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INTRODUCTION

Animal domestication has been important in the development of human societies and today milk and dairy products are included in dietary recommendations by public health organizations around the world. Sustainability in agriculture depends in substantial part on the productivity of plant and animal agriculture. Productivity, also referred to as productive efficiency, is defined as the relationship between food production and resource inputs. Over the last century we have gone from the American farmer producing enough food to feed 15 people a century ago, to today where an average farmer produces sufficient food to feed over 150 people (Bauman and Capper, 2011). In the case of animal agriculture, the application of scientific principles in the dairy industry began following WWII and annual milk yield per cow has increased from about 2100 kg in 1944 to over 10,000 kg today. Improvements in genetics and management, especially nutritional management, are the basis for these gains. Provision of feed represents a major cost in dairy production, and feed efficiency has improved over 400% during this interval. Thus, the dairy industry has made remarkable gains in the efficiency of resource use, and an equally impressive reduction in the environmental impact of producing milk (Capper et al., 2009).

The dairy cow has a remarkable ability to coordinate her biological processes to support mammary gland use of nutrients for milk synthesis, a coordination that must involve all nutrient classes and include most, perhaps all, organs and physiological processes (Bauman and Currie, 1980). While a linear increase in milk yield per cow has occurred over the last century, at some point gains in milk yield will reach a plateau where genetic selection and management practices have optimized the biological processes essential for milk synthesis. But where is this plateau? In the Northeast we have many herds that annually average over 29,000 lbs per cow. The top Jersey cow, Mainstream Barkley Jubilee, calved at 4 yr 8 mo and produced 55,590 lbs of milk (4.6% fat, 3.2% protein). The Holstein record is held by EverGreen View My 1326 with an annual production of 72,170 lbs milk (3.9% fat, 3.0% protein); Evergreen had to have an average daily NE intake which was over 6.5x maintenance. Finally, Guinness World Records recently recognized Gillette E Smurf as the lifetime record holder; Smurf’s production of 478,167 lbs milk in 10 lactations represents an amazing average of 85.5 lbs of milk for every day of life. These few examples indicate dairy cows are capable of extraordinary milk yields. Indeed, there must be a plateau, but that plateau is not evident and increases in productive efficiency will clearly continue in the future.

The following sections identify the biological processes that are associated with the remarkable gains in productive efficiency that have occurred over the last century.
and discuss concepts of regulation in lactating dairy cows. Genetics and management are key to these gains and this leads to a discussion of new applications of genomics, including the recent focus on residual feed index (RFI) to examine the genomics of feed efficiency and a recognition of nutrigenomics and the role nutrients can play in regulating metabolism.

BIOLOGICAL BASIS FOR IMPROVEMENTS IN PRODUCTIVE EFFICIENCY

Sources of Variation

The biology of a dairy cow involves a series of chemical reactions in which food is transformed and used to support body tissues and activities. Feed is consumed by the dairy cow and following digestive processes the nutrients are absorbed. Absorbed nutrients are then utilized by body tissues and this in turn plays a key role in regulating feed intake. Some nutrients are used to meet the maintenance requirement while other nutrients are used to maintain body reserves. Depending on physiological state, a major portion of the nutrients are utilized for productive functions such as milk synthesis (lactation) or fetal development (pregnancy). These biological processes comprise the “Cycle of Life” (Figure 1) and provide the framework to consider the basis for the historic gains in productive efficiency (Bauman et al., 1985).

Figure 1. Basis for animal differences in productive efficiency as illustrated by the Cycle of Life. Sources are: a) digestion and absorption, b) maintenance requirement, c) partial efficiency of milk synthesis, and d) nutrient partitioning for milk synthesis.

Digestion and Nutrient Absorption. The chemical and physical characteristics of feedstuffs have major effects on digestibility, and this forms the basis for current extension recommendations. Likewise, effects of feeding level, diet composition and
other dietary factors markedly alter digestibility and nutrient absorption (Huhtanen et al., 2009; Nousiainen et al., 2009). In contrast, studies with lactating cows have observed that genetic merit and digestibility were independent (Grieve et al., 1976; Custodio et al., 1983) and that genetically diverse lines of dairy cows differed little in digestibility, especially when intake was similar (Trigg and Parr, 1981; Davey et al., 1983; Belyea and Adams, 1990; Gordon et al., 1995). Thus, management practices can markedly affect digestibility and nutrient absorption, but genetic advancements have played little or no role in the historic improvements in productive efficiency (Table 1).

Table 1. Sources of variation in productive efficiency in lactating dairy cows

<table>
<thead>
<tr>
<th>Efficiency component</th>
<th>Among-animal variation</th>
<th>Sources of possible improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestion and nutrient absorption</td>
<td>Low</td>
<td>Minor</td>
</tr>
<tr>
<td>Maintenance</td>
<td>Low</td>
<td>Minor</td>
</tr>
<tr>
<td>ME use for milk synthesis</td>
<td>Low</td>
<td>Minor</td>
</tr>
<tr>
<td>Nutrient partitioning and intake</td>
<td>High</td>
<td>Major</td>
</tr>
</tbody>
</table>

Sources: Selection, Management

1Based on Bauman et al. (1985) and Reynolds (2004) with supporting references as cited in text.

Maintenance Requirement. The maintenance requirement can be markedly affected by stress (e.g. overcrowding) or environmental factors (e.g. heat and humidity). However, the maintenance requirement of mammals is remarkably similar across species when expressed on a metabolic body weight, although some variation does exist (Reid, 1974). Relatively few studies have evaluated the variation associated with the maintenance requirement of dairy cows. Van Es (1961) estimated that among animal coefficient of variation in maintenance requirement was 5 to 10% across 237 energy balance studies with dairy cows and steers. Flatt et al. (1969) also found remarkably good agreement in estimates of maintenance requirements of dairy cows across studies. Consistent with this Davey et al. (1983) concluded that the maintenance requirement was not influenced by genetic merit for milk production in Friesens. Thus, the maintenance requirement is only a minor source of variation among animals and changes in maintenance requirement have contributed little to the historic gains in productive efficiency (Table 1).

Utilization of ME for Milk Production. The partial efficiency (PE) of metabolizable energy (ME) utilization varies according to use (maintenance, lactation, growth, fattening or pregnancy) and type of diet (Garrett and Johnson, 1983). This variation undoubtedly relates to differences in the pattern of absorbed nutrients and the specific products being formed. However, under typical conditions in nutrient supply and milk composition there appears to be little difference among cows in the PE of milk synthesis (Table 1). Indeed, studies comparing low- and high-milk yielding genetic lines of dairy cows found no differences in the PE for milk synthesis (Belyea and Adams, 1990; Gordon et al., 1995). The biochemical pathways for synthesis of major milk components involve a series of enzymatic reactions, each having a specific energetic efficiency. Thus, similarities in PE estimates are expected because all cows use the same biochemical pathways and enzyme reactions for the synthesis of milk components.
Nutrient Partitioning. The major source of historic gains in productive efficiency is nutrient partitioning (Table 1). High-yielding cows direct a greater portion of absorbed nutrients to the mammary glands for milk synthesis and associated with this is a greater voluntary intake. Low-yielding cows have a lower voluntary intake: if they do consume more feed they use it for excessive body fat accretion rather than milk synthesis. Thus, selection for high milk production results in dairy cows that not only utilize more nutrients for milk synthesis but also have a higher intake to support a greater milk yield (Bauman et al., 1985; Reynolds, 2004).

Productive efficiency is a key component of sustainability because a greater milk yield per cow results in less resource use per unit of milk, a concept often referred to as “dilution of maintenance”. Dilution of maintenance is usually considered in terms of feed resources per unit of milk, but it applies more broadly to all of the costs of producing milk including renewable and non-renewable resources as well as the costs for facilities and labor.

Concepts of Regulation

The regulation of nutrient use for productive functions is a key biological principle in animal production with the overall goal of maintaining the animal’s well-being regardless of the physiological or environmental challenges that are encountered. The use of nutrients by the mammary gland represents a tremendous demand such that total maternal metabolism must be coordinated to support the demands for milk synthesis. From a conceptual basis, this involves two types of regulation, homeostasis and homeorhesis (Bauman and Currie, 1980; Bauman, 2010).

