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Science and the Agricultural Experiment Station

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2011 Cornell Nutrition Conference for Feed Manufacturers

Conference Program

Tuesday, October 18, 2011

Pre-Conference Symposium hosted by Novus International

- 1:00 PM Welcome and Introduction
Ed Galo, Novus International
- 1:15 PM Understanding Oxidative Balance and its Impact on Animal Performance
Julia Dibner, Novus International
- 2:00 PM Inflammation and Oxidative Stress in Transition Cows
Barry Bradford, Kansas State University
- 2:45 PM Oxidative Stress and Mastitis Susceptibility – The Immunology Link
Lorraine Sordillo, Michigan State University
- 3:30 PM Break
- 3:45 PM The Science Behind Cow Comfort
Marina Von Keyserlingk, University Of British Columbia
- 4:30 PM Novus COWS Program – On-Farm Assessments To Improve Cow Comfort
Kiyomi Ito, Novus International
- 5:30 PM Wrap-Up And Questions
All Speakers

Wednesday, October 19, 2011

6:30 AM Breakfast, sponsored by JEFO

- B Vitamin Supply: Could the Cow Rely on Ruminant Micro-Flora?
Christiane Girard, Agriculture and Agri-Food Canada
- B Vitamins: Improving Efficiency of Cow Metabolism
Essi Evans, Technical Advisory Services

Morning Session – Larry Chase Presiding

- 8:30 AM Early Lactation Diets For Dairy Cattle – Focus On Starch
Heather Dann, Miner Institute
- 9:10 AM What Genomics Will Mean to Nutritionists
Curt Van Tassell, USDA Agricultural Research Station
- 9:50 AM Presentation of Maynard Award
Dale Bauman, Cornell University
- 10:00 AM Break
- 10:20 AM Opportunities and Challenges in Calf Housing and Management for the Next Decade
Nina Von Keyserlingk, University of British Columbia
- 11:00 AM Heifer Growth Rates, Body Composition and Mammary Development: Integrating Literature Data to Describe First Lactation Milk Production
Mike Van Amburgh and Fernando Soberon, Cornell University
- 11:40 AM Influence of Social Environment on Feed Intake of Dairy Cattle

Rick Grant, Miner Institute
12:20 PM Lunch

Afternoon Session – Tom Overton Presiding

1:30 PM Formulating Dairy Rations with Non-Forage Fiber Sources: Where to Begin?
Barry Bradford, Kansas State University

2:10 PM Algal Biofuel Biomass as a Feed Supplement
Xingen Lei, Cornell University

2:50 PM Break

The Dale Bauman Symposium

(An additional full day symposium to be held at Cornell on Friday, October 21)

3:15 PM The Bauman Retrospective
Robert Collier, University of Arizona

4:00 PM Milk Fat and Human Health – Separating Fats From Fiction
Adam Lock, Michigan State University

4:45 PM Future Challenges and Opportunities in Animal Nutrition
Dale Bauman, Cornell University

5:30 PM Evening Dinner Reception

Thursday, October 20, 2011

6:30 AM **Breakfast, sponsored by Alltech**
Global Agriculture: The Future Needs the Ag Minded
T. Pearse Lyons, Alltech

Morning session – Mike Van Amburgh presiding

8:30 AM Alternative Models of Digestion and Passage: Descriptions and Practical Implications
David Mertens, Mertens Innovation And Research LLC

9:10 AM Ammonia Emissions from Dairy Operations – What Do We Know?
Larry Chase, Cornell University

9:50 AM Break

10:10 AM State, Regional, and Farm-Scale Nutrient Balances: Tools for Enhanced Efficiency of Whole-Farm Nutrient Use
Quirine Ketterings, Cornell University

10:50 AM The Link Between Body Condition Score (BCS) and Lameness
Rodrigo Bicaño, Cornell University

11:30 AM Managing The Dynamics of Feed Intake and Body Condition Score During the Transition Period and Early Lactation
Tom Overton, Cornell University

12:10 PM Adjourn – Have A Safe Trip Home!

Posters of Cornell University graduate students will be on display throughout the conference.

Proceedings are available for download at www.ansci.cornell.edu/cnconf.

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UNDERSTANDING OXIDATIVE BALANCE AND ITS IMPACT ON ANIMAL PERFORMANCE

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INTRODUCTION

Oxidation and reduction reactions involving biomolecules play a critical part of both normal and abnormal biological processes (Jones, 2006). At a fundamental level, oxidation and reduction of protein-bound sulfur governs the tertiary structure of proteins and thus the levels of activity of enzymes, transcription factors, and immunoglobulins (Sies, 1997; Poole and Nelson, 2008; Reddie and Carroll, 2008). In addition, oxidation of macromolecules such as cell membrane phospholipids and nucleic acids affects the function of these molecules and can influence both surface receptor function and cell multiplication frequency and fidelity (Chandra et al., 2000; Haddad, 2004).

Reactive oxygen metabolites (ROM) are constantly produced as a byproduct of mitochondrial respiration (Aw, 1999) and periodically during inflammatory responses. The gastrointestinal (GI) system is both metabolically active and the site of encounters between the host and the microbiota, which often lead to inflammation. The addition of oxidized ingredients in the diet can cause an imbalance, and overwhelm the capacity of the endogenous antioxidants, leading to increased apoptosis and local tissue damage.

FORMATION OF REACTIVE OXYGEN METABOLITES AND INITIATION OF OXIDATIVE DAMAGE

Free radicals, also referred to as ROM are formed during normal metabolism of the cells and during the innate immune response. First, the active metabolism of gut epithelium is itself a source of ROM, associated with activity of the electron transport chain (Ojano-Dirian et al., 2007). The reactive species produced include the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\cdot OH$). These are considered to be an inevitable result of oxidative phosphorylation by mitochondria (Chance et al., 1979). Another endogenous source of oxidative stress includes the nitric oxide (NO) generated by the gut innate and acquired immune systems as they react to the numerous commensal and pathological microbial species that are inevitably introduced during ingestion of feed and water. While it is not certain that NO itself is damaging to inflamed tissues or expressed by inflammatory cells, its reaction with superoxide anions creates peroxynitrate ($ONOO^-$), a considerably more damaging reactive nitrogen metabolite with a long half-life and very lipid soluble allowing it to diffuse from its original site of production (Kruidenier and Verspaget, 2002). These metabolites attack a variety of macromolecules including lipid membranes, proteins (e.g. enzymes) and DNA, affecting both structure and function. In addition, in vitro exposure to low doses of ROM or a depletion of endogenous antioxidants (AOX) has

been reported to result in apoptotic cell death. Apoptosis is different from tissue necrosis in that it has distinct morphological and biochemical features that ultimately lead to breakdown and autodigestion of the cell. There is in vitro evidence that apoptotic cell death can be blocked by the addition of AOX compounds (Kruidinier and Verspaget, 2002).

Cells are protected from damage by these ROM through the action of endogenous antioxidant defenses. These defenses consist of three main groups of antioxidants (Miller and Brezeinska-Slebodizinska, 1993). The first group, enzymatic antioxidants, is represented by mitochondrial Mn dependent superoxide dismutase (SOD), Cu-Zn SOD and Se dependent glutathione peroxidase (GSH-Px; Aw, 1999). They are the main intracellular antioxidant defense system and the first defense system against ROM such as H_2O_2 and $\cdot OH$. The second group consists of protein antioxidants in the intracellular fluids such as the sulfhydryl groups of albumin, cysteine, and homocysteine. The third group consists of low molecular weight chain-breaking antioxidants such as the water-soluble vitamin C, glutathione and the lipid-soluble vitamins E and A. In the event that the oxidative stress overwhelms the antioxidant capacity of the animal tissue damage can be extensive (Weiss, 1989).

CAUSES OF OXIDATIVE STRESS

There are a variety of conditions common to production agriculture that promote oxidative stress. During disease challenges, the first immune response of the animal involves generation of ROM by the macrophages and neutrophils to kill the bacteria. During this event, antioxidant enzymes such as SOD and GSH-Px are involved in removing the ROM so they don't damage the host cell. The inflammatory response of the GI system can often play a role in reducing the antioxidant capacity of the animal as a result of malabsorption of certain nutrients, particularly fat soluble vitamins (Miller et al., 1993).

Environmental conditions can also play a role in the oxidative stress of the animal. Bernabucci et al. (2002) evaluated the oxidative stress on cows calving during spring (39.1°C; 56 temperature humidity index) or summer (39.5°C; 73 temperature humidity index). Levels of oxidized lipids (TBARS) and SOD in blood erythrocytes were higher in cows calving during the summer than spring. Similarly, Lin et al. (2006) demonstrated that acute heat stress induced oxidative stress in broilers as measured by accumulation of TBARS in the plasma and reduction of AOX enzymes in the plasma and the liver (SOD). Mugahid et al. (2007) reported an accumulation of ROM from mitochondria in the muscle of broiler cockerels subjected to heat stress whereas there was no similar effect in layer chicks subjected to the same conditions. Bottje et al. (1998) reported an increase in the ratio of oxidized to reduced glutathione (GSSG/GSH) in broiler chickens subjected to poorly ventilated environmental conditions compared with those reared in well ventilated battery cages suggesting an accumulation of oxidized glutathione due to conditions of greater oxidative stress.

SOURCES OF FREE RADICALS IN THE DIET

Supplemental fat is a significant vehicle for bringing free radicals or ROM into the diet. In a survey of different types of fat collected in the U.S. from 2000 to 2005 by Novus International, it was found that 40 to 50% of the fat sold for use in animal feeds was unstable with higher percentages of instability in the warmer summer months. There are other dietary sources of unstable fat besides the fat supplemented in diets. Approximately 50% of the dietary lipids come from feedstuffs other than fat. Feedstuffs such as cottonseed, distiller's grains, soybean products and fish meal significantly contribute to the total dietary lipids. Interestingly, most of the lipids from these ingredients contain high levels of unsaturated fatty acids that are prone to oxidation. For example, distillers' grains from the ethanol industry are sources of unsaturated fatty acids and the heating process during distillation and high water content of the wet distillers grains can exacerbate the oxidation process of the unsaturated fatty acids which can result in a highly oxidized and unstable fat in the distiller grains.

Dietary antioxidants are used to reduce the load of peroxides in the diet by reacting with the free radicals and converting them to non-toxic metabolites. In the case of lipid membranes, the antioxidants are able to bind to the oxidized fatty acid and stabilize the molecule, controlling the propagation of lipid oxidation and further formation of lipid peroxides. The hydrogen of the active group in the dietary antioxidants such as ethoxyquin, tertiary butyl hydroquinone (tBHQ), butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) binds to the unpaired electron of the free radical in the fatty acid or in the media to block oxidation. Addition of an AOX to wet distiller grains stabilized the fat, preventing further oxidation and loss of unsaturated fatty acids such as C18:2 and C18:3 (Andrews and Vázquez-Añón, personal communication). During oxidation the energy content of the fat is significantly reduced, but this loss can be controlled in the presence of an AOX. The energy content as measured by bomb calorimetry was found to be reduced by 35% for oxidized purposely oxidized fat compared to fresh fat. However, in the presence of an effective AOX the energy value was maintained. Oxidation of fat not only reduces the energy and biological value of the fat, but also propagates oxidation to other lipid-based ingredients such as vitamins and dietary pigments.

ANIMAL RESPONSE TO DIETARY ANTIOXIDANTS

Oxidation of dietary ingredients has relevance beyond simply reducing nutrient value of the diet. Lipid hydroperoxides react aggressively with living tissue and can disrupt the redox balance in epithelial cells. This has been observed to lead to apoptosis in vitro in CaCo-2 cells (Wang et al., 2000). These studies indicated that an early step in the process involved a reduction in the ratio of reduced glutathione to oxidized glutathione (GSH/GSSG). This is consistent with observations in human T lymphocytes, in which glutathione depletion triggered apoptosis in activated T cells (Chang et al., 2002). The importance of AOX stabilization of the feed is that, by providing AOX protection in the intestinal lumen, synthetic AOXs may spare endogenous AOXs such as glutathione and

vitamins A and E which are absorbed by gut cells. This concept is supported by studies in broilers showing that adding ethoxyquin to diets containing normal or oxidized fat was associated with increased levels of glutathione in duodenal tissue (Wang et al., 1997). The effect was independent of fat oxidation, suggesting that levels of ROM in the gut lumen are sufficient to consume a significant amount of AOX activity even in diets containing fresh fat. Glutathione and other endogenous AOXs would then be spared to control the ROS resulting from enterocyte metabolism. The importance of maintaining redox balance in gut epithelial cells is related to the role of ROM as regulators of apoptosis signaling pathways (Haddad, 2004). As such, they can reduce the half life of host cells resulting in a range of consequences from poor feed efficiency to susceptibility to inflammation and infection (Ojano-Dirain et al., 2007). The objective of this study was to describe the effect of oxidized fat on the GI system and to determine whether the addition of a feed AOX could ameliorate ROS associated cellular changes. The data to be described here focus on the early post hatch period and suggest that AOXs are not just beneficial for the function of the intestine and its immune system but also for its development in the neonate.

Dibner et al. (1996) examined the primary effects of feeding oxidized fats to broiler chickens using a variety of metabolic, microbiological and histological techniques to help identify any associated functional changes occurring in the GI system, the first site of exposure to dietary insult. Histopathology was used as an indicator of changes in GI structure, and nutrient uptake as a measure of GI function. In addition, stem cell proliferation, both in the GI epithelium and in the gut associated lymphoid tissue, was used to evaluate cytotoxicity and immune status. In their experimentation birds received a standard corn soy broiler starter diet and fat was provided as a blend of oxidized and non-oxidized fats to achieve the desired level of peroxide in the feed. The non-oxidized poultry fat had an initial peroxide value (IPV) of 1.04 meq/kg, the oxidized poultry fat 212.5 meq/kg, and the lard 3.2 meq/kg. Treatments included non-oxidized fat, non-oxidized fat with ethoxyquin (Santoquin[®] ethoxyquin feed preservative) at a rate of 125 ppm (125 g/ton), and oxidized fat with or without ethoxyquin. The fat source for this work was poultry fat and lard, and was added to achieve the final peroxide level of 4.2 meq/kg of feed for both oxidized fat treatments. Three pens of eight birds per pen were fed the four diets ad libitum. Body and feed weights were measured on days 7, 14 and 21. Birds were randomly selected for intestinal microflora, nutrient uptake and histopathology studies. Some of the data from the two and to three week period of this study have previously been published and these reports include a more detailed description of the methods (Shermer et al., 1995; Dibner et al., 1996).

Figure 1 illustrates the effect of fat oxidation and AOX protection on the growth of the neonatal ileum. Feeding the dietary treatments for 11 days resulted in significant differences in GI development. The feeding of oxidized fat resulted in retarded growth of intestinal villi (single degree of freedom contrast, $P < 0.05$) but no difference in crypt depth (contrast, $P = 0.27$). This indicates that proliferative activity of crypt stem cells supported more villus growth in fresh than oxidized fat. In addition, the presence of AOX

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in the diet significantly reduced the magnitude of this effect (contrast, $P < 0.05$). These observations are consistent with a reduction in epithelial cell life span previously reported (Dibner et al., 1996) in older birds from this study. In addition, the reduction in cecal tonsil diameter at 11 days (Figure 1) suggests that the development of this secondary immune organ was also retarded (fresh vs. oxidized fat, $P < 0.17$; control vs. ethoxyquin, $P = 0.05$). Histology examination suggests that reduced bursal lymphocyte proliferation may have contributed to this reduction in tonsil development (data not shown).

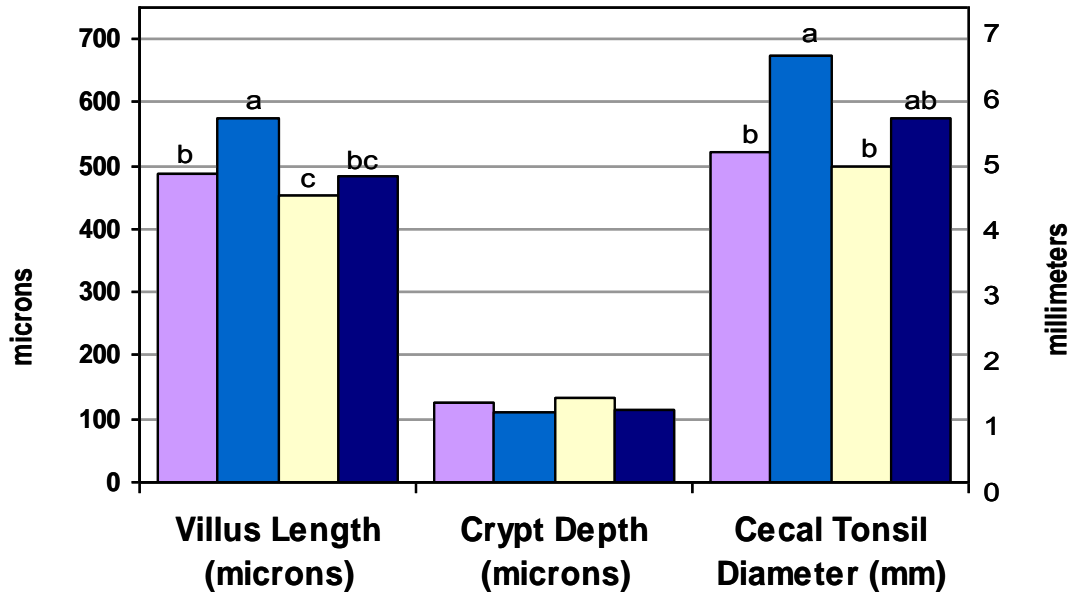






Figure 1. Morphometry of the ileum of birds fed fresh fat without  or with  ethoxyquin compared to birds fed oxidized fat without  or with  ethoxyquin at day 11. (a,b; $P < 0.05$)

The observation that oxidized feed ingredients are associated with a reduction in cell lifespan is consistent with the role of ROS in cell apoptosis (Haddad, 2004). The observation that AOXs are beneficial even in fresh fat suggests that the metabolic ROS generated during mitochondrial respiration are sufficient in and of themselves to put substantial oxidative stress on the system. Support for the hypothesis that synthetic AOXs can spare endogenous AOXs was seen in this study where providing ethoxyquin to either fresh or oxidized fat diets was associated with a significant increase in liver vitamin A (Table 1). Thus, even in the diet containing fresh fat, providing a dietary AOX spared endogenous AOX capacity.

Table 1. Liver Vitamin A content in 21-day old broilers

Fat Source	Antioxidant	Liver Vitamin A (ug/gm liver)	Statistics:	
Fresh	None	5.155 a	Fat	P=0.05
Fresh	Ethoxyquin (125 ppm)	5.475 a	Antioxidant	P=0.04
Oxidized	None	3.512 b	Fat*Antioxidant	P=0.19
Oxidized	Ethoxyquin (125 ppm)	5.077 a		

SUMMARY

The need to preserve dietary ingredients containing high levels of fat has long been recognized. In those cases the primary benefit from AOX inclusion was assumed to be that of preservation of nutrient and energy content, and the economic impact of maximizing the feeding value of ingredients. The potential effect of dietary ROM on the structure and function of the GI tract has generally not been considered. There is currently ample evidence to confirm the ability of feed AOXs to ameliorate some of the effects of oxidative stress in the GI tract and to preserve endogenous supplies of AOX under conditions of oxidative stress.

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INFLAMMATION AND OXIDATIVE STRESS IN TRANSITION COWS

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Inflammation encompasses a critical set of tools in the immune system's arsenal. These non-specific responses contribute to the innate immune system's ability to clear invading pathogens. However, inflammation is a double-edged sword, and there is growing recognition of the harmful effects of excessive or chronic inflammation for the animal, especially with respect to its metabolic function. Although numerous factors can promote inflammation, oxidative stress may be the most common initiator of metabolic inflammation in transition cows. Recent evidence suggests that the resulting inflammation may promote metabolic disorders through decreases in feed intake and maladaptive changes in liver metabolism of carbohydrates and lipids. Treatments that prevent oxidative stress and/or directly inhibit inflammatory cascades hold promise for improving the health and productivity of dairy cows in early lactation.

INFLAMMATORY RESPONSES TO INFECTION

During infections such as mastitis or metritis, immune cells in the body recognize invading pathogens and become activated. When the infection is caused by Gram-negative bacteria, lipopolysaccharide (LPS) released by the bacteria also activates immune cells. The activation of local and systemic host defense mechanisms requires cross-talk between numerous types of immune cells, and one component of this response is inflammation. The host of signaling molecules released by activated immune cells includes inflammatory mediators such as nitric oxide, prostaglandins, and cytokines. While many of these molecules promote local inflammation and increased blood flow to the infected tissue, inflammatory cytokines play a key role in stimulating systemic inflammatory responses, including increased body temperature, increased heart rate, and decreased feed intake. Cytokines are able to alter many physiological systems because nearly all cell types express cytokine receptors. Key inflammatory cytokines include tumor necrosis factor alpha (TNF α), interleukin (IL) 1 β , and IL-6; these inflammatory cytokines act through many of the same signaling cascades and often produce similar responses in cells.

One effect of cytokines is to activate production of acute phase proteins. Primarily produced by the liver, this class of proteins includes haptoglobin, serum amyloid A, and C-reactive protein. Proteins that participate in the acute phase response to infection are generally found in very low abundance in the bloodstream, but are greatly elevated during systemic inflammation. The importance of acute phase proteins in the response to infection is somewhat unclear, but they have gained widespread acceptance as markers of inflammation.

It is clear that mammary and uterine infections result in both local and systemic inflammation. Coliform mastitis results in release of LPS into the bloodstream and increased plasma concentrations of cytokines and acute phase proteins (Hoeben et al., 2000). Likewise, metritis is associated with an acute phase response in transition cows (Huzzey et al., 2009); in fact, plasma haptoglobin is elevated prior to clinical signs of metritis. Furthermore, monocytes are known to become more responsive to inflammatory stimulants during the transition period, resulting in greater secretion of inflammatory cytokines when stimulated (Sordillo et al., 1995). Mastitis and metritis can therefore result in systemic inflammation.

