance of *Salmonella* in Cattle

- ✓ **(B) Physical invasion of the gut epithelium and entrance into the systemic circulation**
- ✓ **Some *Salmonella* can invade and dislodge cells lining the bovine gut epithelium- causing a hemorrhagic diarrhea.**



(I) Importance of *Salmonella* in Cattle

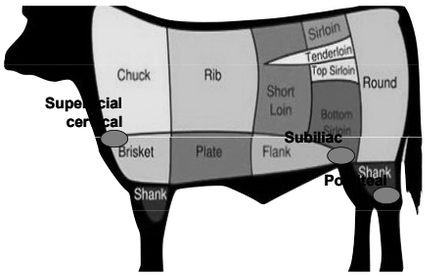
(B) Invasion of intestinal cells



(i) Attachment
(ii) Injection of bacterial proteins
(iii) Rearrangement of actin
(iv) Formation of a "ruffle"
(v) Bacterial entry into the cell
(vi) Cell is damaged and sloughs
(vii) Underlying vasculature is exposed
(viii) Hemorrhagic diarrhea

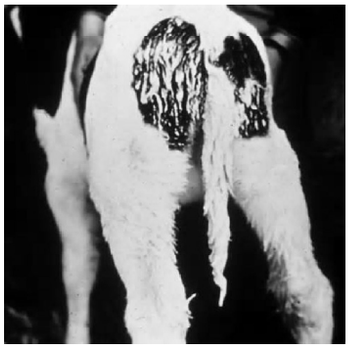
(I) Importance of *Salmonella* in Cattle

- ✓ **Recent studies indicate that up to 30% of peripheral cattle lymph nodes contain *Salmonella.***
- ✓ **These lymph nodes are not excised and are unfortunately incorporated into hamburger at slaughter- hard to prevent.**



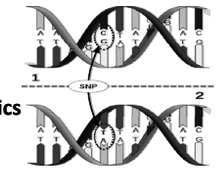
(I) Importance of *Salmonella* in Cattle

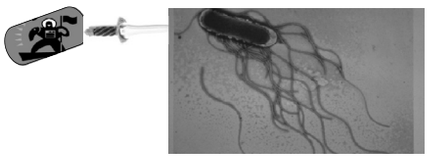
- ✓ **Less invasive strains will cause a moderate diarrhea in calves.**
- ✓ **Costly because of medical costs and hampered growth and development**
- ✓ **The animal becomes a life-long carrier.**



(II) Strategies to mitigate *Salmonella* in cattle

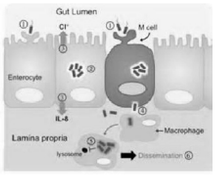
- ✓ **(A) Selecting for cattle that are naturally resistant**
- ✓ **(B) Altering gene expression in cattle**
- ✓ **(C) Altering gene expression in *Salmonella***
- ✓ **(D) Directly killing *Salmonella* with antibiotics**

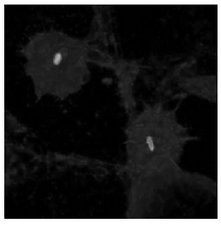




(I) Importance of *Salmonella* in Cattle

- ✓ **(C) Survival within bovine macrophages (white blood cells)**
- ✓ **Macrophages will engulf *Salmonella* on the basal side of the intestinal lining.**
- ✓ ***Salmonella* survives within the macrophages.**
- ✓ **The macrophages carry the *Salmonella* to organs like the lung (pneumonia) or brain (encephalitis).**
- ✓ **The macrophages also carry the *Salmonella* to lymph nodes, causing a food safety hazard.**

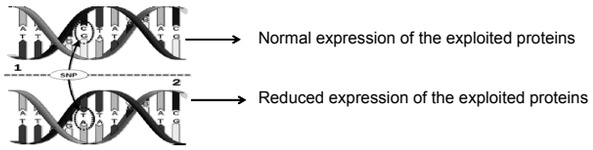




(II) Strategies to mitigate *Salmonella* in cattle

(A) Selecting for cattle that are naturally resistant

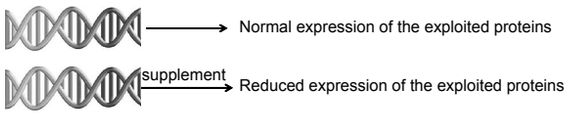
- ✓ ***Salmonella* exploits proteins within intestinal cells and macrophages during the intestinal invasion and macrophage survival processes.**
- ✓ **Minimizing these proteins will lead to a decreased susceptibility to *Salmonella* infections.**
- ✓ **Certain cattle harbor unique genes that lead to minimized expression of these proteins exploited by *Salmonella*.**



(II) Strategies to mitigate *Salmonella* in cattle

(B) Altering gene expression in cattle

- ✓ **It is possible that exogenous compounds (*e.g.*, from supplements) will reduce the expression of the proteins exploited by *Salmonella*.**
- ✓ **This phenomenon is termed an "epigenetic" change where the genetics of an animal are not altered but the gene expression pattern is transiently altered.**



(III) Newly available approaches for mitigating *Salmonella* in cattle

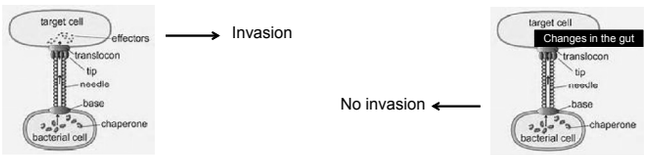
- ✓ **(A) Selecting for cattle with natural resistance- PSR Genetics LLC**
- ✓ **(B) Altering gene expression in cattle- Diamond V products**
- ✓ **(C) Altering genes in *Salmonella*- Diamond V products**
- ✓ **These cost-effective approaches mitigate *Salmonella* without instigating a biofilm, thus avoiding the potential for resistance.**

SmartCare® is registered trademark and Original XPC™ is a trademark of Diamond V Mills, Inc.

(II) Strategies to mitigate *Salmonella* in cattle

(C) Altering gene expression in *Salmonella*

- ✓ **As mentioned, *Salmonella* injects a series of proteins into the intestinal cells during the invasion process.**
- ✓ **Minimizing the expression of these proteins will lead to a decrease in *Salmonella* virulence.**
- ✓ **This specific decrease in expression is an outcome of changes in the chemical and/or microbiologic profile of the gut.**



(III) Newly available approaches for mitigating *Salmonella* in cattle

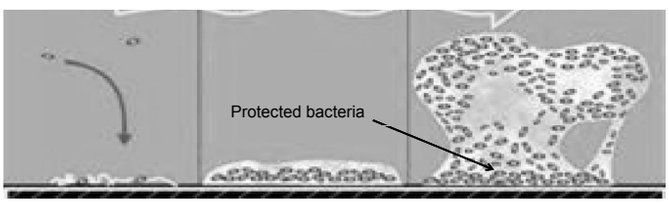
(A) Selecting for cattle that are naturally resistant

- ✓ **PSR Genetics LLC identified a cattle genotype conferring natural resistance to *Salmonella*.**
- ✓ **This genotype leads to reduced expression of the intestinal and macrophage proteins exploited by *Salmonella*.**
- ✓ **About 35% of non-black cattle possess this genotype, while 5% of black cattle have an analogous resistance-conferring genotype.**

(II) Strategies to mitigate *Salmonella* in cattle

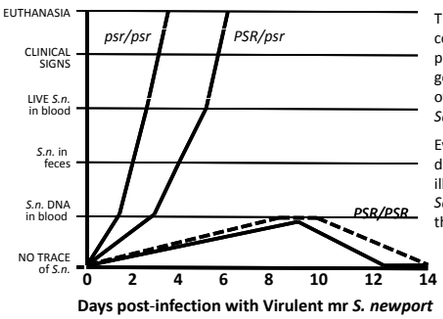
(D) Directly killing *Salmonella* with antibiotics

- ✓ **Antibiotics kill *Salmonella* by perturbing vital processes in the bacteria.**
- ✓ **Unfortunately, *Salmonella* will form a biofilm in which a subpopulation are protected from exposure to the antibiotic.**
- ✓ **Ultimately, this will lead to antibiotic resistance.**



(III) Newly available approaches for mitigating *Salmonella* in cattle

(A) Selecting for cattle that are naturally resistant

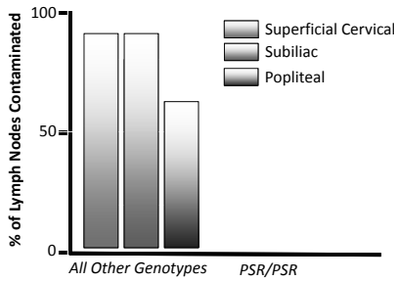


There is a direct correlation between the presence of the *PSR* gene and susceptibility of live cattle to *Salmonella* infection.

Even 10X the challenge dose could not produce illness or any shedding of *Salmonella* in the feces in these cattle.

(III) Newly available approaches for mitigating *Salmonella* in cattle

(A) Selecting for cattle that are naturally resistant



The peripheral lymph nodes of *PSR/PSR* cattle are not susceptible to contamination by two of the *Salmonella* strains implicated in this emerging problem.

(III) Newly available approaches for mitigating *Salmonella* in cattle

- ✓ **Proprietary active compounds- DV Bioactives™ that likely support immune function**
- ✓ **Available for incorporation into milk replacer (SmartCare @ 0.15%) and starter feed (Original XPC @ 3.5gm/head/day)**
- ✓ **Recent blinded studies demonstrate that these products prevent the untoward effects of *S.* Typhimurium in dairy calves on milk.**

*Product image courtesy of Diamond v®

*Product image courtesy of Diamond v®

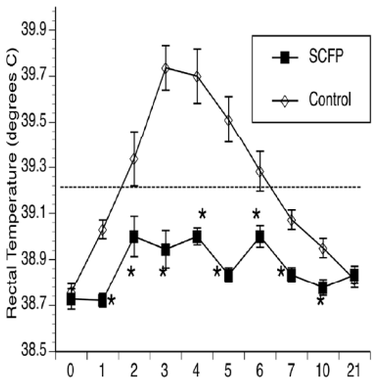
How does the *PSR/PSR* genotype inhibit *Salmonella*?

- ✓ **For non-black cattle, these animal lack one protein exploited by *Salmonella* during the invasion process and they lack one protein exploited by *Salmonella* during the macrophage survival process.**
- ✓ **For black cattle, these animals have a diminished expression of 3-6 proteins exploited by *Salmonella*.**
- ✓ **A genetic test is available for identifying and propagating cattle containing the genotype.**

PSR GENETICS

Diamond V's SmartCare & Original XPC

- ✓ **Calves were fed these products for two weeks then challenged with *S.* Typhimurium.**
- ✓ **Calves were fed these products for another three weeks during which various clinical parameters were measured.**
- ✓ **The DV Bioactives (SCFP) significantly diminished the incidence of fever.**

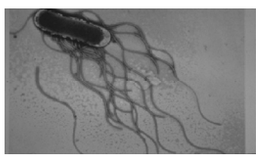


Brewer *et al.* 2014

(III) Newly available approaches for mitigating *Salmonella* in cattle

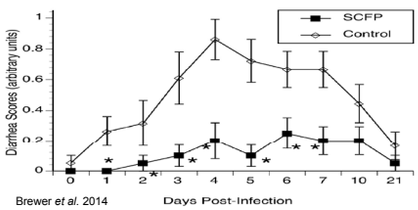
(B) Altering gene expression in cattle- Diamond V products
(C) Altering genes in *Salmonella*- Diamond V products

- ✓ **(i) Calf diarrhea (*Salmonella* and *E. coli* K99)- Diamond V SmartCare® & Original XPC™**
- ✓ **(ii) Adult salmonellosis- Prototype NaturSafe**
- ✓ **(iii) Lymph node persistence- Prototype NaturSafe**

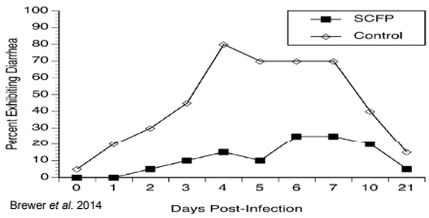


Diamond V's SmartCare & Original XPC

- ✓ **DV Bioactives significantly diminished the severity and incidence of diarrhea throughout the study.**



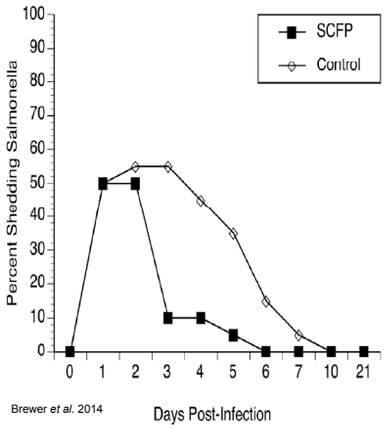
Brewer *et al.* 2014



Brewer *et al.* 2014

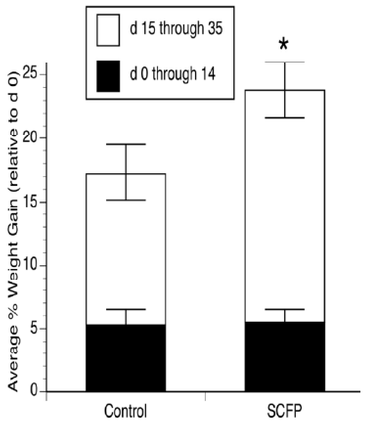
Diamond V's SmartCare & Original XPC

- ✓ **DV Bioactives significantly diminished the prevalence of calves shedding *Salmonella* in their feces.**



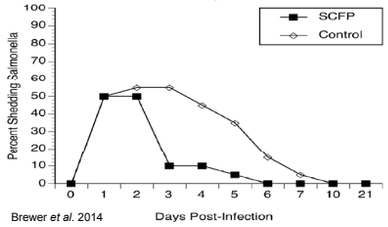
Diamond V's SmartCare & Original XPC

- ✓ **DV Bioactives significantly increased weight gain during the trial.**

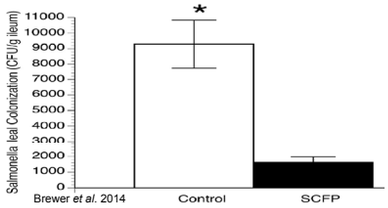


(I) Diamond V's SmartCare & Original XPC

- ✓ **DV Bioactives significantly diminished the prevalence of calves shedding *Salmonella* in their feces.**



- ✓ **Despite the finding that the DV-fed calves harbored fewer *Salmonella* in their ilea at the end of the study.**



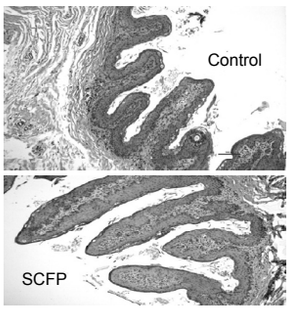
Mitigating Adult Bovine Salmonellosis

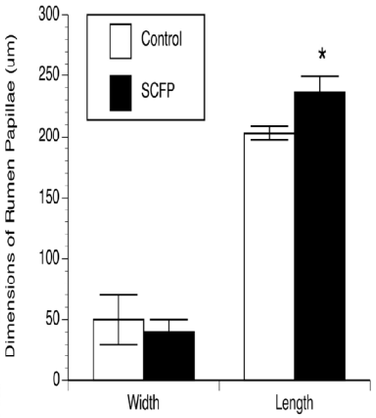
- ✓ **Diamond V is launching a "next generation" product (NutriTek®) for use in dairy cattle.**
- ✓ **Diamond V is working on a similar technology to be used in beef cattle (NaturSafe).**
- ✓ **Our investigator-blinded studies with NaturSafe reveals a protective effect against salmonellosis.**
- ✓ **N=200 animals per group; naturally infected with various *Salmonella*; Control includes an antibiotic**

NutriTek® is registered trademark of Diamond V Mills, Inc.

Diamond V's SmartCare & Original XPC

- ✓ **DV Bioactives significantly increased the length of rumen papillae.**



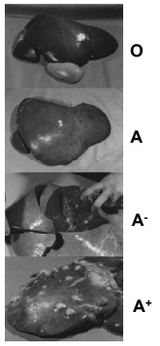


Materials and Methods

- Treatments
  - Positive Control
    - Monensin at 300 mg/head/day,
    - Tylosin at 90 mg/head/day, and
    - Direct fed microbial at 50 mg/head/d
  - NaturSafe
    - Supplemented at rate18 g/head/d
    - No monensin, tylosin, or a direct fed microbial

Materials and Methods

- Liver abscess
  - Classifications
    - With O
      - No abscesses
    - With A- or A
      - 1 to 2 small abscesses
      - Up to 2 to 4 well organized abscesses
    - With A+
      - Multiple large abscesses
      - Tissue inflammation around abscess and adhesions



Brown et al. 1975. J. Anim. Sci. 40: 207-213

Liver Abscesses

	Positive Control[1]	NaturSafe	SEM	*P*-value
Carcasses, n	740	735	--	--
Liver abscesses, %				
A -	6.9	3.3	0.93	0.02
A	5.0	3.5	1.06	0.35
A +	7.4	7.7	1.19	0.81
Total Condemned	19.3	14.5	1.92	0.11

[1]Positive Control contains monensin, tylosin, and a direct fed microbial. These are not included in diet of DV Prototype.

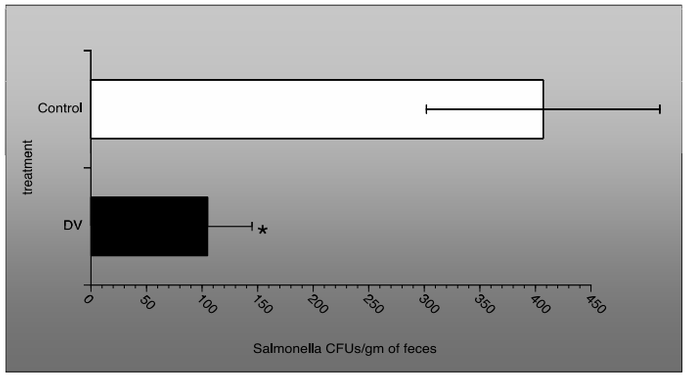
Overview

	Positive Control[1]	NaturSafe
Heifers, n	748	747
Pens, n	10	10
Days on feed	136	136
Bunk space, in/head	14.4	14.4
Pen space, ft²/head	231	231

[1]Positive Control contains monensin, tylosin, and a direct fed microbial. These are not included in diet of DV Prototype.