Homeostatic controls operate on an acute basis so that different tissues and organs are “working cooperatively” to maintain physiological equilibrium. There are many well-established examples of the multiple compensatory mechanisms functioning to maintain physiological equilibrium despite challenges from the external environment. One important example in dairy cows is the maintenance of circulating glucose. Glucose supply is critical for many tissues so over the short term the reciprocal actions of insulin and glucagon ensure the proper balance in glucose supply and for use in milk synthesis and other processes (Bauman and Currie, 1980).

Homeorhesis was defined as the “orchestrated changes for priorities of a physiological state” (Bauman and Currie, 1980). This was first applied to lactation and pregnancy, but the general concept has been extended to include different physiological states, nutritional and environmental situations, and even pathological conditions (Collier et al., 2005; Bauman, 2010). Key features of homeorhetic control are: 1) its chronic nature, hours and days vs. seconds and minutes required for most examples of homeostatic regulation; 2) its simultaneous influence on multiple tissues and systems that results in an overall coordinated response, and; 3) its mediation through altered response to homeostatic signals (Bauman, 2010). The concept of homeorhesis was the basis for our original focus on bovine somatotropin which lead to the commercialization
of rbST; this hormone remains the best characterized example of the homeorhetic control of nutrient partitioning during lactation.

APPLICATION OF GENOMICS TO NUTRITION

Genomic Developments

In April 2009, SCIENCE heralded new developments in animal agriculture with its issue cover and two articles announcing the sequencing of the bovine genome (The Bovine Genome Sequencing and Analysis Consortium et al., 2009; The Bovine HapMap Consortium, 2009). The bovine genome is similar in size to the genomes of humans and other mammals, containing about 3 billion base pairs and ~22,000 genes. These genes code for the specific proteins involved in the cycle of life discussed earlier. Thus, sequencing of the bovine genome offers new opportunities to understand the biology of dairy cattle and provides the framework to identify the genetic basis for the historic improvements and animal differences in productive efficiency.

Single nucleotide polymorphisms represent nucleotide base changes within the DNA sequence. Recently, USDA-ARS scientists genotyped over 5000 Holstein cows and bulls and identified over 38,000 SNP markers which they related to selection traits such as milk production and longevity (VanRaden et al., 2009; Cole et al., 2009). SNPs will undoubtedly be associated with the greater productivity efficiency observed for high producing dairy cows. However, it seems likely that small differences in variation may exist at many sites rather than few sites capturing large portions of the variation in productive efficiency among animals.

Each gene codes for a particular sequence of amino acids that constitute a specific protein. A SNP can result in a different amino acid in the protein sequence and this may affect the biological function of the protein. At this point in time there has been only limited success in identifying specific SNPs that correspond to functional differences the productive efficiency of dairy cows, but this area should offer exciting applications in the future. Nevertheless, there are two areas where the developments in genomics are already affecting our understanding related to nutrition, feed efficiency and the biology of milk production. These are 1) the use of residual feed index to identify cows with superior genetics for feed efficiency, and 2) the application of nutrigenomics to understand and trouble shoot problems in milk fat synthesis.

Residual Feed Index

Feed efficiency is defined and measured in several different ways. Over 50 years ago Koch et al. (1963) proposed the use of residual feed index (RFI) as a measure of feed efficiency in growing cattle, the cited advantage being that RFI is phenotypically independent of the production traits used to calculate feed intake (Herd and Arthur, 2009; Berry and Crowley, 2012). The RFI for growing cattle is calculated as the difference between an animal's actual feed intake and its expected feed intake, which was determined by regression of dry matter intake against mean body weight and
growth rate. An example is shown in Figure 2. Thus, the value for RFI represents the extent to which an animal’s actual feed intake deviates from expected feed intake, with efficient animals having a RFI where predicted intake is greater than observed feed intake and less efficient animals having the reverse (Williams et al., 2011).

Recently scientists at USDA-ARS (Connor et al., 2012) and a multi-university research group (VandeHaar et al., 2012) have applied RFI to evaluate the feed efficiency of individual dairy cows. In this case, feed intake is predicted from a regression that adjusts for parity, body size, body weight changes, body reserve changes and production of energy-corrected milk. Of interest is the potential to use RFI as a tool to identify more efficient animals or to derive associations between SNPs and RFI for use in a selection program. As defined earlier, productivity or productive efficiency in dairy cows is calculated as milk per unit of resource input, so it also represents a measure of feed efficiency. How does the earlier discussion on the “cycle of life” and sources of variation in productive efficiency relate to the variation depicted by RFI values?

Figure 2. Example of residual feed index (RFI). Data represents predicted feed intake versus actual feed intake for Holstein dairy heifers (Williams et al., 2011). RFI equals deviation of a calf’s data point from the unity line with more efficient animals above the line and less efficient calves below the regression line. Williams et al. (2011) used solid dots to indicate the top and bottom 10% of animals from each cohort with respect to RFI.

The calculation of RFI is designed to remove animal differences in nutrient partitioning via the adjustments related to individual animal differences in milk yield and changes in body weight and body composition. Thus, animal differences in nutrient partitioning, the area identified in the preceding section as representing the main basis for historical gains and major source of variation among today’s top herds and genetically superior cows, is not a component of the RFI value. However, RFI would include animal differences in digestibility and nutrient absorption, maintenance requirement and the partial efficiency of nutrient use for milk synthesis – areas which represent only a minor portion of animal differences in productive efficiency (see
previous section and Table 1). Indeed, in selection based on RFI, cows identified as having the best feed efficiency (as reflected by a lower RFI) can be at any level of milk production. Thus, the impressive gains in feed efficiency that are a consequence of achieving higher milk yields and the “dilution of maintenance” in gains in feed efficiency are not components of RFI. The application of RFI to lactating dairy cows is a recent concept and thus far there is a paucity of data to evaluate this approach. However, the failure of RFI to capture the important increases in milk yield and the dilution of maintenance represents an important limitation to its potential use to identify cows with a higher feed efficiency (i.e. productive efficiency). Of additional interest is to evaluate the extent to which RFI is repeatable; variables used in the regression adjustments are all determined with variable accuracy so a demonstration that the rank order of RFI among cows is repeatable in successive lactations would provide insight as to its potential for use in selection systems.

Nutrigenomics

Nutrigenomics is an umbrella term that refers to the impact of dietary components on physiological process by altering gene expression, epigenetic effects, proteins or metabolites (Bauman et al., 2011). Nutrition research has characteristically centered on identifying essential nutrients, establishing dietary requirements, and developing feeding systems to meet these requirements. Thus, past nutrition research has focused on designing diets that supply a quantity and pattern of nutrients to provide nourishment and allow for normal body function and health maintenance. Nutrigenomics represents a new dimension in nutrition research; results provide clear evidence that in addition to a nutritive role, nutrients also regulate the expression of genes thereby affecting metabolic pathways and homeostatic control, including diet-environment interactions. Nutrigenomics has been most vigorously studied in humans where it is hypothesized that the diversity in human genotypes provides differences that may allow an optimization of diets on an individual basis to maintain health and prevent chronic diseases (Müller and Kersten, 2003).

Among the best characterized examples of nutrigenomics is diet-induced milk fat depression in dairy cows (Bauman et al., 2011). While diet-induced MFD continues to be a challenge for commercial dairies, in the context of this paper it serves to illustrate how absorbed nutrients can effect gene expression and alter metabolic processes. The problem of diet-induced MFD perplexed producers, consultants and scientists for over a half-century, and many theories and practices have been purposed and found inadequate (Harvatine et al., 2009). A key development in understanding MFD was the recognition that it involved an interaction between rumen fermentation and mammary fatty acid (FA) synthesis. Thus, under certain dietary conditions rumen biohydrogenation is altered so that unique FA intermediates are produced and some of these are potent inhibitors of milk fat synthesis. To date three conjugated linoleic acid (CLA) isomers have been identify as bioactive FAs that inhibit milk fat synthesis – \textit{trans}-10, \textit{cis}-12 CLA, \textit{cis}-10, \textit{trans}-12 CLA and \textit{trans}-9, \textit{cis}-11 CLA. This inhibition involves a coordinated down regulation of gene expression for key enzymes in the synthesis of milk fat. This has been best studied for \textit{trans}-10, \textit{cis}-12 CLA and the cellular mechanism involves the
SREBP1 transcription factor family. The genes for key enzymes in FA synthesis have a base sequence in their DNA code that is referred to as a SRE element. The SRE element binds the active fragment of SREBP1 thereby reducing gene expression (Bauman et al., 2011). Table 2 lists the specific lipogenic genes that are coordinately down-regulated during diet-induced MFD and CLA-induced MFD, and the DNA sequence for all of these genes has a SRE element in the promoter region.