IS THERE A ROLE FOR INFLAMMATION IN METABOLIC DISORDERS?

Inflammation has been proposed as a missing link in the pathology of metabolic disorders in transition cows (Drackley, 1999). Recent findings have documented relationships between inflammatory mediators and metabolic disorders. Plasma concentrations of haptoglobin and serum amyloid A were increased in cows that developed fatty liver (Ametaj et al., 2005), and Ohtsuka and colleagues (2001) observed increased serum TNF α activity in cows with moderate to severe fatty liver. A retrospective study of cows on 3 commercial Italian dairies suggested that liver inflammation is associated with a problematic transition to lactation (Bertoni et al., 2008). Cows were classified in quartiles for degree of liver inflammation based on plasma concentrations of acute phase proteins. Those cows with the strongest inflammatory profiles were at 8-fold greater risk for experiencing one or more transition disorders, had lower plasma calcium concentrations, took longer to re-breed, and produced less milk in the first month of lactation (Bertoni et al., 2008). These correlations have driven strong interest in potential mechanisms underlying an inflammation-based pathogenesis of transition cow disorders.

INFLAMMATORY PATHWAYS THAT ALTER NUTRIENT METABOLISM

Inflammatory Cytokines

Consistent with their role in responses to infection, cytokines generally have catabolic effects on metabolism. Cytokines promote the breakdown of fat stores through decreased feed intake, impaired insulin sensitivity, and direct stimulation of lipolysis. All of these conditions are associated with ketosis and fatty liver in dairy cattle. Inflammatory cytokines also directly alter metabolic function of the liver. For example, TNF α decreases liver glucose production in some scenarios (Kettelhut et al., 1987) and promotes triglyceride accumulation once mobilized non-esterified fatty acids (NEFA) reach the liver (García-Ruiz et al., 2006). Triglyceride accumulation is likely due in part to decreased FA oxidation in the liver after exposure to TNF α (Nachiappan et al., 1994). TNF α also decreased production of apolipoproteins (Ettinger et al., 1994), which may impair triglyceride export in VLDL and contribute to hepatic triglyceride accumulation. Adipose tissue is a key source of circulating cytokines in obese animals of several species, but some recent evidence suggests that chronic release of cytokines by adipose tissue in transition cows may be minimal (Schoenberg et al., 2011).

Nevertheless, cytokine signaling in cows with clinical infections may provide a critical “first strike” of liver inflammation.

Oxidative Stress

Lipid peroxides are also emerging as likely mediators linking plasma lipids to inflammation. Lipid peroxides are produced when intracellular lipids encounter reactive oxygen species (ROS) such as hydrogen peroxide. Some ROS are always produced in the liver; however, events occurring in early lactation likely contribute to enhanced ROS production. One adaptation to increasing delivery of NEFA to the liver in early lactation is an increase in the capacity of peroxisomal oxidation (Grum et al., 1996), an alternative pathway for FA oxidation. Enhanced peroxisomal oxidation increases total oxidative capacity of the hepatocyte. However, the first step in this pathway produces hydrogen peroxide rather than NADH, and therefore it contributes to ROS production to a greater extent than mitochondrial oxidation. Increased ROS production in early lactation cows, coupled with increased NEFA concentration, increases lipid peroxide formation; both the transition to lactation and high body condition are associated with increased plasma markers of lipid peroxidation (Bernabucci et al., 2005). Similar mechanisms may underlie the fact that withdrawal of feed and water for just 24 hours induced an acute-phase response in steers (Cappellozza et al., 2011).

In vivo observations support a role for oxidative stress in metabolic disorders. Dairy cows with fatty liver have lower antioxidant status and higher hepatic lipid peroxide concentrations than healthy cows (Mudron et al., 1999). Despite these data suggesting a metabolic effect of oxidative stress, transition cow studies employing antioxidants as treatments have looked almost exclusively at effects on infectious disorders such as mastitis and metritis. In rodent models, however, studies have demonstrated that antioxidants improve metabolic function in animals challenged with high-fat diets (Mao et al., 2010) and endotoxin (Sakaguchi and Furusawa, 2006). In a recent phase 3 clinical trial, vitamin E supplementation significantly improved liver health in steatohepatitis patients compared to placebo (Sanyal et al., 2010).

LPS Translocation from the Gut

Toll-like receptor 4 (TLR4) was initially identified as a protein expressed in immune cells that is critical for inflammatory responses to LPS (Poltorak et al., 1998). There is now growing recognition that TLR4 is expressed in many cell types, including muscle cells (Frisard et al., 2010), adipocytes (Schaeffler et al., 2009), and hepatocytes (Galloway et al., 2008). Although immune cell-dependent mechanisms have been shown to alter liver function (Saber et al., 2009), direct activation of TLR4 in hepatocytes can also influence metabolism.

Activation of TLR4-dependent pathways by LPS has numerous effects on metabolic function. TLR4 activation decreases insulin sensitivity in adipose tissue and liver (Shi et al., 2006). Additional studies have demonstrated that LPS signaling via TLR4 increases adipose tissue lipolysis (Zu et al., 2009) and decreases FA oxidation in muscle (Frisard

et al., 2010). In addition, despite relative insulin resistance in liver, LPS activation of this pathway can also suppress hepatic glucose production (Carl et al., 2009). Collectively, these effects have many similarities to the fatty liver / ketosis complex in transition cows.

It has long been debated whether acidosis promotes release and translocation of LPS from the rumen and into the bloodstream. Khafipour et al. (2009) nicely demonstrated that induction of sub-acute ruminal acidosis increased both ruminal and plasma LPS concentrations. This treatment also significantly elevated plasma concentrations of acute-phase proteins, presumably mediated by TLR4 sensing of the translocated LPS. Although no indices of hepatic metabolism were measured in this study, it is likely that if LPS was sufficiently elevated to induce an acute phase response, expression of metabolic genes was also altered. Studies in other species suggest that numerous physiological stressors, including heat stress, can disrupt tight junctions between gastrointestinal epithelial cells and allow translocation of LPS (Lambert, 2009). If this phenomenon is common in dairy cattle, it may play a significant role in metabolic responses to parturition, heat stress, diet transitions, and other stressors.

NET EFFECTS OF INFLAMMATION ON METABOLISM OF LACTATING COWS

Strong evidence has emerged from 2 recent studies where inflammatory mediators directly induced metabolic problems. Trevisi and colleagues (2009) orally administered interferon- α (a cytokine) daily during the final 2 weeks of gestation, which caused liver inflammation and release of acute phase proteins. Compared to control cows, treated cows had significantly higher plasma ketone concentrations in the first 2 weeks after calving. Our own lab recently reported that subcutaneous injection of TNF α for 7 days doubled liver triglyceride content in late-lactation dairy cows (Bradford et al., 2009). We also observed changes in mRNA abundance consistent with transcriptionally-mediated increases in fatty acid uptake and esterification and decreased FA oxidation. These results strongly support the hypothesis that inflammation disrupts normal metabolism, because although both of the above treatments were considered low-dose and short-term, they nevertheless promoted ketosis and fatty liver, respectively.

Beyond direct promotion of ketosis and fatty liver, hepatic inflammation may also impair glucose production. Endotoxin-induced mastitis was shown to alter expression of metabolic genes in the liver, including decreased expression of genes important for glucose production (Jiang et al., 2008). Our TNF α injection protocol also decreased expression of several of the same glucose synthesis genes (Bradford et al., 2009). In early lactation cows, impaired glucose production would likely lead to increased adipose tissue breakdown, elevated plasma NEFA, and increased ketone production by the liver.

OPPORTUNITIES TO OVERCOME OXIDATIVE STRESS AND INFLAMMATION

Antioxidants

Dietary antioxidants, notably vitamin E and selenium, are important for their ability to contribute to ROS neutralization, thereby impeding the progression toward inflammation. Interestingly, plasma concentrations of α -tocopherol (vitamin E) decrease through the transition period (Weiss et al., 1990a), and low antioxidant status is associated with transition cow disorders (LeBlanc et al., 2004; Mudron et al., 1997). Supplementing vitamin E prepartum improves antioxidant status (Weiss et al., 1990a). Given the importance of antioxidants in modulating inflammation, it is not surprising that multiple studies have shown that supplementing vitamin E in excess of traditional recommendations decreases the incidence and severity of clinical mastitis (Smith et al., 1984; Weiss et al., 1990a). Recently, a meta-analysis showed that supplemental vitamin E is also effective at preventing retained placenta (Bourne et al., 2007).

Low plasma vitamin E concentrations are associated with increased incidence of fatty liver and displaced abomasum (Mudron et al., 1997). Surprisingly, no published studies have evaluated the effects of supplemental vitamin E on liver metabolism or incidence of metabolic disorders. Given that supplemental vitamin E can decrease inflammatory cytokine production (Poynter and Daynes, 1998) and improve liver antioxidant status in mice with fatty liver (Soltys et al., 2001), supplemental vitamin E may improve liver function in transition cows. Beta carotene, a precursor of vitamin A, can also function as an antioxidant (Spears and Weiss, 2008), and concentrations of both vitamin A and β -carotene typically decrease during the transition period (LeBlanc et al., 2004).

Although much of the literature on antioxidants in transition cows demonstrates positive effects, these nutrients must be used with caution. In an effort to maximize the odds of observing a response, most studies are designed with rather dramatic treatments; for example, one classic study (Weiss et al., 1990b) compared vitamin E intakes of 574 IU/day (no supplemental vitamin E) to 1474 IU/day (supplementing 88 IU/lb dry matter). In many such scenarios, the control group is fed a diet that is marginally deficient in the nutrient of interest. On most dairies, this is not the case. As a result, adding large amounts vitamin E, for example, can sometimes push the supply of the nutrient high enough to cause mild toxicity. Supplementing 3000 IU/day vitamin E to transition cows with adequate vitamin E status resulted in pro-oxidant responses, increasing markers of lipid peroxidation and the incidence of mastitis (Bouwstra et al., 2010). Any treatment that alters oxidative balance should be evaluated carefully.

Finally, even non-nutritive antioxidants may serve to limit oxidative stress. In one recent study, supplementation of a feed antioxidant decreased peroxide and tended to increase total antioxidant capacity in plasma when fed to cows in early lactation (Wang et al., 2010). These responses were observed despite the presumed lack of absorption

of these antioxidants, suggesting that simply limiting the absorption of unstable oxidized lipids from the diet can help to control oxidative stress. Such an approach would presumably also avoid the risk of toxicity inherent in feeding high amounts of lipid-soluble antioxidant vitamins.

Non-steroidal anti-inflammatory drugs (NSAIDs)

Because pathways other than oxidative stress can cause metabolic inflammation in transition cows, there may be merit to more directly combating inflammation to promote improved metabolic function. The NSAID class of drugs works in just this manner, by preventing the amplification of inflammatory mediators that leads to full-blown inflammation. In fact, several studies have already suggested that NSAIDs hold promise for improving transition cow health and productivity. Cows treated with acetyl-salicylate (aspirin) for the first 5 days of lactation had significantly lower plasma concentrations of acute phase proteins and tended to have greater peak milk production than controls (Bertoni et al., 2004). In a similar study, aspirin treatment for 5 days postpartum improved milk yield in the first 2 months of lactation and increased first service conception rates (Trevisi and Bertoni, 2008). A relatively small number of cows was included in the study (23/treatment); however, ketosis incidence appeared to decrease with aspirin treatment (4.4% vs. 22.7%) while metritis incidence appeared to increase (30.4% vs. 13.6%). These results point to the tradeoffs between metabolic and immune function associated with decreased inflammation.

Our lab recently completed a study in which 78 transition cows were alternately provided with drinking water containing either 0 or 2.5 g/L sodium salicylate for the first 7 days postpartum (Farney and Bradford, unpublished). Consistent with our hypothesis, cows treated with sodium salicylate tended to produce 8% more energy-corrected milk over the first 3 weeks of lactation, with no overall difference in feed intake or incidence of metabolic or infectious diseases. However, the production response was driven primarily by an increase in milk fat content among the salicylate-treated cows, and metabolic profiling revealed that these cows had sustained elevations of plasma NEFA and ketone concentrations compared to control cows. Nevertheless, salicylate treatment still decreased liver triglyceride content at 3 weeks postpartum. These findings suggest still more complicated roles of inflammatory pathways; it may be that inflammation provides a “release valve” for the metabolic system, allowing the cow to slow the rate of lipolysis even as negative energy balance continues, albeit at the risk of impairing liver function. We hope that continued investigation of the metabolic and signaling responses to this treatment will help to uncover the role that inflammation plays in regulating metabolism in early lactation.

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OXIDATIVE STRESS AND MASTITIS SUSCEPTIBILITY: THE IMMUNOLOGY LINK

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INTRODUCTION

Dairy cattle are susceptible to increased incidence and severity of disease during the periparturient period. A major contributing factor to increased health disorders is thought to be alterations in bovine immune mechanisms. Indeed, uncontrolled or impaired inflammatory responses are a major contributing factor to several economically important disorders such as mastitis. Dairy cows undergo several physiological changes during the onset of lactation that can impact the magnitude and duration of mammary gland inflammatory responses. Oxidative stress, for example, occurs when there is an imbalance between the production of oxygen radicals during times of high metabolic demand and the reduced capabilities of the host's antioxidant defenses. The progressive development of oxidative stress in transition dairy cattle is thought to be a significant underlying factor leading to dysfunctional inflammatory responses both systemically and in mammary gland tissues. Dairy cows undergo several metabolic adaptations during the onset of lactation, which together with oxidative stress, can further impact the magnitude and duration of inflammation. Specifically, the dramatic increase in energy requirements needed for the onset of lactation in transition cows is often accompanied by the release of nonesterified fatty acids (NEFA) into the blood stream. The ways in which altered lipid metabolism, increased sera NEFA concentrations, and oxidative stress can interact to initiate and promote uncontrolled inflammatory responses in transition cows will be discussed. Understanding more about the underlying causes of oxidative stress during the periparturient period may facilitate the design of nutritional regimes that will reduce the severity and duration of mastitis as a function of dysfunctional inflammatory responses.

MASTITIS AND MAMMARY GLAND IMMUNITY

Mastitis is a significant disease of adult dairy cattle affecting up to 40 percent of cows within a herd at any given time. Recent surveys show that udder health problems are consistently the most frequent cause of morbidity with the US dairy cattle population (Table 1). The U.S. dairy industry loses an estimated \$2 billion every year due to mastitis, with reduced milk production accounting for the majority of the total economic loss.

The incidence of mastitis is directly related to changes in the composition, magnitude, and efficiency of the mammary gland defense system. However, many different aspects of bovine mammary gland defenses are suboptimal during distinct periods of the lactation cycle, particularly around the transition periods (Sordillo and

Streicher, 2002). Most notably, the two weeks prior to calving through the first three weeks of lactation have long been recognized as a period when key host defense mechanisms are altered dramatically. As a consequence, dairy cattle are more susceptible to mastitis during the periparturient period and through peak milk production. New intramammary infections occurring during the periparturient period are especially problematic as they may greatly impact the productive efficiency of dairy cattle in the ensuing lactation. Therefore, it is not surprising that considerable research efforts have been focused on defining how mammary gland defenses change as a consequence of lactation cycle and understanding those factors that may contribute to immune-dysfunction during this critical period.

Table 1: Health Problems of US Dairy Cattle (NAHMS Surveys)

	1996	2002	2007
Mastitis/Udder Problems	13.4 ± 0.3	14.7 ± 0.3	16.5 ± 0.5
Lameness	10.5 ± 0.3	11.6 ± 0.3	14.0 ± 0.4
Infertility	11.9 ± 0.3	11.9 ± 0.3	12.9 ± 0.3
Retained fetal membranes	7.8 ± 0.2	7.8 ± 0.2	7.8 ± 0.2

Morbidity expressed as percentage of all cows in the US ± standard deviation of the mean. <http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/index.htm>

The mammary gland immune system consists of a diverse array of physical, cellular and molecular factors that function within innate or adaptive (acquired) immune responses (Sordillo and Streicher, 2002). The innate immune system, however, constitutes the primary line of defense during the initial stages of infection and is a key determinant of infection outcome. Components of the innate defense system of the mammary gland include nonspecific physical barriers of the teat end, pattern recognition receptors, phagocytes (i.e., neutrophils and macrophages), and various soluble factors (i.e., cytokines, eicosanoids, complement, and lactoferrin). Innate defense mechanisms are always present within the mammary gland or are activated quickly upon exposure to bacteria. Depending on the efficiency of the innate defense mechanisms, pathogens may be eliminated within minutes to hours following invasion.

Inflammation is a critical component of the innate defense system that involves complex biological responses of vascular tissues to harmful stimuli such as bacterial pathogens. The inflammatory process is initiated by cells already present within the mammary tissues. Resident cells that express pattern molecule receptors are activated by bacterial factors and release various inflammatory mediators such as cytokines that initiate the inflammatory cascade. These inflammatory mediator molecules initially increase vasodilation resulting in enhanced blood flow. The permeability of blood

vessels also changes causing the leakage of plasma components, such as serum albumin, complement, and acute phase protein, into localized areas of affected tissues and possibly resulting in edema. Cytokines and eicosanoids also act directly on vascular endothelial cells to enhance the adhesion and migration of leukocytes from the blood to the site of injury. Neutrophils are the predominant cell type to undergo this extravasation process during the early stages of inflammation. Neutrophils first marginate and then adhere to the local endothelium near the site of infection. Cytokines, eicosanoids, and other mediator molecules stimulate adherent neutrophils to move between endothelial cells and pass the basement membrane into the damaged tissue areas. The movement of neutrophils within the tissues is facilitated by chemotaxis gradients created by inflammatory mediator molecules at the localized site of infection. Both newly recruited and pre-existing leukocyte populations act cooperatively to eliminate mastitis-causing pathogens. Macrophages are the predominant leukocyte type found in healthy mammary tissues and are likely one of the first cell types to respond to bacterial invasion by the release of immune-regulatory cytokines and eicosanoids. Both macrophages and the newly recruited neutrophils also function to phagocytize and kill invading bacteria. The process of phagocytosis involves the internalization of bacteria within phagosomes that contain bactericidal reactive oxygen species (ROS) and hydrolytic enzymes. The ROS are formed by respiratory burst activity that involves the activation of NADPH oxidase and the subsequent production of superoxide radicals and hydrogen peroxide. Myeloperoxidases can further combine hydrogen peroxide with chloride to produce hypochlorite that is associated with bacterial activities. In addition to phagocytosis, neutrophils can kill bacteria through extracellular mechanisms. Activated neutrophils can form neutrophil extracellular traps (NETs) that consist of a web of fibers composed of chromatin and serine proteases that trap and kill bacteria. Studies suggest that NETs provide a highly concentrated foci of antibacterial substances that bind and kill bacteria independently of phagocytic uptake in the mammary gland (Grinberg et al., 2008; Lippolis et al., 2006). NETs also may serve as a physical barrier that prevents further spread of bacteria throughout the mammary gland.

The innate defense mechanisms of the mammary gland function optimally when invading bacteria are recognized promptly, the inflammatory response is adequate to rapidly eliminate the infections, and the mammary gland is returned to normal function quickly without any noticeable clinical symptoms. Factors that adversely affect any aspect of mammary innate defense mechanisms can influence the establishment of new intramammary infections.

OXIDATIVE STRESS AND MAMMARY IMMUNOPATHOLOGY

During the inflammatory process, the vascular endothelium and other mammary gland cells release a number of pro-inflammatory molecules (i.e., cytokines, eicosanoids, reactive oxygen species, and reactive nitrogen species) that function not only to escalate local antimicrobial factors, but also directly kill bacteria. Depending on the severity or duration of the inflammatory response, mammary tissue damage may occur as a consequence of the release of these potent bactericidal components. Therefore, inflammation must be tightly regulated to avoid bystander damage to the milk

synthesizing tissues of the mammary gland. A precarious balance between pro-inflammatory and pro-resolving mechanisms is needed to ensure optimal bacterial clearance and the prompt return to immune homeostasis. Situations that contribute to uncontrolled inflammation are responsible for the tissue damage associated with the pathogenesis of mastitis.

Dairy cows undergo substantial metabolic and physiological adaptations during the transition from pregnancy to lactation that are thought to contribute to dysfunctional host inflammatory responses (Sordillo, 2005). Physiological stresses associated with rapid differentiation of secretory parenchyma, intense mammary gland growth, and the onset of copious milk synthesis and secretion are accompanied by a high energy demand and an increased oxygen requirement. This increased oxygen demand augments the production of oxygen-derived reactants, collectively termed reactive oxygen species (ROS). Although molecular oxygen is required for normal cellular functions in mammals, accumulation of ROS can cause cell and tissue injury and can lead to a condition referred to as oxidant stress (Sordillo and Aitken, 2009). For example, high concentrations of ROS can compromise cellular function by damaging nucleic acids and by altering proteins, carbohydrates and membrane phospholipids. Host tissues do have several enzymes and small molecules that can reduce ROS to less reactive metabolites and it is this antioxidant capability that will help to protect cells from the damaging effects of oxidant stress. Therefore, the imbalance between increased production of ROS and reduced availability of antioxidant defenses around the time of parturition results in increased oxidant stress during this transitional period. Oxidative stress is thought to be a significant underlying factor to dysfunctional host immune and inflammatory responses that can increase the susceptibility of dairy cattle to a variety of health disorders, particularly during the periparturient period (Sordillo and Aitken, 2009). Indeed, oxidative stress can exacerbate several inflammatory-based diseases by increasing the expression of several redox-regulated proinflammatory factors such as eicosanoids and cytokines.