Mitigating Adult Bovine Salmonellosis

✓ **NaturSafe led to a decrease in the presence of *Salmonella* in the feces.**

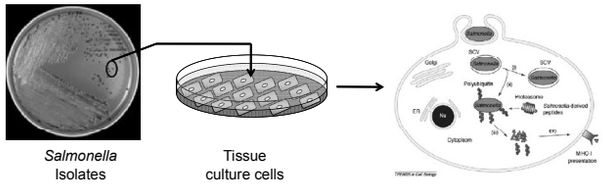


Performance

	Positive Control[1]	DV Prototype	SEM	*P*-value
Body weight, lb				
Initial	790	793	7.50	0.33
Final[2]	1,278	1,280	7.10	0.80
ADG, lb/day	3.61	3.59	0.043	0.73
DMI, lb/day	22.7	23.0	0.33	0.09
Feed efficiency (DMC)	6.29	6.40	0.074	0.16
Yield adjusted				
Final BW, lb[3]	1,266	1,269	9.30	0.64
ADG, lb/day	3.51	3.51	0.041	0.94
Feed efficiency	6.46	6.57	0.122	0.20

[1]Positive Control contains monensin, tylosin, and a direct fed microbial. These are not included in diet of DV Prototype;
[2]Final BW shrunk 4%; [3]Yield adjusted BW calculated by dividing HCW by a common dressing yield of 63.75.

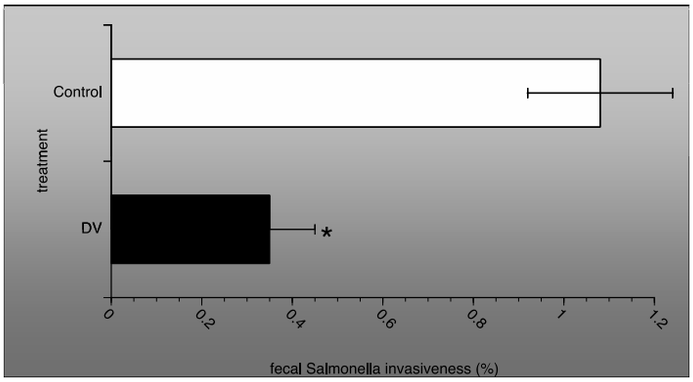
Salmonella invasion assays



Recover and count *Salmonella* in cells

Mitigating Adult Bovine Salmonellosis

- ✓ **NaturSafe led to a decrease in the invasiveness of *Salmonella* recovered from feces.**

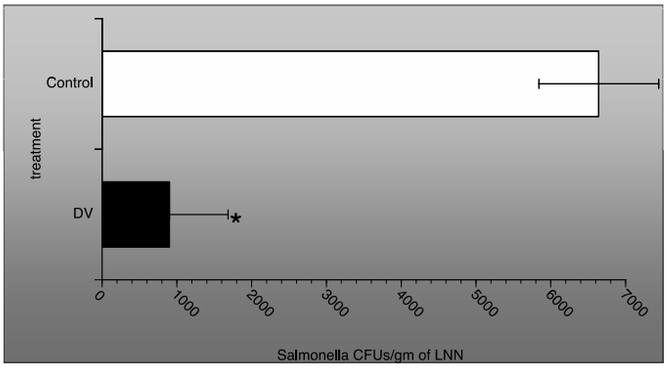


How are the Diamond V products reducing the presence of *Salmonella*?

- ✓ **Studies from other species reveal that the Diamond V products rebalance the immune system, by epigenetically activating gene expression events.**
- ✓ **This rebalancing allows the immune system to appropriately and efficiently respond to pathogens.**
- ✓ **This likely facilitates an enhanced killing of *Salmonella* within macrophages.**
- ✓ **Since *Salmonella* cannot form biofilms within macrophages, the chance for resistance is minimal.**

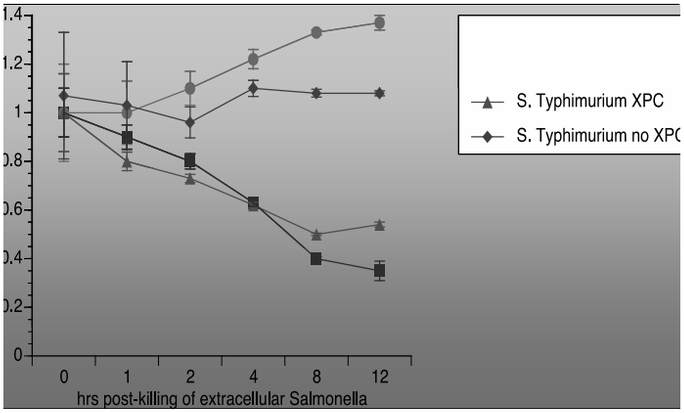
Mitigating Lymph Node Infiltration

- ✓ **NaturSafe led to a decreased load of *Salmonella* in subiliac lymph nodes.**



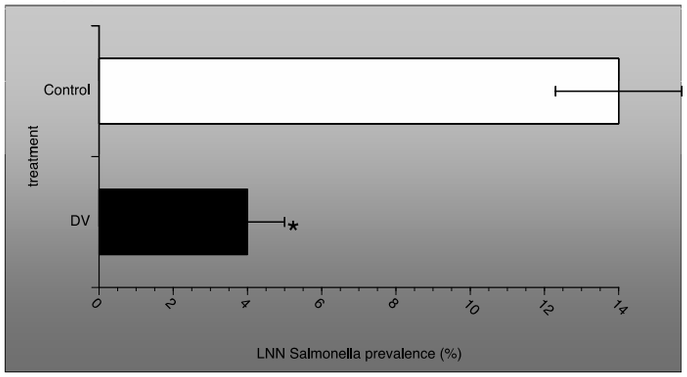
Diamond V's SmartCare & Original XPC

White blood cells from XPC-fed calves were more efficient at killing *S.* Dublin and *S.* Typhimurium.



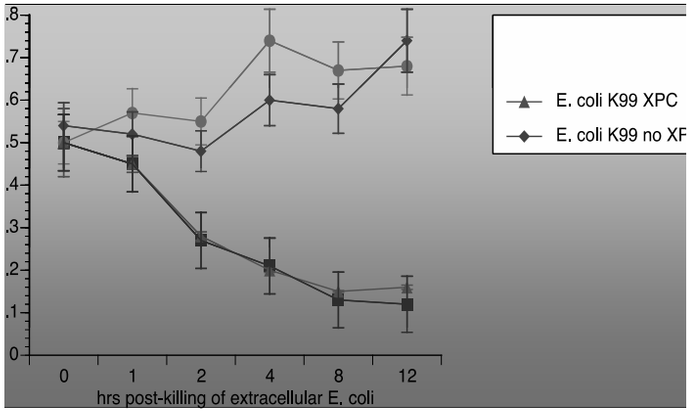
Mitigating Lymph Node Infiltration

- ✓ **NaturSafe led to a decreased prevalence of *Salmonella* in subiliac lymph nodes.**



Diamond V's SmartCare & Original XPC

White blood cells from XPC-fed calves were more efficient at killing *E. coli* K88 and K99.



How are the Diamond V products inhibiting the invasiveness (and thus virulence) of *Salmonella*?

- ✓ **Diamond V Bioactives alter the ruminal and intestinal microbiomes.**
- ✓ **The microbial alterations lead to epigenetic changes in *Salmonella*, altering its invasiveness and virulence.**
- ✓ **Our studies revealed that multiple Diamond V products are capable of inhibiting the expression of the major *Salmonella* invasion-regulating gene designated as *hilA*.**

(IV) Cost Effectiveness of the New Mitigation Approaches for *Salmonella* in Cattle

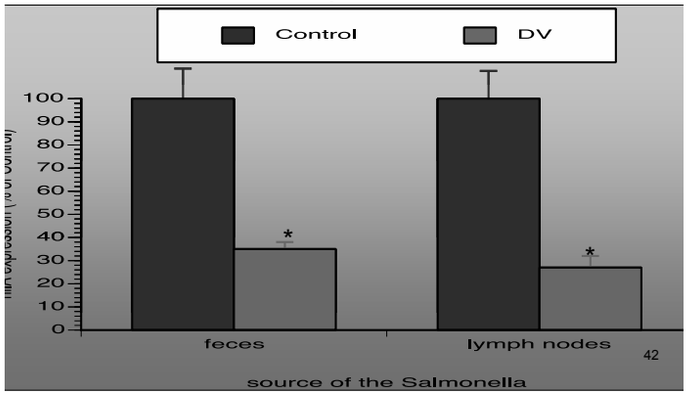
- ✓ **The *PSR* gene appears to be correlated with enhanced production benefits.**
- ✓ **Cattle with the *PSR* gene are healthier than other cattle.**
- ✓ ***PSR/PSR* cattle ranked the best in regards to marbling, average daily gain, longevity, and fertility.**

Genotype	Marbling	Daily Gain	Longevity	Fertility
PSR/PSR	1st	1st	1st	1st
PSR/psr	2nd	2nd	2nd	2nd
psr/psr	3rd	3rd	3rd	3rd

PSR GENETICS

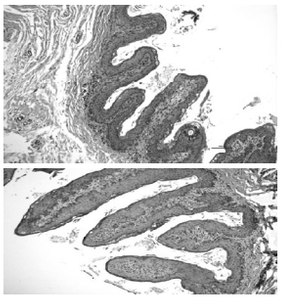
hilA expression of *Salmonella* recovered from cattle

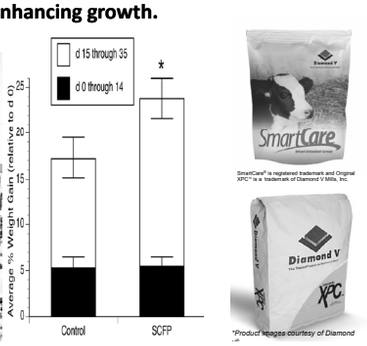
- ✓ **The DV-mediated decrease in *Salmonella* invasion appears to be due to a repression of *hilA* expression.**



(IV) Cost Effectiveness of the New Mitigation Approaches for *Salmonella* in Cattle

- ✓ **SmartCare and Original XPC are useful antibiotic-free feed and milk replacer additives for combating salmonellosis in pre-weaned dairy calves, while also enhancing growth.**





SmartCare® is registered trademark and Original XPC™ is a trademark of Diamond V Mills, Inc.

*Product images courtesy of Diamond V

Anti-Resistance Properties of the New Approaches for *Salmonella* in Cattle

- ✓ **Cattle lacking a protein exploited by *Salmonella*- the bacteria will simply go elsewhere.**
- ✓ **Killing *Salmonella* in macrophages where biofilms cannot exist- no chance for resistance.**
- ✓ **Inhibiting virulence- not adverse for *Salmonella* since it has the option to adopt or not adopt the pathogenic lifestyle; no resistance response.**

PSR GENETICS

*Product image courtesy of Diamond V®

(IV) Cost Effectiveness of the New Mitigation Approaches for *Salmonella* in Cattle

- ✓ **NaturSafe has similar anti-*Salmonella* properties in adult cattle.**

	Positive Control[1]	NaturSafe	SEM	*P*-value
Carcasses, n	740	735	--	--
Liver abscesses, %				
A -	6.9	3.3	0.93	0.02
A	5.0	3.5	1.06	0.35
A +	7.4	7.7	1.19	0.81
Total Condemned	19.3	14.5	1.92	0.11

(v) Summary of the New Mitigation Approaches for *Salmonella* in Cattle

- ✓ **The *PSR* gene is a cost-effective selection tool for *Salmonella*-resistant cattle.**
- ✓ **SmartCare and Original XPC are useful antibiotic-free feed and milk replacer additives for combating salmonellosis in pre-weaned dairy calves, while also enhancing production.**
- ✓ **NaturSafe has similar properties and is for use in beef cattle.**
- ✓ **NutriTek is the adult dairy-specific version of the aforementioned products and, given its relatedness to these products, has the same production and anti-pathogen traits.**

*Product image courtesy of Diamond v®

PSR GENETICS

Now that you've heard the science, how do you apply it to your practical feeding situations?

By Bill Sanchez, PhD., Dipl. ACAN,
Diamond V; bsanchez@diamondv.com

This presentation will start with an applied summary of the previous speakers talks. I will discuss how to apply this science to the application of two new products from Diamond V, SmartCare® and NutriTek®. Two key objectives will be covered.

1. Delivering Diamond V's new fermentation metabolites (we call DV Bioactives™) into the dairy calf. These new products provide the opportunity of delivering these Bioactives earlier in the diet of the newborn calf as well as including them in the starter and weaning diet. We will look at the in vivo studies that form the foundation of our recommendations and include a summary of commercial farm experiences.
2. I'll then take you through the application of feeding NutriTek from close-up to dry off. We will compare and contrast research from transition only, early lactation and post-peak feeding. I will share some new information we discovered on the timing of the response, how these DV Bioactives affect the microbiome of the gut, and how the anti-inflammatory compounds within this product affect the immune system. Finally, I'll summarize the on-farm controlled trials and long-term feeding experiences we have on the product.

Immunology and the Diamond V Experience

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Traditionally Diamond V products have been known as nutritional additives. Over the last 15 years the company have been exploring the effects of these products on the immune system, and the resultant impacts on animal health. In this presentation this history will be discussed, and the advances in experimental methods that have taken place over this period of time will be mentioned. The impact of these studies on understanding the mode of action of Diamond V products on health and safety of finished products will be demonstrated, as will the pending patent on pre-harvest food safety.

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 (uNDF240) 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 ($uNDF_{240}$) 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 a 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 kd=0 and the potentially digestible NDF (pdNDF) has a kd that varies among feeds. The equation for the 2-pool model of NDF digestion is:

$uNDF_{(t)} = pdNDF*\exp^{(-kd*[t - lag])} + iNDF_2$; 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, iNDF2 is reliably estimated by $uNDF_{72}$, which was measured after 72-h of fermentation (Smith et al., 1972).

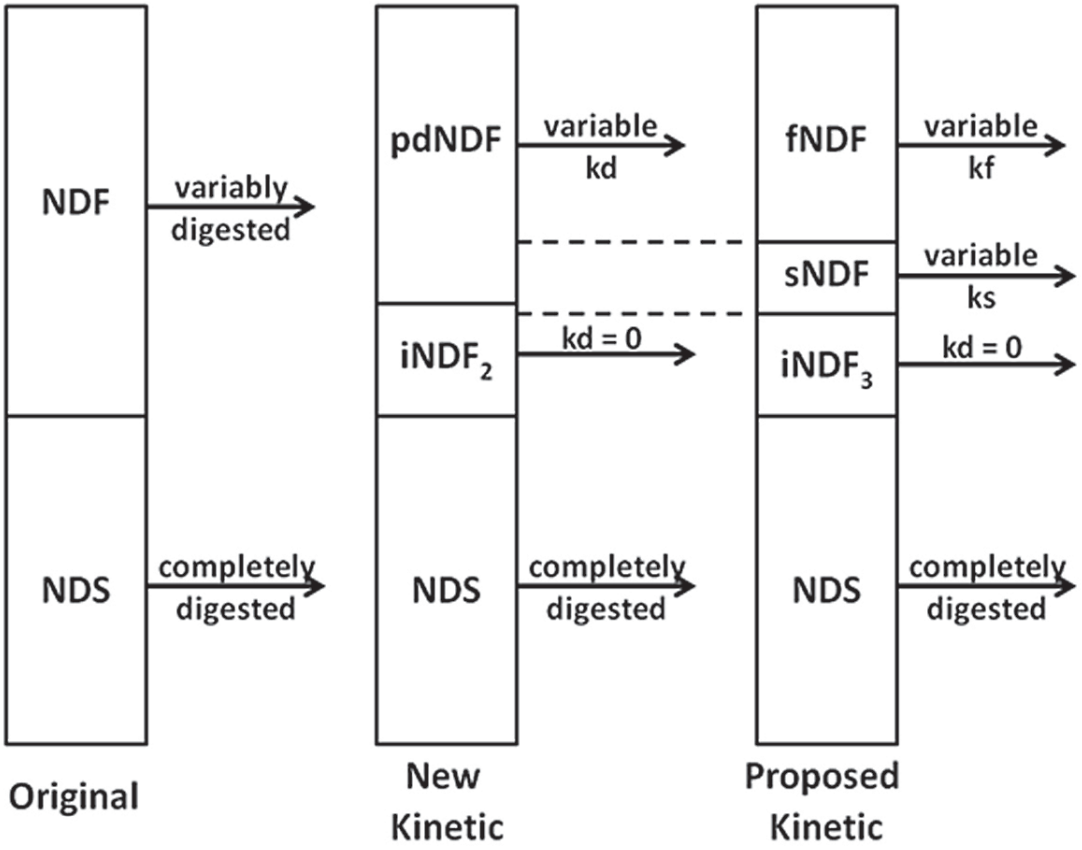


Figure 1. Illustration of the changes in modeling feed digestibility based on NDF (NDS = neutral detergent solubles, pdNDF = potentially digestible NDF, iNDF = indigestible NDF, fNDF = fast-digestion NDF, sNDF = slow-digesting NDF and k = fractional rate for each pool).

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 $uNDF_{240}$ 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 $iNDF_3$ is the indigestible NDF in a 3-pool model. Note that iNDF is a hypothetical pool defined by the model and that $iNDF_2$ and $iNDF_3$ are estimated by different uNDF ($uNDF_{72}$ and $uNDF_{240}$, 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 NDFD24 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 uNDF72, which was used to estimate iNDF2, 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 % 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 $uNDF_{240}$ or non-lignin characteristics of cell walls can affect NDF indigestibility. Mertens (2016) observed that the relationship between $NDFD_{30}$ and $uNDFOM_{240}$ (as a fraction of NDF) was better than that between $NDFD_{30}$ 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 $NDFD_{48}$, 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 uNDF48 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 in DM) = pdNDF_pool % kd + pdNDF_pool % kp, solving for ruminal pdNDF_pool,

pdNDF_pool = [DMI/h % (pdNDF in DM)] / (kd + kp) and

DMI/h % ($iNDF_2$ in DM) = $iNDF_2$_pool % kp, solving for ruminal $iNDF_2$_pool,

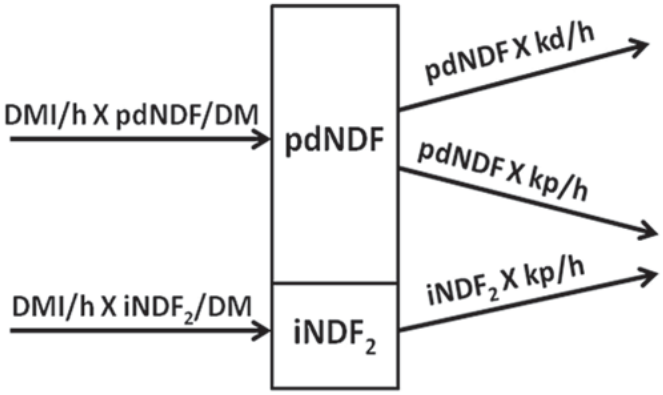
iNDF2_pool = [DMI/h % ($iNDF_2$ in DM)] / kp.

Alternatively, if we measure intakes of fiber fractions and measure pools by emptying rumens, we can rearrange the equations to solve for rates of digestion 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 in DM) / (NDF in DM)] % [kd/(kd + kp)]

= [(NDF in DM) - ($iNDF_2$ in DM)] / (NDF 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 iNDF2 (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 $iNDFOM_2$ 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 $iNDFOM_2$. For comparison, Oba and Allen (2003) reported ruminal pools of 6.71, 4.57, and 11.28 lb for $uNDF_{120}$, 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 $uNDF_{240}$, 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 $uNDF_{120}$ or $uNDF_{240}$ to estimate iNDF, which are smaller than the $iNDF_2$ of the 2-pool model (Figure 1, new kinetic model) that is estimated most appropriately by $uNDF_{72}$. 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 (iNDF2). The base ration is defined in the text.