Table 2. Summary of SREBP1-regulated lipogenic genes in which mammary expression is coordinately reduced during milk fat depression

<table>
<thead>
<tr>
<th>Biochemical process/enzyme</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesis \textit{de novo}</td>
<td></td>
</tr>
<tr>
<td>Acetyl CoA carboxylase</td>
<td></td>
</tr>
<tr>
<td>Fatty acid synthase</td>
<td></td>
</tr>
<tr>
<td>Preformed fatty acids</td>
<td></td>
</tr>
<tr>
<td>Lipoprotein lipase</td>
<td></td>
</tr>
<tr>
<td>Fatty acyl CoA ligase</td>
<td></td>
</tr>
<tr>
<td>Desaturation</td>
<td></td>
</tr>
<tr>
<td>Stearoyl-CoA desaturase</td>
<td></td>
</tr>
<tr>
<td>Esterification</td>
<td></td>
</tr>
<tr>
<td>Acylglycerol phosphate acyl transferase</td>
<td></td>
</tr>
<tr>
<td>Glycerol phosphate acyl transferase</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1}Harvatine et al. (2009)

In the case of MFD, an understanding of how unique rumen biohydrogenation intermediates are able to regulate milk fat synthesis is already being applied in the formulation of diets to minimize the problem and for troubleshooting MFD when problems are encountered on commercial dairy farms. Improving our understanding of the role that specific nutrients play in the regulation of gene expression and metabolism represents a developing area that should offer exciting new opportunities to improve productive efficiency of dairy cows.

FINAL THOUGHTS

Our challenge in animal agriculture is to understand the regulation of physiological processes and their role in animal productivity, well-being and disease prevention. Estimates are that two-thirds of the historic gains in productive efficiency have come from genetic improvement and one-third from advances in nutrition and management. Presentations at this and previous Cornell Nutrition Conferences provide invaluable information on the latest developments in nutrition and management. But it's important to recognize that this is a package deal – sound nutrition and management practices are essential to fully actualize improvements in genetics and vice versa. While past gains have been impressive, recent developments indicate that the opportunity for future gains in productive efficiency may be even greater.
REFERENCES


Dairy cattle experience a remarkable shift in metabolism after calving, after which milk production typically increases so rapidly that feed intake alone cannot meet energy requirements (Baird, 1982, Bauman and Currie, 1980). Cows with a poor adaptive response to negative energy balance may develop hyperketonemia (ketosis) in early lactation. Cows that develop ketosis in early lactation lose milk yield and are at higher risk for other postpartum diseases and early removal from the herd.

DEFINING KETOSIS

Subclinical ketosis (SCK) is defined as an excess of circulating ketone bodies without clinical signs of ketosis (Andersson, 1988). The circulating ketone body most commonly used to diagnose SCK is blood \( \beta \)-hydroxybutyric acid (BHBA). The lower threshold concentration of BHBA for SCK is 1.2 mmol/L (1200 \( \mu \)mol/L or 12.4 mg/dl; note that multiplying by 10.3 converts BHBA concentrations from mmol/L to mg/dl). Other studies have reported lower thresholds for SCK ranging between 1.0 and 1.4 mmol/L. These different thresholds represent different outcomes and time periods; they will be explained in more detail later in this review.

The upper threshold of BHBA concentration for SCK should (by definition) be the onset of clinical signs. However, the detection of clinical signs of ketosis varies greatly from herd to herd. Therefore, \( \geq 3.0 \) mmol/L blood BHBA has been used as the upper threshold for SCK (McArt et al., 2011, Oetzel, 2004). Cows above this threshold probably should have been detected as having clinical ketosis, although my clinical experience indicates that this is not always the case.

The clinical signs of ketosis in early lactation dairy cows are decreased appetite, weight loss, decreased milk production, and (perhaps) a positive cowside test result for ketosis. These clinical signs are mostly quite subjective in nature, and the cowside tests for ketosis commonly used by dairy producers have considerable variability in their sensitivity and specificity for detecting ketosis. Thus, the incidence of clinical ketosis in a herd (as determined by dairy producers) is of very limited value in assessing the true ketosis status of a herd. Producers in smaller herds tend to overestimate the incidence of clinical ketosis (Simensen et al., 1990), and producers in larger herds tend to underestimate the incidence of clinical ketosis (based on my own clinical observations). Using blood BHBA testing to measure the incidence or prevalence of SCK in a herd is a powerful and useful clinical tool.
INCIDENCE AND PREVALENCE OF SUBCLINICAL KETOSIS

Incidence of Subclinical Ketosis

The incidence of SCK in a herd is the number of new cases of SCK (blood BHBA between 1.2 and 2.9 mmol/L) that occurred during the risk period (early lactation) divided by the number of cows who completed the risk period. Most new cases of SCK occur within the first 2 to 3 weeks after calving in herds that manage cows in groups and feed a TMR. Cows that are component-fed and housed in individual stalls appear to develop ketosis later (3 to 6 weeks after calving).

The time period over which the incidence of SCK is measured must be specified (e.g., a week, a month, or a year). Determining the incidence of SCK requires repeated testing of cows throughout this risk period. Testing must occur twice or more weekly in order to accurately assess the incidence of SCK. This is necessary because the median time for the resolution of SCK is about 5 days (McArt et al., 2011). If testing occurs only once a week, a cow could potentially develop and resolve her SCK between test intervals (McArt et al., 2012a). Because of the need for repeated testing, the incidence of SCK is usually determined only in research trials.

Published studies that did repeated testing of early lactation cows report early lactational incidence rates of ketosis between about 40% and 60% (Duffield et al., 1998, Emery et al., 1964, Simensen et al., 1990). These rates could have been even higher because some of these studies evaluated incidence based on once weekly blood BHBA testing. We (my Cornell coworkers and I) reported an overall SCK incidence rate of 43.2% for 1,717 cows in 4 large commercial herds (McArt et al., 2011). The SCK incidence ranged from 26.4% to 55.7% by herd. We also found that new cases of SCK occur remarkably soon after calving; peak incidence was at 5 DIM (see Figure 1 on the next page).

Prevalence of Subclinical Ketosis

Prevalence is a ‘snapshot’ measure the current of the SCK status of a group of cows and is defined as the proportion of cows with blood BHBA concentrations between 1.2 and 2.9 mmol/L at a given point in time. Repeated testing of individual cows is not necessary for determining prevalence. It is usually done for a subset of the early lactation cows within a herd. Herds can be tested repeatedly for SCK and the results pooled into a cumulative prevalence; this increases the reliability of the estimate of the herd’s prevalence of SCK. For practical reasons, almost all herd-level evaluations for SCK are conducted as prevalence testing.

The peak prevalence of SCK occurred at 5 DIM in the large field study mentioned above (McArt et al., 2012a) (see Figure 2 on the next page). At 5 DIM, 28.9% of cows were positive for SCK. This finding underscores the observation that SCK occurs very soon after calving.
Figure 1. Histogram of the incidence of SCK (first blood BHBA test between 1.2 to 2.9 mmol/L) on any one of 5 or 6 tests between 3 and 16 DIM.

Figure 2. Histogram of prevalence of SCK in 1,717 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. A positive test for SCK was defined as a blood BHBA concentration of 1.2 to 2.9 mmol/L.
Estimating the Incidence of Subclinical Ketosis from its Prevalence

Although the prevalence of SCK in a herd is much easier to determine than its incidence, the incidence of SCK must be known in order to estimate the overall negative impacts of SCK on herd performance. Knowledge of the herd’s prior incidence of SCK is also needed before determining the best ketosis testing strategy for a herd (McArt et al., 2012a).

The repeated testing necessary to determine the incidence of SCK within a herd is daunting and requires testing a large number of cows twice weekly for about the first two weeks of lactation. Fortunately, the incidence of SCK can be estimated from its prevalence. The incidence of ketosis has been reported to be 2.2 X the prevalence (Duffield et al., 1998, Oetzel, 2004) and was 2.4 X the prevalence in our recent field study (McArt, et al., unpublished data, 2012).

NEGATIVE IMPACTS OF SUBCLINICAL KETOSIS

Reduced Milk Yield

The negative impacts of SCK on milk yield are well-known. Estimates of milk yield loss due to ketosis represent the difference between ketotic and non-ketotic cows and do not fully account for how much milk the ketotic cow might have produced had she not developed ketosis. Thus, actual milk lost may be underestimated because cows with ketosis may have been higher producing cows prior to the onset of their ketosis.