Several recent studies have documented important changes in the antioxidant potential and prooxidant status in the transition dairy cattle (Bernabucci et al., 2002; Castillo et al., 2005; Sordillo et al., 2007). Lower antioxidant potential as a consequence of lactation stage can result from an excess accumulation of ROS, a depletion of antioxidant defenses, or a combination of both. One way to determine if ROS-mediated damage is occurring within host tissues is to measure end products of free radical oxidative processes. For example, when ROS react with polyunsaturated fatty acids, lipid peroxidation occurs. Peroxidation of lipids within cellular membranes can lead to changes in fluidity and cause damage to intracellular organelles. The determination of lipid hydroperoxide levels in plasma would be an indication of early stages of this lipid peroxidation damage. We showed that measurement of lipid hydroperoxides increased significantly from calving through the first 3 weeks of lactation when compared to the pre-partum measurements (Sordillo et al., 2007). These findings are consistent with other reports in periparturient animals where lipid hydroperoxides and biomarkers of lipid peroxidation, such as thiobarbituric acid-reactive substances (TBARS), were found to increase from calving through early lactation (Bernabucci et al., 2002; Castillo et al.,

2005). Impairment of blood and milk leukocyte function has long been linked with increased susceptibility to mastitis around the time of calving when oxidative stress is increased. However, remarkably few studies have examined in any detail the redox status of important immune cell populations during this time. Results from our laboratory indicate that the antioxidant potential of isolated peripheral blood mononuclear cells (PBMC) remained relatively constant from 3 weeks prior to calving and through calving, but dropped by 21 days in milk (Sordillo et al., 2007). These findings are consistent with reports in both humans and dairy cows that showed a relationship between the physiological changes during the periparturient period with a loss in overall antioxidant potential in several different tissue compartments (Aitken et al., 2009; Gitto et al., 2002).

The ability to control the degree of oxidant stress can be effective in ameliorating the severity of several pro-inflammatory-based diseases, such as mastitis. For example, it is well established that certain antioxidant micronutrients, such as selenium (Se), can dramatically impact the progression of acute coliform mastitis. Studies by Smith et al., (Smith et al., 1984) who showed that dairy cattle with existing deficiencies in Se had more severe clinical symptoms of coliform mastitis when compared to cows supplemented with adequate levels of this micronutrient. While supplementing dairy cattle with antioxidants is now a widely accepted management practice to avoid deficiencies, it is important to note that the underlying mechanisms for the benefits of Se are not completely known. Better understandings of how Se exerts its beneficial effects are needed for several reasons. Negative energy balance and increased production demands during the transition period results in an accumulation of ROS that far exceeds the cow's current antioxidant capabilities when supplemented with the maximum allowable (non-toxic) levels of Se. This trend of increased oxidant stress will likely continue as cows continue to be selected for increased milk production and therefore, the ways in which animals receive safe levels of antioxidant supplementation will need to change accordingly. Unfortunately, there is no information to suggest how Se plasma levels may translate to increased antioxidant potential within targeted cell populations of the mammary gland. The lack of information concerning which seleno-metabolites are critical for optimal health benefits has hampered the design of nutritional regimes that would maximize the effectiveness of either organic or inorganic sources of Se.

OXIDATIVE STRESS, LIPOMOBILIZATION, AND INFLAMMATION

The dramatic increase in energy requirements needed for the onset of lactation in transition cows is often accompanied by a decrease in voluntary dry matter intake that causes a negative energy balance (NEB). Energy requirements that cannot be met by the diet must then rely on tissue energy reserves. Therefore, NEB during the periparturient period causes mobilization of fat from tissue stores and the release of nonesterified fatty acids (NEFA) into the blood stream. Numerous studies clearly document an association between oxidative stress, elevated sera NEFA concentrations, compromised immunity, and increased disease susceptibility in dairy cattle during the periparturient period (Contreras and Sordillo, 2011; Sordillo and Aitken, 2009). However, the mechanisms by

which fatty acids may influence bovine host defenses and disease susceptibility in metabolically challenged cows are not known.

Essential fatty acids, including the omega 6 (n-6) linoleic and omega 3 (n-3) α -linolenic acid, are metabolized into long chain polyunsaturated fatty acids (PUFA). Linoleic acid can be converted through enzymatic reactions involving elongase and desaturase into the n-6 PUFA, arachidonic acid. These same enzymatic reactions also can convert α -linolenic acid into n-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Research in humans and various animal models suggest that these n-3 and n-6 PUFA are important modulators of immune and inflammatory reactions (Serhan, 2009). PUFA are incorporated into membrane phospholipids and can influence cellular functions by controlling membrane fluidity, interacting with nuclear transcription factors, affecting cellular signalling mechanisms, and regulating gene expression (Calder and Yaqoob, 2007). Most notably, however, certain PUFA can serve as precursors for the synthesis of bioactive lipid mediators, such as the eicosanoids, that are key inflammatory regulators in a variety of cell types. For example, arachidonic acid is released from membrane phospholipids through the action of phospholipases and is then oxidized by either the cyclooxygenase (COX) pathway to yield prostaglandins (PG) and thromboxins (TX) or the lipoxygenase (LOX) pathway that leads to the formation of hydroxyeicosatetraenoic acids (HETEs), leukotrienes (LT) and lipoxins (LX).

Depending on the timing and magnitude of expression, certain eicosanoids can either enhance or resolve the inflammatory response (Figure 1). The COX pathway is composed of 2 major isoforms. COX1 is constitutively expressed in most tissues and synthesizes low levels of PGs, such as prostacyclin (PGI_2), that are thought to function in the maintenance of normal physiological functions. Conversely, COX2 is highly inducible in response to pro-inflammatory stimuli and it traditionally has been associated with the biosynthesis of pro-inflammatory mediators such as PGE_2 , $\text{PGF}_{2\alpha}$ and TXA_2 . Non-steroidal anti-inflammatory drugs can inhibit PG biosynthesis by targeting COX activity and are used widely to treat a variety of inflammatory-based diseases including coliform mastitis in dairy cows (Erskine et al., 2003). Suppression of these enzymes, however, also can cause undesirable side effects. The most common consequence of prolonged COX1 inhibition is the development of abomasal ulcers. Although selective COX2 inhibitors minimize the risk of gastrointestinal events such as stomach ulcers, these drugs have been related to fatal cardiovascular reactions in humans, possibly by decreasing vascular PGI_2 production. Previous assumption that all COX2 metabolites are solely responsible for propagating the inflammatory response is not supported by current literature. Indeed, there is evidence to suggest that both COX1 and COX2 isoforms can contribute to agonist-induced inflammatory responses whereas some COX2-derived metabolites may be critical in mediating the resolution of acute and chronic inflammation (Serhan, 2009).

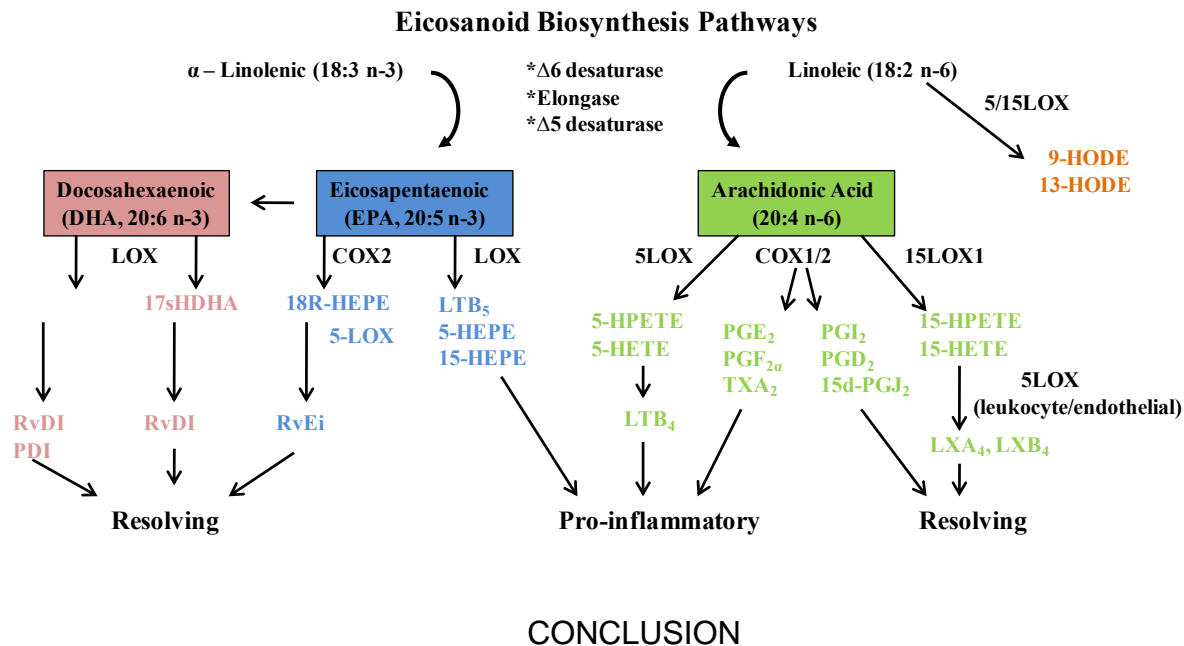
LOX is a heterogeneous family of non-heme enzyme dioxygenases with the ability to oxidize PUFA. There are several different LOX isoforms including 5LOX and 15LOX where the nomenclature is defined by the capability of each enzyme to introduce molecular oxygen on a specific carbon of the fatty acid structure. Metabolism of

arachidonic acid by the 5LOX pathway gives rise to hydroxyl and hydroperoxy derivatives (5HETE and 5HPETE), respectively that are often elevated in acute and chronic conditions. The 15LOX1 isoform is characterized as an inducible enzyme expressed in endothelial, epithelial, reticulocytes, monocytes, and macrophages with the ability to oxygenate PUFA during inflammation. The initial oxygenated product formed during arachidonic acid metabolism by 15LOX is 15HPETE, which is the biosynthetic precursor of 15HETE and other leukotrienes. Increased expression of 15LOX1 is observed in diseases where oxidative stress plays important roles such as atherosclerosis, Alzheimer's disease, and prostate cancer (Kuhn and O'Donnell, 2006). Previous in vitro studies showed that both 15HPETE and 15HETE can enhance adhesion molecule expression within vessel walls during disease progression in humans (Natarajan and Nadler, 2004). These data suggest that 15LOX1 may facilitate the development of inflammatory-based diseases in cattle, at least in part, by enhancing the pro-inflammatory phenotype of endothelial cells.

The biosynthesis of either pro-inflammatory or resolving eicosanoids through either the COX or LOX pathways depends, in part, on the n-6 vs n-3 PUFA composition of membrane phospholipids. Dietary linoleic acid generally is consumed in greater quantities by humans and food animal species than α -linolenic acid. Therefore, it is not surprising that arachidonic acid is the most abundant 20 carbon polyenoic fatty acid found in the phospholipids of mammalian tissues. As such, the relative fatty acid composition within the membrane phospholipids is largely determined by dietary intake of linoleic and α -linolenic acid. Increased consumption of n-3 fatty acids has long been recognized to benefit human health, in part, by controlling inflammation and improving cardiovascular health. One reason for this beneficial effect is likely from the production of pro-resolving lipid mediators derived from the metabolism of n-3 fatty acids through the COX and LOX pathways. Resolvins (Rv) and protectins (PD) are newly discovered fatty acid metabolites of the COX and LOX pathways in which the n-3 fatty acids eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), rather than arachidonic acid, serve as the enzyme substrate (Serhan, 2009). Studies are currently underway to determine if alterations in the composition of various fatty acids could impact bovine host immune defenses as well (Contreras et al., 2010). A better understanding of how elevated blood lipids may affect dairy cattle immunity during the transition period may lead to innovative approaches to control increased disease susceptibility through nutritional intervention.

Linoleic acid is the parent compound of the n-6 family of fatty acids and α -linolenic acid is the n-3 fatty acid precursor. These fatty acids compete for a microsomal enzyme system that desaturates (desaturase) and lengthens (elongase) them to form long-chain PUFA including arachidonic acid, eicosapentanoic acid and docosahexanoic acid. These PUFA are incorporated into membrane phospholipids, but serve as important substrates for the biosynthesis of eicosanoids through the cyclooxygenase (COX) and lipoxygenase (LOX) pathways.

Figure 1. Enzymatic pathways leading to the production of biologically active lipid mediators.



Oxidation and the production of free radicals are an integral part of aerobic metabolism. Considerable evidence supports the contention, however, that oxidative stress during the periparturient and early lactation period may contribute to a number of health disorders in dairy cattle. The performance of high producing dairy cattle can be optimized to a certain extent by supplementing diets with optimal levels of micronutrients with antioxidant capabilities. However, oxidative stress continues to be a problem in transition cows. Increased NEFA concentrations in transition cows also are closely linked with disease susceptibility. Changing the composition of fatty acids may be an effective way of altering the cow's response to infectious and metabolic diseases during the transition period. The development of innovative feeding strategies that could enhance host immunity is an attractive approach when considering transition cow management.

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THE SCIENCE OF COW COMFORT: BUILDING BETTER BARNs – SEEING THE FREESTALL FROM THE COW’S PERSPECTIVE

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INTRODUCTION

Poorly designed and managed facilities cause injuries and increase the risk of health problems including lameness and transition cow disease, arguably two of the most serious welfare challenges facing the dairy industry (von Keyserlingk et al., 2009). Producers spend millions of dollars building indoor housing for dairy cattle, with the aim of providing a comfortable environment for their animals - one that ensures adequate rest, protection from climatic extremes, and free access to an appropriate, well-balanced diet. Despite these laudable aims, housing systems do not always function well from the perspective of the cow – poorly designed and maintained facilities can cause injuries, increase the risk of disease, and increase competition among herd mates for access to feed and lying space. In this paper, we review research on the feeding, standing and lying areas with particular emphasis on the work undertaken by graduate students and visiting scholars working in our laboratory. Our aim is to provide science-based solutions that can facilitate better designs and improvements in management that will prevent some of these problems. Our work has generally evaluated housing systems from the cow’s perspective by asking how the housing affects cow health (e.g. by reducing the risk of hock injuries), what housing the cow prefers, and how the housing affects behavior (e.g. by reducing competition and increasing feeding time).

BETTER LYING AREAS

The issue of cow comfort has received considerable interest within the dairy industry, with the bulk of research having focused on the design of freestalls and the effect of stall design on stall occupancy and the time spent resting. Our work has shown that the commonly used tool to assess comfort such as the Cow Comfort Index is not a reliable method; but instead, monitoring 30 cows/farm for 3 days gives an accurate estimate of the true lying behavior (Ito et al., 2009). These research-based knowledge on stall design and its effect on cow behavior - are now beginning to be implemented in the design of new barns (LeBlanc et al., 2006).

Our work on lying areas for cattle has focused on two aspects: the surface cows lie down upon, and how the stall is configured.

Lying surface

A growing body of research has now demonstrated that the surface we provide for cows is one of the most important factors in designing a suitable lying area. First and foremost, the housing we provide should not cause injuries or other health risks to the cow. Although this sounds obvious, too often poor design leads to preventable health problems. An important first step in assessing cow comfort is an understanding of how a cow behaves when she is comfortable (Ceballos et al., 2004). Several researchers have measured stall usage, when the animals have no choice between surfaces, to assess how different bedding types affect behavior. For example, Haley et al. (2001) used a simple comparison between a space considered “high comfort” (a large box stall with mattresses) and a stall that represented “low comfort” (a tie stall with concrete flooring). They measured many behaviors including lying, standing, and eating times, the number of times the cows stood up, and various leg positions during lying. Lying times were 4 h longer and cows were more willing to stand up and change positions in the high-comfort housing. Cows also spent more time standing idle in the low-comfort stalls. There is some evidence that cows prefer lying down on straw rather than sand (Manninen et al., 2002), but this can be altered with greater experience of sand (Norrington et al., 2008). Furthermore, the reduced risk of mastitis or lameness (Cook et al., 2004; Espejo et al., 2006; Norring et al., 2008) with sand bedding may compensate for the reduced preference. Collectively these studies tell us which behavioral measures are likely to change if a cow is uncomfortable, namely, time spent lying and standing, and the number of times she is willing to stand up.

In some of our group’s first work on cow comfort, we found that cows on farms with mattresses (and little bedding) have more severe hock lesions than do cows on farms that using deep-bedded stalls (Weary and Tazskun, 2000). Although similar results have now been found in other research (Wechsler et al., 2000), and most dairy professionals are aware of the risks of poorly-bedded mattresses, too often this surface continues to be used.

Cows also clearly prefer lying surfaces with more bedding, and spend more time lying down in well-bedded stalls. In a more recent experiment, we examined the effect of the amount of bedding on the time spent lying and standing by cows housed in free stalls (Tucker and Weary, 2004). Each stall was fitted with a geotextile mattress, and bedded with one of three levels of kiln-dried sawdust (0, 1, and 7 kg). Cows spent 1.5 h more time lying down in the heavily bedded stalls. In addition, cows spent less time standing with only the front legs in the stall when the mattresses were heavily bedded. These changes in both standing and lying behavior indicate that cows are hesitant to lie down on poorly-bedded mattresses.

These differences in stall comfort may also account for a second important health problem; cows housed on mattresses also have a higher incidence of severe lameness than those housed in deep-bedded stalls (Ito et al., 2010). The lying surface can also affect udder health, and many studies have now shown the advantages to cows of using

sand or other inorganic bedding as a way of reducing the growth of bacteria associated with environmental mastitis (Zdanowicz et al., 2004).

Making the decision to provide a well-bedded surface is just the first step in achieving a reasonable level of cow comfort – this surface must also be properly maintained. In a series of experiments, we documented how the sand level declines in stalls that are not maintained, and how this decline reduces stall use by cows (Drissler et al., 2005). Sand levels in deep-bedded stalls decreased over a 10-day period, with the deepest part at the center of the stall. Lying time by cows also declined as the stall empties; every inch decline decreased lying time by about half an hour per day. Contact with concrete while lying down may explain lower lying times in deep-bedded stalls with less sand, and this concrete also affects leg health. Lesions on the point of the hock are common in deep-bedded stalls (Mowbray et al., 2003), likely due to contact with the concrete curb when stalls are not well maintained. Cows also showed a strong preference for lying on dry bedding during the summer months and when forced to lie down on wet bedding showed a 5 h reduction in lying time (Fregonesi et al., 2007).

Stall configuration

Most indoor housing provides more than just a lying surface for the cows. Typically the space is designed to encourage the cow to lie down in a specific location, and to use the stall in such a way that feces and urine do not soil the stall. Unfortunately, most attempts to constrain how and where the cow lies down also reduce cow comfort as illustrated by the studies described below.

Although some excellent recommendations for stall dimensions are now available, too often new constructions and renovated barns fail to provide appropriate space. We have conducted several experiments that show how stall size and configuration affect standing and lying times. For example, in one study we tested the effect of stall width on cow behavior (Tucker et al., 2004), by providing cows access to free stalls measuring 42, 46, or 50” between partitions. Cows spent an additional 42 min/day lying in the widest stalls, likely because they had less contact with the partitions in these larger stalls. Cows also spent more time standing with all four legs in the wider stalls, reducing the time they spent standing partially (i.e. perching) or fully on the concrete flooring available elsewhere in the barn.

STANDING AREA

One challenge in creating suitable freestalls for cows is that this one structure is supposed to do it all. In addition to stall width, neck rail placement is important for managing standing behavior. According to popular thinking, when cows are not in the parlor they should be eating or lying down. Unfortunately, no one seems to have explained this to the cows. In a number of studies, we have found that even when cows have access to well-designed stalls they spend only about half of the day lying down. Cows spend the other 12 h a day on their feet, and we need to take this into account in designing suitable housing.

In most barns, the surface for standing outside of the stall is wet concrete – a known risk factor for hoof health (Borderas et al., 2004). Cows can use the stall as a refuge, providing a dry, softer surface for standing. However, this increases the likelihood that cows will urinate and defecate in stall. The common response by barn designers has been to make the stalls more restrictive (as described above), forcing cows back into the concrete alley, and explaining in part why lameness is now the most prevalent and costly health problem for cows housed in freestall barns. With our current barn designs, we are stuck with two bad choices: use restrictive stalls that keep the stall surface cleaner but force cows back onto the wet concrete, or use more open designs and increase frequency of stall maintenance. Of these two options we favor the latter, but there may also be a third approach – improving the standing surface elsewhere in the barn. Both the height of the neck rail and its distance from the curb affect standing behavior (Tucker et al., 2005); more restrictive neck rail placements (lower and closer to the rear of the stall) prevent cows from standing fully inside the stall, again increasing the time cows spend on concrete flooring elsewhere in the barn. Recent work has also shown that gait scores improve when neck rails are moved to a less restrictive position so that cows can stand with all four feet in the stall, and worsen when neck rails are more restrictive (Bernardi et al., 2009). The neck rail is designed to „index“ the cow in the stall while she is standing, but the brisket board achieves this function while cows are lying down. Unfortunately, brisket boards also discourage stall use – cows spend 1.2 h per day less time lying down when stalls have a brisket board compared to when using stalls without this barrier (Tucker et al., 2006a).

Keeping cows out of the stall obviously helps keep the stalls clean. We found that both the narrow free stalls and the more restrictive neck rail placements reduced the amount of fecal matter that ended up in the stall (Tucker et al., 2005; Bernardi et al., 2009). Although dirty stalls are undesirable, readers should be aware that stall cleanliness alone is a poor measure of stall design. Free stalls that have higher occupancy rates are most likely to contain feces. Thus well-used stalls require more stall maintenance, just like other equipment used on the farm.

Research suggests that cow comfort plays an important role in whether or not cows become lame and how long they stay lame (Hernandez-Mendo et al., 2007; Bernardi et al., 2009), but assessing cow comfort on-farm can be a challenge. Some of our most recent work also provides the first evidence that increased standing time in the pre-partum period is a key risk factor for hoof health problems later on in lactation (Proudfoot et al., 2010).