Model variables	Base Ration	Decr NDF	Incr pdNDF	Incr kp	Incr Kd	Incr $iNDF_2$
Model inputs						
Ration aNDFOM (% DM)	28.32	**25.49**	28.32	28.32	28.32	28.32
Ration $iNDFOM_2$ (% 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
Model responses						
Rumen Pool $iNDFOM_2$ (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
$iNDFOM_2$ 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

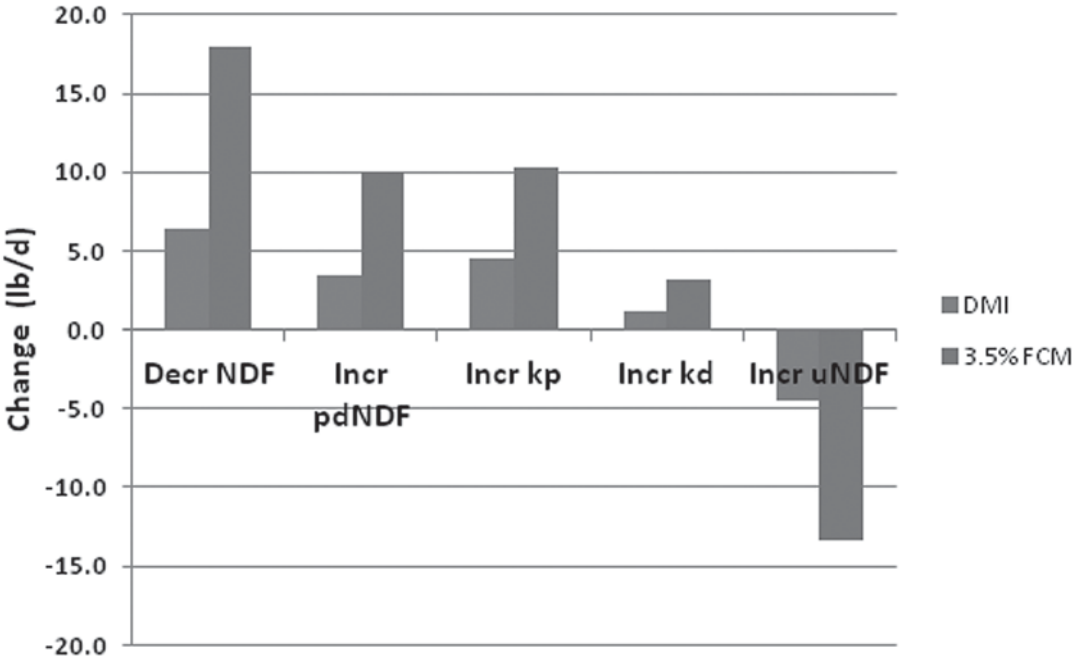


Figure 3. Changes in intake and 3.5% FCM when intake is adjusted to obtain the same total ruminal load (NDFOM pool) as the model base diet.

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

HEAT STRESS ETIOLOGY

Mechanisms responsible for altered nutrient partitioning during HS are not clear; however, they might be mediated by HS effects on gastrointestinal health and function as we and others have demonstrated HS compromised intestinal barrier function (Lambert et al., 2002; Dokladny et al., 2006; Pearce et al., 2013; Sanz-Fernandez et al., 2014). During HS, blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat leading to intestinal hypoxia (Hall et al., 1999). Enterocytes are particularly sensitive to hypoxia and nutrient restriction (Rollwagen et al., 2006), resulting in ATP 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 (Ceciliani et al., 2012). For a detailed description of how livestock and other species detoxify LPS see our recent review (Mani et al., 2012). Endotoxin infiltration during HS into the bloodstream which was first observed by Graber et al. (1971), is common among heat stroke patients (Leon, 2007) and is thought to play a central role in heat stroke pathophysiology as survival increases when intestinal bacterial load is reduced or when plasma LPS is neutralized (Bynum et al., 1979; Gathiram et al., 1987). It is remarkable how animals suffering from heat stroke or severe endotoxemia share many physiological and metabolic similarities to HS, such as an increase in circulating insulin (Lim et al., 2007). Infusing LPS into the mammary gland increased (~2 fold) circulating insulin in lactating cows (Waldron et al., 2006). In addition, we intravenously infused LPS into growing calves and pigs and demonstrated >10 fold increase in circulating insulin (Rhoads et al., 2009; Stoakes et al., 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 (Loor et al., 2005; Bertoni et al., 2008), and this assumption was recently reinforced when TNFα infusion decreased productivity (albeit without overt changes in metabolism; Yuan et al., 2013; Martel et al., 2014). Additionally, in late-lactation cows, injecting TNFα increased (>100%) liver TAG content without a change in circulating NEFA (Bradford et al., 2009). Our recent data demonstrates increased inflammatory markers in cows diagnosed with ketosis only and no other health disorders. In comparison with healthy controls, ketotic cows had increased circulating LPS prior to calving and 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.

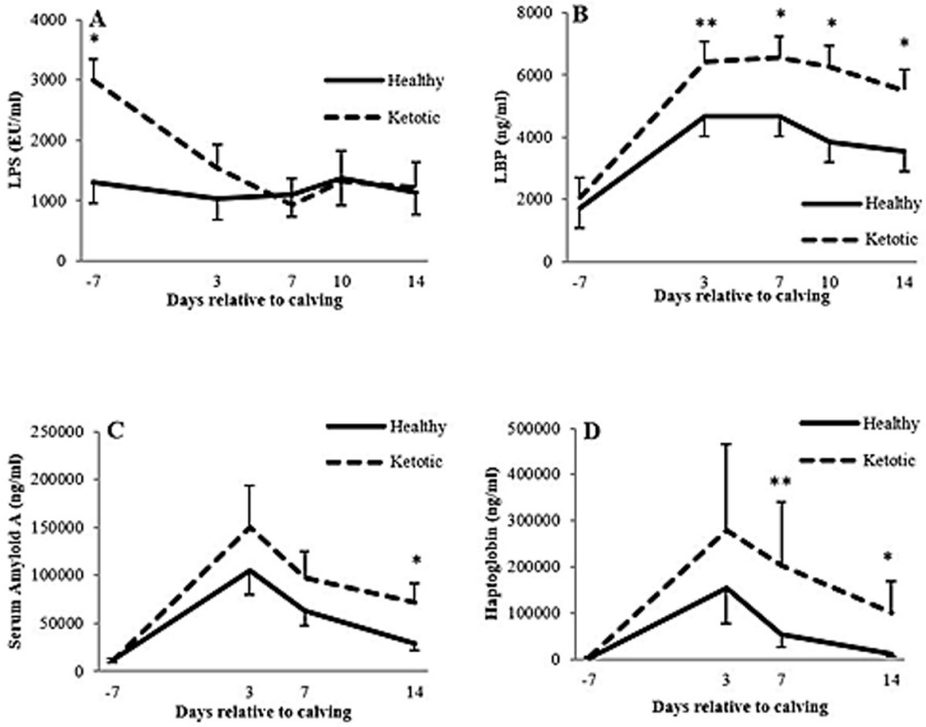

Figure 1. Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows.

METABOLISM OF INFLAMMATION

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

Endotoxin has previously been recognized to be involved with metabolic dysfunction. In humans, both obesity and high fat diets are linked to endotoxemia (Cani et al., 2007, Gregor and Hotamisligil, 2011). Furthermore, LPS is involved with the development of fatty liver (Ilan, 2012), and cytokines are linked to lipid accumulation and cholesterol retention (Ma et al., 2008; Clément et al., 2008). Experimentally-induced endotoxemia in dairy cattle has been linked to several metabolic and endocrine disturbances including decreased circulating glucose, termination of pregnancy, leukopenia, disruption of ruminal metabolism, and altered calcium homeostasis (Griel et al., 1975; Giri et al., 1990; Waldron et al., 2003; Jing et al., 2014). The aforementioned pathological conditions are likely mediated by LPS-induced inflammation and the subsequent changes in nutrient partitioning (Fig. 2) caused by immune system activation.

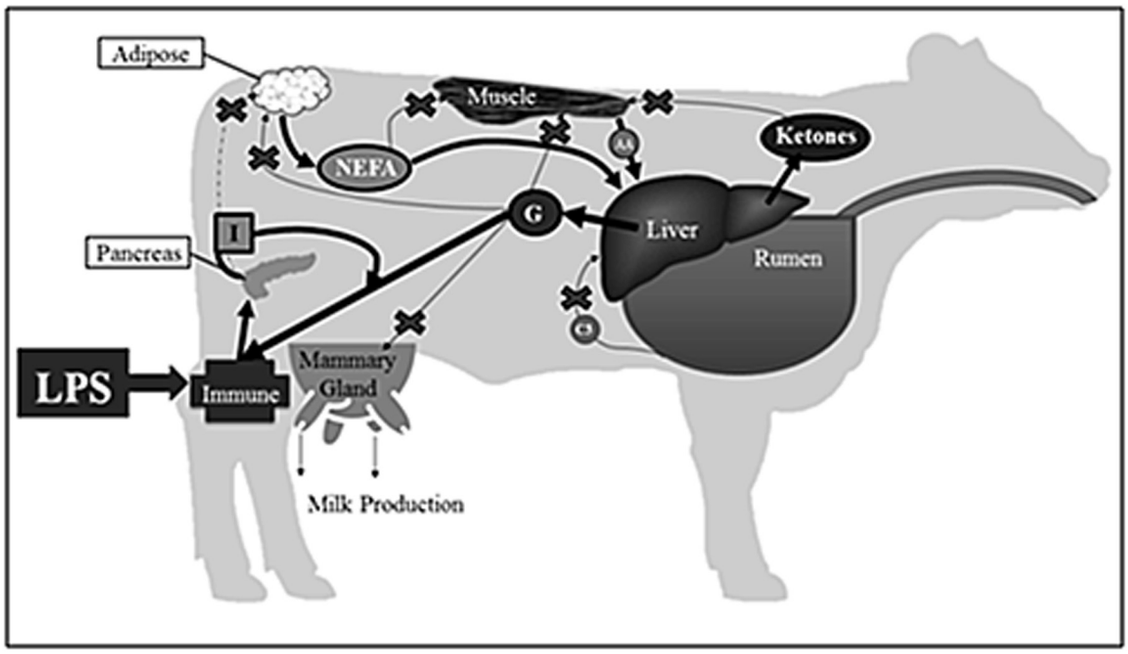

Figure 2. LPS induced alterations in glucose metabolism and insulin sensitivity.

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 $BW^{0.75}$/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 (R2 = 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 ± 88 microns (Range = 630 - 865 microns), 3 as "Medium" with a MPS of 1,220 ± 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 Powel-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-ruminally 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 ± 233 microns (Range = 405 to 1379 microns) and 92.7 ± 20.8 cm^2/g (Range = 50 to 139 cm^2/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% ± 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_3 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_3 hybrids. The second experiment (Ferraretto et al., 2015a) compared 3 hybrids (bm_3, 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_3 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 28th 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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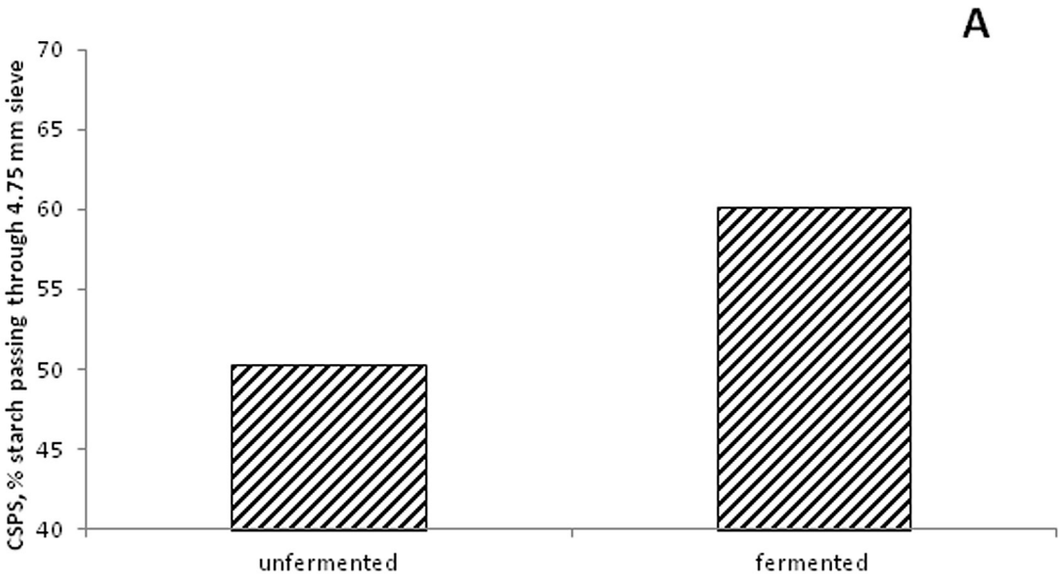

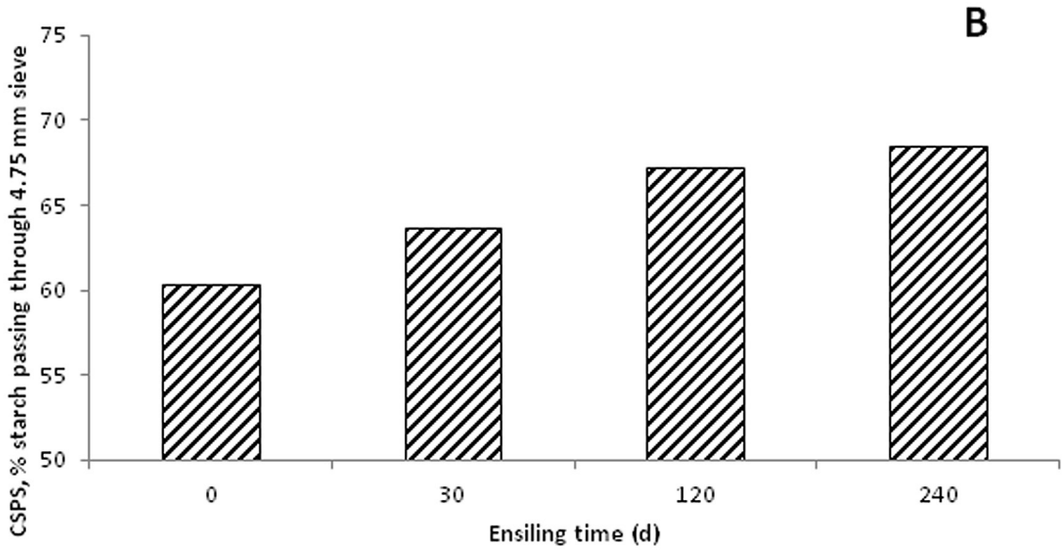

Figure 1. A. Effect of ensiling on corn silage processing score (CSPS) of whole-plant corn silage; n = 12, SEM = 3.1, P = 0.01. B. Effect of ensiling time on corn silage processing score (CSPS) of whole-plant corn silage; n = 3, SEM = 2.0, P = 0.08. Source: Ferraretto et al., 2015b.

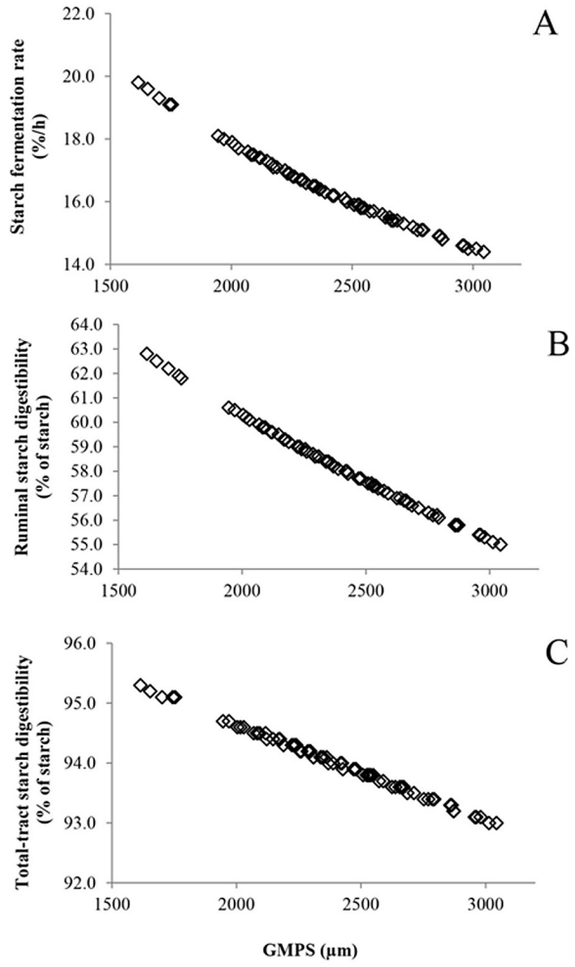

Figure 2. Simulations (Dias Junior et al., 2016) of the effect of kernel fraction geometric mean particle size (µm) on starch fermentation rate (%/h; A) and ruminal and total-tract starch digestibilities (% of starch; B and C, respectively) performed using the Feed Grain V2.0 Evaluation System (Hoffman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

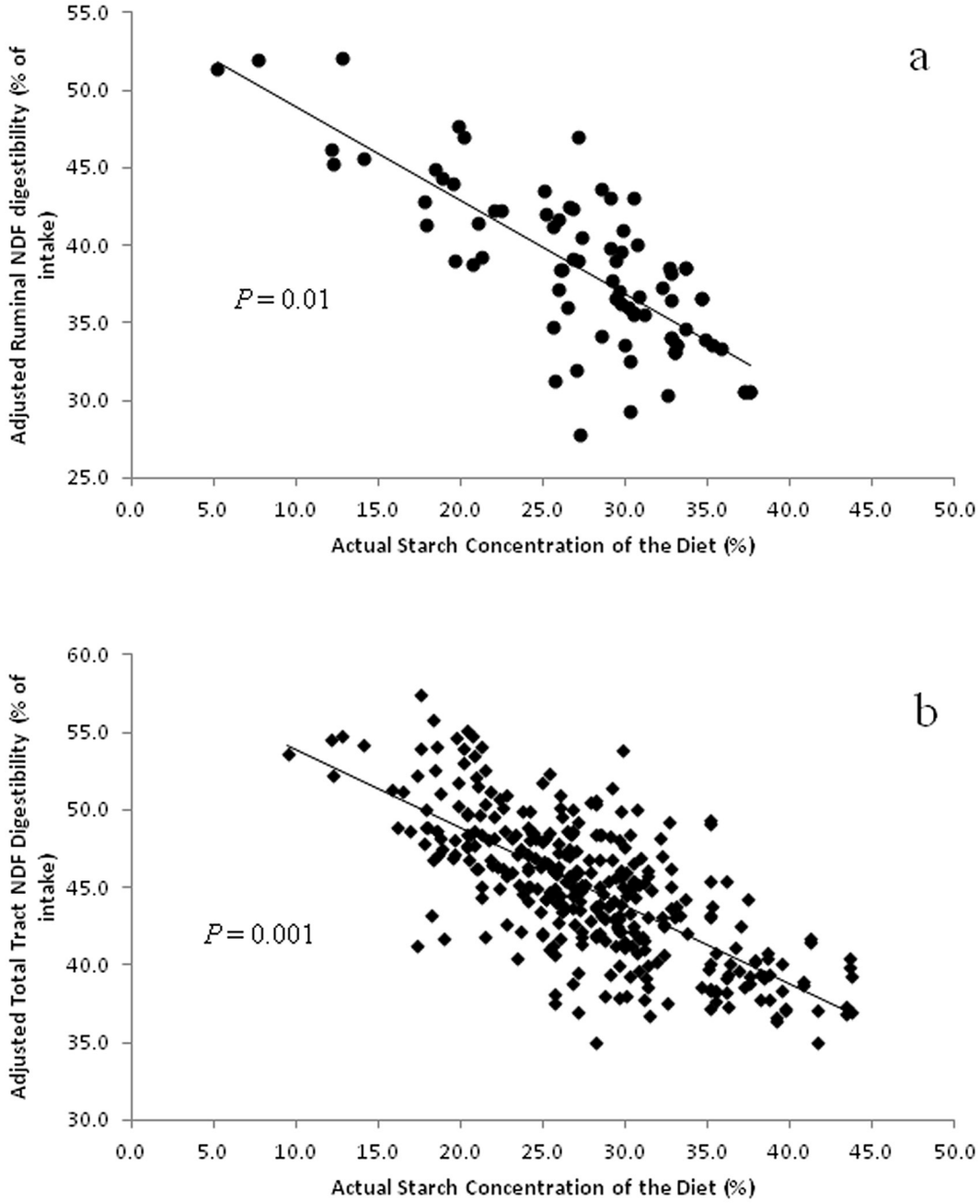

Figure 3. Effect of starch concentration of the diet on ruminal and total-tract digestibility of diet NDF adjusted for the random effect of trial. Ruminal digestibility data (Panel a) predicted from equation: y = 54.9746 + (-0.605*starch concentration) + (0.063 + 3.524); n = 70, RMSE = 3.55. Total-tract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

	Year		
Variable	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 ouput/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[a]	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

[a]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.