Previous studies have reported milk yield losses in ketotic vs. non-ketotic cows of 2.2 to 3.1 lbs of daily milk (4.4 to 6.6%); a milk ketone test was used to diagnose ketosis in this study (Dohoo and Martin, 1984). Duffield et al. (2009) reported a 4.1 lb decrease (about 5.5%) in milk yield at first DHIA test for cows with blood BHBA ≥ 1.4 mmol/L during the first week after calving. Ospina et al. (2010a) used a cutpoint of ≥ 1.0 mmol/L of blood BHBA to define SCK and reported that cows (≥ lactation 2) with SCK lost 865 lbs of 305-day ME milk (about 7.0%). Chapinal et al. (2012) reported a 5.3 lb reduction in milk yield (about 6.9%) at the first DHIA test for cows with blood BHBA ≥ 1.4 mmol/L during the first week after calving.

In our recent field study, cows (any parity) with SCK produced 2.6 lbs less daily milk (about 3.4%) for the first 30 DIM compared to non-ketotic cows (McArt et al., 2012a). Early detection and treatment of SCK with propylene glycol (300 ml orally once daily until the ketosis resolved) improved milk production by about 1.5 lbs of daily milk compared to cows whose SCK was left untreated (McArt et al., 2011).

The severity of the milk yield loss due to SCK was associated with magnitude of the elevation in BHBA at the first diagnosis of SCK (McArt et al., 2012a). Each additional 0.1 mmol/L increase in BHBA (beyond 1.2 mmol/L) was associated with 1.1 lbs more lost milk for the first 30 DIM. The difference between modest SCK (1.2 mmol/L BHBA)
and more severe SCK (2.4 mmol/L) was 13.2 lbs of daily milk for the first 30 DIM (see Figure 3 below).

Figure 3. Regression plot of mean predicted daily milk yield for the first 30 DIM by blood BHBA concentration of first positive BHBA test (1.2 to 2.9 mmol/L) for 369 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. The solid line represents the best fit; 95% confidence intervals are shown for each predicted milk yield by BHBA concentration.

Days in milk at the first onset of SCK also affects the severity of the milk yield loss. Cows first diagnosed with SCK between 3 and 7 DIM produced 4.6 lbs less daily milk (about 6.0%) in the first 30 DIM compared to cows first diagnosed with SCK between 8 and 16 DIM (McArt et al., 2012a). This was the first study to report that earlier onset of SCK resulted in more detrimental effects to the cow. Other problems associated with SCK were more severe in cows that were first diagnosed between 3 and 7 DIM; these will be discussed later in this review.

Increased Risk for Early Lactation Removal

In our recent field study, we reported that cows with SCK were 3.0 X (95% CI 2.2 to 4.2) more likely to be removed from the herd (sold or died) in the first 30 DIM compared to non-ketotic cows (McArt et al., 2012a). No previous studies have reported the effect of SCK on herd removal.

Increasing severity of ketonemia at the onset of SCK increased the risk for herd removal in the first 30 DIM (McArt et al., 2012a). Each 0.1 mmol/L increase in BHBA increased the risk for herd removal by 1.4-fold (95% CI = 1.1 to 1.8). For example, increasing blood BHBA at the onset of SCK from 1.2 to 2.4 mmol/L increased the risk for early lactation herd removal by 56.7 X (1.4^{12}). Note that this study only considered
cows with blood BHBA < 3.0 mmol/L; cows with higher BHBA concentrations could have had an even greater risk for herd removal in the first 30 DIM.

Early detection and treatment of SCK with oral propylene glycol reduces the risk for early lactation removal. We reported that cows not treated for their SCK were 2.1 times (95% CI = 1.2 to 3.6) more likely to die or be sold by 30 DIM than cows treated with oral propylene glycol (McArt et al., 2012b)

Increased Risk for Displaced Abomasum

The association of SCK with increased risk for displaced abomasum (DA) is well-established. Duffield et al. (2009) reported that blood BHBA ≥ 1.2 mmol/L in the first week after calving increased the odds for DA by 2.6 (95% CI = 1.3 to 5.2). Ospina et al. (2010b) reported 6.9-fold higher risk for DA (95% CI = 3.7 to 12.9) for cows with postpartum BHBA ≥ 1.0 mmol/L. Interestingly, Chapinal et al. (2011) reported no effect of postpartum BHBA on the risk for DA, although elevated blood NEFA and low blood calcium were associated with increased odds for DA.

In our recent field study we found even more profound effects of SCK on the risk for subsequent DA. Cows with SCK were 19.3 times more likely to develop a subsequent DA (95% CI = 13.8 to 27.0). The very large risk ratio reported here reflects the very low rate of DA in non-ketotic cows in the study (0.3% in the non-ketotic cows vs. 6.5% in the cows with SCK).

We also reported that cows with more severe hyperketonemia at the onset of their SCK had increased risk for DA (McArt et al., 2012a). Each 0.1 mmol/L increase in BHBA at the first SCK-positive test increased the risk for developing a DA by a factor of 1.1 (95% CI = 1.0 to 1.2). A cow with an initial blood BHBA of 2.4 at the onset of her ketosis would have a 3.1-fold (1.1^{12}) increased risk for a subsequent DA compared to a cow with an initial BHBA concentration of 1.2 mmol/L at her first SCK diagnosis.

Days in milk at the first onset of SCK also affects the risk for subsequent DA (McArt et al., 2012a). Cows who first developed SCK between 3 and 5 DIM were 6.1 time more likely (95% CI = 2.3 to 16.0) to develop a DA compared to cows first testing positive for SCK between 6 and 16 DIM.

Early detection and treatment of SCK with oral propylene glycol reduces the risk for subsequent DA. We reported that cows not treated for their SCK were 1.6 times (95% CI = 1.3 to 2.0) more likely to develop a DA than cows treated with oral propylene glycol (McArt et al., 2012b).

Increased Risk for Metritis

Duffield et al. (2009) reported that blood BHBA ≥ 1.2 mmol/L in the first week after calving increased the odds for metritis 3.4-fold (95% CI = 1.6 to 7.2). The authors suggested that impaired immune function due to ketosis could explain the increased risk
for metritis. Ospina et al. (2010b) reported a 2.3-fold higher risk for metritis (95% CI = 1.1 to 5.2) for cows with postpartum BHBA ≥ 0.7 mmol/L. We did not formally evaluate the association between metritis and SCK in our recent field study (McArt et al., 2012a), but instead offered it to the models as a potential confounding variable. Because metritis occurs very soon after calving and may not be diagnosed promptly, it is particularly difficult to infer whether the associations between SCK and metritis are cause or effect.

Impaired Fertility

Associations between SCK and fertility have been inconsistent. Walsh et al. (2007) evaluated cows from mostly small and medium-sized herds in Ontario in 1990’s and reported that ketosis (defined as blood BHBA ≥ 1.0 mmol/L) in the first week after calving reduced the risk for pregnancy at first service (OR = 0.73, 95% CI = 0.54 to 0.99). In the second week after calving, ketosis (defined as blood BHBA ≥ 1.4 mmol/L) reduced the odds for pregnancy even more (OR = 0.60, 95% CI = 0.40 to 0.88). Ospina et al. (2010a) studied larger herds (> 250 cows) in New York in the late 2000’s and found a less profound effect of ketosis on reproduction. They reported that the risk for pregnancy with 70 days of the voluntary waiting period tended to be lower (hazard ratio = 0.87, P = 0.10) for cows with blood BHBA ≥ 1.0 mmol/L after calving.

Chapinal et al. (2012) followed cows from 55 herds (herd size >100 cows) from 2006 to 2007 and found no association between blood BHBA before or after calving on first service conception rates. In our recent field trial in 4 large commercial dairies, we found no overall effect of SCK on first service conception rates (McArt et al., 2012a). We did find that cows first diagnosed with SCK between 3 and 7 DIM were 0.7 times as likely to conceive at first service (95% CI = 0.6 to 0.8) compared to cows first testing positive between 8 and 16 DIM. We also reported reduced pregnancy rates by 150 DIM for cows that first developed their SCK earlier in lactation.

Early detection and treatment of SCK with oral propylene glycol increases first service conception. We reported that cows with SCK who were treated with oral propylene glycol were 1.3 times (95% CI = 1.1 to 1.5) more likely to conceive at first insemination than control cows (McArt et al., 2012b).