We have now completed a series of studies on alternative flooring surfaces in dairy barns. In this work, we have concentrated on the area where cows stand to eat, as cows spend about half of their standing time in this area. A number of studies have shown that access to pasture improves hoof health, likely because under good grazing conditions the pasture is a more comfortable and healthier surface for standing upon. We showed that a relatively brief period on pasture could help lame cows recover (Hernandez-Mendo et al., 2007). Non-concrete surfaces can also provide better traction

and be more comfortable for cows to walk upon. Cows will typically choose to walk upon a rubber surface and avoid concrete if the option is available, and our research shows that cows slip less frequently and show improved gait when walking on rubber compared to concrete, a difference that is especially clear for lame cows (Flower et al., 2007).

Other work has shown that cows prefer to stand on softer surfaces and moving the neck rail further from the curb reduces perching behavior and can reduce lameness cases. Bernardi et al. (2009) provided some of the first experimental evidence that aspects of stall design can reduce the risk of lameness and hoof disease. This study assessed the effect of the position of the neck rail and found that over a 5 wk period, although we noted little change in lying times, gait scores improved for cows kept in pens without the neck rail compared to pens equipped with the neck barrier. However, these results also illustrate that some changes in design that result in improvements in hoof health come at the expense of cow hygiene and udder health. Although removing the neck rail comes at a hygiene cost (cows standing with all 4 feet in the stall will defecate and urinate more into the stall) there is no clear evidence that it increases the risk of mastitis. However, if this practice is utilized, particularly during the transition period, it is recommended that stalls be cleaned often, as fresh cows are at high risk for mastitis.

No work to date however has looked at the interaction between stall maintenance and injuries, and we encourage more work in this area. In one study, we gave cows the choice of standing on concrete or on softer surfaces, and cows spent the majority of their time standing on the softer flooring (Tucker et al., 2006b). This study also showed that when cows did not have the choice, they spent more time standing when they had access to the softer surface. In this study and in a previous experiment (Fregonesi et al., 2004), we also found that standing times increased when cows had access to a rubber standing surface in front of the feeder. These effects on standing times are only modest, so the development of new standing surfaces remains an important area for future work.

A high standing time could suggest a deficit in the cow's environment; for instance, cows housed in pens with insufficient number of lying stalls, low bedding, wet bedding, or restrictive neck rails spend more time standing than those with ample dry stalls and less restrictive neckrails (Tucker and Weary, 2004; Fregonesi et al., 2007; Fregonesi et al., 2009). Cows that perch with their 2 front feet in the stall during transition are also at increased risk for lameness (Proudfoot et al., 2010); as stated above, this behavior has been linked with restrictive stall design (Tucker et al., 2005; Fregonesi et al., 2009).

BETTER FEEDING AREAS

There are several aspects of the feeding environment that affect the cow's ability to access feed, including the amount of available feed bunk space per animal and the physical design of the feeding area. Reduced space availability increases competition in cattle. For example, a recent study by DeVries et al. (2004) showed that doubling feed bunk space from 20 to 40" reduced by half the number of aggressive interactions while

feeding. This reduction in aggressive behavior allowed cows to increase feeding activity by 24% at peak feeding times, an effect that was strongest for subordinate animals.

In addition to the amount of available feed bunk space, the physical design of the feeding area can also influence feeding behavior. One of the most obvious features of the feeding area is the physical barrier that separates the cow and the feed, and research shows that some designs can reduce aggressive interactions at the feed bunk. For example, Endres et al. (2005) compared the effects of a post-and-rail versus a headlock feed line barrier on the feeding and social behavior of dairy cows. Average daily feeding time (about 4.5 h per day) did not differ, but during periods of peak feeding activity (90 min after fresh feed delivery), subordinate cows had lower feeding times when using the post-and-rail barrier. This difference in feeding times was likely due to positive effects of the headlock barriers in reducing competitive interactions; there were also 21% fewer displacements at the feed bunk with the headlock barrier compared to the post-and-rail barrier. These results suggest that using a headlock barrier reduces aggression at the feed bunk and improves access to feed for subordinate cows.

In a second study, we retested the effects of these two types of feed bunk barriers, but did so over a range of stocking densities (Huzzey et al., 2006). Cows were tested with the barriers described above but using stocking densities of 32, 24, 16 and 8 in/cow (corresponding to 1.33, 1.00, 0.67 and 0.33 headlocks/cow). Daily feeding times were higher and the duration of inactive standing in the feeding area was lower when using a post-and-rail compared to a headlock feed barrier. As well, regardless of barrier type, feeding time decreased and inactive standing increased as stocking density at the feed bunk increased. Providing adequate feed bunk space during the pre-partum period is also essential as work has shown that overstocking during this period reduces dry matter intake (Proudfoot et al., 2009), and that cows that consume less are at higher risk for post-partum disease (Huzzey et al., 2007).

Cows were displaced more often from the feeding area when the stocking density was increased, and this effect was greater for cows using the post-and-rail feed barrier. Again we found that this effect was greatest for subordinate cows, particularly at high stocking densities. Clearly, overstocking the feed bunk decreases time spent at the feed bunk and increases competition, resulting in poor feed access. We have recently found very similar effects (less usage and more competition) when lying stalls are overstocked (Fregonesi et al., 2007). Moreover, we have observed that cows on average left the feed bunk 30 min earlier when stalls were stocked at 150% compared to when they were stocked at 100% (Fregonesi et al., 2007).

New work has now shown that providing additional partitions (“feed stalls”) between adjacent cows provides additional protection while feeding and allows for improved access to feed (DeVries and von Keyserlingk, 2006). Providing a feed stall resulted in less aggression and fewer competitive displacements, effects that were again greatest for subordinate cows. This reduced aggression allowed cows to increase daily feeding time, and reduced the time they spent standing in the feeding area while not feeding. Thus, the provision of more bunk space, especially when combined with feed stalls,

improves access to feed and reduces competition at the feed bunk, and this effect is strongest for subordinate cows. These changes in feed bunk design and management could help reduce the between-cow variation in the composition of ration consumed; under conventional systems, subordinate cows can only access the bunk after dominant cows have sorted the feed (DeVries et al., 2005). The use of a barrier that provides some physical separation between adjacent cows can reduce competition at the feed bunk. A less aggressive environment at the feed bunk may also have longer-term health benefits; cows engaged in aggressive interactions at the feed bunk are likely at higher risk for hoof health problems (Leonard et al., 1998).

BARN LAYOUT

Cow comfort may also be affected by overall layout of the barn. For example, some work has shown that cows rarely use certain stalls in a pen, while seemingly identical stalls are occupied more than 80% of the available time. One study showed that stalls in the row closest to the feed alley were occupied 41% more frequently than were stalls in more distant rows (Gaworski et al., 2003). In addition, stalls located within the centre of each row were used 12% more often than those stalls located on the periphery of the row (i.e. either near a wall or fence). Natzke et al. (1982) also found that stalls on the periphery were used less than stalls in the interior of the row. These results suggest that certain stalls, particularly those farther from the feed bunk and on the periphery, are less desirable to dairy cattle, perhaps because cows need to walk farther, or because of they have to navigate past certain physical (e.g. narrow alleys) or social obstacles (e.g. dominant cows) on their way to the more distant stalls. Indeed, earlier work has indicated that the movements of subordinate animals are prevented by the location of dominant cows (Miller and Wood-Gush, 1991). Such factors may partly explain reduced user satisfaction and lower production in those barn designs consisting of more rows (e.g. 6 and 4 row verses 2 and 3 row barns; Bewley et al., 2001).

We strongly encourage producers to evaluate their facilities on an individual resource basis - the lying, feeding and standing areas. For example, large differences in usage can occur even among identically configured stalls within the same barn. The fact that stalls within a pen vary in their popularity suggests that stall availability from the cows' perspective is not the same as from the producer's perspective - what looks to us as 1:1 cow-to-stall stocking density may seem considerably worse to the cows if some stalls are unacceptable. Another example is providing adequate feed bunk space on a per cow basis; in a 6 row barn, the amount of feed bunk space per cow is often far less than that recommended. A number of lines of evidence now suggest that providing adequate feed bunk space is essential to maintain dry matter intake, and reduced feed bunk space can have profound effects on rates of illness, particularly during the transition period (Huzzey et al., 2007; Goldhawk et al., 2009).

TAKE HOME MESSAGES

1. Cows like softer surfaces, for both lying down and for standing upon. Deep-bedded stalls work well for cow comfort, but require maintenance.

2. When it comes to the physical structures used to build freestalls, less is more – the hardware we place in the stall is for our benefit and not the cows". The more restrictive we design stalls, the less attractive they become for the cow.
3. Use of restrictive stall designs can help keep stalls clean, but to avoid problems with hoof health, these designs need to be accompanied by better flooring options, such as softer and drier flooring.
4. The design and management of the feeding area are important. High stocking densities at the feed bunk increase aggressive competition, and keep subordinate cows away from feed.
5. Physical barrier between cows, including headlocks and feed stalls, can help reduce this competition, and increase feeding time.

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NOVUS C.O.W.S. PROGRAM: ON-FARM ASSESSMENTS TO IMPROVE COW COMFORT

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INTRODUCTION

Over the past decade, the issue of cow comfort and the importance of non-dietary factors on productive performance of dairy cows have received increasing attention. For instance, Bach et al. (2008) showed that 47 herds fed the same TMR and shared similar genetics, varied in average milk yield between 45 to 75 lb/cow/day. Among the environmental and management factors identified as explanatory of this variation were stocking density and stall maintenance. Increasing productive efficiency is not only a nutritional problem, but is a multi-factorial one. The cows' environment must be designed and managed in such a way that allows them to maintain health, well-being, and productivity.

Novus COWS (Comfort · Oxidative Balance · Well-being · Sustainability) program brings these factors together and acts as a vehicle for engagement on topics of cow comfort. It is driven by the desire to provide a service to dairy producers, while contributing to optimizing animal well-being, productive efficiency, and the sustainability of the industry. Our ultimate goal is to drive change. In order to do so, we must be able to 1) identify problems; 2) create motivation for change; and 3) provide recommendations with practical solutions.

OVERVIEW: WHAT IS C.O.W.S. PROGRAM?

In 2010, Novus International Inc. partnered with The University of British Columbia's (UBC) Animal Welfare Program to undertake a nationwide cow comfort benchmarking study. UBC Animal Welfare Program is a globally recognized and respected research group contributing to the development of science-based solutions to improving dairy cattle welfare. This collaboration resulted in the COWS program, expanding on a project initially developed by UBC and piloted on 43 dairies in British Columbia.

The COWS program assesses individual dairies on several cow comfort measures (e.g. lying time, lameness, and hock injuries), and facility and management measures (e.g. stall design, bedding quality, and stocking density). Initial benchmarks for these measures have been created from data collected on 118 dairies in California, New York, Pennsylvania, Vermont, Texas, and New Mexico (Barrientos et al., 2011). Since then, Novus has committed to the implementation of the program as a service to dairy producers in the United States.

Participating producers receive individualized reports (Appendix), comparing their data against the benchmarks from that region. This process brings awareness to the issues on the topic of cow comfort, while highlighting opportunities for improvement. This is done in a confidential manner, and in terms of several objective measures, to encourage producers to participate with no external judgment. The regional and system-specific benchmarks show not only the industry averages but also the potential for success. In this way, the COWS program offers an alternative approach to on-farm assessment as a knowledge sharing vehicle, rather than a pass-or-fail auditing scheme. Producers and their advisors are then encouraged to identify priority areas and plans of action that are specific to their dairy. If they decide to make certain changes, they have the opportunity to participate in a reassessment to evaluate the effectiveness of the change. At this early stage of the program, we have already seen promising outcomes where participating producers have made real changes (some are small and inexpensive, while others are larger scale involving significant investment) that translated into improved cow comfort as well as productive performance.

C.O.W.S. ASSESSMENT: WHAT DO WE MEASURE AND WHY?

The on-farm assessment involves collecting a number of animal-based measures and facility-based measures, previously developed and tested (Ito, 2009).

Animal-based Measures

Lying time

The measuring of lying time is a unique feature of the COWS assessment. Dairy cows are highly motivated to lie down for up to 12 hours per day (Jensen et al., 2005) and lying is a high priority behavior compared with feeding and social contact when opportunities to perform these behaviors are restricted (Munksgaard et al., 2005). Lying time, along with the frequency of lying bouts and the duration of individual lying bouts, has been identified as a sensitive measure of stall comfort (Haley et al., 2001).

Traditionally, stall comfort has been estimated by indices based on one-time observation at a quick walk-through of the barn. For example, the Cow Comfort Index (CCI) is calculated as the proportion of the number of cows lying in a stall out of the total number of cows „touching“ a stall (standing fully inside or perching in a stall). However, these indices do not reflect actual lying time (Ito et al., 2009), and cannot be used as a replacement for this measure.

In the COWS assessment, lying time is measured using electronic data loggers (Ledgerwood et al., 2010). The loggers are attached to 40 randomly selected cows from the assessment group, and record if the cows are lying or standing at 1-min intervals for 72 consecutive hours (Ito et al., 2009). Farm average is calculated as the mean of individual daily lying times (h/d), and is reported with the minimum and maximum lying times from the group.

Prevalence of Lameness

Lameness has been recognized as a serious production and welfare issue in the dairy industry for many years. Recent studies have estimated the lameness prevalence in North America to be 25-30%, but ranging widely from farm to farm (Cook, 2003; Espejo et al., 2006; Ito et al., 2010). The management strategy for lameness will depend on the extent and severity of the problem on each dairy. For example, a dairy that has 10% lameness and another dairy that has 50% lameness would need different plans of action; similarly, a dairy that has mostly mildly lame cows would benefit from a different strategy than a dairy that has many severely lame cows. Therefore, the producer must know what the status of lameness is on their dairy specifically, and not act on the industry average.

Despite its importance, lameness detection has been challenging for dairy producers; as a result lameness is often underestimated (24.6% identified by trained observer vs. 8.3% estimated by producers; Espejo et al., 2006). As lameness becomes increasingly more common, abnormal locomotion may become normalized, resulting in the cows showing subtle signs of lameness being perceived as sound. By the time a cow is diagnosed as lame, the damage has already manifested in reduced performance (Green et al., 2002; Garbarino et al., 2004; Bicalho et al., 2008), and compromised welfare (Whay et al., 2003). Gait (or locomotion) scoring, a method that identifies subtle behaviors exhibited by lame cows, requires training and additional time commitment; however, it can be a valuable tool for early detection of lameness.

During the COWS assessment, all cows in the group are gait scored upon exit from the parlor, after their routine milking. Gait scoring categorizes cows on a 5-point scale based on six gait attributes: back arch, head bob (jerky head movement), tracking up (stride length), joint flexion (joint stiffness), asymmetric steps, and reluctance to bear weight (Flower and Weary, 2006) as follows:

- 1: **“Sound”** – walks with a smooth and fluid locomotion, a flat back and even steps.
- 2: **“Imperfect gait”** – walks with a slightly uneven gait and slight joint stiffness but with no limp.
- 3: **“Mildly lame”** – walks with shortened strides, an arched back and a slight limp.
- 4: **“Moderately lame”** – walks with an obvious limp, a severely arched back and a jerky head bob.
- 5: **“Severely lame”** – not bearing weight on at least one limb and/or must be vigorously encouraged to stand or move; extremely arched back when standing and walking.

For the purpose of our assessment, cows scored as 1 or 2 are considered „not lame“, 3 are „mildly lame“, and 4 or 5 are „severely lame.“

Prevalence of hock lesions

Inappropriately designed and managed free-stalls often cause injuries that compromise cow comfort. Hock injuries are often caused by rubbing of the leg on abrasive lying surface; in particular, mattress or rubber mats with minimal bedding are associated with the highest risk of hock lesions (Weary and Tazskun, 2000; Lombard et al., 2010). Hock condition of the same 40 cows selected for lying time assessment are scored, on a 3-point scale where 1 = healthy, 2 = hair loss, and 3 = swollen or injured (Lombard et al., 2010).

Facility-based Measures

Facility design and management practices are recorded through an interview with the manager or herdsman of each dairy, and by direct measurements in the barn where the assessment group of cows is housed. The measures include:

- Stall dimensions (free-stall herds) – length, width, neckrail and brisket board placement
- Bedding type and maintenance
- Stocking density
- Feedbunk design and management
- Milking management – distance to parlor, time away for milking

These measures, when reported to the producer, serve as guidelines for troubleshooting management. For example, if cows are not lying down, there may be several factors that are responsible. Factors that can affect lying time include: stall dimensions, type of lying surface, and the quantity and quality of bedding material. Cows spend more time lying down on well-bedded stalls compared with poorly bedded mattresses (Tucker et al., 2003; Tucker and Weary, 2004), and on wider stalls with no brisket board (Tucker et al., 2004; 2006). Lying time decreases as the dryness of the bedding material decreases (Fregonesi et al., 2007b; Reich et al., 2010), and as the stocking density increases (Fregonesi et al., 2007a). Producers can use these measures provided in the report (Appendix) to begin to identify risk factors for reduced lying time.

Management factors such as feeding and milking procedures influence the time budget of the cow. Cows spend about half of their time lying down, and divide the rest for milking, feeding, and standing (in alleyway or inside stalls) (Gomez and Cook, 2010). The time the cows spend waiting to get milked or to gain access to feed is the time taken away from what is available for lying down. Therefore, the management protocols must be considered together with the functionality of the stalls when interpreting lying time.

All of these measures are multi-dimensional issues that require multi-dimensional approach to troubleshoot. For instance, lameness is a function of the environment, management, and physiology of the cow (Cook and Nordlund, 2009). Stall features that affect lying time may also affect lameness. Mattress stalls are associated with lower

lying time (Tucker et al., 2003) and also higher risk of lameness than deep-bedded stalls (Cook et al., 2004; Espejo et al., 2006; Ito et al., 2010). Prolonged standing time is a risk for lameness (Cook et al., 2004; Galindo and Broom, 2000), regardless of its cause: uncomfortable stalls, overstocking, or inappropriate feeding and milking management. However, providing cows with a comfortable place to stand as an alternative to concrete can reduce the risk of lameness (Bernardi et al., 2009). Moreover, a complex relationship exists between lameness and lying time, depending on the type of the stall surface as well as time available for rest (Gomez and Cook, 2010; Ito et al., 2010). This complexity demonstrates that an effective on-farm assessment must take a comprehensive approach encompassing a multitude of factors.

C.O.W.S. DISCUSSION: WHERE DO WE GO FROM HERE?

COWS benchmarking project has revealed a number of opportunities for improvement for the industry; however, many producers have already achieved considerable success in various areas of cow comfort (Barrientos et al., 2011). We aim to create a program where knowledge and experience can be shared, so that producers can learn from each others' successes (or mistakes), and to collectively develop „best management practices“. Novus continues to collaborate with UBC on research and data analysis to identify risk factors, and to provide scientifically sound recommendations for improved management. Future work is required in developing the most effective method for driving change and sustaining the effort.

TAKE HOME MESSAGES

- Novus COWS Program is a science-based, comprehensive assessment aimed at optimizing cow comfort and well-being, while removing limitations to production through improved management.
- The program brings awareness to cow comfort issues and facilitates discussion. It provides an ideal vehicle for engaging the producers, advisors, researchers, and the industry as a whole, with the common goal to develop practical solutions.
- COWS benchmarking project has revealed a number of opportunities for improvement, but many producers have already achieved considerable success in various areas of cow comfort. We aim to develop a program in which knowledge and experience can be shared for the collective progress of the industry.

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APPENDIX

Excerpt from COWS Report (Novus International Inc., 2010)



C.O.W.S. Individualized Report Benchmarking Region: New York, Vermont, Pennsylvania

Specifically designed for:
Example Dairy
Anytown, USA

Thank you for participating in the C.O.W.S. (Comfort • Oxidative Balance • Well-Being • Sustainability) benchmarking project. The data collected on your farm was combined with data from 39 other farms we visited and was used to develop the benchmarking information for your region.

In this report, you will be able to compare the data from your farm to the regional benchmarks. The report shows the summary of the benchmarking information from participating herds in New York, Vermont and Pennsylvania, and how your herd compares to the others in the region.

Please use the C.O.W.S. handbook for instructions on how to interpret your report. The handbook also provides information on factors known to affect cow comfort and lameness that may help improve the conditions on your farm and enhance the performance of your herd.

C.O.W.S. Benchmarking

Background of Benchmarking Segment

Region: New York, Vermont, Pennsylvania

Timing: July – October, 2010

Farms compared: 40 free stall dairy herds in the Northeastern United States

Measurements summarized in this report: lying time, prevalence of lameness, prevalence of hock and knee injuries, housing environment – freestall dimensions, bedding quality, stocking rates, etc.

Lying Time: On your dairy



Your Farm

Average: 9:58 hrs

Min: 5:02 hrs Max: 16:12 hrs

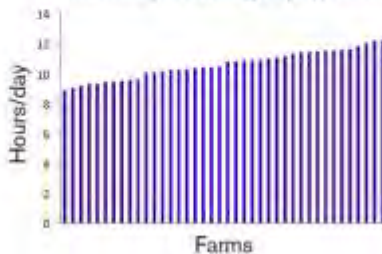
Comments from your dairy:

Increased time spent standing on concrete is a risk factor for lameness. The more time cows spend lying down in the stall, the less likely that they are standing on concrete. All cows should have the opportunity to lie down for 10-12 hours per day.

Lying Time: Summary of all herds

- On average, cows were lying down for approximately 10.5 hours per day.
- Herd average lying times ranged from 9 to 12 hours per day.
- Individual cows' lying times across all herds ranged from 3 to 20 hours per day.