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 protoleolysis 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.

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 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).

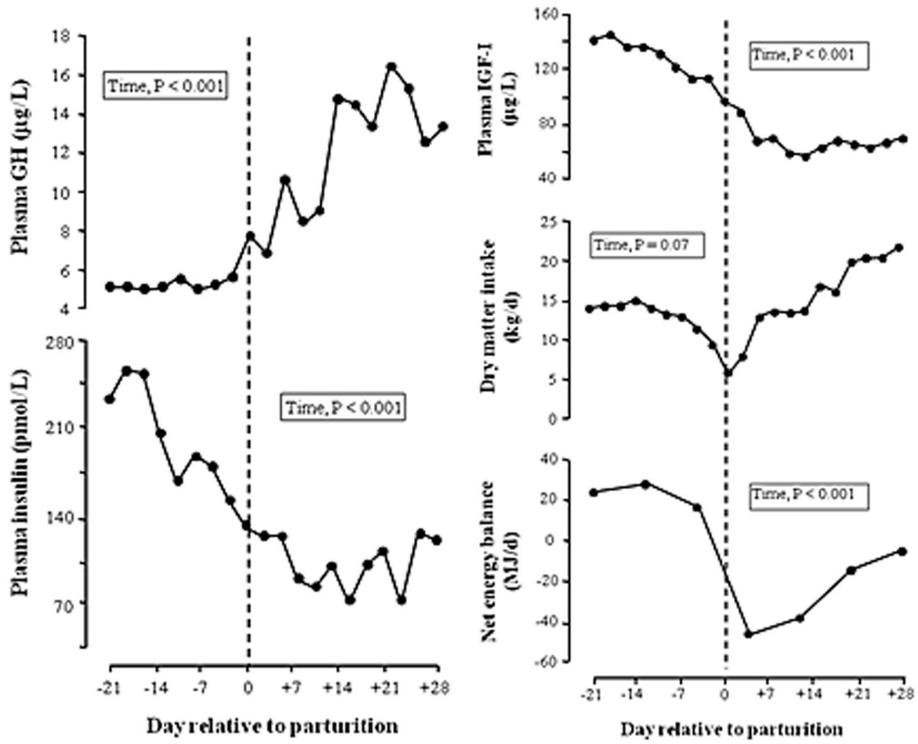

Figure 1. Temporal pattern of whole-animal energetics and key hormones responsible for nutrient partitioning in transitioning lactating Holstein cows.

During NEBAL, somatotropin promotes non-esterified fatty acids (NEFA) export from adipose tissue by accentuating the lipolytic response to β-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.

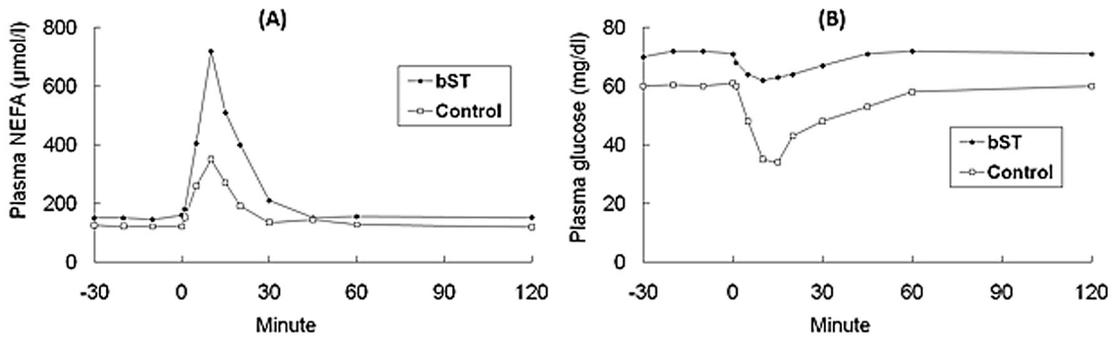

Figure 2. Effects of rbST on (A) the non-esterified fatty acid (NEFA) response to an epinephrine challenge and (B) the glucose response to an insulin tolerance test in lactating Holstein cows. Adapted from Sechen et al., 1990.

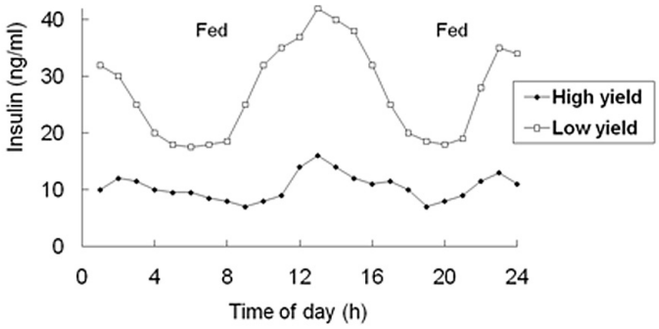

Figure 3. Plasma insulin levels in high and low yielding dairy cows. Adapted from Bines and Hart (1982).

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-lacting 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 represent 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).

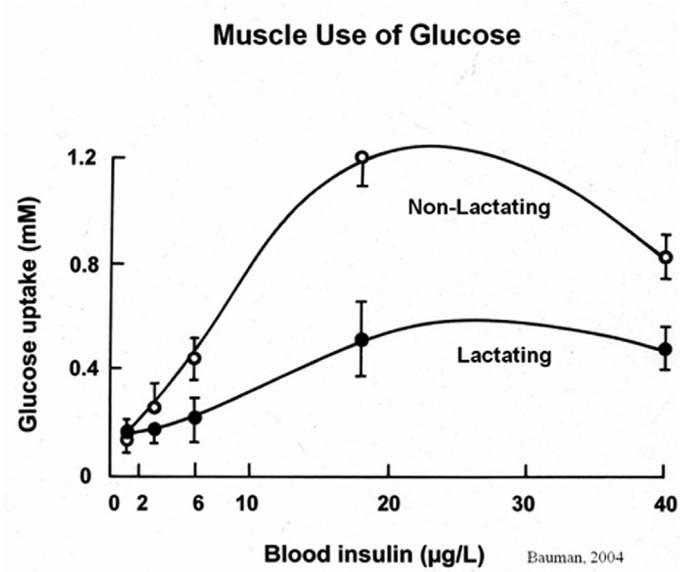

Figure 4. Effects of physiological state on insulin action in skeletal muscle. Adapted from Bauman, 2004.

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.

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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 attending 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[1]	HF	SBS	TMR	SEM	*P*-value
Body weight, lb					
d 28[2]	398.4	388.3	389.0	5.67	0.36
d 133	607.3[a]	572.4[b]	576.4[b]	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[a]	1.83[b]	1.85[b]	0.064	0.06
d 0 to 133	2.09[a]	1.90[b]	1.87[b]	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[a]	15.7[b]	15.6[b]	0.19	<0.01
d 0 to 133	14.9[a]	14.0[b]	14.1[b]	0.16	<0.01
Feed efficiency[3]					
d 0 to 28	0.252[a]	0.246[a]	0.205[b]	0.014	0.06
d 29 to 133	0.123	0.116	0.117	0.003	0.41
d 0 to 133	0.151[a]	0.145[abx]	0.137[by]	0.003	0.03

[1]HF = hay feeder; SBS = side-by-side; TMR = total mixed ration; SEM = standard error of the mean.
[2]Day of study.
[3]Feed efficiency expressed as lb of ADG per lb of daily DMI.
[ab]Means differ at $P < 0.05$ level.
[xy]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[1]	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

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

[1]Grain:forage ratio.

[2]Day of study.

[3]Feed efficiency expressed as lb of ADG per lb of daily DM intake.

[abc]Means with differing superscripts are significantly different at $P \leq 0.05$ level.

[xy]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.

	CONV		HIGH			*P*-value[1]		
Item	HNFC	LNFC	HNFC	LNFC	SEM	MILK	GRWR	MILK × GRWR
Body weight, lb								
28 wk[2]	516.4[a]	503.0[ab]	522.1[a]	494.8[b]	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[ab]	13.9[ab,x]	14.1[a]	13.7[b,y]	0.10	0.85	0.15	0.08

[1]MILK = effect of pre-weaning milk treatment; GRWR = effect of post-weaning diet; MILK × GRWR = interaction of milk treatment vs. post-weaning diet effects.
[2]Weeks of age.
[ab]Means with differing superscripts significantly differ at $P \leq 0.05$ level.
[xy]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 (Coblentz et al., 2010a), and the NDF concentration by mid-August is about 75 to 80% (Coblentz 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; Coblentz 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 (Coblentz 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 disbursals were adjusted to allow for ad-libutum 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).

	Blended Diet[1]				
Item	EGG0	EGG9	EGG18	EGG27	Limit-Fed
Ingredients, % of DM					
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
Nutrients[2]					
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
Intake[3]					
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
Performance					
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

[1] 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.
[2] Expressed as % of DM, unless otherwise indicated.
[3] 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; Coblentz et al., 2015).

	Diet[1]			
Item	Control	EGG	Wheat Straw	Corn Fodder
Ingredients, % of DM				
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
Corn Fodder	0	0	0	14.9
Nutrients[2]				
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
Intake[3]				
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
Performance				
Gain, lbs	309	258	209	256
ADG, lbs/d	2.56	2.16	1.74	2.14
CV, %[4]	10.4	11.5	14.4	15.5
Feed:Gain, lbs/lbs	9.6	10.8	12.1	10.5

[1] 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.
[2] Expressed as % of DM, unless otherwise indicated.
[3] Expressed as lbs/d, unless otherwise indicated.
[4] 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; Coblentz et al., unpublished).

	Diets		Stocking Rate[1]		
Item	Well-Processed Straw	Poorly-Processed Straw	100%	125%	150%
Performance					
Gain, lbs	183	184	189	181	181
ADG, lbs/d	2.02	2.02	2.07	1.99	1.99
CV, %[2]	18.7	14.3	13.3	17.3	18.9
Behavior					
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
Hygiene Score[3]					
Flanks	1.9	2.1	1.7	2.0	2.2
CV[2], %	32.5	26.2	18.0	30.6	39.5
Legs	2.2	2.5	2.1	2.4	2.5
CV[2], %	13.6	15.0	9.4	13.9	19.7

[1] 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.
[2] Coefficient of variation (%) for within-pen total gain or ADG, hygiene of flanks, and hygiene of legs.
[3] Lying and standing behaviors determined by data logger (HOBO Pendant® G Acceleration Data Logger; Onset Computer Corp., Bourne, MA), as calculated per Ledgerwood et al. (2010).
[4] 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.

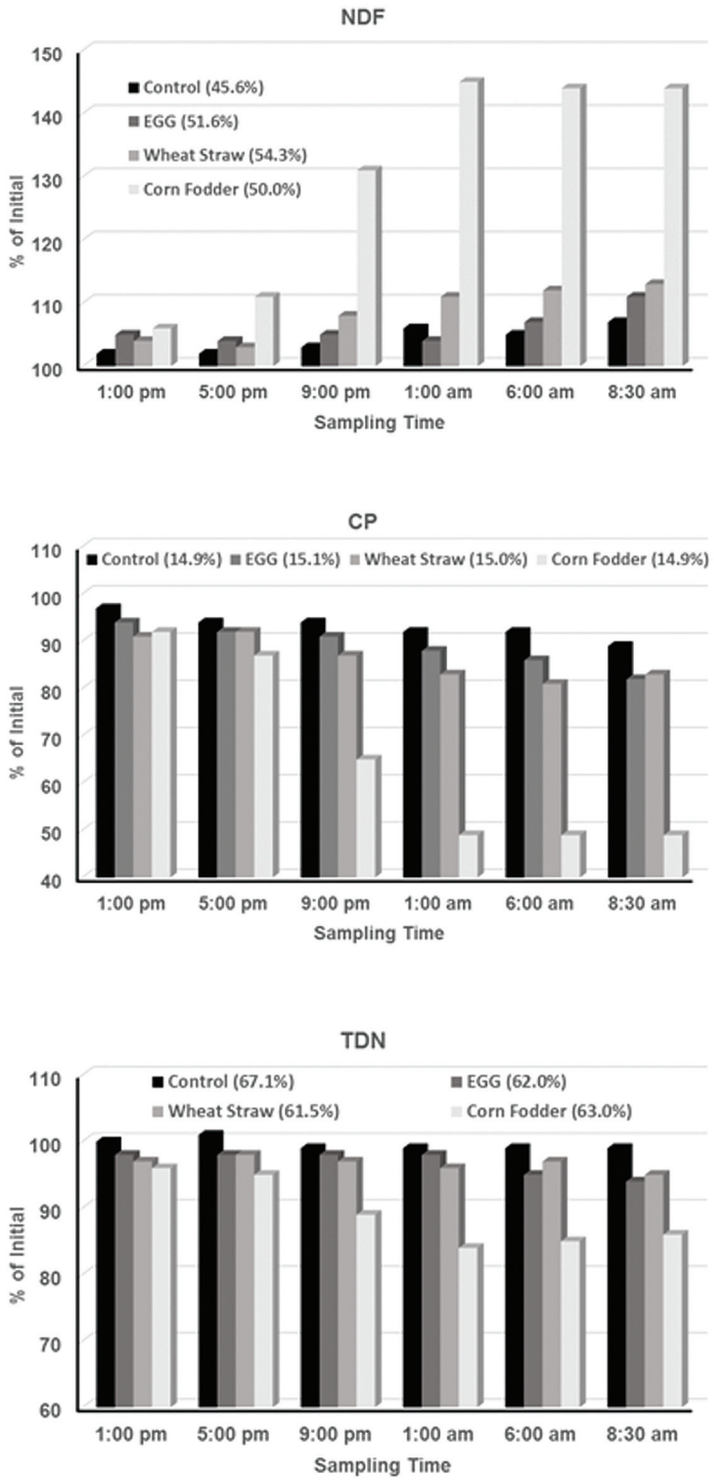
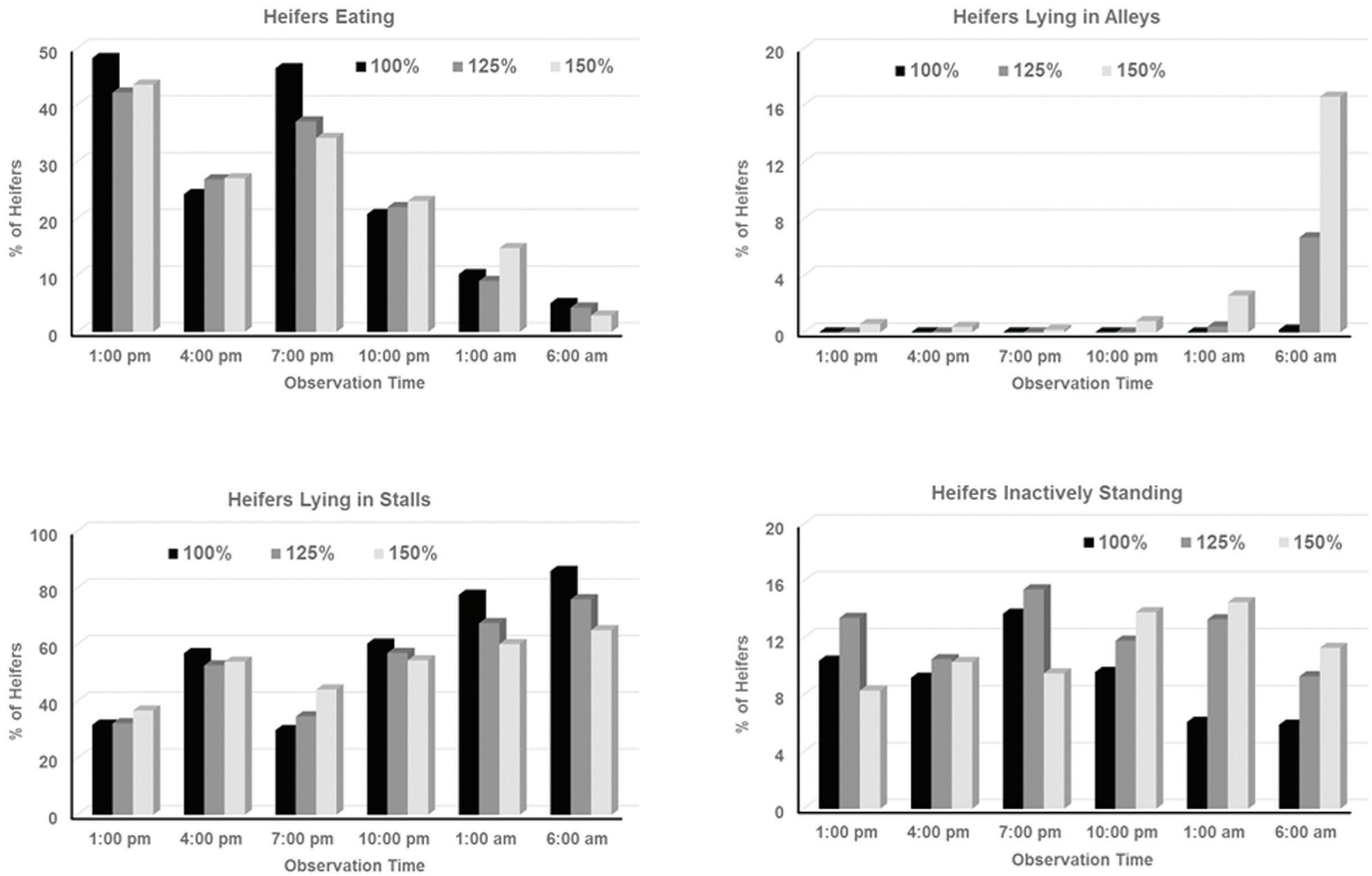


Figure 2. Eating and resting behaviors by 900-lb Holstein dairy heifers at 100, 125, and 150% of stocking capacity in freestall housing (Experiment 3).