ECONOMIC IMPACTS OF SUBCLINICAL KETOSIS

The economic impact of SCK can be quantified, but is dynamic and dependent on the expected milk yield loss, feed costs, feed efficiency, expected increase in the occurrence of postpartum diseases, the background incidence of these diseases, expected costs of these diseases, expected increase in early lactation herd removals, slaughter value of sold cows, disposal costs of dead cows, treatment costs for sick cows, and the cost of herd replacements. Duffield (2000) estimated the cost of a case of SCK to be CAN $50 to CAN $100, which is approximately US $46 to US $92 when adjusted for inflation and the exchange rate. Geishauser et al. (2001) derived a similar estimate of CAN $78 per case of SCK (approximately US $68 after adjusting for inflation.
and the exchange rate). We are currently working on detailed models to estimate the cost of a case of SCK based on the results from our recent field trial.

The high incidence of SCK, in combination with even a moderate cost per case, results in very high overall costs to the dairy producer. We have seen large variations in herd-level incidence of SCK (from about 25 to 60%), which suggests that there are important economic opportunities in many of our dairy herds for SCK prevention.

COWSIDE BLOOD BHBA TESTING WITH A HAND-HELD KETOMETER

Our understanding of SCK and the ability of veterinarians and dairy producers to diagnosis ketosis has been greatly enhanced by the availability of a rapid and accurate cowside test for blood BHBA. The Precision Xtra® meter (Abbott Laboratories) was developed to measure either whole blood BHBA or whole blood glucose in human patients. As far as we know, no other human glucometer can also function as a ketometer (i.e., able to measure blood BHBA). The Precision Xtra® meter gives excellent results for measuring whole blood BHBA in cows. No additional calibration or adjustment from the human system is needed.

The Precision Xtra® ketone monitoring system is a simple and direct electrochemical test (which may explain why it works well for both human and bovine blood). The ketone test strip contains the enzyme β-hydroxybutyrate dehydrogenase, which oxidizes BHBA to acetoacetate. This reduces NAD+ to NADH. The NADH is then reoxidized to NAD+ by an electron transfer mediator molecule. The electrical current generated by this conversion is measured by the meter and is directly proportional to the BHBA concentration.

These meters retail in human pharmacies for about $80 (USD). Veterinary suppliers carry the meters for about $50 each. The blood ketone test strips (which measure BHBA) are sold in boxes of 10 strips each. Veterinary suppliers sell these for about $13 to $15 for a box of 10 strips, or $1.30 to $1.50 per test. Human suppliers will typically sell them for about $4.00 to $5.00 per strip. Most pharmacies do not stock the blood ketone strips routinely but can order them for you.

The BHBA results on blood from cattle are surprisingly accurate using the Precision Xtra® system. Three initial studies (Burke et al., 2008, Iwersen et al., 2009, Oetzel and McGuirk, 2008) all gave very similar results. These studies involved a total of 622 cows with a 14.1% prevalence of ketosis. The average R² between hand-held meter and laboratory BHBA results was 0.94. The meter was 91% sensitive and 94% specific for diagnosing ketosis (pooled results from all three trials). The positive predictive value for the meter was 73% and the pooled negative predictive value was 98%.

The most recent evaluation of the Precision Xtra™ blood ketone system showed exceptionally high sensitivity and specificity (>98%) for ketosis diagnosis (Oetzel, 2010) using the threshold of ≥1.3 mmol/L. The R² between hand-held meter and laboratory BHBA results was 0.86, which was lower than for previous reports. This may have
been the result of the cold conditions during this study. Although the meter and strips can be used as a cowside test year-round, it is important to keep the meter and strips as warm as possible (e.g., in an inside pocket) during cold weather.

The most rewarding use of cowside blood BHBA testing is for herd-based ketosis monitoring. Strategies for herd-based testing have been explained in detail (Oetzel, 2004). The cowside BHBA test with the hand-held meter can be used in place of submitting serum or plasma samples to a laboratory for BHBA testing. In summary, the protocol involves testing 12 or more cows in early lactation. If more than 10% of the cows tested have blood BHBA ≥1.2 mmol/L) the group is considered to have a ketosis problem.

ACKNOWLEDGMENTS

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CHOLINE: A LIMITING NUTRIENT FOR TRANSITION DAIRY COWS

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INTRODUCTION

Choline has been shown to be a required nutrient for many animals including rats, mice, dogs, pigs, guinea pigs, chickens, and trout. Choline is often referred to as a vitamin, however, it doesn’t fit any of the classical definitions for a vitamin. It is not a co-factor in enzymatic reactions, it can be synthesized endogenously, and it is required in larger amounts than vitamins. The ability to synthesize choline endogenously does not mean it is a dispensable or non-essential nutrient. Deficiency symptoms include suppressed growth rates, renal dysfunction, and development of fatty liver. Choline is crucial for normal function of all cells. The most common form of choline in biological systems is phosphatidylcholine (PC), a phospholipid that is a component of all cell membranes and lipoproteins that function to transport lipids through the circulatory system. Choline is a source of methyl groups, therefore, it can spare methionine and have interactions with other nutrients involved in one-carbon metabolism (e.g. folate). Choline is also a component of acetylcholine, an important neurotransmitter.

The NRC (2001) wrote: “The establishment of a choline requirement, either for lactating dairy cow, or a transition cow in the late dry period and in early lactation, will require more extensive feeding experiments than available at the time of this publication.” It has now been 11+ years since publication of the last NRC and at this time there has not been an announcement for the formation of a new committee to author the next NRC. That means it will probably be at least 2016 until publication of the 8th revised edition. Since publication of the last NRC, numerous studies have been conducted to examine the effects of feeding ruminally protected choline to dairy cows, particularly as they transition from the dry period to early lactation. In light of new research and because a revised NRC is not on the immediate horizon, it seems appropriate to initiate discussion on whether choline should be considered a required nutrient in dairy diets.

TRANSITION COW AND CHOLINE BIOLOGY

Several studies have shown 50 to 60% of transition cows experience moderate to severe fatty liver (Boe et al., 2004). These studies have been conducted in numerous countries across different genetic lines of cattle and varying management systems and the data do not represent problem cows or herds. The consistency amongst these studies suggests that development of fatty liver is a “normal” part of the cow’s biology.
Because fatty liver is a classic deficiency symptom for choline, it is reasonable to question if transition cows are typically deficient in choline.

At calving there are hormonal changes that trigger an intense period of lipid mobilization from adipose tissue and as a result, blood nonesterified fatty acid (NEFA) concentrations typically increase 5- to 10-fold (Grummer, 1993). NEFA remain elevated, although to a lesser extent, during early lactation when cows experience negative energy balance. Blood flow to the liver doubles as a cow transitions from the dry period to lactation (Reynolds et al., 2003). NEFA concentration and blood flow are the two biggest factors affecting how much NEFA is taken up by the liver. As a result, daily fatty acid uptake by the liver increases 13-fold at calving, from approximately 100 to 1300 g/day (Reynolds et al., 2003). Not all of the fatty acids taken up by the liver will be stored and contribute to fatty liver. However, Drackely (2001) estimated that during peak blood NEFA concentration, approximately 600 g might be deposited in 24 hours which would correspond to an increase in liver fat of 6-7% by weight. As a reference, fat above 5% in the liver (wet basis) is considered by the veterinary community to be moderate to severe fatty liver. It is important to understand that this dramatic increase in NEFA uptake by the liver is part of the normal biology of transition cows and is not restricted to fat cows, poorly fed cows, or cows housed in suboptimal environments.

The most desirable fate of fatty acids entering the liver would be complete oxidation to provide energy to the liver or reesterification and export as triglyceride from the liver as part of a very low density lipoprotein (VLDL). Hepatic oxidation increases approximately 20% during the transition period (Drackley et al., 2001). This increase does not represent a strategic move by the cow’s liver to cope with the sudden surge of NEFA uptake at calving. It occurs because the liver becomes metabolically more active. Unfortunately, the increase in oxidation is not sufficient to cope with the increased load of fatty acid being presented to the liver. Research conducted nearly 25 years ago at the University of Wisconsin (Kleppe et al., 1988) and Michigan State University (Pullen et al., 1990) revealed that ruminants have a low capacity to export triglyceride from the liver as very low density lipoprotein (VLDL) as compared to nonruminants. This and the inability to markedly increase fatty acid oxidation is why transition dairy cattle develop fatty liver when experiencing elevated blood NEFA.