Summary of Average Lying Time



Facility Design and Management Measures

1. Lying Area

Lying Area	Your Measurement	Average	Comments
Bedding frequency	3.0 days	3.6 days	Increased frequency and quantity of bedding may lead to increased lying time.
Bedding quantity – on a scale of 1 (ample bedding) to 3 (less than 50% of stall base covered)	1.0	2.3	Stall use improves with the addition of more bedding. The stall base or type of bedding is as important as bedding quantity or quality.
Bedding maintenance – number of times stalls cleaned/raked per day	2	2.9	Well-used stalls are drier. Stall design, frequency of new bedding and maintenance all contribute to stall cleanliness.
Bedding Cleanliness – on a scale of 1 (clean) to 3 (dirty)	2.0	1.5	
Bedding dry matter	75.0%	75%	Bedding dry matter less than 60% has been shown to reduce lying time.
Number of cows/stall *100 (stock rating)	130.0%	113% (Target: 100%)	Stocking rates of more than 100% (not enough stalls for every cow) can reduce lying times and milk yield.

2. Stall Dimensions

Stall Dimensions	Your Measurement	Target*	Comments
Curb height	9 inches	8 inches or less	High curbs are associated with increased risk of lameness.
Stall width	47 inches	48 inches	Cows spend more time lying and are less likely to perch in wider stalls. Larger cows require larger stalls.
Stall length	90 inches (single row) 90 inches (double row)	108 inches (single row) 102 inches (double row)	Stalls should have adequate lunge space, with no obstruction, to allow cows to easily stand up and lie down.
Neck rail height from bedding	49 inches	48 inches or greater	Neck rails increase the time cows spend standing in the alley or perching. Moving the neck rail up and further from the curb reduces these behaviors and the risk of lameness.
Neck rail distance from the curb	69 inches	68 inches or greater	
Brisket board height above bedding	5 inches	4 inches or less	Brisket board reduces the amount of time cows spend lying in the stalls, and may increase the risk of knee lesions. High brisket boards prevent the forward thrust of the front leg when the cow rises.
Brisket board distance from curb	69 inches	68 inches or greater	

* Target is based on 1400 lb. dairy cow – larger cows will require larger stalls.

3. Feeding Area

Feeding Area	Your Measurement	Target	Comments
Number of cows/headlock *100 (stocking rate)	120.0%	100%	Overstocking at the feed bunk increases competition. In turn, this reduces the time cows spend feeding and increases the time cows spend standing in the alley waiting to feed.
Bunk space per cow	20.0 inches	24 inches (mid lactation) 30 inches (close up and fresh)	

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EARLY LACTATION DIETS FOR DAIRY CATTLE – FOCUS ON STARCH

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INTRODUCTION

Feeding and management practices for transition dairy cows can have a substantial impact on a cow's well-being and farm's profitability. Suboptimal transitions from the dry cow diet to the fresh cow diet can decrease milk yield, lactation persistency, and reproductive performance. During the postpartum phase of the transition period, a cow undergoes rapid increases in dry matter intake (DMI) and milk yield coordinated by a homeorhetic mechanism involving many biological processes in several tissues (Bauman and Currie, 1980; Ingvarsten, 2006). Typically, a cow will experience 1) a period of insulin resistance, reduced DMI, negative energy balance (NEB), lipolysis, and weight loss in early lactation, 2) hypocalcemia in the day(s) after calving, 3) reduced immune function for 1 to 2 weeks before and 2 to 3 weeks after calving, and 4) bacterial contamination of the uterus for 2 to 3 weeks after calving (LeBlanc, 2010).

The time to reach peak DMI varies and depends on the composition of the lactation diet and the body condition score of the cow (Ingvarsten and Andersen, 2000). However, peak DMI usually occurs well after peak milk production. Thus, mobilization of body adipose and muscle occur to support the energy requirement in early lactation. Implementation of proper nutritional and management strategies is critical to support non-compromised lipid mobilization and prevent compromised lipid mobilization and disease (Ingvarsten et al., 2003). Periods of extreme NEB during early lactation are associated with increased digestive, locomotive, and reproductive problems (Collard et al., 2000).

The most rapid decrease in energy balance and NEB nadir usually occur in the first 3 wk postpartum with most cows reaching positive energy balance by 6 to 9 wk postpartum (Grummer et al., 2010; Grummer and Rastani, 2003). Factors other than energy output are responsible for variability in time to reach positive energy balance. There are stronger relationships between days to positive energy balance and dietary energy density or energy intake than peak milk yield, days to peak milk, or milk energy output (Grummer and Rastani, 2003; Santos et al., 2010). Minimizing the severity and duration of NEB is most likely to be accomplished through successful feeding rather than through decreasing milk yield.

There are many studies that have evaluated the carryover effect of prepartum diets on postpartum metabolism and performance. However, there are fewer studies that have evaluated nutritional strategies immediately postpartum to support metabolic adaptations and optimize lactational and reproductive performance in early lactation. Strategies have focused on increasing the dietary energy density, altering the source of fermentable carbohydrates, and changing the availability of glucogenic nutrients relative

to lipogenic nutrients. Many of the early lactation feeding recommendations (Block, 2010; Drackley, 1998; Overton and Boomer, 2010) are based on field experience and limited research.

FRESH COW FEEDING STRATEGIES

Nutrient Density

In order to maximize energy intake during a time of lower DMI, researchers have evaluated increasing the energy density of the diet during the transition period by incorporating higher proportions of concentrates. This approach was evaluated initially when complete feeds (i.e. TMR) were first being recommended. Cows were changed from dry diets of nearly all forage ($\geq 80\%$) to a high concentrate (60%) lactation diet compared with a high forage (60%) lactation diet without negative effects on DMI, milk yield, and ruminal fermentation during the first 4 weeks of lactation (Hernandez-Urdaneta et al., 1976). However, cows were producing ≤ 28 kg/d. More recently, Rabelo et al. (2003; 2005) fed cows either low or high energy diets for 28 d prepartum (1.58 vs. 1.70 Mcal/kg) and 20 d postpartum (1.57 vs. 1.63 Mcal NEL/kg; 60 vs. 40% forage; 20 vs. 36% corn meal; 30 vs. 25% neutral detergent fiber (NDF)) and then fed all cows the high energy diet for 21 to 70 d postpartum. The prepartum diets had minor effects on the postpartum metabolic status of cows compared with the postpartum diets fed during the first 20 d of lactation. Cows fed the high postpartum diet had the more favorable metabolic profile, had higher DMI and energy intake during the first 20 d of lactation, and had a higher rate of increase in milk yield. One concern with feeding diets with more fermentable carbohydrates and less NDF is the risk of ruminal acidosis may be increased. Cows that were fed the high energy density diet had lower ruminal pH and higher propionate concentrations than cows fed a low energy density diet (Rabelo et al., 2003). Cows fed Florida-style diets ($\leq 39\%$ forage with several byproduct feeds) that had a large change in energy content (+0.26 Mcal NEL/kg) between the prepartum and postpartum diets that were fed for 21 d before and after calving had more hemorrhages and ulcers in the sole suggestive of subclinical laminitis (Donovan et al., 2004). However, Guo et al. (2007) demonstrated the positive effects on energy balance and lipid metabolism of abruptly changing the diet from the prepartum (1.54 Mcal NEL/kg, 53% NDF) to postpartum (1.77 Mcal NEL/kg, 35% NDF) periods compared with maintaining the same diet (1.71 Mcal NEL/kg, 35% NDF) for 2 wk before and after parturition. Aghaziarati et al. (2011; 33:67 foraged to concentrate ratio (F:C)) and Andersen et al. (2002; 25:75 F:C) showed that cows that are milked more frequently (3 vs. 6 times/d and 2 vs. 3 times/d, respectively) can benefit from increased dietary energy and protein content through improved hepatic oxidation capacity and production potential.

Source of Fermentable Carbohydrates

The optimal dietary concentration of fermentable carbohydrates (i.e. fiber, sugar, and starch) is being refined for early lactation. Allen et al. (2009) suggested that optimizing DMI requires different diets at different stages of the lactation because DMI is controlled

by oxidation of fuels (fatty acids, propionate, lactate, and amino acids) in the liver in very early lactation and by gut fill as lactation proceeds towards its peak. Limiting dietary starch content and starch fermentability may increase DMI during the very early lactation period (≤ 7 to 21 days) since there will be less rapid production and absorption of propionate (Allen et al., 2009). However, more fermentable carbohydrates (i.e. starch, nonforage fiber sources, and highly digestible forages) should be fed as lactation proceeds and plasma nonesterified fatty acids (NEFA) and β -hydroxybutyrate (BHBA) decrease.

In an attempt to maximize DMI and minimize the risk of ruminal acidosis, Penner and Oba (2009) maintained the F:C ratio (50:50) but replaced starch (cracked corn grain) with sucrose in barley silage-based diets. Cows fed the lower starch diet (19 vs. 21%) over the first 4 wk postpartum had increased DMI and increased milk fat yield but lower plasma glucose and increased adipose tissue mobilization. However, the lower starch diet reduced the severity of ruminal acidosis. In alfalfa silage-based diets (40:60 F:C), replacement of ground corn with 1.5% sucrose caused a transient increase in DMI during the first 2 wk postpartum but did not affect DMI or milk yield over the first 12 wk postpartum (Nombekela and Murphy, 1995).

Increasing the ruminal starch availability in a diet containing adequate physically effective NDF fed during the first 2 to 3 months may improve lactational and reproductive performance. Cows fed steam-flaked corn (24% of ration dry matter) compared with cows fed cracked corn in postpartum corn silage-based diets (51:49 F:C; $\geq 31\%$ NDF) consumed similar dry matter, produced 2.3 kg/d more milk, had a lower plasma (NEFA) concentration, and had a lower ruminal acetate to propionate ratio with a similar 24-h mean ruminal pH during the first 9 wk postpartum (Dann et al., 1999). Santos et al. (1999; 2000) fed cows alfalfa hay-based diets (45:55 F:C, $\geq 28\%$ NDF) containing either 39% steam flaked sorghum (SFS; 31% dietary starch) or steam rolled corn (SRC; 28% dietary starch) for the first 90 d postpartum. Feeding SFC compared to SRC increased digestibility of starch and organic matter but not NDF, improved metabolic (energy) status but not milk yield, and increased luteal activity.

Starch is usually increased in the diet through the addition of grain, but can also be increased by replacement of legume silage with cereal silage. During the first 70 d postpartum, cows were fed diets containing 45% barley-based concentrate, 10% alfalfa hay and either 45% alfalfa silage, 45% barley silage, or 41% barley silage plus 4% corn starch (Dyck et al., 2011). Forage contributed 4, 24, and 19% of the total starch in the alfalfa silage (25% starch), barley silage (23% starch), and the barley silage plus corn starch (27% starch) diets, respectively. Dietary starch source and concentration had little effect on lactational performance and metabolism. However, starch supplementation to the barley silage-based diet tended to decrease the interval from calving to first ovulation, but did not affect subsequent estrous cycles or fertility.

Dietary starch content can be reduced in diets by using byproduct feeds. Partial replacement of forage with nonforage fiber sources that are high in readily available NDF may improve voluntary DMI and energy intake. Cows that were fed soyhulls (15%

of dietary dry matter) as a partial replacement for corn silage and vetch hay during the first 90 d postpartum increased DMI by 7%, *in vivo* NDF digestibility by 23%, and milk yield by 7%, but had no change in production efficiency (Adin et al., 2009). Lactational performance was not affected when glycerol (≤ 0.86 kg/d) was topdressed with or without corn starch (≤ 0.86 kg/d) on corn silage and alfalfa-based diets from 14 d prepartum (18% starch, 39% NDF) to 21 d postpartum (29% starch; 31% NDF; DeFrain et al., 2004). However, glycerol did decrease plasma glucose and increase BHBA concentrations at a time when cows are at greatest risk for ketosis. In contrast, glycerol fed at ~11% of dietary dry matter was a suitable replacement for high moisture corn in corn silage-based diets fed 28 d prepartum (15 vs. 23% starch) to 56 d postpartum (19 vs. 27% starch) since there was no treatment effect on lactational performance and postpartum blood metabolites (Carvalho et al., 2011).

Glucogenic/Insulinogenic Diets

Increasing the supply of glucogenic nutrients relative to lipogenic nutrients in early lactation may improve energy balance, decrease metabolic disorders, and improve reproduction through earlier resumption of the estrus cycle. Gong et al. (2002) showed that feeding a higher starch diet (26 vs. 10%) increased blood insulin concentration in early lactation and increased the proportion of cows that ovulated with the first 50 d postpartum. An increase in dietary glucogenic nutrients (27% starch) in grass and corn silage-based diets fed through 9 wk postpartum improved energy status assessed by calculated energy balance, plasma NEFA and BHBA concentrations, and liver triglyceride content, but did not affect DMI or milk yield (van Knegsel et al., 2007). Although increasing insulin by dietary manipulation can be beneficial for resumption of the estrous cycle, there is evidence that a high insulin status might have a detrimental effect on oocyte quality and embryo development (Santos et al., 2010). Garnsworthy et al. (2009) demonstrated that pregnancy rate was improved when a glucogenic diet that stimulated plasma insulin was fed before the first ovulation postpartum followed by a lipid-rich diet that lowered plasma insulin during the breeding period. Feeding a high starch (27%) diet for 50 d postpartum followed by a high fat (7%) diet until 120 d postpartum compared with an UK industry standard diet tended to increase the proportion of cows cycling by 50 d postpartum but did not affect conception rate (Gilmore et al., 2011). The diet switch was made at 50 d postpartum instead of at first ovulation so the higher starch diet may have been detrimental to embryo development. Caution is advised when formulating early lactation diets to stimulate the recrudescence of ovarian activity since highly fermentable starch diets fed immediately after calving may decrease DMI and prolong NEB (Allen et al., 2009).

Research at Miner Institute

Controlled-energy dry diets are recommended for use in the far-off dry period in a 2-group management system or in a 1-group management system. The controlled-energy dry diet approach has been successful in some but not all dairies. Some of the failures may be attributed to a transition to an inappropriate fresh cow diet. Unfortunately, there is a paucity of research data with fresh diets, especially following a controlled-energy

diet fed for a 60 or 40 day dry period. Nelson et al. (2011) used multiparous Holstein cows (n = 72) to evaluate the effect of dietary starch content in corn silage-based diets fed in early lactation on performance and blood metabolites following a shortened (40 day) dry period where a controlled-energy diet was fed. Typically, controlled-energy dry diets contain between 12 to 16% starch on a dry matter basis, which is much less than lactation diets (e.g. $\geq 23\%$ starch). A phase feeding or step-up approach to feeding during the prepartum and postpartum periods is often recommended but the optimal increase in starch from a controlled-energy dry diet to a lactation diet is unknown. Dietary treatments (Table 1) were 1) a low-starch diet (L; 21.0%) for the first 91 d postpartum (LL), 2) a medium-starch diet (M; 23.2) for first 21 d postpartum and a high-starch diet (H; 25.5) for the next 70 d postpartum (MH), and 3) a high-starch diet (H; 25.5%) for the first 91 d postpartum (HH). Corn meal was replaced partially with soyhulls and wheat middlings in the L and M diets.

Table 1. Ingredient and analyzed chemical composition (mean \pm standard error) of low, medium, and high starch diets fed to early lactation Holstein cows.

Item	Low	Medium	High
Ingredients, % of DM			
Corn silage	34.6 \pm 0.1	34.6 \pm 0.1	34.6 \pm 0.1
Haylage	11.4 \pm 0.4	11.7 \pm 0.3	11.4 \pm 0.4
Wheat straw	4.1	4.1	4.1
Corn meal	6.9 \pm 0.4	11.1 \pm 0.1	16.7 \pm 0.4
Soybean meal	11.4 \pm 0.1	11.9 \pm 0.1	11.9 \pm 0.1
Soybean hulls	9.7	6.5 \pm 0.2	3.2
Wheat middlings	6.1	3.9 \pm 0.1	1.8 \pm 0.1
Canola meal	3.1	6.1	6.1
AminoPlus	2.5	-	-
Other	10.2 \pm 0.3	10.1 \pm 0.3	10.2 \pm 0.2
Chemical composition			
DM, %	49.5 \pm 0.7	50.1 \pm 0.9	49.6 \pm 0.7
CP, %	17.3 \pm 0.1	17.0 \pm 0.2	16.7 \pm 0.2
NDF, %	35.7 \pm 0.3	33.9 \pm 0.4	31.9 \pm 0.3
Sugar, %	6.1 \pm 0.1	5.8 \pm 0.1	5.9 \pm 0.1
Starch, %	21.0 \pm 0.3	23.2 \pm 0.3	25.5 \pm 0.3
Rumen fermentable starch, %	16.8 \pm 0.5	18.9 \pm 0.6	20.2 \pm 0.5
Digestibility			
24-h NDF, % NDF	58.4 \pm 0.6	57.3 \pm 0.5	54.0 \pm 0.8
7-h starch, % starch	76.5 \pm 1.4	76.7 \pm 1.2	74.5 \pm 1.2

Lactational performance is summarized in Table 2. During the first 13 wk postpartum, DMI tended to be higher for cows fed LL than cows fed HH; cows fed MH were intermediate. During the first 3 wk postpartum, cows fed M consumed similar starch and rumen fermentable starch as cows fed L. However, when the MH cows were fed the higher starch diet after 3 wk postpartum, they consumed more starch and rumen fermentable starch than LL cows. During the 2nd wk postpartum, feeding and meal times

Table 2. Lactational performance from wk 1 to 13 postpartum of multiparous Holstein cows (n = 72) fed either a low-starch (21%) diet for the first 91 d postpartum (LL), a medium-starch (23%) diet for first 21 d postpartum and a high-starch (26%) diet for the next 70 d postpartum (MH), or a high-starch (26%) diet for the first 91 d postpartum (HH).

Item	Treatment (TRT)				SE	TRT	P - value	
	LL	MH	HH				Time	TRT × Time
DMI, kg/d	25.2 ^x	24.9 ^{xy}	23.7 ^y	0.5	0.06	<0.001	0.09	
DMI, % body weight	3.72 ^x	3.66 ^{xy}	3.50 ^y	0.07	0.06	<0.001	0.09	
Starch intake, kg/d	5.3 ^b	6.3 ^a	6.1 ^a	0.1	<0.001	<0.001	<0.001	
Rumen fermentable starch, kg/d	4.4 ^b	5.2 ^a	5.0 ^a	0.1	<0.001	<0.001	<0.001	
Neutral detergent fiber intake, kg/d	9.0 ^a	8.1 ^b	7.6 ^b	0.2	<0.001	<0.001	0.85	
Sugar intake, kg/d	1.5 ^a	1.5 ^{ab}	1.4 ^b	<0.1	0.02	<0.001	0.25	
Body weight, kg	681	682	682	12	0.99	<0.001	0.59	
Body condition score	3.13	3.04	3.16	0.07	0.46	<0.001	0.37	
Serum NEFA, μ Eq/L (wk 1-3)	452 ^{abv}	577 ^{ax}	431 ^{bv}	43	0.03	<0.001	0.11	
Serum BHBA, mg/dL (wk 1-3)	9.3	8.8	7.8	1.1	0.15	0.46	0.97	
Milk, kg/d	47.9 ^{ab}	49.9 ^a	44.2 ^b	1.6	0.04	<0.001	0.75	
3.5% Fat-corrected milk, kg/d	51.9	52.2	47.4	1.7	0.09	<0.001	0.40	
Solids-corrected milk, kg/d	47.4	47.9	43.5	1.5	0.09	<0.001	0.39	
Fat, %	3.88 ^x	3.64 ^y	3.79 ^{xy}	0.08	0.08	<0.001	0.59	
Fat, kg/d	1.91 ^x	1.86 ^{xy}	1.71 ^y	0.06	0.09	<0.001	0.93	
True protein, %	2.90	2.92	2.97	0.04	0.52	<0.001	0.08	
True protein, kg/d	1.42 ^{ab}	1.50 ^a	1.34 ^b	0.04	0.03	<0.001	0.48	
Milk urea nitrogen, mg/dL	15.2 ^a	12.7 ^b	11.9 ^b	0.3	<0.001	<0.001	0.88	
Week 2 fat to protein ratio	1.31	1.35	1.27	0.04	0.45	-	-	
Milk/DMI kg/kg	1.92	2.02	1.87	0.06	0.18	<0.001	0.08	
Milk nitrogen efficiency, %	34.2 ^b	37.6 ^a	35.6 ^{ab}	0.7	0.005	<0.001	0.31	

^{ab} Least squares means within a row without a common superscript differ ($P \leq 0.05$).

^{xy} Least squares means within a row without a common superscript differ ($P \leq 0.10$).

per day increased over time for cows fed L compared with cows fed the M or H but there was no difference in meal duration or the number of meals per day (Krawczel et al., 2011). The cows fed MH had higher milk yield than cows fed HH, indicating the benefit of a step-up feeding approach for starch when a controlled-energy dry diet is used. Cows fed LL had higher milk urea nitrogen than cows fed MH and HH, indicating less efficient use of nitrogen presumably due to less rumen fermentable starch intake and (or) excess dietary crude protein intake. Milk nitrogen efficiency was highest for cows fed MH because of high milk true protein yield and intermediate crude protein intake relative to the other treatments. Lipid mobilization to support NEB was not compromised based on acceptable losses of body weight and body condition, and concentrations of serum NEFA and BHBA. Serum NEFA tended to be higher for cows fed MH than cows fed LL or HH. Insulin sensitivity, assessed by a glucose tolerance test at d 15 postpartum, was not affected by dietary starch. This study demonstrated that lower starch ($\leq 23\%$) diets can support lactational performance. The step-up diet approach (MH) may be preferred over the 1-group diet approach (LL and HH) because of improvements in nutrient use (i.e. milk nitrogen efficiency). However, the 1-group lactation diet approach (LL) may be preferred when energy from corn starch is expensive relative to energy from nonforage fiber sources or a facility does not have the ability to have 2 groups in early lactation.