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 to1 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.

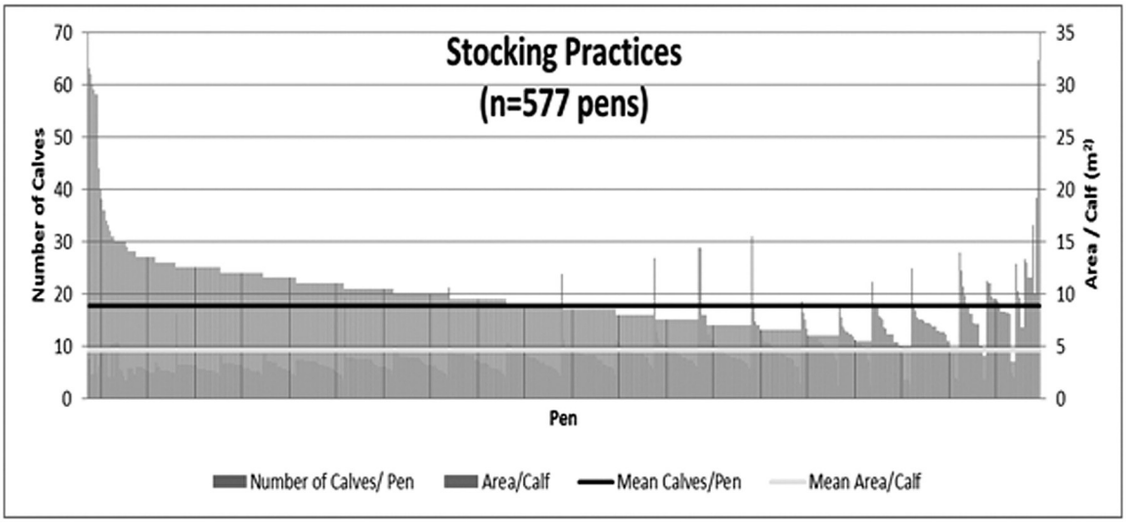

Figure 1. Stocking density as number of calves per pen and area per calf

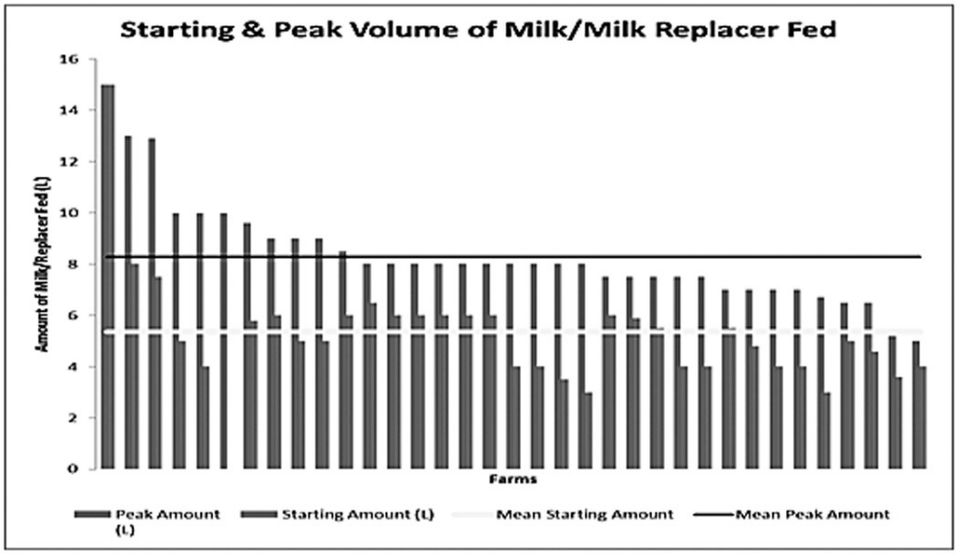

Figure 2. Starting and peak amounts of milk/milk replacer fed

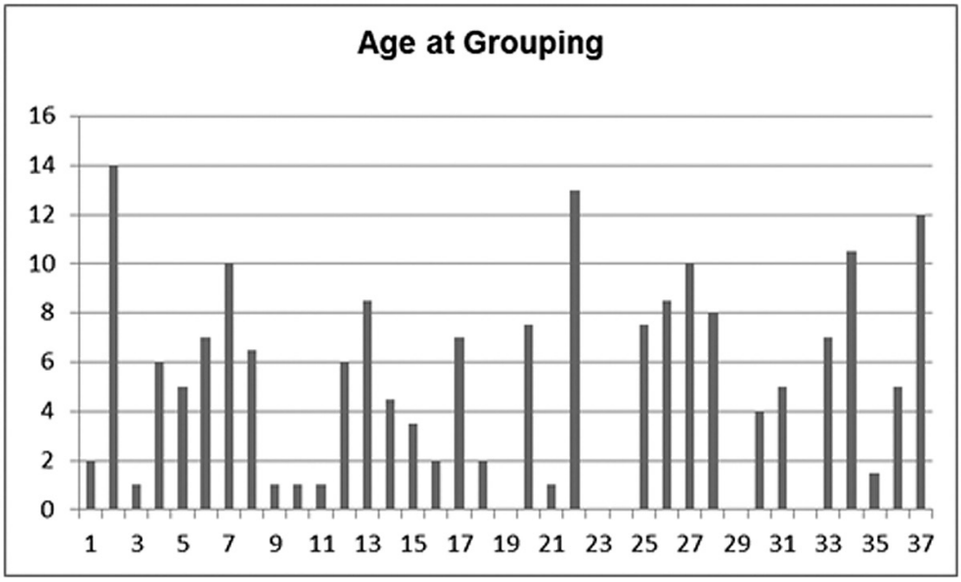

Figure 3. Age calves are introduced to group feeding

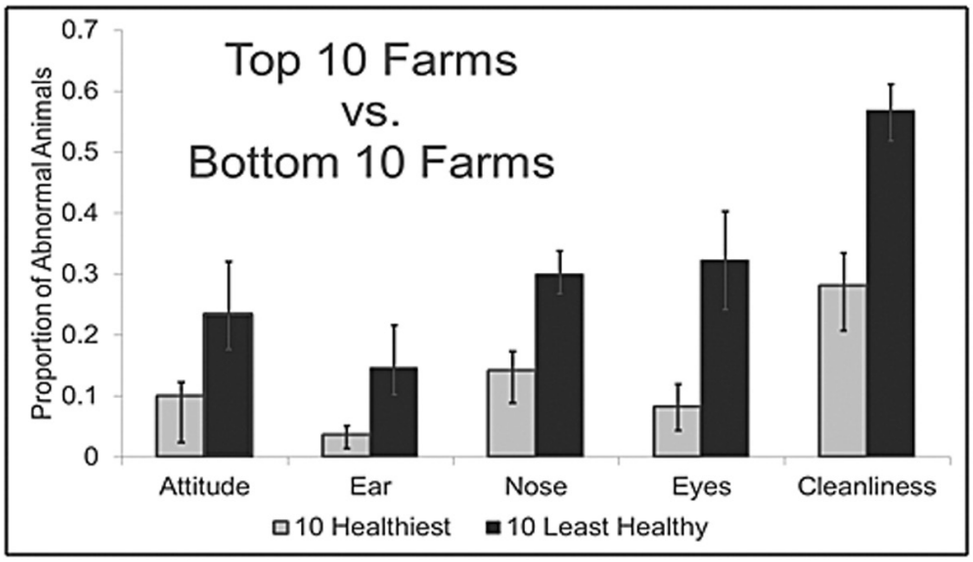

Figure 4. Average proportion of abnormal scores (indicating potential disease presence)

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 autofeeder 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 preweaned calves as long as appropriate management and maintenance of equipment are emphasized and implemented.

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- Dairy farm cooperators
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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 bromegrass, 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 be 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

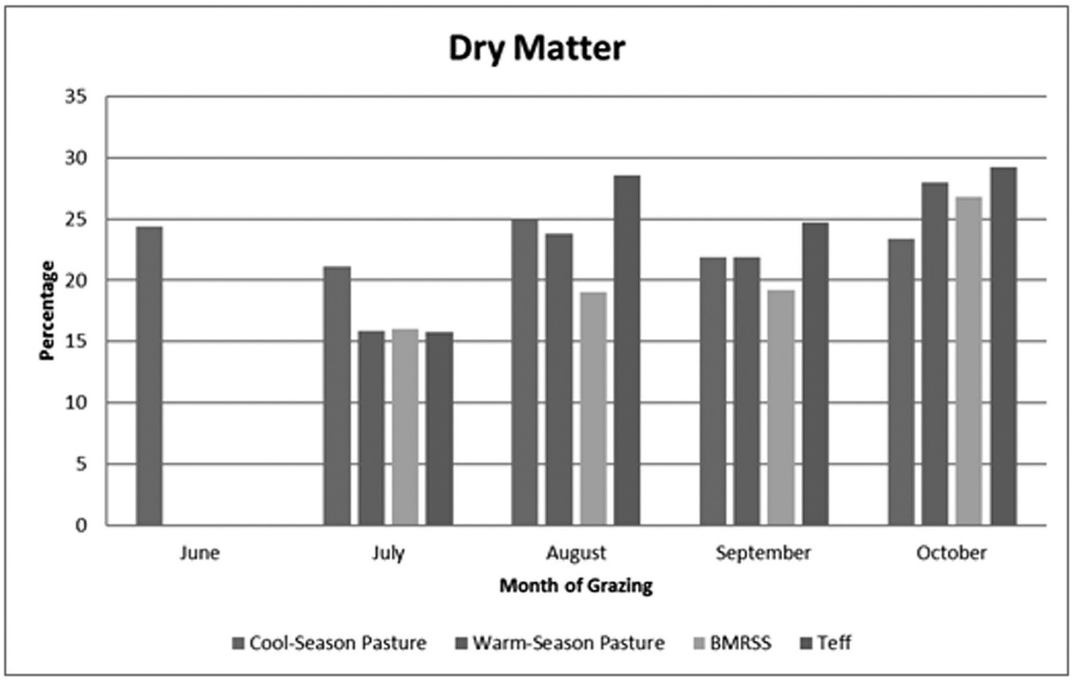
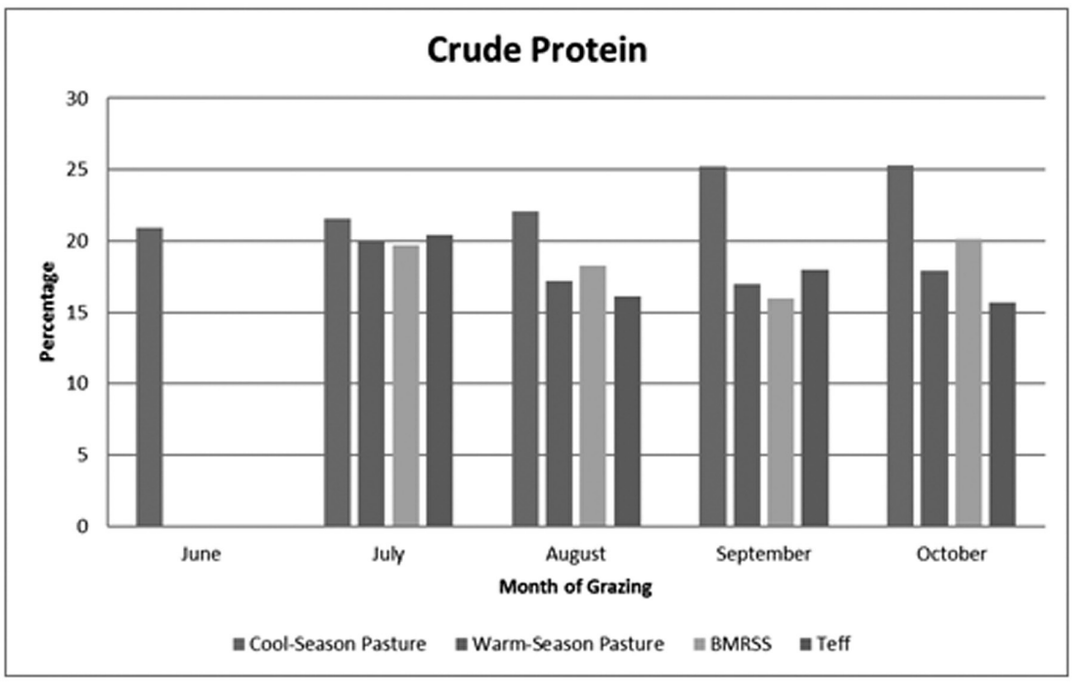


Figure 2. Crude protein of pasture grass species across the grazing season



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

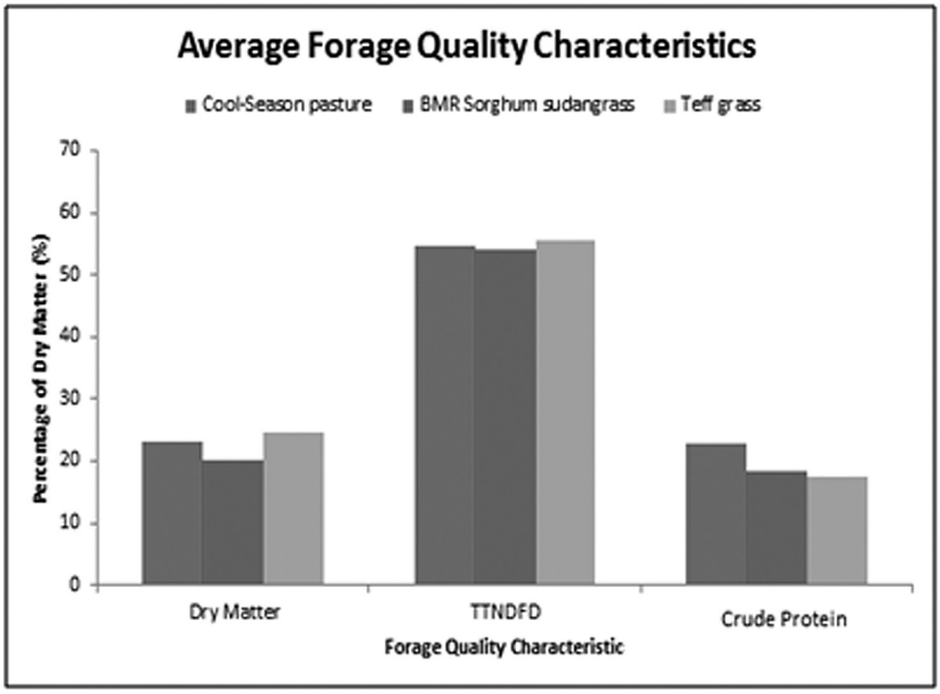
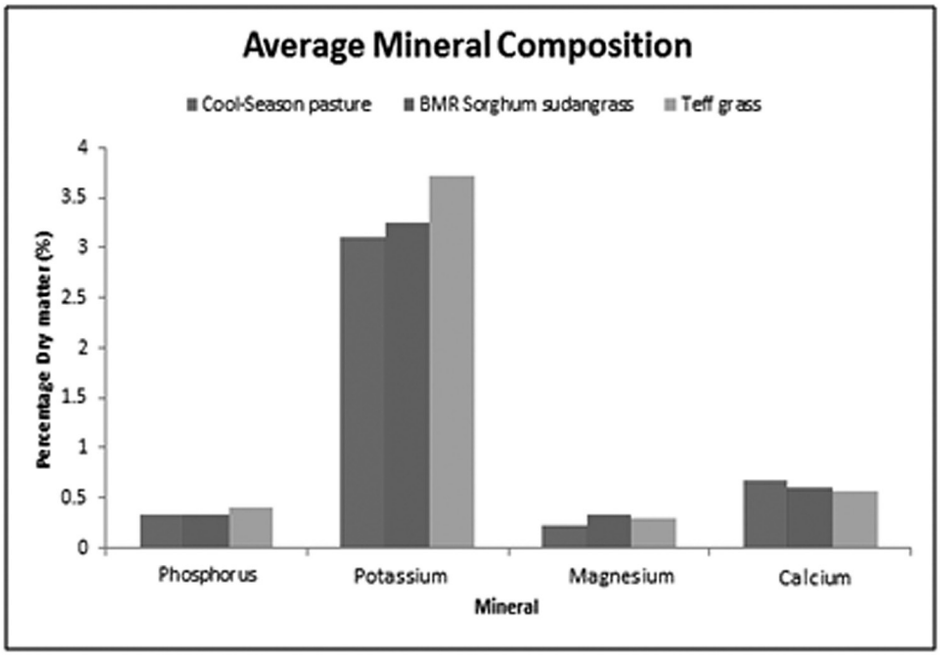
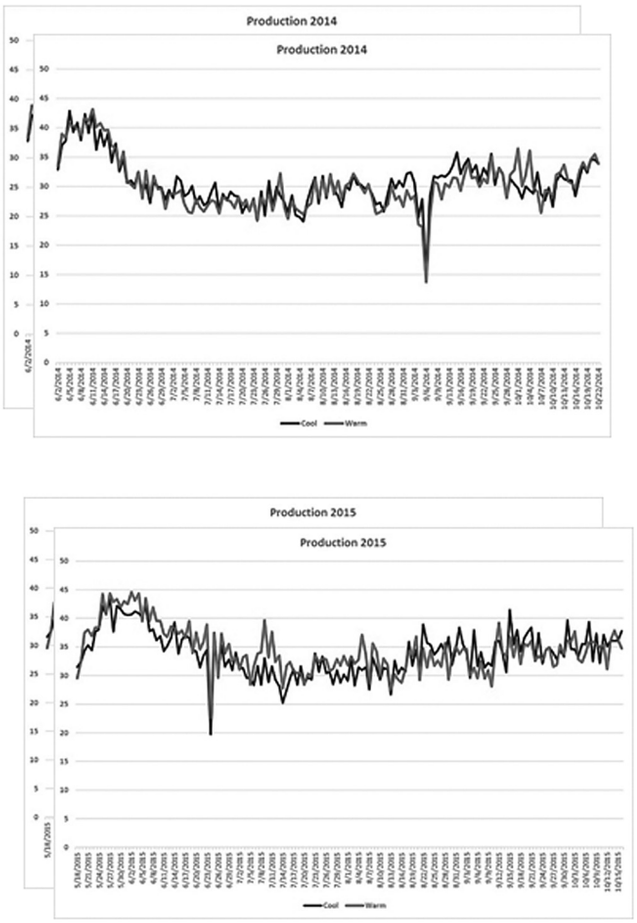