It is now apparent that choline deficiency is a limiting factor for VLDL triglyceride export from the liver. It has been shown in many species, using a wide variety of experimental approaches, that rate of VLDL export is highly related to the rate of hepatic PC synthesis (Cole et al., 2011). Models include monogastrics fed choline deficient diets, isolated hepatocytes cultured in choline and methionine deficient media, and in knock out mice for genes involved in PC synthesis (Cole et al., 2011). Interestingly, there is no evidence that synthesis of any other phospholipid is required for hepatic VLDL assembly and secretion. In addition to direct PC synthesis from
dietary choline, there is endogenous hepatic synthesis of PC via methylation of phosphotidylethanolamine (PE). Sharma and Erdman (1988) demonstrated dietary choline is extensively degraded in the rumen of dairy cows and very little is available to the small intestine for absorption. Choline flow to the duodenum increased less than 2 g/day, even when free choline intake was increased to more than 300 g/d. Therefore, ruminants are more highly dependent than nonruminants on endogenous synthesis of PC from PE. Is endogenous synthesis of PC from PE sufficient during the transition period or do cows require choline supplementation? The high proportion of transition cows developing moderate to severe fatty liver during the transition period suggests that endogenous synthesis is not sufficient in many cows.

EVIDENCE FOR A CHOLINE DEFICIENCY IN TRANSITION DAIRY COWS

The first piece of evidence that transition cows are deficient in choline is the development of fatty liver during the periparturient period (Grummer, 1993; Bobe et al., 2004). More compelling evidence is the alleviation of fatty liver when supplying cows with choline that is protected from ruminal degradation (Cooke et al., 2007; Zom et al., 2011). Dutch researchers (Goselink et al., 2012) recently demonstrated greater gene expression for microsomal triglyceride transfer protein (MTTP) in liver of transition cows supplemented with rumen-protected choline (RPC). MTTP is an important protein required for hepatic VLDL synthesis. This provided solid evidence that choline limitation is a causative factor for inadequate fat export out of the liver.

The reduction in liver fat content when feeding transition cows RPC is accompanied by improved health and production. Lima et al. (2011) observed reduced incidences of clinical ketosis, mastitis, and morbidity when feeding RPC from 25 days prepartum to 80 days postpartum. It has been known for years that elevated fat in the liver is associated with poor reproductive performance (Bobe et al., 2004). First service conception rate was increased by feeding RPC in one study (Oelrichs et al., 2004) but not another (Lima et al., 2011). We (Grummer and Crump, unpublished) recently completed a meta-analysis for 13 studies that fed RPC to transition cows. Feed stability or evidence of bioavailability of choline source was not a criterion for study selection. Studies were not screened for “soundness” of research. Treatment means and sample size (standard error of the mean) had to be available for the analysis. Ten of the thirteen trials were published in peer-reviewed journals. For studies to be included in this analysis, RPC had to be fed prior to calving. Time when RPC supplementation was started varied between 28 to 7 days prior to expected calving. RPC supplementation was terminated anywhere from the day of calving (one study) to 120 days in milk. Response variables included DMI, milk yield, energy corrected milk yield, fat %, protein %, and fat and protein yield. Insufficient data was available for analysis of liver fat or energy-related blood parameters. Analysis revealed a significant increase of 4.9 lb milk/day and 1.6 lb of dry matter intake/day (Table 2; Figure 1). Milk fat and protein yield percentage were
not significantly affected by treatment but yields were (Table 2). These studies were conducted in several countries under a variety of management conditions and they did not target problem herds or cows. This implies that benefits to supplementing protected choline can be realized by a wide variety of herds. Alleviating a choline deficiency not only reduces liver fat but also improves parameters that are economically important to dairy producers.

Table 1. Studies used in the Meta-Analysis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Choline Dose, g/d</th>
<th>Product</th>
<th>Duration</th>
<th>Exp.Units</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hartwell et al., 2000</td>
<td>0,6,12</td>
<td>Capshure</td>
<td>-21 to 120</td>
<td>24</td>
<td>M</td>
</tr>
<tr>
<td>Zom et al., 2011</td>
<td>15</td>
<td>ReaShure</td>
<td>-21 to 42</td>
<td>19</td>
<td>M</td>
</tr>
<tr>
<td>Lima et al., 2007¹</td>
<td>15</td>
<td>ReaShure</td>
<td>-25 to 80</td>
<td>4 (pen)</td>
<td>M, P</td>
</tr>
<tr>
<td>Lima et al., 2007¹</td>
<td>15</td>
<td>ReaShure</td>
<td>-22 to 0</td>
<td>5 (pen)</td>
<td>P</td>
</tr>
<tr>
<td>Oelrichs et al., 2002¹</td>
<td>15</td>
<td>ReaShure</td>
<td>-28 to 100</td>
<td>32</td>
<td>M, P</td>
</tr>
<tr>
<td>Zahra et al., 2006</td>
<td>14</td>
<td>ReaShure</td>
<td>-25 to 28</td>
<td>91</td>
<td>M, P</td>
</tr>
<tr>
<td>Piepenbrink et al., 2003</td>
<td>11,15, 19</td>
<td>ReaShure</td>
<td>-21 to 63</td>
<td>12</td>
<td>M</td>
</tr>
<tr>
<td>Janovick et al., 2006</td>
<td>15</td>
<td>ReaShure</td>
<td>-21 to 21</td>
<td>21</td>
<td>M</td>
</tr>
<tr>
<td>Elek et al., 2008</td>
<td>25/50 Pre/Post</td>
<td>Norcol-25</td>
<td>-25 to 60</td>
<td>16</td>
<td>M, P</td>
</tr>
<tr>
<td>Ardalan et al.</td>
<td>14</td>
<td>Col 24</td>
<td>-28 to 70</td>
<td>20</td>
<td>M, P</td>
</tr>
<tr>
<td>Pinotte et al.</td>
<td>20</td>
<td>Overcholine</td>
<td>-14 to 30</td>
<td>13</td>
<td>M</td>
</tr>
<tr>
<td>Xu et al. #1</td>
<td>7.5</td>
<td>Not reported</td>
<td>-7 to 21</td>
<td>7</td>
<td>M</td>
</tr>
<tr>
<td>Xu et al. #2</td>
<td>11,22,33</td>
<td>Not reported</td>
<td>-15 to 15</td>
<td>9</td>
<td>M, P</td>
</tr>
</tbody>
</table>

¹Studies have not been published in a peer-reviewed journal. Standard errors were not reported in abstracts but were obtained from the authors.
Table 2. A Meta-analysis of 13 studies examining the effects of feeding RPC to transition cows on dry matter intake and milk.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>RPC</th>
<th>SEd</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, lb/d</td>
<td>39.98</td>
<td>41.60</td>
<td>.46</td>
<td>.0042</td>
</tr>
<tr>
<td>Milk, lb/d</td>
<td>70.88</td>
<td>77.75</td>
<td>.75</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ECM, lb/d</td>
<td>76.87</td>
<td>82.78</td>
<td>1.33</td>
<td>.0038</td>
</tr>
<tr>
<td>Fat yield, lb/d</td>
<td>2.788</td>
<td>3.042</td>
<td>.086</td>
<td>.021</td>
</tr>
<tr>
<td>Protein yield, lb/d</td>
<td>2.300</td>
<td>2.467</td>
<td>.053</td>
<td>.010</td>
</tr>
</tbody>
</table>

Figure 1. Individual study results from a meta-analysis of 13 transition cow trials that examined the effects of feeding rumen-protected choline (Grummer and Crump, unpublished).

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**Milk Yield (lb/d) by Study**

Average Response = 4.9 lb/d  
P < 0.0001

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**CAN PROTECTED METHIONINE SUBSTITUTE FOR PROTECTED CHOLINE?**

Protected methionine has often been suggested as a possible alternative to protected choline for supplementation to transition dairy cows. Methionine and choline both serve as methyl donors. Methionine methyl groups can be used for endogenous synthesis of PC from PE. As an amino acid, methionine is needed for the synthesis of apolipoproteins. Therefore, there is a conceptual basis for methionine substitution for
choline. There have been three studies that have examined the role of methionine in prevention of fatty liver in either feed restricted dry cows (Bertics and Grummer, 1999) or transition cows (Piepenbrink et al., 2004; Osorio et al., 2011). Sources of methionine included 2-hydroxyl-4-methylthio-butanoic acid (HMB, a methionine analog that can be absorbed and partially converted to methionine by the liver; Bertics and Grummer, 1999; Piepenbrink et al., 2004), Smartamine (methionine protected from ruminal degradation by a pH sensitive polymer coating; Osorio et al., 2011), or MetaSmart (isopropyl ester of HMB; Osorio et al., 2011). None of these studies indicated that methionine treatment affected liver triglyceride accumulation during negative energy balance.