FRESH COW MANAGEMENT

Many dairies in the U.S. house fresh cows separately from other cows to facilitate monitoring of health problems, minimize social stress, and provide a diet specifically formulated for fresh cows. The use of the objective Transition Cow Index (TCI) has allowed the practice of separating fresh cow to be justified based on the findings that TCI scores of freestall herds are higher when there is an effective screening program for cows needing attention, pen moves and social stress are minimized, bunk space is at least 76 cm (30 in), and cow comfort is provided (Nordlund, 2009). In one of the few studies available on management practices for early lactation cows (Heuwieser et al., 2010), 97% of herds in Germany had a fresh cow exam based on subjective criteria such as general appearance and appetite and objective criteria such as milk yield. On average, only 22% of herds had a designated fresh cow pen. However, the use of a designated fresh cow pen increased with herd size. A fresh cow pen was used on 81% of herds with ≥ 200 cows. The addition of fresh cows to small groups of cows compared to large groups of cows at 100% freestall stocking density resulted in less social stress as indicated by fewer agonistic and non-agonistic interactions within the 3 h post mixing (Burow et al., 2009). Introducing fresh heifers as pairs rather than individuals to a group containing older cows promoted lying behavior in the immediate post mixing period (O'Connell et al., 2008). Cows housed as a separate group for one month after calving with a stocking density of $\leq 100\%$ resulted in improved production and health in primiparous but not multiparous cows (Østergaard et al., 2010). Interestingly, a fresh cow diet was not used in the separate group. An additional benefit of separate grouping may be observed if an appropriate fresh cow diet is used.

During the 1 wk before and the 2 wk after calving, competition at the feed bunk increased the number of displacements and feeding rate of cows, potentially increasing the risk of health problems (Proudfoot et al., 2009). In fresh cows during the first 21 d postpartum, feed bin stocking density did not affect DMI, water intake, or standing behavior in the absence of freestall overcrowding (Krawczel et al., 2009). However, there was a trend for increased feeding rate suggesting that overstocking feed bins may alter feeding behavior and increase the risk for problems associated with slug-feeding. The incidence and severity of ruminal acidosis increases immediately postpartum, emphasizing the need to develop and implement feeding strategies and management practices that reduce the risk (Penner et al., 2007). The severity and duration of NEB may be greater with early lactation ruminal acidosis because of the negative effects on ruminal digestion and nutrient supply to the cow. Based on field observations and limited research, fresh cows should be housed in small, separate groups to minimize social stress, maximize comfort of the physical resting space, properly size the feeding area to minimize slug feeding and other undesirable feeding behaviors, and provide a diet that promotes DMI and prevents health problems. The optimal duration of stay in fresh group pens is unknown and it most likely varies among cows.

CONCLUSIONS

Early lactation diets should be formulated to maximize DMI and energy intake, prevent compromised lipid mobilization, and support a return to positive energy balance in order to optimize lactational and reproductive performance. There is no “one size fits all” early lactation feeding strategy because the interaction of nutrition, environment, and management is unique for every dairy. However, use of a fresh cow group and diet for 2 to 3 wk postpartum is recommended. The fresh cow diet should be formulated within the context of the dry and high group diets. The fresh diet should not exceed ~25% starch or the amount that will be fed in the high group, should avoid inclusion of highly fermentable starch sources, and provide adequate physically effective NDF to maximize DMI and minimize ruminal acidosis. After the fresh period when serum NEFA and BHBA are lower, the diet should contain highly digestible carbohydrates to maximize DMI and milk production. The addition of lipogenic nutrients after first ovulation may improve fertility. The effectiveness of the early lactation feeding and management program should be monitored by reviewing clinical disease records, measuring feed intake and milk yield variation, body condition scoring, using the Transition Cow Index, or metabolic testing (LeBlanc, 2010).

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APPLICATIONS OF GENOMICS TO GENETIC IMPROVEMENT OF DAIRY CATTLE

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ABSTRACT

Implementation of genomic evaluation has caused profound changes in dairy cattle breeding. All young bulls bought by major artificial-insemination (AI) organizations now are selected based on such evaluations. Evaluation reliability can reach about 75% for yield traits, which is adequate for marketing semen of 2-yr-old bulls. Shortened generation interval from using genomic evaluations is the most important factor in increasing rates of genetic improvement. Current genomic evaluations are based on 45,187 single nucleotide polymorphisms (SNP) genotyped with technology that became available in 2007. The first unofficial USDA genomic evaluations were released in 2008 and became official for Holsteins, Jerseys, and Brown Swiss in 2009. Evaluation accuracy has increased steadily from including additional bulls with genotypes and traditional evaluations (predictor animals). Some of that increase occurs as a result of young bulls with genotypes receiving a progeny-test evaluation at 5 yr of age. Cow contribution to evaluation accuracy is increased by adjusting mean and variance of their evaluations so that they are similar to bull evaluations. Integration of US and Canadian genotype databases was critical to achieving acceptable initial accuracy and continues to benefit both countries. Genotype exchange with other countries added predictor bulls for Brown Swiss and will add bulls for Holstein. In 2010, a low-density chip with 2,900 SNP and a high-density chip with 777,962 SNP were released. The low-density chip has increased greatly the number of animals genotyped and is replacing microsatellites in parentage verification. The high-density chip can increase evaluation accuracy by better tracking of loci responsible for genetic differences. To integrate information from chips of various densities, a method to impute missing genotypes was developed based on splitting each genotype into its maternal and paternal haplotypes and tracing their inheritance through the pedigree. The same method is used to impute genotypes of nongenotyped dams based on genotyped progeny and mates. Reliability of resulting evaluations is discounted to reflect errors inherent in the process. Further increases in evaluation accuracy are expected because of added predictor animals and more SNP. The large population of existing genotypes can be used to evaluate new traits; however, phenotypic observations must be obtained for enough animals to allow estimation of SNP effects with sufficient accuracy for application to the general population.

(Key Words: genomic evaluation, SNP effects, reliability)

INTRODUCTION

Genetic evaluation of dairy cattle has provided the means for steady genetic improvement in production, fitness, and conformation traits. The evaluations have been depended on milk recording and breed association programs for data on a broad range

of traits. Widespread use of superior bulls through AI has been the primary vehicle for progress. Identification of superior bulls has been expensive and time consuming because of the need to wait for milking daughters and the cost of collecting their data to achieve an evaluation of adequate accuracy. The great promise of DNA analysis has recently become a reality with the advent of low cost genotyping of large numbers of SNP markers.

The critical development was assays that can genotype large numbers of SNP at low cost. Although SNP are only biallelic (2 states), the large number available allows tracking the inheritance of short chromosomal segments. A consortium of government and academic scientists worked with Illumina (San Diego, CA) to develop a set of SNP to be included on a chip (Van Tassell et al., 2008). A commercial set of 54,001 was included in the original release of the BovineSNP50 BeadChip (Illumina, 2010b). Consortium members had access to the new chip in fall 2007, and it became publicly available in late December 2007. In July 2010, Illumina released two new genotyping chips: a low-density chip (**Bovine3K**) with 2,900 SNP (Illumina, 2010c) and a high-density chip (**BovineHD**) with 777,962 SNP (Illumina, 2010a).

Some SNP were excluded because of low call rate, poor calling properties, or high correlation with other SNP (Wiggans et al., 2009b). Procedures were developed to check for parent-progeny conflicts and other inconsistencies (Wiggans et al., 2010b). Extensive simulation work by VanRaden (2008), which was based on the research of Meuwissen et al. (2001), enabled development of genomic evaluation methods, which were applied once genotypes became available for US dairy cattle. The phenotypic and genotypic information for a predictor population was used to estimate SNP effects. Predictor animals are genotyped animals with traditional evaluations (i.e., they do not include genomic information). The SNP effects estimated from a predictor population are used to calculate genomic evaluations for animals without traditional evaluations (VanRaden, 2008; VanRaden et al., 2009). The first unofficial USDA evaluations based on SNP genotypes were released in April 2008. Genomic evaluations became official for Holsteins and Jerseys in January 2009 and for Brown Swiss in August 2009.

The money to genotype thousands of animals came from research grants and contributions from AI and breed organizations. In return for their support, the AI organizations received the exclusive right to have males genomically evaluated until May 2013. The genotyping is done in the following laboratories GeneSeek (Lincoln, NE), DNA LandMarks (Quebec, Canada), and Genetic Visions (Middleton, WI),.

EVALUATION PROCESS

Nomination

Since genomic evaluations became official in 2009, genotypes that were usable for genetic evaluations have been received by USDA for >125,000 animals as of August 2011 (Table 1). The availability of the Bovine3K chip has greatly increased the number of animals genotyped, and its SNP are replacing microsatellites for parentage verification. From September through December 2010, almost 33,800 Bovine3K chip genotypes were received; 94% of those genotypes were for females. The 8 AI and 4

breed organizations that arrange for genotyping are designated as requesters. They arrange for a DNA sample to be collected and attached to a bar-coded mailer. That mailer is usually sent to the requester but may be sent directly to the genotyping laboratory. The bar code facilitates sample processing at the laboratory. The requester is expected to nominate each animal by making an entry in a database maintained by USDA's Animal Improvement Programs Laboratory (**A IPL**) before the sample reaches the genotyping laboratory. The nomination is either through a web interface or pedigree records containing the bar code. The breed associations use the pedigree record option for nearly all their nominations as do several of the larger AI organizations. All requesters use the nomination query for nomination confirmation and update and for problem resolution. The nomination process ensures that the pedigree for the animal is in the AIPL database before the genotype arrives at AIPL and simplifies matching the identification associated with the genotype with the animal's information in the AIPL database.

Table 1. Numbers of genotyped animals by breed and evaluation date

Breed	Evaluation date ¹	Predictor ²		Young ³			All animals
		Bulls	Cows	Bulls	Cows	Imputed	
Holstein	April 2009	7,600	2,711	9,690	1,943	—	21,944
	August 2009	8,512	3,728	12,137	3,670	—	28,047
	January 2010	8,974	4,348	14,061	6,031	—	33,414
	April 2010	9,770	7,415	16,007	8,630	1,471	41,822
	August 2010	10,430	9,372	18,652	11,020	2,029	49,475
Jersey	December 2010	3	5	21,161	6	2,172	63,615
	February 2010	1,977	479	1,172	197	—	3,825
	April 2010	2,072	637	1,250	202	97	4,161
	August 2010	2,145	792	1,476	258	152	4,671
Brown Swiss	December 2010	2,217	2,189	1,754	1,924	178	8,084
	February 2010	1,168	54	179	15	—	1,416
	April 2010	1,185	98	188	31	47	1,502
	August 2010	1,248	124	228	35	69	1,635
	December 2010	1,596	146	256	40	79	2,038

¹Evaluation dates in boldface are official USDA-DHIA evaluation releases.

²Animals with traditional evaluations (no genomic information included).

³Animals without traditional evaluations.

Genotyping

At the genotyping laboratories, DNA is extracted from the sample. In 2010, DNA sources included hair (82%), nasal swab (12%), blood (5%), semen (<1%), and ear

punches (<1%). The process of DNA amplification and fragmentation, hybridization to the chip, labeling, and genotype detection takes 3 d. Data generated from the laser reader then are clustered to determine SNP genotypes (Illumina, 2010b). Those genotypes and corresponding identification information are transferred to AIPL.

Genotype Storage and Validation

The AIPL database can store multiple genotypes for an animal and relies on chip identification and sample location on the chip to identify a sample uniquely. Multiple samples arise from collection and labeling errors as well as upgrading from lower to higher density. Samples are checked on an animal basis for call rate and parent-progeny conflicts. In addition to conflicts with reported parents, a conflict also is designated if comparison with all other genotypes indicates that an animal has a parent-progeny relationship that is not found in the pedigree (usually the genomically correct parent). A report of SNP with a call rate of <90%, a departure from Hardy-Weinberg equilibrium (difference between number of expected and actual heterozygous SNP), or parent-progeny conflicts of >2% is returned to the submitting laboratory. Laboratories can run these checks using an automated process before they submit the genotypes for loading into the database. Based on the check runs, laboratories often were successful in reclustering problematic SNP to reduce the number of SNP conflicts in those categories. Those checks serve as a measure of the quality of the genotype calls. For BovineSNP50 genotypes, usually <10 SNP were outside those limits for any submission. For Bovine3K genotypes, considerable effort was required to determine which SNP were reliable and to adjust procedures to achieve results similar to those for BovineSNP50 genotypes.

The database allows for storage of genotypes from chips with differing numbers of SNP. Currently, the Bovine3K, BovineSNP50, and BovineHD chips are supported. Comparisons of SNP genotypes from different chips are supported but limited to SNP in common.

Many conflicts can be resolved. For most cases of sire conflict, an alternative sire is suggested. Identical genotypes often are the result of embryo splits or identical twins. Because bulls have only one X chromosome, their genotypes for X-specific SNP appear to be homozygous, and that characteristic is used in sex validation. Some cows inherit both of their X chromosomes from the same male ancestor and, therefore, appear to be males. If a common male ancestor can be found, genotypes for such cows are accepted. The Bovine3K chip includes Y-specific SNP, which are used in sex validation. Usability of genotypes is evaluated whenever pedigree of a genotyped animal changes.

Genotype Preparation

The SNP genotypes for each animal (45,187 SNP for BovineSNP50 genotypes, 40,241SNP for BovineHD genotypes, and 2,683 SNP for Bovine3K genotypes) are extracted from the database. Because the number of animals with high-density genotypes is too few for routine evaluation, only the 40,241SNP that match the BovineSNP50 chips currently are extracted. During extraction, multiple genotype calls

for an individual animal are merged, with preference given to the genotype with the highest call rate. Identical twins and animals from split embryos have their genotypes harmonized. For dams without genotypes, genotypes are imputed (constructed from relatives) if the number of genotyped progeny and mates is sufficient to reach a call rate of 90% on an allele basis. Since April 2010, dams with imputed genotypes have been included in genomic evaluations. Imputation also is used to add genotypes for SNP that are on the BovineSNP50 but not the Bovine3K chip. Imputation involves splitting the genotype into paternally and maternally contributed chromosomes (haplotypes). Haplotype inheritance is traced and used to fill in missing genotypes. When pedigree sources are not available, the most common consistent haplotype for the population is selected. Table 1 shows the number of usable genotypes by breed for most of the genomic evaluations released since April 2009.

Estimation of SNP Effects

The effects of SNP on traditional evaluations are estimated for >30 traits. The traditional evaluations are deregressed so that shrinkage based on amount of information, which is inherent in estimation of random effects, is undone to make the data more like individual records. Cow and bull evaluations must be comparable, because both are used to estimate SNP effects. Therefore, traditional evaluations of cows for milk, fat, and protein yields and component percentages are adjusted to remove overestimation usually associated with cow evaluations for yield traits (Wiggans et al., 2010a). That adjustment makes the mean and variance of the deregressed value for a cow similar to that for a bull with similar accuracy. To do that, the contribution of parent average is removed from the traditional evaluation and then the remainder is deregressed, is multiplied by a number less than 1 to reduce the variance. The mean is adjusted within birth year such that low parent average cows have their evaluations increased and high parent average cows have theirs reduced. This adjustment is applied to all cows and maintains the estimates of genetic trend.

Deregressed traditional evaluations are regressed on each of the 54,187 SNP genotypes (VanRaden, 2008), where the genotypes are expressed as the quantity of one of the alleles (0, 1, or 2). Because the effects are considered to be random, a system with more effects than observations is solvable. The solution is the effect on each trait from replacing 1 allele in the SNP genotype with the other allele. In addition to SNP effects, a polygenic effect is estimated to capture genetic variation not accounted for by SNP.

Most SNP have small effects, which are distributed evenly across all chromosomes. For both Holsteins and Jerseys, the largest effects for milk and fat were found on chromosome 14 and were associated with the DGAT1 (diacylglycerol O-acyltransferase 1) gene (Grisart et al., 2004). An increased effect for protein yield was also found on chromosome 14 for Jerseys. Methods for the visualization of SNP effects were described by Cole and VanRaden (2010), and plots of the absolute values of effects for all 45,187 SNP on 31 traits of economic importance are available at the AIPL website (http://aipl.arsusda.gov/Report_Data/Marker_Effects/marker_effects.cfm).

Calculation of Genomic Evaluation

An animal's genomic evaluation includes a genomic prediction (estimates of SNP and polygenic effects) and information from traditional evaluations that is not already included in the genomic information. A traditional evaluation is calculated for just the subset of animals with genotypes to allow determination of the traditional information that was accounted for by genomics. A selection index is used to combine the genomic prediction, traditional evaluation, and subset evaluation (VanRaden et al., 2009).

Measure of Accuracy

Reliability measures how much information contributes to the evaluation. For genomic evaluations, reliability combines daughter equivalents from genomics, parent average, and information from the traditional evaluation not accounted for through genomics. The genomic contribution is approximated by a function of the weighted sum of the genomic relationships of the animal with the predictor population. The weight is the reliability with the component for parent average removed. The genomic relationship with predictor animals and their evaluation reliability are the primary determinants of accuracy for genomic evaluations. Thus, the genomic contribution is lower for less related animals, such as those with foreign ancestors or subpopulations that contributed little to the current population (Wiggans and VanRaden, 2010).

The increase in evaluation reliability from including genomic information can be demonstrated by comparing August 2006 traditional parent averages for young bulls without daughter information, their August 2006 genomic evaluations that include SNP and polygenic effects estimated from the August 2006 predictor population in addition to their traditional parent average, and their June 2010 daughter deviations deregressed from their traditional evaluations (Table 2). Mean reliability for August 2006 genomic evaluations of young bulls across all yield, health, and fertility (where applicable) traits was 57% for Holsteins, 55% for Jerseys, and 52% for Brown Swiss. Gains in reliability above parent average (Table 2) ranged from 2.7 to 47.6 percentage units for Holsteins, 9.6 to 29.2 percentage units for Jerseys, and 3.0 to 25.8 percentage units for Brown Swiss. Reliability gains were lowest for stillbirth, which had the smallest predictor population because cow evaluations were not included and because fewer bulls had evaluations as data collection had begun more recently than for other traits. Coefficients of determination (R^2) also are provided in Table 2 as a measure of the relationship between 2006 evaluations (either parent average or genomic evaluation) and 2010 daughter deviations (deregressed values). The R^2 ranged from 3.1 to 36.7 for parent average and from 9.6 to 62.1 for genomic evaluation. Reliabilities for both parent average and genomic evaluation are higher than their respective R^2 , because reliability adjusts for error variance (differing amounts of information) and because selection had occurred in the genotyped population. Coefficients for regression of June 2010 daughter deviation on August 2006 genomic evaluations (Table 2) ranged from 0.87 to 1.08 for Holsteins, 0.88 to 1.30 for Jerseys, and 0.84 to 1.09 for Brown Swiss; a coefficient close to 1 indicates that a 1-unit difference in the genomic evaluation results in a 1-unit change in the trait. For bias in genomic evaluation (Table 2), a negative value indicates that the initial August 2006 genomic evaluation was higher than the June 2010

deregressed value. Changes in methodology for genomic evaluation also impact the measure of evaluation accuracy. Implementation of the adjustment for cow evaluations in April 2010 increased the gain in reliability from genomics by about 3 percentage units for Holstein and Jersey yield traits (Wiggans et al., 2010a). The accuracy loss from imputation required to include Bovine3K genotypes required a reliability adjustment. Reliabilities are converted to daughter equivalents and discounted by the lower call rate and loss in accuracy. The adjusted daughter equivalents then are converted back to reliabilities. Predictive ability of genetic merit with a low-density chip with 3,000 equally spaced SNP was reported to be 84 to 89% of that with the BovineSNP50 chip for Holsteins (Vazquez et al., 2010) and around 95% for Jerseys (Weigel et al., 2010). In December 2010, reliabilities for official PTA for milk yield, which included all sources of information, ranged from 74 to 81% for most young Holstein bulls (Figure 1).

Distribution

Genomic evaluations are calculated monthly. At each triannual release of official USDA-DHIA evaluations, all genomic evaluations are released. Between those releases, genomic evaluations are released only for new animals or young bulls that are not being marketed so that evaluations of marketed bulls do not fluctuate between official evaluations. Evaluations of bulls that are less than 2 yr old and not enrolled in the cross-reference program of the National Association of Animal Breeders are distributed only to the owners and requesting AI organizations.

FUTURE

Genomic evaluations are expected to continue to increase in accuracy. The largest contributor to that increased accuracy will be additional predictor animals. Table 2 shows the natural increase in the US predictor population at each official evaluation from bulls with a first progeny-test result at approximately 5 yr of age. The US predictor population also increases the month following evaluation release when newly evaluated foreign bulls can contribute.

In July 2010, Illumina (2010a) released a high-density chip with 777,962 SNP, and Affymetrix (Santa Clara, CA; 2011) released a high-density chip with 648,855 SNP in January 2011. Although such chips can provide genotypes that increase accuracy of genomic evaluations by better tracking of the loci responsible for genetic differences, the accuracy gains are not expected to be large (VanRaden and Tooker, 2010). As with low-density SNP, high-density SNP would be imputed from current genotypes. The first step is to collect enough high-density genotypes so that most haplotypes are represented. Several thousand genotyped animals may be required. The higher density genotypes may also support genomic evaluations of crossbred cattle, because the SNP

Table 2. Observed reliabilities (REL) in August 2006 for traditional parent averages and genomic evaluations¹ of young bulls without daughter information, coefficients of determination ($R^2 \times 100$) between August 2006 evaluations and June 2010 daughter deviations deregressed from traditional evaluations, coefficients (b) for regression of June 2010 daughter deviations on August 2006 genomic evaluations, and bias in genomic evaluation by trait and breed.