Figure 4. Mineral composition of pasture grass species



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

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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 2nd 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 crossbred 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 µM) 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 µM (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 µM), 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®, 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®), 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 µM vs. Met-supplemented = 22.9 µM) 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, Penagaricano et al. (2013), evaluated whether the embryos that were recovered from cows that had been supplemented 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 formulated 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 Specific 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 concentration. Cows fed the methionine enriched diet had a lower pregnancy loss from 21 to 61 after AI (16.7 % RPM cows vs. 10.0% from CON cows). Pregnancy losses between days 28 and 61 were not different in the primiparous cows (12/8% CON and 14.6% RPM), however, pregnancy losses between treatments were significant for the multiparous cows (19.6% CON vs. 6.1% RPM; 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 critical 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 embryo. Methionine supplementation seems to impact the preimplantation embryo in a way that enhances its capacity for survival because there is strong evidence that endogenous lipid reserves serve as an energy substrate. The lower pregnancy losses from cows fed a methionine enriched diets suggest that methionine favors the embryo survival, at least in multiparous cows. Further studies are needed to corroborate 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: × 40; scale bars = 100 µm). 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

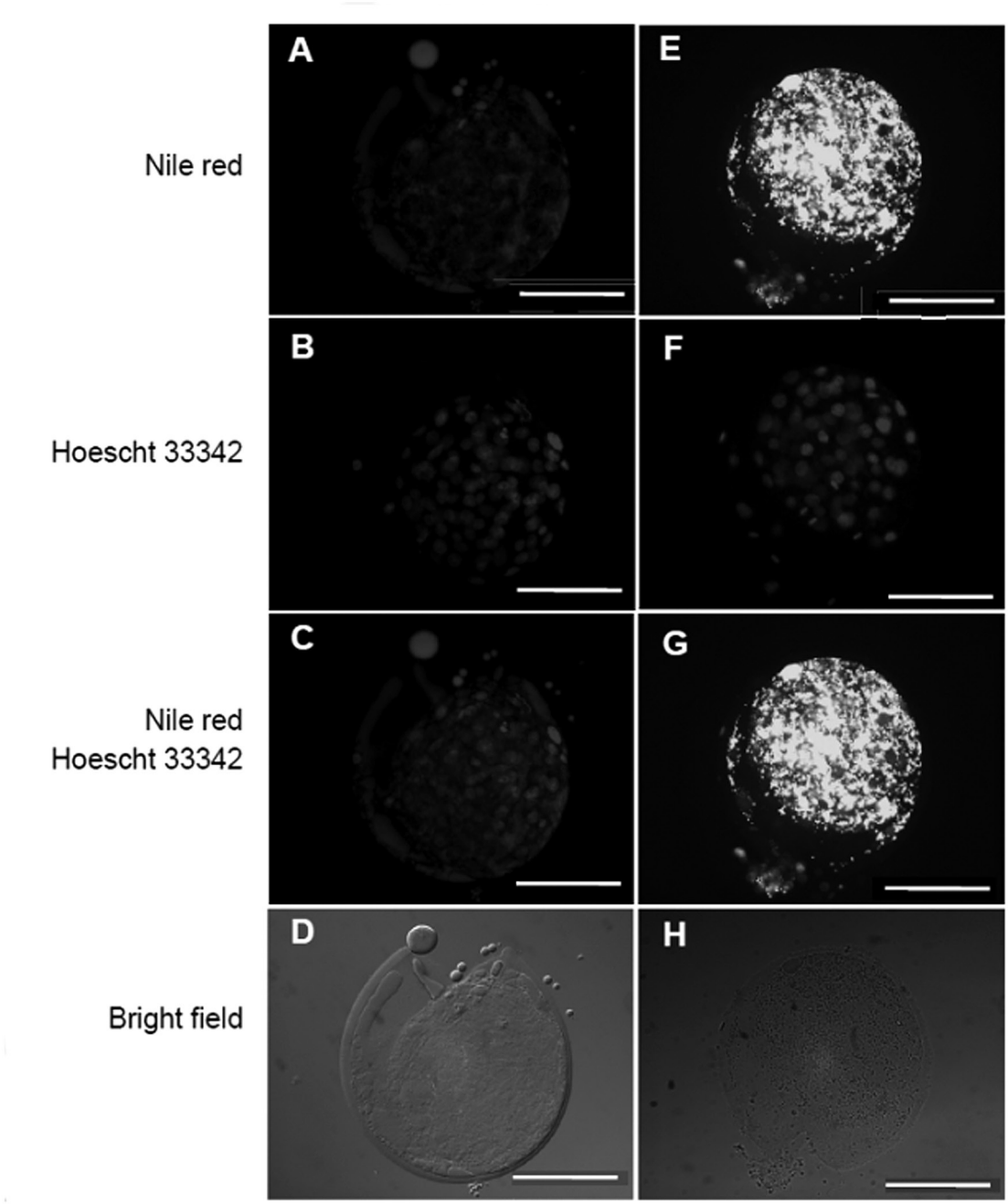
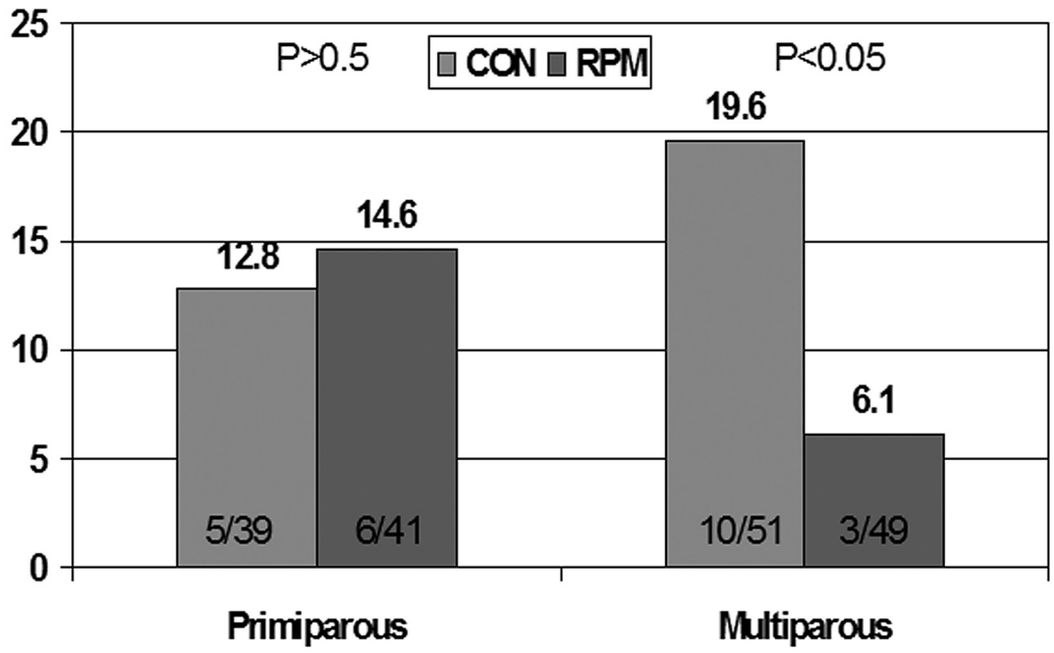


Figure 2: Pregnancy losses between days 21 and 61 after timed AI of primiparous and multiparous cows fed a control diet (CON) or a methionine enriched diet (RPM)



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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 $F_{2\alpha}$ 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 $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ 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 PGF2α 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 $PGF_{2\alpha}$ 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 PGF2α 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 $PGF_{2\alpha}$ 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

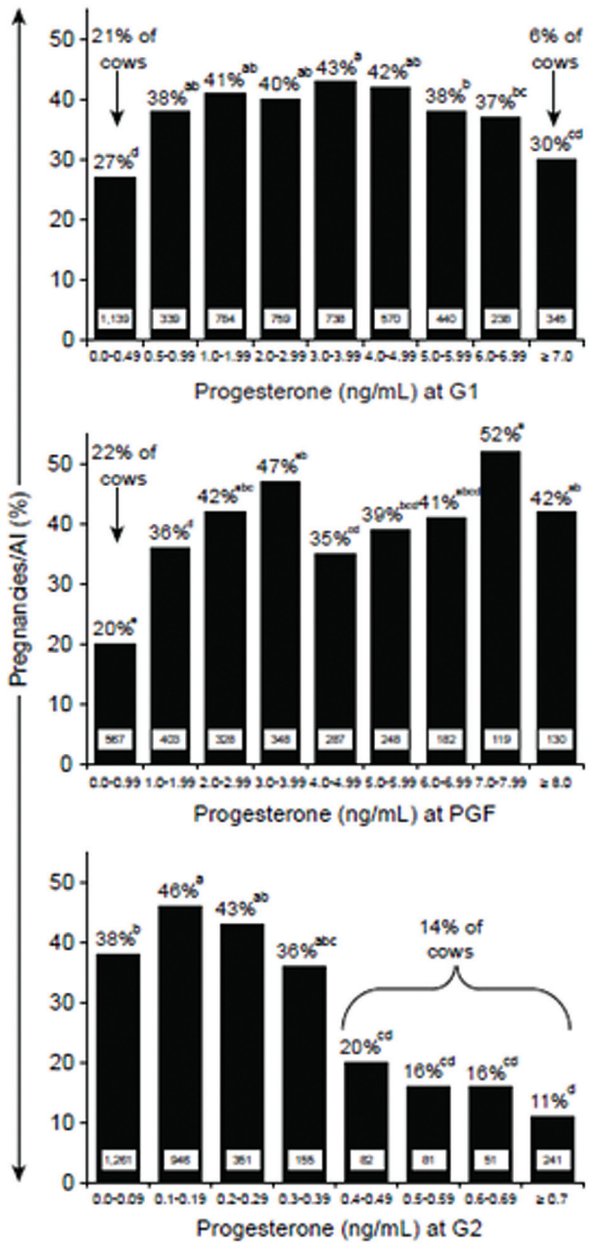

Figure 2. Effect of progesterone at each treatment of an Ovsynch protocol on pregnancies per AI in lactating Holstein cows. At G1, concentrations of progesterone in 6,144 cows were stratified into nine P4 categories from 0 to ≥7 ng/mL using 0.5 ng/mL increments (upper panel). At the $PGF_{2\alpha}$ treatment, concentrations of progesterone in 3,383 cows were stratified into nine P4 categories from 0 to ≥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 ≥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 ≥ 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 oviductal 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 $PGF_{2\alpha}$ 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 ≥ 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 (~6d) at the $PGF_{2\alpha}$ treatment during an Ovsynch protocol fail to fully regress to a single $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ treatment during a Double Ovsynch protocol on the proportion of Holstein dairy cows undergoing complete luteal regression by G2 (P4 < 0.4 ng/mL)[1].

Age and number of CL at $PGF_{2\alpha}$ 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)

[1]Adapted from Giordano et al., 2012c

Several experiments have assessed the effect of adding a second $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ treatment 24 hours after the first (Brusveen et al., 2009). Cows receiving 2 $PGF_{2\alpha}$ treatments during the Ovsynch protocol had a greater incidence of luteal regression than cows receiving 1 $PGF_{2\alpha}$ treatment (98% vs. 86%); however, P/AI to first TAI did not differ between cows receiving 2 vs. 1 $PGF_{2\alpha}$ 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 PGF2α 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 $PGF_{2\alpha}$ treatment and avoids setting up cows with a young CL ~6 days of age at the $PGF_{2\alpha}$ 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 PGF2α 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 $PGF_{2\alpha}$ treatment had incomplete luteal regression (≥ 0.4 ng/mL) than cows receiving 2 $PGF_{2\alpha}$ treatments regardless of P4 concentrations at G1 (Table 4). For cows with P4 concentrations < 1.0 ng/mL at G1, cows receiving 2 $PGF_{2\alpha}$ treatments had more (P = 0.03) P/AI than cows receiving 1 PGF2α 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 $PGF_{2\alpha}$ treatments (Table 2).

Table 2. Effect of 1 vs. 2 $PGF_{2\alpha}$ 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)1.

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

[1]Adapted from Carvalho et al., 2015a.
[a,b]Proportions differ (P < 0.01).
[c,d]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 PGF2α treatment 24 h after the first $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ treatment 32 d after TAI with the addition a second PGF2α treatment 24 h after the first $PGF_{2\alpha}$ treatment. Nonpregnant cows lacking a CL restart an Ovsynch-56 protocol that includes a second $PGF_{2\alpha}$ 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

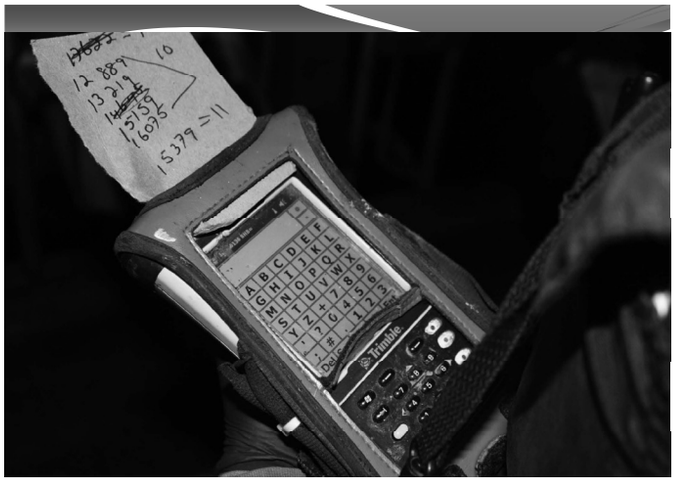
30 is the New 20
What changed?

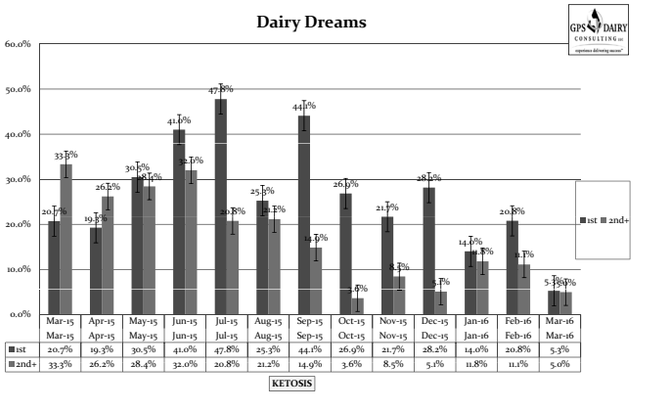
- Nutrition
- TAI Programs
- Genetic strategies

Nutritional Effects

Dr Marty Faldet GPS

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





	Mar-15	Apr-15	May-15	Jun-15	Jul-15	Aug-15	Sep-15	Oct-15	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16
	Mar-15	Apr-15	May-15	Jun-15	Jul-15	Aug-15	Sep-15	Oct-15	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16
1st	20.7%	19.3%	30.5%	41.0%	47.8%	25.3%	44.1%	26.9%	21.7%	28.2%	14.0%	20.8%	5.3%
2nd+	33.3%	26.2%	28.4%	32.0%	20.8%	21.2%	14.9%	3.6%	8.5%	5.1%	11.8%	11.1%	5.0%

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	0	???? Preg Stat
9/29/15	85	66	78		0	0	0	0	???? Preg Stat
Total	1516	989	65		1459	409	28	47	

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	0	???? Preg Stat
9/29/15	318	229	72	0	0	0	0	???? Preg Stat
Total	6725	4512	67	6474	1941	30	253	

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	???? Preg Stat
10/07/15	434	359	83	0	0	0	0	???? Preg Stat
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
6/2/2015	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
10/6/2015	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
12/8/2015	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
Total	100	1068	924	1122	2.0	9.0	

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
Total	100	1068	924	1122	2.0	9.0	30!

2016 Dairy Dreams Genetic Plan (cont)

- Until recently the lactating recipients were 1st breeding
- Currently using the following criteria:
  - 1st and 2nd 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 composit 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
Total	100	5907	330

Milk Urea Nitrogen as a Tool to Assess Efficiency of Nitrogen Utilization in Dairy

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Abstract

Insufficient intake of nitrogen (N) penalizes milk production and milk protein production of lactating dairy cows, but the excessive use of protein supplements, especially commercial sources of rumen undegraded protein (RUP), may translate into unnecessary additional costs and losses of N to the environment in the form of urinary urea-N. Thus the question of *how low in crude protein can a diet be formulated to avoid losses of production, unnecessary expenses, and unnecessary losses of urinary urea-N?* This article focuses on milk urea-N (**MUN**) as one of the tools available to assess N use efficiency (**NUE**) defined as the conversion of dietary N to milk N, and more broadly the adequacy of a diet to deliver nutrients (in proper amounts and proportion) to maximize milk protein production. Extensive literature of experiments conducted in North America indicated that on a daily basis, the modern lactating dairy cow converts 26% of the N consumed into milk N, and she excretes as much urinary urea-N (168 g/d) than she secretes N as milk protein (166 g/d). In general increasing the percentage of dietary crude protein increases N intake and urinary urea-N excretion and decreases NUE, but does not alter milk protein production. For its part, MUN reflects closely the percentage of crude protein in the diet. It is a reliable predictor of urinary urea-N and NUE, but (unfortunately) not a good predictor of milk protein production. For example, analysis of data collected on farm by a dairy herd improvement (**DHI**) association from the Midwest of the United States has indicated that milk protein production can be near or at maximum for any MUN value ranging from 10 to 16 mg/dL. As opposed to common assumptions, MUN does not reflect only an excess rumen degradable protein (**RDP**) in the diet. Milk urea-N should rather be interpreted as an indicator of the overall adequacy of the diet to provide amino acids and energy yielding nutrients (in particular glucose) that support the most efficient use of the N consumed by a cow. Data from DHI have demonstrated that test-day MUN is influenced substantially by numerous cow factors (e.g., breed, body weight, parity and genetics) and many complex interactions among nutritional, animal, and managerial factors. As a result, a within-herd baseline may be more appropriate than an industry standard. Nevertheless, our analysis of studies in which Holstein cows were fed diets typical of the Midwest of the United States (corn silage, alfalfa silage, corn grain and protein supplement based on soybean by-products primarily) revealed that maximum protein yield (1.20 kg/d) occurred at MUN of 11.3 mg/dL, with diets of 16.2% crude protein (diet dry matter basis). Under this dietary situation, expected urinary urea-N excretion would be approximately 134 g/d, which would be a 25% reduction compared with the expected 178 g/d in urinary urea-N excretion with diets of 18% crude protein with essentially no change in milk protein yield. Calculation of income over feed cost (**IOFC**) with prices prevailing in the Midwest of the United States between 2007-2015 indicated that maximum IOFC occurred at MUN of 10.9 mg/dL. Due to possible genetic and permanent management effects on MUN, producers and consultants are encouraged to determine a reference MUN for a herd when fed a ration balanced according to NRC standards and experiment with reducing RUP and (or) RDP in order to determine the most appropriate herd-specific MUN target.