CONCLUSIONS

The time between NRC publications is increasing and when (or if) the next publication will occur is not known. Consequently, discussions outlined in this article become important for providing nutritionists with updates regarding nutrient requirements. Since the last NRC (2001) publication, a significant body of evidence has accumulated to support choline being a required but limiting nutrient in transition cow diets. An analogous situation occurred when the last NRC (2001) committee included a supplemental vitamin E recommendation to improve mammary health and reproduction. The recommendation was made despite the lack of titration trials, knowing the amount of vitamin E in the basal diet would seldom be known, and realizing there could be numerous interactions with other antioxidants. Similarly, our knowledge of availability of choline from rumen-protected sources is incomplete as is our knowledge of interactions between choline and other nutrients involved with one-carbon metabolism. Nevertheless, there is overwhelming evidence that feeding transition dairy cows 15 g choline/day in a form that is protected from ruminal degradation will alleviate choline’s classic deficiency symptom and lead to improvements in health and performance.

REFERENCES


Pronounced seasonal patterns of milk yield and composition are evident in cattle. These seasonal patterns are largely induced by climatological variables, breed effects and management factors, such as feed quality and reproductive management. Month of parturition is known to have a pronounced impact on subsequent milk yield and composition. Highest milk yields in dams occurred when calves were born in January and February while lowest yields occurred following August and September calving, (Figure 1).

The seasonal pattern in milk yield is related to the direct and indirect effects of environment on milk production. Direct effects are related to the effects of elevated temperature on milk yield; indirect effects are due to photoperiod effects and the negative impact of heat stress, during late pregnancy on maternal and fetal metabolism and circulating plasma endocrine patterns which are altered by the stress, (Collier et al. 1982). As is apparent from Figure 1, there is also a seasonal pattern in milk protein which parallels the seasonal pattern in milk yield. Interestingly, the milk protein yield pattern appears to be more directly affected by temperature as the nadir occurs during the hottest part of the summer. This may reflect the need for production of heat shock proteins by mammary epithelial cells during periods of heat stress which would reduce milk protein synthesis rates while the milk yield curve displays both direct and carry-over effects related to indirect effects on pregnancy and metabolic state of the cow. The seasonal
pattern in calf birth weight mimics the seasonal pattern in milk yield and birth weight has been shown to be correlated with milk yield, (Collier et al. 1982, Thatcher and Collier, 1986).

The majority of studies published on climatic effects on milk composition and yield have evaluated effects of temperature. Dairy cattle are sensitive to heat stress because of the high metabolic heat production and feed intake associated with rumen fermentation and milk yield. Likewise, for the same reasons, dairy cattle are relatively resistant to cold stress. Heat stress in cattle is characterized by increased rectal temperature, elevated respiration rates and decreased feed intake which contributes to the decreased milk yield. The environmental temperature range from -5 to 23.9 °C has little impact on milk yield and composition and is referred to as the thermoneutral zone for the lactating dairy cow. However, temperatures above 23.9 °C are known to decrease solids-not-fat (SNF), protein, lactose and fat percentage of milk. Due to its involvement in osmotic regulation of milk, the impact of temperature on lactose and mineral content of milk is much smaller than the impact of temperature on protein and fat yields. Generally, in temperate regions, the fat content may average 0.4% lower and the protein content 0.2% lower in summer as compared to winter months.

**THERMAL THRESHOLD IN HIGH PRODUCING DAIRY COWS**

The interface between the environment and the animal is the hair coat and skin surface. It is not surprising then that physiological responses to thermal load are closely associated with skin surface temperatures (Collier et al 2008). The responses include activation of thermal receptors, sweat gland and secretion of heat shock proteins into blood as well as activation of the autonomic nervous and endocrine systems. These changes are well described in several recent reviews (Collier et al. 2008, Baumgard and Rhoads 2012). However, producers need an on farm tool for estimating when cows need additional cooling. The tool of choice is usually the temperature humidity index (THI) which has been recently revised for high producing dairy cows (Zimbleman et al. 2009). The THI threshold for lactating dairy cows producing more than 35 kg of milk per day is 68. Therefore, cooling methods on commercial dairy farms should be implemented earlier to prevent these effects.

**METABOLIC EFFECTS OF HEAT STRESS**

During periods of heat stress the nutrient requirements of animals are altered resulting in the need to reformulate rations. For example, if dry matter intake decreases then an increase in nutrient density is required along with recalculating mineral and water requirements due to increased potassium loss in sweat (Collier et al., 2005). Reductions in dry matter intake are major contributors to decreased milk production. (Beede and Collier, 1986; Collier et al., 2008). When cows are heat stressed there is also a reduction in rumination and nutrient reabsorption and an increase in maintenance requirements causing a net decrease in nutrient/energy availability for production (Beede and Collier, 1985; Collier, 2005). Studies by Wheelock et al. 2010 have shown the reduction in DMI may only be responsible for ~40-50% of the decrease in milk production when cows are heat stressed and ~50-60% can be explained by other changes induced by heat stress. This raises the possibility that some of the loss in milk yield during thermal stress might be recoverable through appropriate nutritional management. Other approaches to decrease the effect of heat stress nutritionally are to decrease fiber intake to levels where the rumen can function properly, adding fat supplementation because of its high energy content and low heat increment, implementing higher concentrate diets with caution, and more recently adding niacin supplementation (Beede and Collier, 1986).

Niacin, nicotinic acid, is a possible supplement which induces vasodilation therefore transferring body heat to the peripheral (Di Constanza et al., 1997). Transferring body heat to the surface through peripheral or vasomotor function can perhaps alleviate some of the decrease in dry matter intake and thus milk production. Researchers have reported niacin to decrease skin temperatures during periods of mild to severe heat stress when supplementing cows with 12, 24, or 36 g of raw niacin for three consecutive 17 day periods (Di Constanza et al., 1997). When supplementing raw niacin, the amount of niacin degraded or absorbed in the rumen is much larger than the amount that reaches the small intestine (~17-30%; NRC, 2001). Early research observing the effects of niacin in heat stressed dairy cows had only looked at raw niacin, however encapsulated niacin was recently evaluated during two experiments at
the University of Arizona, one in our environmental chambers, and one with larger number on a commercial dairy. In both studies a decrease in core body temperature was detected in cows fed dietary niacin (NIASHURE®) at a dose of 12g/cow/day (Zimbleman et al. 2007 and 2008). We subsequently evaluated impact of heat stress on niacin concentrations in whole blood, plasma and milk (Rungruang et al. 2010). We found that heat stress decreased whole blood and plasma niacin indicating that heat stress increased niacin metabolism and likely increased niacin requirements in lactating dairy cows, (Figure 2).

![Figure 2](image.png)

**Figure 2.** Effect of environment on concentration of niacin in blood, plasma, and milk. Alphabet comparison within samples, $P < 0.05$ From Rungruang et al. 2010.

Thus, addition of 12 g/d of Niashure/day in the diet increased sweating rates and reduced body temperatures of lactating dairy cows exposed to heat stress (Zimbelman et al., 2010). Niacin has been shown to induce prostaglandin D synthase activity (Benyo et al., 2005; Meyers et al., 2007) in Langerhans cells leading to increased blood levels of prostaglandin D (Kamanna and Kashyap, 2008) and increased skin vascularity and sweating rate (Di Constanza et al., 1997, Zimbleman et al., 2010). Therefore, as the supplemental dose of NIA provide to dairy cows is increased there should be a corresponding increase in free and total blood niacin and nicotinamide, plasma prostaglandin D and sweating rate. In addition, we speculated that there would be a dose response relationship between supplemental NIA and sweating rate, water intake and core body temperature (sweating rate and water intake increasing with dose corresponding with a decreased body temperature with increasing NIA). Therefore, we evaluated a dose range of protected niacin (NIASHURE®) at 0, 4, 8, and 12 g/d on body temperature indices, production parameters and niacin concentration in milk, blood, and plasma.