Breed	Trait ²	August 2006 REL, %			R^2		b	Bias ⁴
		Parent average	Genomic evaluation	Gain ³	Parent average	Genomic evaluation		
Holstein	Milk, kg	38.1	67.5	29.4	19.4	41.1	0.91	-4.0
	Fat, kg	38.1	73.1	35.0	17.5	43.3	0.96	-0.9
	Protein, kg	38.1	63.7	25.6	20.3	39.1	0.88	0.6
	Fat, %	38.1	85.7	47.6	26.9	62.1	1.02	0.0
	Protein, %	38.1	77.9	39.8	29.5	58.9	0.90	0.0
	PL, mo	31.0	64.2	33.2	16.4	31.4	1.04	-1.5
	SCS	33.9	60.4	26.5	15.8	31.7	0.88	0.0
	DPR, %	29.8	46.8	17.0	21.8	29.4	1.08	-0.2
	Sire CE	27.1	40.9	13.8	20.5	28.2	0.79	1.0
	Daughter CE	26.2	44.3	18.1	10.1	17.7	0.93	-1.0
	Sire SB	22.7	29.8	7.2	7.6	10.2	0.87	2.1
	Daughter SB	26.6	29.3	2.7	9.3	10.2	0.89	0.3
Jersey	Milk, kg	39.5	53.9	14.3	38.9	49.2	1.03	89.8
	Fat, kg	39.5	49.9	10.4	30.7	38.1	0.88	5.8
	Protein, kg	39.5	49.1	9.6	34.2	41.0	0.94	3.4
	Fat, %	39.5	64.9	25.3	40.2	58.1	0.97	0.0
	Protein, %	39.5	61.4	21.8	36.7	52.6	0.96	0.0
	PL, mo	24.2	50.8	19.1	10.6	19.2	0.97	-0.4
	SCS	18.7	48.9	13.8	10.4	18.3	0.70	0.1
	DPR, %	24.1	60.0	29.2	9.9	22.7	1.30	-0.1
Brown Swiss	Milk (kg)	37.2	53.8	16.7	5.1	24.4	0.61	-163.0
	Fat (kg)	37.2	53.1	16.0	7.5	21.3	0.54	-6.3
	Protein (kg)	37.2	53.0	15.9	6.2	22.4	0.52	-4.1
	Fat (%)	37.2	59.1	22.0	26.4	42.0	1.09	0.0
	Protein (%)	37.2	57.8	20.6	29.8	43.9	1.02	0.0
	PL (months)	28.3	54.2	25.8	9.7	22.0	1.07	-1.2
	SCS	32.2	53.4	21.2	12.1	23.0	1.02	0.0
	DPR (%)	24.9	28.1	3.0	3.1	9.6	0.48	0.0

¹Includes SNP and polygenic effects estimated from the August 2006 predictor population (genotyped animals with traditional evaluations) and August 2006 traditional parent averages.

²PL = productive life, DPR = daughter pregnancy rate, CE = calving ease, and SB = stillbirth.

³Genomic REL – parent average REL.

⁴June 2010 daughter deviation – August 2006 genomic evaluation.

may be close enough to the QTL that the phase of the association persists across breeds. However, even with accurate tracking of QTL alleles, their effects may differ between breeds.

Increased Accuracy through Collaboration

Collaboration is the least expensive way to increase the predictor population and thus increase accuracy. Collaboration between the United States and Canada was quite successful in initially increasing the size of the predictor population and continues to add to it. Research collaboration has helped to improve evaluation methodology, and coordination across countries has aided with producer acceptance by minimizing differences and explaining existing differences. Genotypes from the United States were traded with Switzerland, Germany, and Austria to increase the number of predictor bulls for Brown Swiss. Agreements with groups in Italy and Great Britain have provided more Holstein predictor bulls.

CONCLUSIONS

Genomic evaluations have revolutionized dairy cattle breeding by greatly increasing accuracy of estimates of genetic merit for young animals and could double the rate of genetic progress. Those evaluations are based on genotypes that are extensively checked for quality, and conflicts are resolved. They are becoming more accurate as animals are added to the predictor population. All young bulls purchased by major AI organizations now are selected based on genomic evaluations. The development, implementation, and acceptance of genomic evaluations have allowed extensive marketing of 2-yr-old bulls.

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OPPORTUNITIES AND CHALLENGES IN DAIRY CALF HOUSING AND MANAGEMENT FOR THE NEXT DECADE

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INTRODUCTION

Calf care is possibly the most challenging job on the dairy farm, in part because milk-fed calves are the animals most likely to become ill. New methods of calf rearing are becoming available that can benefit both producers and their calves, providing the potential for widespread improvements in calf care over the next decade. We predict that in the coming years producers will begin feeding dairy calves more milk than they are now commonly fed, increasingly using labour-saving milk delivery systems that facilitate more natural milk drinking behaviour. These improved feeding systems will ease the move towards group housing of calves before weaning, saving producers time and money. However, changes in feeding and housing systems pose new challenges for producers and their calves that require much innovation and research. In this presentation we will describe how new milk feeding methods promote rapid growth and more natural calf behaviour. New feeding systems facilitate keeping calves in groups, but group housing can result in increased competition and increased risk of disease transmission. Therefore, we will also discuss the challenges involved in using new feeding methods, and how to reduce these problems.

CALF FEEDING

Methods of feeding calves in modern dairying differ markedly from those found in nature (von Keyserlingk and Weary, 2007), but knowing more about the natural behavior of cow-calf pairs can help us develop better ways of feeding calves (von Keyserlingk et al., 2009; Khan et al., 2011). On many dairy farms, calves are separated from their mothers within 24h of birth and then fed milk by bucket or bottle until 4 to 12 wks of age. Separating cow and calf early is thought to allow for better supervision of colostrum, milk and solid food intake and help prevent transmission of disease. Early separation also reduced the distress response of both the cow and calf. For example, Flower and Weary (2001) examined some of the effects of the age of separation on cow and calf behaviour and found that cows and calves that were separated (14 days versus 1 day) had higher levels of activity and vocalized more often. However, the calves separated at 14 days gained 16.5 kg over this period, versus just 4.5 kg for those separated early, and the calves maintained this weight advantage even after separation from the dam. The higher growth of calves kept with the cow may have been due, at least in part, to higher milk intakes – the spread between the cow-fed and people-fed calves shows the opportunity we have for improved gains with improved feeding management of dairy calves.

In conventional management schemes, calves are normally provided milk at 10% of their body weight (~ 4 kg / day), are vulnerable to disease, often fail to gain adequate weight and can sometimes experience high levels of mortality. We have tested the effects of feeding calves ad libitum by teat (Appleby et al., 2001; Jasper and Weary, 2002). In each experiment we compared weight gain, milk intake, starter intake and number of days with diarrhoea for calves fed milk conventionally (i.e. twice daily by bucket at 10% of body weight per day) versus ad libitum from a teat. In our first experiment, we found that weight gains during the first 2 weeks after birth were less than 0.4 kg/d for the conventionally fed calves versus 0.85 kg/d for the teat-fed ones; during the next 2 weeks gains were 0.58 and 0.79 kg/d respectively (Appleby et al., 2001). In a second experiment we again found that the teat-fed calves gained weight more quickly (0.78 versus 0.48 kg/d from birth to weaning at 37 days of age) (Jasper and Weary, 2002). We also found that calves maintained their advantage in body weight after weaning. In both experiments the differences in weight gain were likely due to teat-fed calves drinking approximately twice as much milk as the calves fed conventionally. For example, the ad libitum fed calves consumed on average 8.8 litres of milk per day, compared to 4.9 litres per day for the conventionally fed calves (Jasper and Weary, 2002). Calves limit fed according to conventional practices also show behaviours indicative of chronic hunger (de Paula Vieira et al. 2008).

It is commonly thought that feeding less milk will encourage solid feed intake. Indeed, we have found that over the first 5 weeks of life, feeding calves less milk does increase starter consumption (0.17 versus 0.09 kg per day) but this practice also severely limits weight gains (Jasper and Weary, 2002; de Paula Vieira et al. 2008). Moreover, we have found that the ad libitum milk-fed calves quickly caught up to the conventionally fed calves in their intake of starter after weaning; both groups consumed on average 1.9 kg per day during the two weeks after weaning.

Improving access to milk raises practical problems, such as maintaining milk quality throughout the day, especially during warm weather. An alternate approach to continuous access is to provide unlimited availability of milk but only for a few hours each day. Previous research has found that calves provided unlimited access to milk spend just 45 minutes per day drinking milk, and that the largest meals occur just after the delivery of fresh milk (Appleby et al., 2001). In another study, we tested the effects of limited access to milk (4 h/d) versus continuous (24 h/d) access on milk intake, weight gain and behaviour of dairy calves (von Keyserlingk et al., 2004). Calves consumed as much milk in the 4 h/d treatment as they did in the 24 h/d treatment. An added advantage of the 4 h/d treatment, for some facilities at least, is that the same equipment can also be used to supply water to calves.

Much research and on-farm innovation is required to maximize the benefits of these new calf-feeding methods. In particular, little is known about how best to

wean rapidly growing calves fed high milk rations. Current recommendations for weaning age and method are specific to slow growing calves fed conventionally, but new work is showing that slowly reducing milk intakes in the days before weaning can be helpful (Khan et al., 2007). In one study with calves fed up to 12 L/d (Sweeney et al., 2010), we compared calves weaned abruptly with calves weaned gradually over 4, 10, or 22 d. Calves weaned over 22 d ate the most starter, but also had the lowest weight gains before weaning. The abruptly weaned calves ate the least amount of calf starter but had the best weight gains before weaning. After weaning, calves on the 22 and 10 d treatments ate more starter and had better weight gains than calves on the more abrupt treatments. These findings suggest that weaning over 10 d is optimal. This type of gradual weaning is easily accomplished using automated calf feeders.

GROUP HOUSING

For the past decades, common wisdom among North American dairy experts was that calves should be housed individually, in separate pens or hutches (e.g. Quigley, 1997). This practice was considered to maximize performance and minimize the risk of disease. Individual housing also helps avoid behavioural problems such as competition and cross-sucking.

The new calf-feeding methods described above work well for individually housed calves, but also facilitate group housing. Group housing provides more space for calves and allows for social interactions. Research and practical experience show that group rearing of calves can result in considerable benefits through reduced labour requirements for cleaning pens and feeding. One study on a commercial farm in New York State showed that calves kept in groups required one third of the labour that went into caring for the individually housed and fed calves (de Passillé et al., 2004). Calves are social animals that need exercise and keeping dairy calves in groups may provide a number of advantages to both producers and their calves. Successful adoption of group housing will mean avoiding problems such as increased disease and competition. Recent research provides some insights into how these risks can be minimized.

We evaluated the behaviour and growth rates of calves housed in pairs versus individually (Chua et al., 2002); calves gained weight steadily regardless of treatments. Interestingly, during the week of weaning (approximately 5 weeks of age), pair-housed calves continued to gain weight normally but the individually housed calves experienced a slight growth check. There were no differences between groups in the amounts of milk, starter or hay consumed, or in the incidence of scouring or other diseases. Aggressive behaviour and cross-sucking were almost never observed (less than 0.2% of time).

In a more recent study, de Paula Vieira et al. (in press) found that calves housed in pairs vocalized less during weaning than did individually housed

calves. The results of this study also illustrated some longer-term costs to housing calves individually. When all calves were eventually introduced to a group pen after weaning calves that had previously been single housed took on average 50 h to begin feeding, in comparison to just 9 h for the pair-reared calves. These results suggest that individual housing may result in at least temporary deficits in cognitive or social tasks.

Successful group rearing requires appropriate management, including feeding method and group size. Large epidemiological surveys of U.S. and Swedish dairy farms found increased mortality and disease on farms keeping calves in large groups (more than 7 or 8) (Losinger and Heinricks, 1997; Svenson et al., 2000). Thus, small groups are likely a better alternative than large ones.

Calf immunity and the design and management of the housing systems, such as its cleanliness and ventilation, likely affect disease susceptibility more than group housing per se. Our work shows that housing young dairy calves in small groups is viable in terms of calf health, performance and behaviour. New research is now required on management strategies that will help prevent disease. For now, we encourage producers to consider keeping a closed herd (i.e. no new animals entering the herd), keeping groups small and physically separated from one another (e.g. in super hutches), and managing group pens in an all-in-all-out basis.

Calves in groups sometimes compete with pen mates. In one experiment using a simple teat-feeding system, we found that group-housed calves can displace one another from the milk teat many times each day if there are not enough teats (von Keyserlingk et al., 2004). However, giving each calf access to its own teat greatly reduced these displacements. This improved access to teats resulted in longer feeding times and increased milk intakes.

Other research has focused on how computerized feeding stations can be managed to reduce competition between calves. Increasing the daily milk allowance for calves from 5 to 8 liters per day reduced by half the number of times calves visited the feeder, reducing occupancy time and displacements from the feeder, and improving the efficient use of this equipment (Jensen and Holm, 2003; de Paula Vieira et al. 2008). Our research shows that young calves can be introduced into a group with little disruption when they are trained to feed from the computerized feeding station prior to the introduction (O'Driscoll et al., 2006). Although the calves visited the feeder less frequently on the day of mixing, they were able to compensate by increasing both the duration and amount consumed per meal, and established their pre-mixing feeding pattern after just one day.

CONCLUSION

Current research on dairy calves is paving the way for new methods of managing and housing these animals that will facilitate calf care and improve living conditions for these young animals. Calf care is arguably the most difficult

job on the dairy farm. For the good calf manager, the research that we will describe provides opportunities to further improve calf care and reduce labour. However, like any new method, these are best adopted first by the best and most innovative managers. New methods require new skills and a careful eye to ensure that these are implemented in the best ways possible.

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INTEGRATING CONCEPTS OF PRE-PUBERTAL MAMMARY DEVELOPMENT AND RATES OF BODY GROWTH TO DESCRIBE DIFFERENCES IN FIRST LACTATION MILK YIELD

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To optimize first lactation and lifetime milk yield, growth benchmarks have been established to help nutritionists and dairy producers meet the appropriate growth objectives that achieve breeding weight and age in an economically viable time and achieve optimum body composition at first calving. However, there are still concerns that mammary development is impaired by a particular rate of body growth and that this impairment affects first lactation milk yield. This paper will integrate concepts of body growth and composition, mammary development and first lactation milk yield to provide a system based approach to first lactation milk reduction that has been associated with mammary development. The purpose of this review is to discuss how the stage of maturity and the rate of gain at each stage of physiological development can result in changes in body composition that help explain the milk yield observed in previous studies. This information can be used to improve lactation performance by promoting growth at each stage of maturity while considering the final or targeted body composition of the animals.

The goals for raising replacement heifers go beyond achieving a specific weight gain. Given that they are future dairy cows, the final goal of heifer rearing should be to optimize their future milk production potential. Body composition is directly related to growth rate, diet composition and stage of maturity at the time the growth occurred. With this in mind, it is vital to remember the effects of body condition or body composition at calving on milk yield. The effect of greater body condition on performance of dairy cattle was reported as a linear decrease in milk yield (Garnsworthy and Topps, 1982). More contemporary data has refined this observation and associated it with reduced dry matter intake and further, this is the focus of much research into transition cow metabolism, insulin resistance and the interaction between obesity and milk yield (Ingvarsen and Andersen, 2000; Douglas et al. 2006; Allen et al., 2009; Overton, 2011). Thus, when evaluating the data integrating pre-pubertal growth rates, mammary development and milk yield, the composition of growth, and therefore the final body composition of the heifer at calving are essential when comparing studies related to milk production.

MAMMARY DEVELOPMENT AND MILK YIELD

Traditionally, body composition has been overlooked when analyzing the effects of pre-pubertal growth rates on first lactation performance. However, just as body composition and obesity influence the performance of mature dairy cattle, those factors are also a crucial determinant of first lactation heifer performance. As reported by

Swanson (1960), when heifers were fattened and bred to calve at the same age as their non-fattened twins, the fattened heifers produced considerably less milk during their first lactation. Although the goals of that study were to compare fattened vs. non-fattened heifers and their corresponding lactation performance, the data was associated with the concept that something other than body composition was impacting the lactation performance.

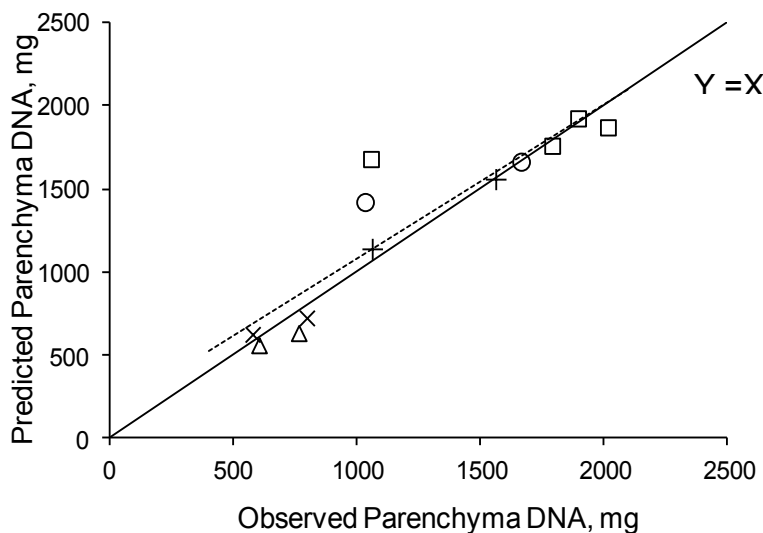
Subsequently, the seminal work by Sejrsen et al. (1982; 1983) describing the effect of high energy intake on mammary development and the relationship with circulating growth hormone linked the relationship between pre-pubertal growth, mammary development and future milk yield. The primary outcome of this work was to provide an intuitive mechanism to explain why rapid growth during the pre-pubertal phase resulted in reduced milk production in the first lactation. The observation of reduced mammary development could be repeated in almost every experiment (Pritchard et al., 1972; Petitclerc et al., 1984; Mäntysaari et al., 1995; Capuco et al., 1995; Meyer et al., 2006ab). These repeatable observations lead to the conclusion that high energy intakes reduced mammary development through altered hormone status or signaling processes. However, Meyer et al. (2006ab) were the first to recognize that mammary development was not reduced by high energy intake, and instead was the time to reach puberty and the associated signals to change allometric mammary growth that were altered. The mammary gland, like all other reproductive organs, grows in proportion to the size of the body and not in proportion to nutrient intake during the post-weaning, pre-pubertal phase.

To evaluate whether the time effect associated with the mammary development observed in Meyer et al. (2006ab) was similar to previous studies, the amount of mammary development (measured in milligrams of DNA accumulation per day) was determined. Meyer (2005) hypothesized that if the observation was consistent among studies, mammary development should be predictable based on days on treatment. The daily DNA accumulation from Meyer et al. (2006b) was compared to five other studies with adequate descriptions of the experimental design (Figure 1). In that comparison, a majority ($R^2=0.83$) of the difference in mammary development could be explained by time on study, suggesting that in all of these studies, energy intake hastened the time to puberty, and earlier puberty and the hormonal changes associated with puberty were responsible for the decreased mammary development.

Tissue harvest was the endpoint in most of these studies of mammary development which precludes evaluation of milk yield. There are a few studies where tissue harvest and pregnancy and milk yield data were collected under similar feeding conditions to be able to measure heifers in a “pair-fed” experimental design. The studies with direct comparisons are those of Capuco et al. (1995), Waldo et al. (1998) and Smith (2002). Other studies with similar but sequential study data are from Radcliff et al. (1997; 2000). In each of these studies, the authors observed significant changes in mammary development, without significant changes in first lactation milk yield.

Capuco et al. (1995) observed a 52% decrease in mammary development at puberty in heifers fed for higher rates of pre-pubertal gain, but in the pair fed animals, there was no significant difference in milk yield (Waldo et al., 1998). Smith (2002) fed a calcium salt of conjugated linoleic acid (Ca-CLA) and measured differences in body composition and pre-pubertal mammary development and in pair-fed animals, measured milk yield. In this study, mammary development was reduced by approximately 60% in heifers fed Ca-CLA, however there was no significant difference in milk yield of the pair-fed heifers.

Figure 1. Evaluation of the prediction of “normal” and “diet impaired” prepubertal parenchyma development in Holstein heifers. The data points are predicted versus observed. Observed data are from previously published papers [Pritchard et al., 1972, (Δ); Sejrsen et al., 1982, (+); Petitclerc et al., 1984, (X); Capuco et al., 1995, (\square); and Mäntysaari et al., (1995), (o)]. Predicted values were generated using the mean daily DNA accretion rate determined by Meyer (2005) and the average age at slaughter as published in the respective papers. Slope of predicted verses observed (dashed line) is 0.92, $r^2 = 83\%$ ($P < 0.01$). Meyer, (2005).



In the studies by Radcliff et al. (1997; 2000), bST was administered from 125 to 336 kg (276 to 740 lbs) of body weight to enhance pre-pubertal mammary development. In the tissue harvest study, mammary development was enhanced approximately 48% by the use of growth hormone (Radcliff et al. 1997). Milk yield from the heifers treated pre-pubertally with growth hormone did increase by approximately 5.9%, but that was not significant and not highly correlated with the increase in mammary parenchyma development (Radcliff et al. 2000).

Thus, mammary development, measured as DNA content of the parenchyma at puberty, varied by about 100% (+48 to -60%) with no significant difference in milk yield. This strongly suggests that mammary development when measured as DNA content at puberty is not a good indicator of future milk yield. This is not to dismiss the concept that mammary development is important, but rather to provide opportunity to consider specific cell types instead of gross measurements using DNA as a proxy for cell number (Sinha and Tucker, 1969; Ballagh et al., 2008).