Introduction

The daily supply of nitrogen (**N**) or crude protein (**CP**), rumen degraded protein (RDP), and rumen undegraded protein (**RUP**) is determined by the choice of feed ingredients, their proportion in the ration, and the total amount of feed consumed by the cow. Insufficient intake of N penalizes milk production and milk protein production, but excess N intake translates into unnecessary N losses as urea in the urine. Urinary urea-N is an environmental concern because during storage and after soil application of the manure, the N or the urea is converted to atmospheric pollutants such as ammonia that contributes to acid rain as well as nitrous oxide, which is a greenhouse gas that contributes to climate change. Furthermore, high levels of urinary urea-N in the manure may contribute also to eutrophication of surface water (rivers and lakes) and nitrate in drinking water (a human health issue). On the other hand, from a dairy farm profitability standpoint, protein supplementation of lactating cows, which is a necessity in many produc-

tion systems, may add substantially to cost of feeding. Thus excess protein in the diet, especially in the form of expensive RUP, has direct negative economic and environmental impacts. The question that an increasing number of researchers, consultants and producers are asking is: *How low of a CP diet can I formulate to avoid losses in milk production, protein yield and income, and at the same time avoid unnecessary losses of urinary urea-N?*

The answer to this question is actually very complex, depends on many nutritional and economic factors, and is beyond the scope of a single article. In general, measuring intake N on farms is difficult and expensive. However, nutritionists have alternative tools at their disposal to assess the adequacy of N intake and N use efficiency (**NUE**) defined as the percentage of intake N converted to milk N. This article focuses on milk urea-N (**MUN**) as one of these tools which allows for a diagnostic without having to measure N intake. Analysis of urea concentration in milk is economical. It is most commonly available through monthly samples of individual cows (test-day MUN) collected by dairy-herd improvement (**DHI**) organizations or as bulk tank MUN from samples collected when milk is hauled to the processing plant. Monitoring the level of MUN and the changes in MUN may help producers identify and diagnose possible problems and make sound decisions to bring them near the best possible nutritional management practices on their farm. Thus, our specific objectives are to discuss the origin of the urea found in milk, to explore the link between N intake, milk protein production and daily excretion of urinary urea-N (as revealed by dairy cattle nutrition research), to review the main known sources of variation in test-day MUN (as revealed essentially by analysis of databases from field data collected by DHI organizations), to explore the relation between MUN and income over feed cost (**IOFC**), and to provide general recommendations to develop herd-specific MUN target associated with low but adequate levels of dietary crude protein.

N Transformations in the Cow: Relation Between Inputs vs. Outputs

Table 1 describes the partitioning of the N consumed into milk N, fecal N, urinary N and urinary urea-N. Milk N reflects primarily milk protein synthesis in the udder [milk protein (g/d) = milk N (g/d) × 6.38]. It is not uncommon for a typical lactating dairy cow to secrete more than 1 kg of milk protein per day, which amounts to 157 g/d of N, but the amount of milk urea-N excreted is typically 4 g/d (ranging from less than 2 up to 9 g/d). Thus there is nearly 40 times more N in the form of protein than in the form of urea in the milk of typical dairy cow. Fecal N reflects the undigested feed N, the undigested microbial N that had been synthesized in the rumen, and microbial N produced in the large intestine. In turn, urinary N includes various end-products of post-absorptive N metabolism. Data of Table 1 indicates that urea-N makes up 82 and 79% of the total N excreted in urine daily, in Northern Europe and North America, respectively. The main message, however, is that on average the modern lactating dairy cow excretes more urinary urea-N (152 g/d and 168 g/d in Northern Europe and North America, respectively) than she secretes N as milk protein (133 g/d and 166 g/d in Northern Europe and North America, respectively). For dairy cows in North America, NUE averages 26.1% (166×100/637) whereas the N lost as urinary urea-N averages 26.4% of N intake; the corresponding percentages for dairy cows in Northern Europe are 27.4 and 31.3%, respectively. These regional differences might be explained in part by the difference in N intake.

Focusing on the main types of dietary ingredients fed in the Midwest of the United States: corn silage, alfalfa silage, corn grain and a protein supplement (usually soybean meal and/or heat-treated soybean by-products), we studied the relationship among key economic and environmental variables, namely intake N, milk N, urinary urea-N, and NUE with dietary CP (Figure 1).

Table 1: Partition of N in lactating dairy cows (Spek et al., 2013a).

	Northern Europe		North America	
	g/d	%	g/d	%
Intake N, g/d	485	100.0	637	100.0
Milk N, g/d	133	27.4	166	26.1
Fecal N, g/d	159	32.8	223	35.0
Urinary N, g/d	185	38.1	212	33.3
Urinary Urea-N,	152	31.3	168	26.4

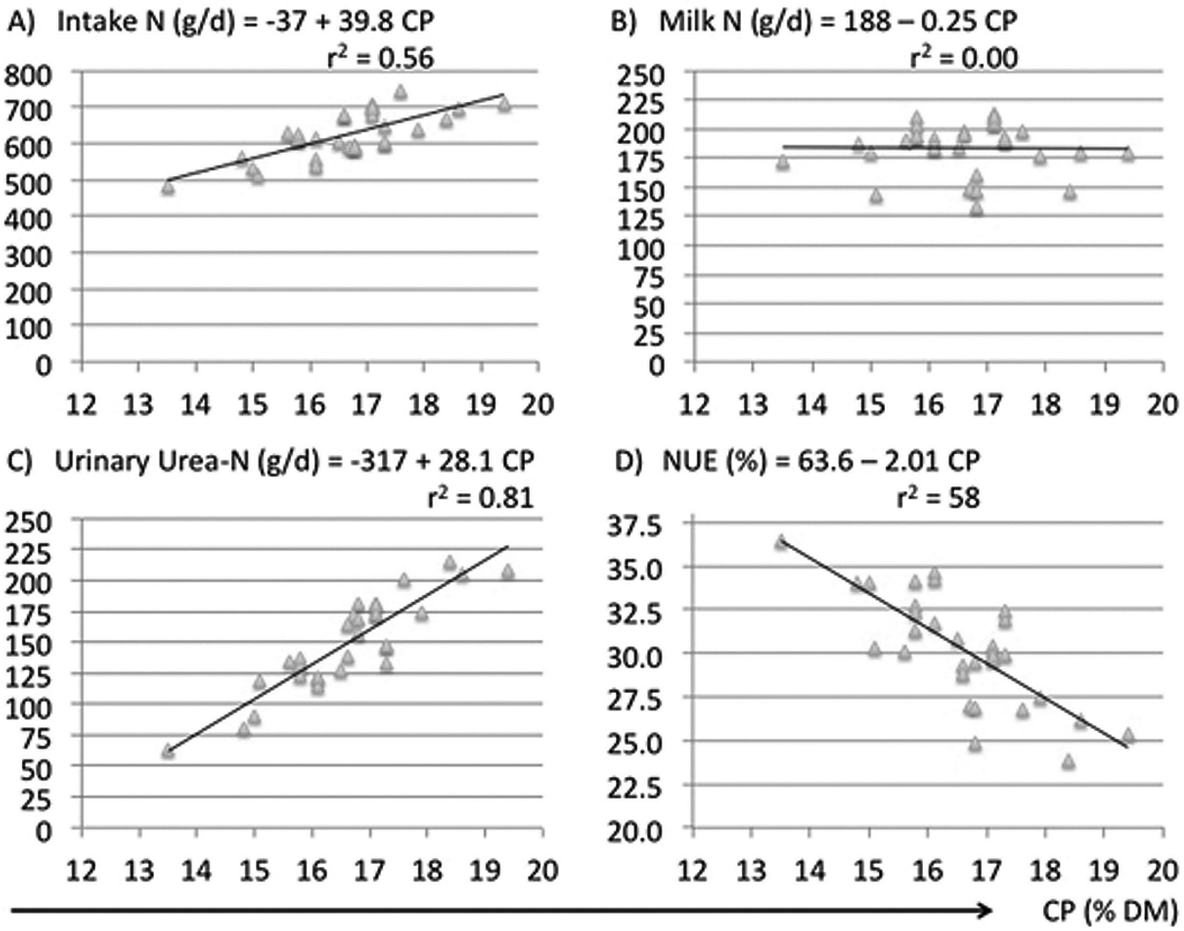

Figure 1: Dietary CP (% of diet dry matter) as a predictor of: A) intake N, B) milk N, C) urinary Urea-N, and D) nitrogen use efficiency (NUE); see text for details.

In these regressions, one data point represents one dietary treatment (n=31) fed to cows in five trials conducted at the University of Wisconsin-Madison (*Broderick, 2003; Olmos-Colmenero and Broderick, 2006a, 2006b; Broderick et al., 2008*; and *Broderick et al., 2009*). The coefficients of determination (r^2) for the four graphs in Figure 1 indicated that the concentration of CP in the diet, as a single predictor, explained 56%, 0%, 81% and 58% of the variation observed in the dataset for intake N, milk N, urinary urea-N and NUE, respectively. Here are important take home messages from the analysis of Figure 1:

- In addition to dietary CP concentration, dry matter intake is an important determinant of intake N and contributed 44% (100-56) of its variation.
- Milk protein production ranged from approximately 0.8 to 1.3 kg/d (i.e., milk N of 125 to 205 g/d) but it was completely independent of dietary CP concentration (within the range of dietary CP used in the trials). This observation may be explained in part by the fact that the diets included in this database were carefully balanced according to the NRC (2001). However, this finding may reflect also the cow's ability to produce milk protein fairly independently of the concentration and sources of CP in the ration.
- Dietary CP can be used to predict fairly accurately the daily excretion of urinary urea-N. Every reduction in dietary CP of one percentage unit between 19 and 13% (dietary dry matter basis) is predicted to reduce urinary urea-N by 28.1 g/d.
- Dietary CP is negatively correlated with NUE. A reduction in dietary CP is a reliable way to increase NUE. Every reduction in dietary CP of one percentage unit between 19 and 13% (dietary dry matter basis) is accompanied by an increase of the conversion of intake N to milk N by 2.01 percentage unit.

Using the same experimental data, we explore the use of MUN as a predictor of intake N, milk N, urinary urea-N and NUE (Figure 2). The coefficients of determination (r^2) for the four graphs in Figure 2 indicates that MUN, as a single predictor, explained 30%, 9%,

78% and 65% of the variation observed in the dataset for intake N, milk N, urinary urea-N and NUE, respectively. Here are important take home messages from the analysis of Figure 2:

- Milk urea-N is a poor predictor of intake N as evidenced by a moderate coefficient of determination (r^2 = 0.30), indicating that only 30% of the total variation in intake N may be associated with the variation in MUN. However, MUN is an accurate predictor of dietary CP (data not shown). The prediction equation was dietary CP (% of diet dry matter) = 10.6 + 0.54 MUN (mg/dL) (r^2 = 0.85). Thus 85% of the variation in dietary CP can be captured in the corresponding variation in MUN. Thus, MUN can be used as a tool to monitor unexpected or expected changes in dietary CP even under farm conditions (Jonker et al., 2002).
- Similarly to CP, MUN does not help explain any variation in milk protein synthesis. In other words, the mammary gland machinery responsible for the synthesis of milk protein from amino acids does not contribute significantly to the variation in concentration of urea-N found in milk.
- Similarly to dietary CP, MUN can be used to predict fairly accurately the daily excretion of urinary urea-N. Every reduction in MUN of one percentage unit between 16 and 8 mg/dL is predicted to reduce urinary urea-N by 16.1 g/d.
- Milk urea-N is negatively correlated with NUE and is a slightly better predictor of NUE than dietary CP. Milk urea-N captures 65% of the variation in NUE (Figure 2) but dietary CP captured only 58% of the variation in NUE (Figure 1). Based on this dataset, every reduction in MUN of 1 mg/dL between 16 and 8 mg/dL is accompanied by an increase of the conversion of intake N to milk N by 1.24 percentage unit (Figure 2).

It is important to note that some of the findings discussed here are likely not generalizable to all regions of the world. The data used to construct the regressions presented in Figures 1 and 2 were from similar types of diets fed experimentally at one university only (university of Wisconsin-Madison). Thus the data presented here does not include the variation that is likely to occur across regions because of: a) types of diets (level of fiber, starch, RDP and RUP that may vary with the type of forages and concentrates used to balance the ration), b) cow population (e.g., breeds, genetics), c) feeding management practices (e.g., pasture vs. confinement feeding systems), milk sampling technique (e.g., number of milking sampled per day), and d) methods and accuracy laboratory analyses.

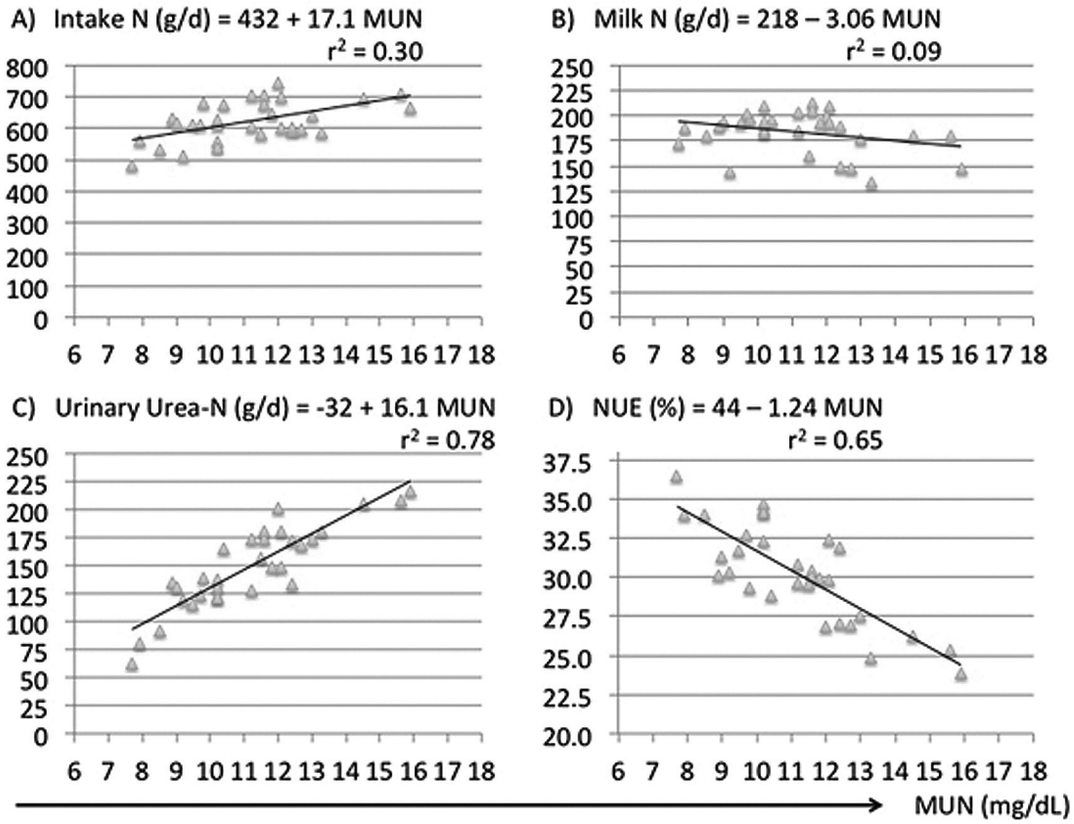

Figure 2: Concentration of urea-N in the milk (MUN) as a predictor of: A) intake N, B) milk N, C) urinary urea-N, and D) nitrogen use efficiency (NUE); see text for details.

N Transformations in the Cow: Sources of Urea in Milk

Protein nutrition in dairy cows is extremely challenging because of the profound transformation of the N during digestion and metabolism and because of the profound interactions that exist between protein and energy. The key organs for protein, amino acids and urea metabolism include the reticulo-rumen, the liver, the mammary gland, and the kidney. Regardless of the type of N-containing compounds that can be fed to the cow, ammonia absorbed through the ruminal wall and amino acids absorbed trough the small intestinal wall are by far the two main N-containing compounds that appear in the blood. The liver removes the ammonia from the blood, synthesizes proteins, converts one amino acid into another (transamination), and degrades amino acids (deamination). In addition, the liver synthesizes urea utilizing the N from ammonia (coming from the gastro-intestinal tract) and the N from unutilized amino acids for milk production and other biological functions such as muscle growth and turnover, or fetal growth during pregnancy. In addition, the urea released in the bloodstream is partially recycled to the digestive tract with the saliva secreted during eating and rumination as well as directly as the arterial blood brings nutrients to the digestive tract. Thus, as opposed to common assumptions, MUN does not reflect only an excess RDP in the diet, but rather it should be interpreted as an indicator of the overall adequacy of the ration to provide amino acids and energy yielding nutrients (in particular glucose) that support the most efficient use of the N consumed by the cow. Additional evidence that support this conclusion can be found in the work of Broderick and Clayton (1997) indicating that the r^2 between MUN and ruminal ammonia concentration was 0.57, which is a value that is much lower than the 0.84 r^2 value that these authors observed between MUN and dietary CP. Similarly, the work of Spek et al. (2013a) indicated the RDP and RUP were relatively poor predictors of urinary urea-N excretion (r^2 = 0.36 and 0.38, respectively). Therefore, one needs to be cautious in associating high urinary urea-N excretion with high RDP in the diet. In contrast, the same authors reported an r^2 of 0.79 between urinary urea-N and dietary CP and an r^2 of 0.72 between urinary urea-N and MUN, indicating that 79% and 72% of the variation in urinary urea-N may be explained by the variation in dietary CP and MUN, respectively.