In this study we reported that Niashure fed to dairy cows increased plasma niacin in a dose responsive manner, (Rungruang et al. 2011, (Figure 3). Dietary Niashure also increased skin temperature in a dose responsive manner, (Figure 4) indicating that cutaneous vasodilation was increased as dose of dietary Niashure was increased in heat stressed lactating dairy cows, Rungruang et al. 2011.
We also evaluated impact of Niashure dose and heat stress on dry matter intake, water intake, milk yield, and milk composition under thermoneutral and heat stress conditions (Table 1). Heat stress decreased feed intake and milk yield at all doses of Niashure fed. However, milk protein and milk fat concentrations (Table 1, Figures 5 and 6) were increased in a dose responsive manner in cows fed Niashure resulting in increases in fat and protein yield in thermoneutral but not heat stress conditions because of the impact of heat stress on overall milk yield.
Table 1. Effect of encapsulated niacin on dry matter intake, water intake, milk yield and milk composition under thermoneutral and heat stress conditions (From Rungruang et al. 2010)

<table>
<thead>
<tr>
<th>Item</th>
<th>TN</th>
<th>HS</th>
<th>P-value</th>
<th>Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0g</td>
<td>4g</td>
<td>8g</td>
<td>12g</td>
</tr>
<tr>
<td>DMIs</td>
<td>23.77</td>
<td>25.03</td>
<td>24.42</td>
<td>23.02</td>
</tr>
<tr>
<td>Water Intake</td>
<td>76.5</td>
<td>80.7</td>
<td>91.8</td>
<td>99.7</td>
</tr>
<tr>
<td>Milk Yield (%)</td>
<td>40.62</td>
<td>39.67</td>
<td>40.73</td>
<td>40.79</td>
</tr>
<tr>
<td>Milk protein, %</td>
<td>2.76</td>
<td>2.81</td>
<td>2.83</td>
<td>2.83</td>
</tr>
<tr>
<td>Milk fat, %</td>
<td>3.68</td>
<td>3.45</td>
<td>3.78</td>
<td>4.03</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.78</td>
<td>4.82</td>
<td>4.79</td>
<td>4.71</td>
</tr>
<tr>
<td>SNF, %</td>
<td>8.38</td>
<td>8.50</td>
<td>8.50</td>
<td>8.42</td>
</tr>
<tr>
<td>SCC</td>
<td>168.9</td>
<td>144.9</td>
<td>106.9</td>
<td>203.6</td>
</tr>
</tbody>
</table>

1 = Thermoneutral
2 = Heat stress
3 = Standard error of the mean
4 = Environment
5 = Environment and dose interaction
6 = linear
7 = quadratic
8 = No significant with cubic relationship
9 = Dry matter intake, kg/cow/day
10 = L/day
11 = 4% FCM, kg/cow/day
12 = Solid Not Fat
13 = Somatic cell count (X 1,000 cells/mL)

We also measured changes in evaporative heat loss, core body temperature (rectal and vaginal) and respiration rates in these cows (Table 2). In this study we failed to demonstrate an effect of Niashure on evaporative heat loss. However, this study was done in January and it was clear that sweating rate in all animals was 10 fold lower than sweating rates reported in an earlier study utilizing cows that were summer adapted (Zimbleman et al. 2007). It is possible that evaporative heat loss in winter adapted cattle is considerably lower than summer adapted cattle although this is not yet proven.
Table 2. Effect of encapsulated niacin on heat parameters and evaporative heat loss in lactating dairy cows exposed to heat stress

<table>
<thead>
<tr>
<th>Item</th>
<th>TN 1</th>
<th>HS 2</th>
<th>SEM 3</th>
<th>P-value</th>
<th>Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 g</td>
<td>4 g</td>
<td>8 g</td>
<td>12 g</td>
<td>Dose</td>
</tr>
<tr>
<td>Body Temp (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Env 3</td>
</tr>
<tr>
<td>Rectal Temp</td>
<td>38.3</td>
<td>38.4</td>
<td>38.5</td>
<td>38.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Skin Temp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E*D 5</td>
</tr>
<tr>
<td>Shaved skin</td>
<td>30.8</td>
<td>31.1</td>
<td>30.5</td>
<td>30.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Un-shaved skin</td>
<td>28.2</td>
<td>28.7</td>
<td>28.4</td>
<td>29.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Vaginal Temp</td>
<td>38.6</td>
<td>38.6</td>
<td>38.6</td>
<td>38.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Evaporative Heat Loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L 6</td>
</tr>
<tr>
<td>Respiration rate 9</td>
<td>31.1</td>
<td>32.2</td>
<td>31.2</td>
<td>27.9</td>
<td>2.2</td>
</tr>
<tr>
<td>Sweating rate 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q 7</td>
</tr>
<tr>
<td>Shaved skin</td>
<td>26.2</td>
<td>24.9</td>
<td>21.9</td>
<td>23.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Un-shaved skin</td>
<td>19.2</td>
<td>18.0</td>
<td>17.5</td>
<td>17.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Calculated Evaporative heat loss 11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C 8</td>
</tr>
<tr>
<td>Shaved skin</td>
<td>75.2</td>
<td>68.8</td>
<td>66.4</td>
<td>56.5</td>
<td>8.6</td>
</tr>
</tbody>
</table>

1: Treatment; 2: Heat Stress; 3: Standard Error of Mean; 4: L = Linear; 5: Q = Quadratic; 6: C = Cubic

Note: Comparisons: a,b = Dose; x,y = Enviroment; * = Dose x Enviroment
<table>
<thead>
<tr>
<th></th>
<th>57.6x</th>
<th>50.4x</th>
<th>52.8x</th>
<th>42.0x</th>
<th>83.3y</th>
<th>92.2y</th>
<th>83.5y</th>
<th>89.1y</th>
<th>7.7</th>
<th>0.83</th>
<th>&lt;0.01</th>
<th>0.46</th>
<th>0.40</th>
<th>0.70</th>
<th>0.81</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un-shaved skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 = Thermoneutral  
2 = Heat stress  
3 = Standard error of the mean  
4 = Environment kcal/h  
5 = Environment and dose interaction  
6 = linear  
7 = quadratic  
8 = cubic  
9 = Respiration rate, breath/min  
10 = grams/square meter/hour  
11 = Average for all 4 measurement times (sweating rate/h x surface area x 0.90 x 580 cal/g of sweat),  
a-b Values within a row with differing superscripts denote differences (P<0.05) within environments (TN or HS)  
x-z Values within a row with differing superscripts denote dose x environment interactions (P<0.05)
Niacin, also known as vitamin B₃, has been used for decades to prevent metabolic disease and maintain energy balance in dairy cows resulting in increased milk production (Kung et al., 1980; Dufva et al., 1983; Muller et al., 1986; Jaster and Ward, 1990; Schwab et al., 2005). Niacin receptors, GPR 109A and GPR 109B, have been identified in adipose tissue, immune cells, liver, spleen, lung, and brain in humans (Maciejewski-Lenoir et al., 2006; Kamanna and Kashyap, 2008; and Lukasova et al., 2011). Maciejewski-Lenoir et al. (2006) reported neutrophils have the highest gene expression levels of GPR109A when compared to adipose tissue, spleen, skin, heart, kidney, liver, colon, and brain in humans. Titgemeyer et al. (2011) reported that niacin receptors in cattle are distributed differently from human tissues and found niacin receptors in bovine fat, muscle, brain and the highest amount in liver. Niacin coupled with GPR109A stimulates synthesis of the prostaglandins D₂ (PGD₂) and E₂ (PGE₂) from Langerhans cells (Maciejewski-Lenoir et al., 2006; Gille et al., 2008; Kamanna et al., 2009). In addition, PGE₂ can stimulate HSP27 and HSP70 in osteoblast-like MC3T3-E1 cells and HSP72 in A549 human lung epithelial-like cells (Tokuda et al., 2002 and Shah et al., 2010). Zimbleman et al. 2007 demonstrated that prostaglandin D and niacin increased heat shock protein production in mammary epithelial cells and increased survival of mammary cells exposed to heat stress.

**SUMMARY**

Heat stress decreases plasma and whole blood niacin concentration in lactating dairy cows indicating that niacin requirements are likely increased during thermal stress. Feeding protected niacin (Niashure) to lactating dairy cows increases plasma niacin concentration as well as milk fat and protein concentration in lactating dairy cows. Feeding niashure to lactating dairy cows exposed to heat stress is associated with increases in skin temperature and water intake indicating increased vasodilation and in some studies an increase in sweating rate and a decrease in body temperature. Further studies are warranted to examine niacin requirements in lactating dairy cows exposed to heat stress.
REFERENCES


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