Main Sources of Variations in MUN

One of the advantages of controlled nutritional studies is to increase our understanding of the biology of the cow. These studies contribute to improving the tools (e.g., ration formulation software) available to feed dairy cows for greatest benefits to the producer with minimum negative impact on the environment. As indicated above, nutritional experiments have shown that MUN is highly influenced by dietary CP and both are reliable predictors of urinary urea-N excretion. However, one of the limitations of these nutritional studies is that they do not reflect non-nutritional factors that affect MUN when collected on farms. Field studies (*Eicher et al., 1999*) and the analysis of DHI-type database (*Johnson and Young, 2003; Wattiaux et al., 2005*) have shown that on-farm test-day MUN (cow-level MUN) is highly variable and influenced by a great number of factors. Such factors with additional references, which sometimes reached contradictory conclusions, are highlighted here:

- Breed: Average MUN differed among breeds as follows: Holstein (12.0 mg/dL), Jersey (14.0 mg/dL), and Brown Swiss (14.8 mg/dL) in *Wattiaux et al. (2005)*, but Holstein MUN was 15.5 mg/dL compared to 14.1 mg/dL for Jersey MUN in *Johnson and Young (2003).*
- Body Weight: Regardless of breed (Holstein or Jersey), MUN increases with an increase in cow body weight (*Kauffman and St Pierre, 2001*).
- Parity: Significant but numerically small differences due to parity were found in *Johnson and Young (2003)*, but large differences in pattern of change in MUN with days in milk for primiparous and multiparous Holstein were found in *Wattiaux et al. (2005;* Figure 3);

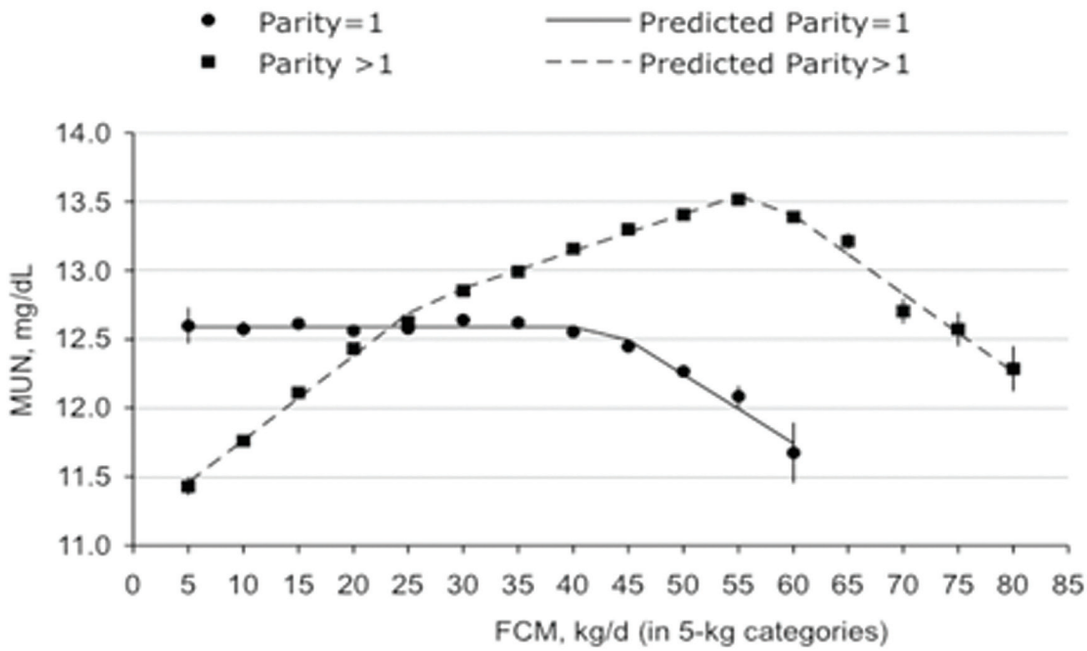

Figure 3: Interaction between cow parity (number of calving) and level of 3.5% fat-corrected-milk (FCM) production in Holstein cows (*Wattiaux et al., 2005*).

- **Stage of lactation and level of milk production:** MUN varies with days in milk in a parallel pattern to the variation in milk production throughout the lactation.
- **Udder health:** *Johnson and Young (2003)* have reported a negative correlation between MUN and somatic cell counts, but *Stoop et al. (2007)* have reported a high genetic correlation between MUN and somatic cell score (r = 0.85).
- **Seasons:** Large seasonal variations have been reported in many publications, but they are more likely to reflect an interaction between seasonal feeding practices and other management factors (e.g., animal health) then an effect due to change in weather condition (temperature, humidity or day length).
- **Sampling schedule:** Morning samples yield lower MUN than evening samples (when milking frequency is two), which may be associated with sampling time in relation to feeding and milking time *(Spek et al., 2013b).*
- **Laboratory equipment** *(Peterson et al., 2004; Kohn et al., 2004)* **and analytical method** *(Broderick, 2003)* are important to consider as a source of variation. Given the small amount of urea in the milk, it is essential that commercial lab engaged in routine calibration and to guaranteed precision and accuracy of measurements.
- More recently, researchers have attempted to better understand on-farm MUN variation focusing on **herd effects and cow-within-herd effects** *(Aguilar et al., 2012)*. These authors found large deviations in MUN from expected values (based on dietary predictions) among cows fed the same diet, as well as among herds. The authors argued that part of these variations may originate from genetic difference because a high heritability of MUN has been reported (0.5-0.6 in *Wood et al. (2003)*, and 0.4 in *Miglior et al. (2007)*. However, other publications have reported considerably lower heritability [0.14 in *Stoop et al. (2007)* and 0.15 in *Mitchell et al. (2005)*]. A recent analysis of phenotypic variation in MUN indicated that between-cow variation in MUN had a smaller effect on NUE compared with published responses of MUN to dietary CP concentration *(Huhtanen et al., 2015),* suggesting that nutritional management has greater potential to improve NUE and lower MUN on farm than genetic selection.

Some of the factors listed here have permanent effects but others have temporary effects. The magnitude of these effects may vary regionally and over time. It is clear, however, that none of the factors listed here should be considered the cause of changes observed in monthly test-day MUN. Furthermore, as illustrated in our earlier research *(Wattiaux et al., 2005),* it appears that test-day MUN are influenced strongly by many interactions rather than a series of single factors acting independently of one another. Thus one might speculate that the commonly accepted "optimal" MUN of 10-12 mg/dL (see below), which reflects high NUE may have to be adjusted for the genetic variation or other permanent effects that may exist among herds. Regardless of the reasons for differences among herds, researchers have argued that a within-herd baseline may be more appropriate than an industry standard *(Wattiaux et al., 2005; Aguilar et al., 2012).*

Regardless of these scientific arguments, our analysis of herd-level MUN with 539 distinct herds from the Midwest of the United States revealed that the top 200 herds had an average protein yield of 1.14 kg/d with a. average herd-level MUN of 12.6 mg/dl. In contrast, the remaining herds had an average protein yield of 0.94 kg/d with an average herd-level MUN of 13.2 mg/dL. In other words, top performing herds were able to obtain 200 g/d more milk protein yield with 0.5 mg/dL less MUN (Figure 4). The absence of MUN value greater than 17 mg/dL suggested that the top herds for milk protein yield do not feed blatant excess of dietary CP. Note, however that the variation in MUN among the top herds remained substantial and within the range of 10 to 16 mg/dL.

MUN for Maximum Protein Yield, Maximum IOFC, and Minimum Urinary Urea-N

Although high milk protein yield is not synonymous with high NUE, dairy nutritionists are most concerned with formulation of diets that allow for maximum protein yield at the lowest possible cost. As illustrated in Figure 4, protein yield can be near or at maximum for any MUN value ranging from 10 to 16 mg/dL. In other words, MUN is a poor indicator of protein yield. However, as discussed earlier MUN is a good predictor of dietary CP and urinary urea-N excretion. Thus the question stated at the onset: *How low of a CP diet can I formulate to avoid losses in milk production, protein yield and income, and at the same time avoid unnecessary losses of urinary urea-N?*

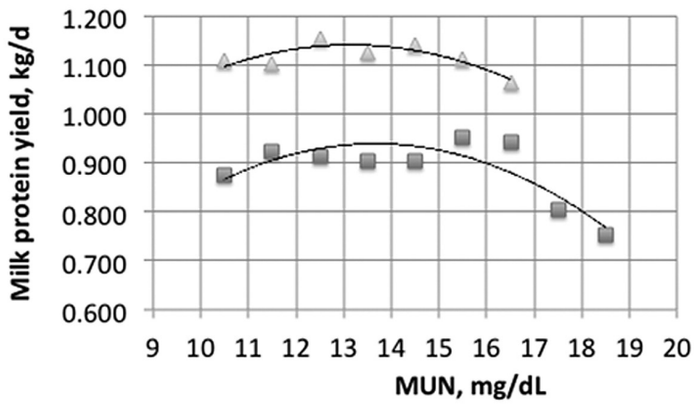

Figure 4: Relationship between MUN and protein yield from DHI database analysis for top producing herds (triangles) compared with average producing herds (squares); Wattiaux, unpublished; see text for details.

losses of urinary urea-N?

To help answer this question, we decided to return to nutritional studies (instead of DHI databases) to identify published research where the authors tested different levels of dietary CP, and reported MUN, urinary urea-N excretion and protein yield. This database focused primarily on the type of diet fed in the Midwest of the United States where a combination of alfalfa silage and corn silage comprised approximately 55% of the ration DM, with corn grain as the main energy (starch) source and a variety of protein supplements were used to balance the diet for RDP and RUP. Twenty-one studies were identified, however, in order to include results obtained with low dietary CP (less than 13% of dietary DM), two of our recent (yet unpublished) experiments were added to the database. Thus in total, results of 23 studies comprising 80 dietary treatments were summarized in nine dietary CP categories. The CP categories and the number of treatments in each category were as follows: <12.0% (n=3), 12.1-14.4% (n=3), 14.5-15.4% (n=12), 15.5-15.9% (n=10), 16.0-16.4% (n=9), 16.5-16.9% (n=17), 17.0-17.4 (n=14), 17.5-17.9 (n=5), and >18.0% (n=7). The IOFC of each dietary treatment was calculated using the equation: IOFC ($/cow/d) = [milk price ($/kg of milk) × daily average milk production (kg/cow/d)] – daily feed cost ($/cow/d). Average milk price was calculated using annual prices of milk components (milk fat, milk protein, and other solids) from 2007-2015 (http://future.aae.wisc.edu/). Feed cost was calculated as the average feed ingredients from multiple sources for the period of 2007 to 2015.

Figure 5A showed that within the range of dietary CP of approximately 12 to 18%, MUN is a reliable predictor of dietary CP. The linear equation with a high r^2 of 0.95 was: dietary CP (% of diet dry matter) = 8.47 + 0.68 × MUN (mg/dL) indicating that for every unit change (increase or decrease) in MUN in the range of 5 to 16 mg/dL, dietary CP is expected to change in the same direction by 0.68 units (% of dietary CP). Similarly, the relationship between MUN and urinary urea-N was linear (Figure 5B) with a high r^2 of 0.95. The equation was: Urinary urea-N (g/d) = -48.6 + 16.2 × MUN (mg/dL) indicating that for every unit change (increase or decrease) in MUN in the range of 5 to 16 mg/dL, urinary urea-N is expected to change in the same direction by 16.2 units (g/d). Figure 5C showed that the relationship between MUN and milk protein yield was curvilinear (similar to that observed in DHI database; Figure 4). The r^2 of the quadratic relationship was 0.93 and the equation was: protein yield (kg/d) = 0.18 + 0.24 MUN – 0.011 MUN^2 (where MUN is expressed in mg/dL). This relationship was driven, however, by the low protein yield observed on the few treatments with dietary CP less than 14.4% (the two lowest categories). Similar to the relationship observed between MUN and milk protein yield, there was a curvilinear relationship between MUN and IOFC (Figure 5D). The r^2 of the quadratic relationship was high (0.84) and the equation was: IOFC ($/cow/d) = -5.72 + 2.71 MUN – 0.12 MUN2 (where MUN is expressed in mg/dL). The maximum IOFC ($9.03/cow/d) occurred when MUN was 10.9 mg/dL

and coincided closely with the maximal protein yield. Thus, this data indicated that given feed and milk price structures in the Midwest of the United States, the maximum protein yield coincides essentially with the maximum IOFC.

Clearly, there is a need to generate additional data points from research including low dietary CP treatments. However, using the available data it appears that an MUN near 11 mg/dL corresponds to the maximum IOFC (~ $9.00/cow/d) and the maximum protein yield (~1.20 kg/d) when diets of 16.2% CP are fed to the cows. Under these optimal dietary situations, expected urinary urea-N excretion would be 134 g/d. This value is 25% lower than the expected excretion of 178 g/d for a diet of 18% CP for which MUN would be 14 mg/dL and IOFC less than $8.0/cow/d (Figure 5). Note that these relationships may vary with the level and type of energy present in the diet and thus may not be generalized to all feeding situations.

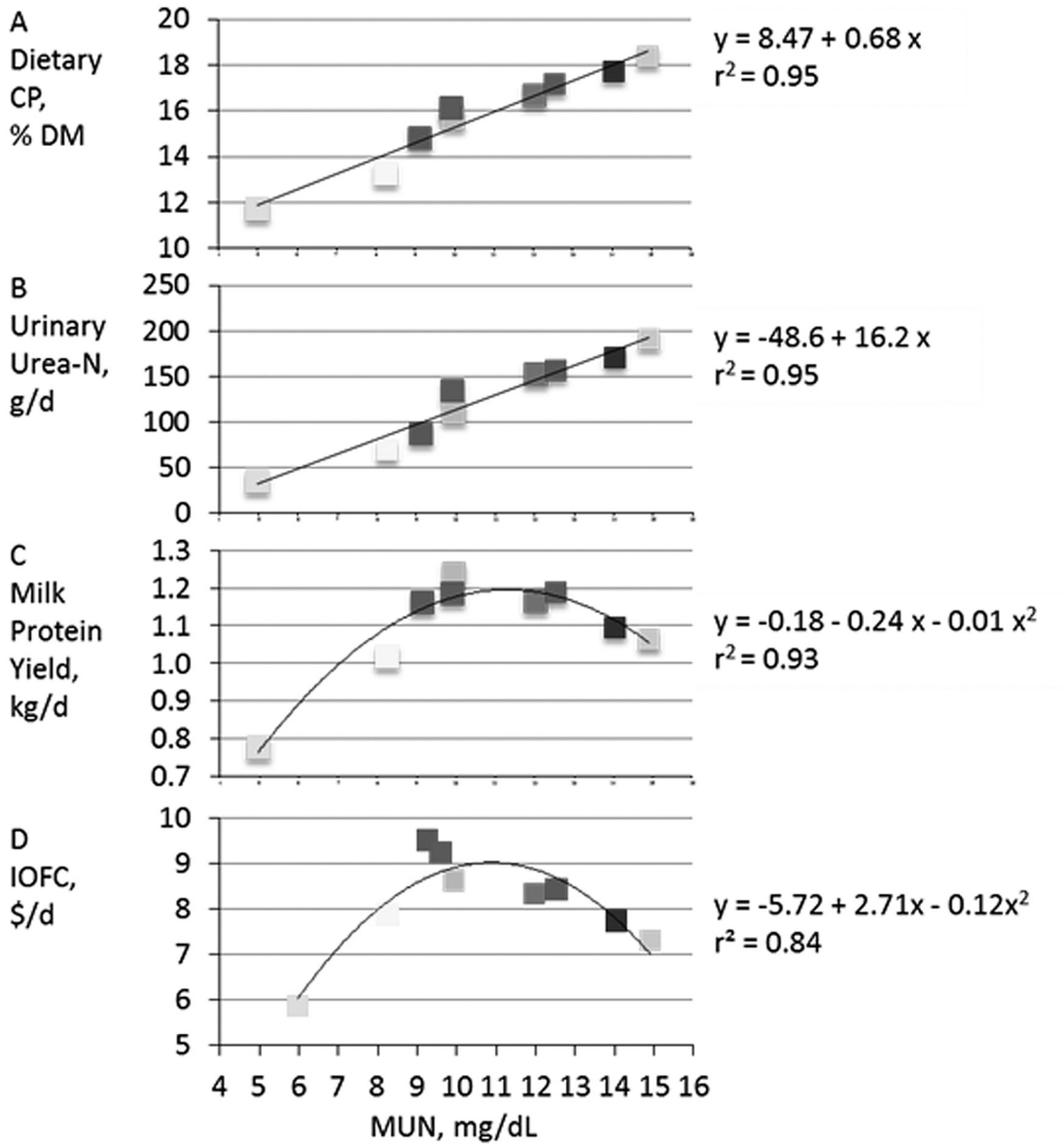

Figure 5: MUN as a predictor of dietary CP, urinary urea-N and milk protein yield; Data from 23 studies and 80 dietary treatments summarized in 9 dietary CP categories ranging from less than 12% to more than 18% of dietary dry matter (see text for details).

Target MUN of a Herd

The nutritional studies discussed above have helped us understand that high MUNs are likely to reflect an excess N relative to cows' need but provide little insight to the reason for the excess (RDP, RUP, or both). As discussed above, not only nutritional effects but also genetic and other permanent effects are likely to influence MUN test results on farms. Thus the optimal MUN of approximately 11 mg/dL discussed above may not apply to all dairy herds. In other words, a reference or baseline MUN above 11 mg/dL obtained through monitoring may not necessarily reflect excess dietary CP when measured on a particular farm. Still, if one attempts to use MUN as decision-making criteria to adjust dietary CP, one will have to decide whether to adjust the RDP or the RUP in the diet. Assuming a dietary CP adjustment is made in order to lower MUN, recalibrating the RUP in the diet should be the first step because RUP usually cost considerably more than RDP. *Hannigan et al. (2013)* encouraged producers and consultants to consider the following steps to find the lowest dietary CP that does not penalized milk (protein) production on a particular farm:

1. Make sure the diet is balanced to avoid excess CP and has sufficient energy according to *NRC (2001)*. In absence of long-term monitoring data, feed such a diet for at least 3 weeks to obtain a reference MUN for the herd;
2. Lower the RUP content of the diet by 0.25 or 0.5% units while holding energy and RDP content constant. Feed the diet for two to three weeks before determining whether dry matter intake and milk production have been reduced along with the (expected) reduction in MUN;
3. If no loss of production occurred continue the process of reducing RUP;
4. When loss of production occurs, add back the last reduction in RUP and record the target RUP and the corresponding RUP-adjusted target MUN for the herd;
5. Then (if desired), lower the RDP content of the diet by 0.25 or 0.5% units while holding RUP constant;
6. If no loss of production occurred continue the process of reducing RDP;
7. When loss of production occurs, add back the last reduction in RDP and record the target RDP and the corresponding RUP-and-RDP-adjusted target MUN for the herd.

Conclusions

Our review of the literature indicated that MUN (mg/dL) is highly correlated with dietary CP (% of diet DM), and both variables are reliable predictors of urinary urea-N excretion (g/d) when cows are fed diets typical of the Midwest of the United States. However, MUN and dietary CP are not good predictors of milk protein yield. Compared to CP analysis of feed ingredients or total mixed ration, the analysis of milk for urea-N is simple, more convenient and much more economical. If feasible, producers and consultants are encouraged to follow a (bulk tank) MUN monitoring protocol in combination with dietary interventions to determine herd-specific dietary CP and target MUN that do not penalize milk (protein) production, maximize IOFC, and at the same time minimize urinary urea-N excretion. In absence of such protocol, our best answer to the question of *"how low in CP can a diet be formulated?"* is that —based on data collected from nutritional studies— well-balanced diets of approximately 16.2% CP results in MUN of 11.3 mg/dL, which correspond to maximum protein yield (~1.20 kg/d) and IOFC (~ $9.00/cow/d). Furthermore, when cows are fed a 16.2% CP diet, expected urinary urea-N excretion would be 25% lower compared to the same cow fed a diet of 18% CP (for which MUN would be 14 mg/dL and IOFC less than $8.00/cow/d). Much remains to be discovered on how to best use MUN as a management tool for the benefit of the dairy industry. For example, critical information is needed to determine factors affecting cow-level (test-day MUN) and herd-level (bulk tank MUN). Research is also needed to determine whether excretion of urea-N in milk (i.e., MUN expressed in g/d) would provide additional predictive value compared with concentration of urea-N in milk (i.e., MUN expressed in mg/dL). Finally, additional fieldwork is still necessary to determine the relationship between MUN and IOFC under contrasting feeding systems.

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