BODY COMPOSITION AND MILK YIELD

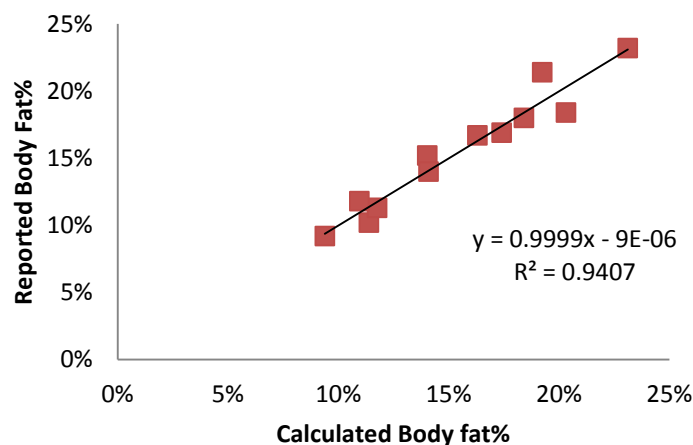
One aspect that is harder to quantify is the difference in body composition among heifers at calving in studies investigating the effect of age at first calving on milk yield. Again, for example, Swanson (1960) compared the milk yield of fat versus moderately conditioned heifers and observed that the fatter heifers did not perform as well. Based on data describing the productivity of dairy cattle calving at higher than desired body condition scores, dry matter intake, milk yield and post-partum health are usually at greater risk of being compromised (Grummer et al. 2004; Allen et al 2005; Douglas et al., 2006; Ospina et al. 2010). Thus, body composition at calving as it relates to energy balance is as important for first lactation cattle as multiparous cattle. Further, any difference in body composition of heifers at puberty or pregnancy will most likely be maintained or enhanced since under most conditions the animals remain in positive energy balance from puberty to calving. Thus, experiments evaluating rapid growth prior to puberty are potentially measuring the long-term effect of altered body composition at calving.

There are currently data to make accurate predictions of the maintenance and growth requirements of dairy heifers, as well as to model growth and body composition while taking into consideration stage of maturity of the heifer. In this paper, we used published equations describing energy and protein requirements and body composition to predict body composition at various stages of growth up to calving. Predictions were made using the current Nutrient Requirements of Dairy Cattle 7th edition (NRC, 2001) publication, as well as optimizations of requirements calculated from data generated after the publication of the NRC (Van Amburgh and Drackley, 2005). In addition, equations from Fox, et al. (1999) and the Nutrient Requirements of Beef Cattle (NRC, 1996) were also used to predict nutrient requirements or body composition. The predictions for body fat percent were evaluated using data from Meyer (2005) and resulted in a R^2 of 0.94 (Figure 2). Protein and lean tissue composition were also considered and the body composition of the protein content was also predicted by the model.

The model was evaluated with data from Gibb et al. (1992), where post-calving body composition was available for cows fed to grow at three different ADG pre-calving. A distinctive characteristic of this study was that the mature body weight of the cattle used could be described as approximately 700 kg since the study utilized cattle with 3 and greater lactations. The reported body fat content of cattle grown at the 3 different pre-calving body growth rates were 18.6%, 19.4% and 21.2%. When accounting for the mature weight of the population, the estimates from our model for post-calving body fat content were 18.5%, 19.5% and 20.8% for each of the respective ADG.

To better understand the relationship between rate of gain, composition of gain and age at first calving (AFC), the model was used to develop estimates of the body composition at calving of heifers who were grown at different rates of gain, bred when they had achieved 55% of their mature body weight and had calved at 82% of their

Figure 2. Regression of calculated body composition with measured body composition of dairy calves and heifers at different weights for calves grown at two different rates of gain. Measured body compositions taken from Meyer et al., 2005.



mature body weight as per current recommendations (Fox et al. 1999; NRC, 2001). In the base scenario, all animals were assumed to double their birth weight by 60 days and have an ADG of 0.6 kg (1.3 lbs) during pregnancy, excluding the weight of the gravid uterus. Three different pre-pubertal growth rates were used: 0.75 kg/d (1.7 lbs/d), 0.64 kg/d (1.4 lbs/d) and 0.56 kg/d (1.2 lbs/d) that allowed for AFC of 22, 24 and 26 mo., respectively. Given these growth rates, the three groups of animals were estimated to calve at 25% body fat and 15% protein, and would not be expected to have differences in milk production, although the animals that calve at 22 months would be producing milk 4 months sooner than those set to calve at 26 months.

Subsequently, several different scenarios were created based on published data to represent studies and potential on-farm conditions that describe various management approaches to decision making for AFC and BW at or post-calving. When pre-pubertal growth rates were adjusted but heifers were bred by age, the predicted body composition of heifers in each group changed significantly. Using similar assumptions, if calves double their birth weight by 60 d and grow at 0.7 kg/d (1.5 lbs/d) during pregnancy (without the weight of pregnancy), and all heifers are bred at 16 months for an expected AFC of 25 months, but during pre-puberty had ADG of 1 kg (2.2 lbs), 0.8 kg (1.8 lbs) or 0.6 kg (1.3 lbs) they would calve at 30%, 27% or 23% body fat and 14%, 15% and 16% protein, respectively. Data are not available to fully characterize the body composition at calving that provides the most optimum energy balance for first lactation cattle, however the difference in body fat from 23 to 30% would be enough to increase the BCS by at least 1 score, equivalent to 40 kg (88 lb) body fat in a 560 kg (1,250 lb) Holstein heifer. These calculations are consistent with data where heifers were bred to calve at the same age but at different body weights; consequentially, heavier (fatter) heifers produced less milk during first lactation (Swanson, 1978).

To better understand the effects of pre-pubertal ADG on future milk production, we estimated the body composition at calving for heifers from published studies where milk yield was evaluated.

Valentine et al. (1987) reported growth rates from 0.18 kg (0.40 lbs) to 0.94 kg (2.07 lbs), where AFC ranged from 26.9 mo for the slowest treatment to 22.4 mo for the treatment gaining over 0.9 kg/d. After calving, the estimated body fat percent for all groups was 22% and researchers reported no difference in milk production among any of the groups. This data suggests that if little difference exists in body composition at calving, and BW are reasonably similar, dry matter intake, energy balance and milk yield will not be negatively impacted.

Hohenboken et al. (1995) compared three different growth rates from 6 wk of life to 300 kg (661 lb). In this study, all heifers were bred at the same age generating AFC of 29, 26 and 23 mo for heifers raised at ADG of 0.6 kg (1.3 lbs), 0.7 kg (1.6 lbs) or 0.9 kg (1.9 lbs) respectively. These treatments resulted in a predicted body composition of 17% and 25% body fat and 18% and 16% protein for calves raised at 0.6 kg and 0.9 kg respectively. The treatment heifers with 17% predicted body fat produced 500 kg (1,103 lbs) more milk than the group with higher body fat percent. This is consistent with the data describing the potential impact of greater body condition score on dry matter intake, energy balance and milk yield (Garnsworthy and Jones, 1987; Allen et al., 2005; Janovick and Drackley, 2010)

In agreement with these calculations are the results from Hoffman et al. (1996), who reported the effects of different growth rates post-puberty (~45% of mature body weight) and during pregnancy on first lactation milk yield. Heifers on this study were fed to achieve an ADG of 0.97 or 0.79 kg (2.14 or 1.74 lbs) from 10 mo of age until calving. The group fed for higher gain was bred at 10 mo while the control group was bred at 14 mo. At calving, both groups had similar body weights but researchers reported that the group with higher gains had lower wither height and pelvic area. The interpretation of these results suggest that calves with higher ADG during pregnancy had a higher fat composition given the fact that they were smaller framed animals but had similar weights than control. Furthermore, milk production of the calves with higher ADG during pregnancy was 2 kg/d (4.4 lbs) lower than control calves but their milk fat yield was higher during the first 2 months of lactation. These observations are consistent with the lactation performance of over-conditioned cattle.

One of the most crucial and overlooked variables in the effects of growth rate on future performance is mature size. As previously mentioned, the composition of the gain is dependent on the stage of maturity, therefore, when evaluating growth rates pre-puberty, it is important to characterize the growth rates within the stage of physiological maturity. This concept was described for dairy cattle by Fox et al. (1999), where they described the percent of mature BW at pregnancy (55%) and post-calving BW (minimum 82%) necessary to optimize first lactation milk yield. The key factor in this approach is utilizing the mature BW of the herd to adjust for stage of maturity for nutrient requirements instead of using a population value. In all of the studies

conducted on heifers prior to the publication of the Dairy NRC (NRC, 2001), no consideration was given to the mature size of the cattle, thus most data were not adjusted for stage of growth and under those conditions, energy intake is almost always greater than required for dairy replacements (Van Amburgh and Meyer, 2005).

Foldager and Sejrsen (1987) concluded that the optimal growth rate of dairy calves between 90 and 350 kg (200 and 770 lbs) live weight should be 0.6 kg/d (1.3 lbs/d). However, representative animals from that data set are shown in Figure 3. From this picture, over- conditioning of the fastest growing heifers was not included in the analysis and was probably a confounding factor in milk production. To better describe this, the growth data from Foldager and Sejrsen (1987) were used to predict body composition at calving, however, we had to make assumptions about the mature body weight of the animals represented and chose a range of mature weights for comparison. Predicted body composition at calving for cattle with mature body weights from 500 to 700 kg (1,103 to 1,544 lbs) are presented in Table 1. As mature weight increased, body fat

Figure 3. Three 18 months old heifers grown at ADG of 400, 600 and 800 g (0.88, 1.32 and 1.76 lb). Live weights were 250, 402 and 540 kg (551, 886, 1,190 lb) respectively (Foldager and Sejrsen, 1987).



decreased at similar calving weights. The cattle represented in the study appear to be small framed cattle with mature body weights between 500 and 550 kg (1,103 to 1,213 lbs). If this study had been performed with larger framed cattle, conclusions on the effects of growth rate on milk performance might have been different due to the composition of the gain of the animals. Again, depending on the mature size of the cattle, the differences in fat percent translate into differences in BCS of at least 1 unit and this would have a significant effect on post-partum DMI, and milk yield. Milk production on this study differed by 500 kg (1,102 lbs) in the first 250 d of lactation where heifers grown at 0.6 kg/d (1.3 lbs/d) produced 5,100 kg (11,245 lbs) of milk compared with 4,600 kg (10,143 lbs) produced by heifers grown at 0.8 kg/d (1.76 lbs/d) during the pre-pubertal period.

Table 1. Calculated body composition at calving of heifers grown at different pre-pubertal rates with different mature body weights.

Mature body weight, kg (lbs)	ADG from 90 to 350 kg, g/d	Calculated body fat, %	Calculated body Protein, %
700 (1,544)	400	18.5%	18.0%
700 (1,544)	600	19.5%	17.7%
700 (1,544)	800	20.8%	17.4%
600 (1,323)	400	20.8%	17.3%
600 (1,323)	600	22.0%	17.0%
600 (1,323)	800	23.6%	16.6%
550 (1,213)	400	22.2%	16.9%
550 (1,213)	600	23.6%	16.5%
550 (1,213)	800	25.3%	16.1%
500 (1,103)	400	23.9%	16.4%
500 (1,103)	600	25.4%	16.0%
500 (1,103)	800	27.2%	15.5%

The overall goal of heifer rearing is to provide the management and nutrition that allows for optimum milk yield in the first and subsequent lactations. Research has evaluated many aspects of heifer rearing. However, most of the focus has been on pre-pubertal growth rate and its effects on mammary development. Little to no attention has been placed on the effects of such growth rates on body composition at calving. Transition cow research has unequivocally shown the negative effects of over conditioned cattle at the time of calving on DMI, metabolic problems and milk yield. These findings also apply to first lactation heifers. When accounting for predicted body composition at calving, we are able to explain most of the variation in milk production observed in different studies. Body composition explains both the lack of differences in production observed in some studies (Valentine et al., 1987; Waldo et al., 1998) as well as the differences in milk production observed in others (Swanson, 1978; Foldager and Sejrsen, 1987; Hohenboken et al., 1995). Thus in many studies evaluating mammary development and milk yield, directly or indirectly, the outcome was most likely better predicted by body composition at calving and not mammary development.

Moreover, body composition during growth is greatly influenced by mature size. When mature size is not accounted for in ration formulation, energy is often over-fed, resulting in greater fat deposition in growing heifers in subtle but significant outcomes.

SUMMARY

Data presented in this paper support the current growth benchmarks for heifer rearing (Fox et al., 1999; NRC, 2001) to achieve a body composition by calving that does not compromise post-partum energy balance or milk yield and allows for earlier age at first calving. Heifers should be bred between 55 and 60% of their mature body weight to achieve a post-calving weight of 82 to 85% of the mature body weight of the herd. When these targets are attained, heifers can successfully calve earlier without a negative impact on milk production, with the added benefit of having reduced the length of the non-productive stage.

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INFLUENCE OF SOCIAL ENVIRONMENT ON FEED INTAKE OF DAIRY CATTLE

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DRY MATTER INTAKE PREDICTION: IMPORTANCE OF MODELING CATTLE MANAGEMENT

Accurate prediction of dry matter intake (DMI) enhances ration formulation and is a critical component of nutritional models. Feeding behavior of dairy cattle determines DMI which is controlled by ruminoreticular fill and physiological mechanisms but modulated by the animal's feeding environment. The combination of housing facilities and management routines define the physical and social environment within which dairy cattle consume feed. Social interactions, palatability and other feed characteristics such as moisture content, as well as learning behavior are all integral components of psychogenic modulation of DMI (Grant and Albright, 1995; Grant and Albright, 2001).

Commonly used DMI prediction equations include factors for milk production or some measure of productivity, BW, stage of lactation, and dietary energy density (NRC, 2001). For example, the 2001 Dairy NRC publication predicts DMI as a function of 4% FCM yield and $BW^{0.75}$ with an adjustment for depressed DMI during early lactation. There are no adjustments to the DMI prediction equation for parity, temperature and humidity conditions outside the thermoneutral zone, dietary nutrient content, or management factors such as feeding frequency, grouping, or feed-bunk stocking density. Some nutritional models include inputs for the physical environment that directly or indirectly influence maintenance requirements and (or) DMI. As an example, the Cornell Net Carbohydrate Protein System model (CNCPS) allows inputs for temperature, humidity, wind speed, degree of lot muddiness, hair coat, standing time, and distance walked (Fox et al., 2004; Tylutki et al., 2008). Although some nutritional models incorporate important components of the animal's physical environment, to-date none have inputs for key components of the social environment.

Over the next decade, nutritional models need to incorporate inputs for the feeding environment such as feeding frequency, stocking rate, grouping strategy, and other critical psychogenic components to more accurately predict DMI. For example, we know that greater stocking density at the feed bunk and free-stall increases aggressive interactions, displacements, and alters meal patterns, rumination, and resting behavior especially for subordinate cattle (Hill et al., 2009). These changes in behavior may certainly affect DMI, but nutritional models currently do not incorporate these environmental inputs. The primary objective of this paper is to review important social components of the feeding environment (stocking density, grouping by parity, time budgets, and feeding frequency) and their effect on feeding behavior and DMI. Potential approaches for improving the prediction of DMI will be proposed and discussed with a primary focus on the lactating dairy cow using the CNCPS nutrition model.

SOCIAL ENVIRONMENT AND DRY MATTER INTAKE

When cattle are grouped, social behavior modifies DMI and productivity (Grant and Albright, 1995). Cattle are social animals and readily form dominance hierarchies, especially at the feed bunk which may have a substantial impact on feeding behavior and DMI. Highly competitive times at the feed bunk for dairy cattle occur when fresh feed is delivered and when cows return from the parlor (Friend and Polan, 1974; DeVries et al., 2004). Maximal effect of social dominance on feeding behavior lasts for about 45 min after delivery of fresh feed (Friend and Polan, 1974). Often the subordinate animals in a pen are most negatively affected when competition for feed, free stalls, or some other resource is high (DeVries et al., 2004).

Stocking Density

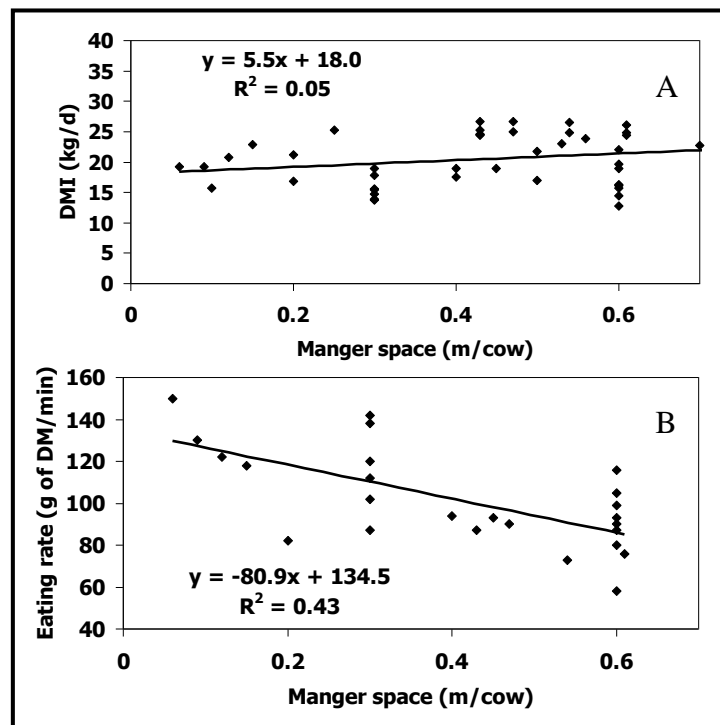
As stocking density within a pen increases, the frequency of aggressive interactions increases, cows spend less time lying down and more time standing outside the free stall, they consume feed up to 25% faster, and take less time to lie down after milking (Fregonesi et al., 2007; Hill et al., 2009). Competition at the feed bunk is responsible for 88% of displacements indicating that gaining access to feed is a high priority for cows (Val-Laillet et al., 2008). Competitive success by dairy cows at the feed bunk varies according to each cow's motivation to eat. In addition to altered feeding behavior, overstocking may also suppress rumination activity, lower milk fat percentage, and increase SCC under some conditions (Batchelder, 2000; Krawczel et al., 2008; Hill et al., 2009).

We summarized data from 10 studies that measured DMI response to variable stocking densities. In addition to DMI, some studies also measured components of feeding behavior such as number of meals, eating rate, meal size, and eating time. Greater feeding time has been assumed to be related to greater DMI. However, greater feeding time does not necessarily reflect greater DMI because feeding or meal time has been found to be poorly correlated ($r = 0.179$) with total daily DMI for dairy cows (Kauffman et al., 2007). This weak relationship constitutes a major constraint on data sets that can be used to model feeding behavior and DMI. All studies fed TMR and the DIM ranged from 1 wk postpartum to late lactation. Both pen feeding and individual cow feeding studies were included in this data set. Many studies did not differentiate response by parity. Some studies imposed stocking density treatments only on the feed resource, whereas others imposed various stocking densities on both the feed bunk and free stalls. Additionally, all studies were short-term (periods of < 4 wk) and the inference to chronic overcrowding on a commercial farm remains unclear. Published research has used feed bins, headlocks, and post-and-rail feeding systems. Feed bins capture feeding behavior and meal sizes, but how they relate to headlock and post-and-rail systems commonly used on commercial farms is unknown. Nonetheless, all studies have been converted to the common basis of bunk space availability (m/cow). Our approach is based on Proudfoot et al. (2009) who used a feed-bin system to evaluate stocking density and suggested that 100 or 200% stocking of the bin would be

equivalent to 0.30 or 0.60 m/cow of bunk space, respectively (width of the feed bin was 0.6 m).

Figure 1 illustrates the relationship between manger space available (m/cow) and DMI and eating rate found in this database. There was not a strong relationship between manger stocking density and short-term DMI response across a wide range of stocking densities. In contrast, over the same range of manger space, eating rate increased markedly as manger space was reduced by overcrowding. Number of meals per day increased, particularly as manger space fell below 0.4 m/cow, and meal size and total eating time decreased. Although some of the studies overstocked free stalls as well as manger space, which likely strengthened the relationship, it is interesting that a positive relationship existed between manger space and resting time. These observed relationships suggest that future approaches to modeling the effect of stocking density on DMI will likely need to be dynamic to reflect changes in eating rate and meal patterns throughout the day.

Figure 1. Relationship of feed manger space with (A) dry matter intake and (B) eating rate.



Grouping by Parity

Lactating primiparous cows may benefit from separate grouping (Grant and Albright, 2001; Østergaard et al., 2010). They have greater growth requirements, smaller body size, greater persistency of lactation, and frequently a lower position in the group's dominance hierarchy. Phelps (1992) reported that separately grouped primiparous cows

produced 729 kg more milk per lactation than heifers that had to compete with older cows in commingled groups. Grant and Albright (2001) reviewed the research on grouping dairy cattle by parity and concluded that, when first-calf heifers were separated from mature cows, eating time increased by 11.4%, meals per day increased by 8.5%, silage DMI increased by 11.8%, lying time increased by 8.8%, and lying periods increased by 19% per day. There is a differential response in DMI by parity within commingled pens as manger space is reduced with primiparous cows decreasing in DMI more rapidly than multiparous cows (Hill, 2006; Azizi et al., 2009). Eating rate increased as manger space was reduced for both parities, but the eating rates were lower for primiparous cows and they did not increase to the same extent as for multiparous cows. Number of meals per day was slightly reduced for multiparous cows, but it was much more obvious for primiparous cows. The responses in DMI and feeding behavior by parity within commingled pens at different stocking densities have been variable and more research is needed to better model the effect of grouping by parity.

FEEDING ENVIRONMENT AND DRY MATTER INTAKE

The effect on feeding behavior and DMI of various components of the physical and social environments are integrated into the specific feeding environment that the cow experiences.

Feeding System and Feeding Strategy

Feeding system components include feed barrier design, feeding surface and height, feed availability, and feed bunk space; these factors have been extensively reviewed (Albright, 1993). Influence of feed barrier on DMI has been variable among studies with some studies showing no difference in DMI between headlocks and post-and-rail systems (Brouk et al., 2001) and others showing greater DMI with post-and-rail (Batchelder, 2000). Several studies have found that headlocks may confer more protection from displacement from the feed bunk versus a post-and-rail feeder, especially for subordinate animals (Endres et al., 2005; Huzzey et al., 2006). Additionally, pen arrangement such as 2- or 3-row free-stall pens influences feeding behavior (Mentink and Cook, 2006). Dry matter intake was not measured by Mentink and Cook (2006), but feed bunk use was greater especially later in the day with the 3-row pen. The authors suggested that the greater feeding space per cow allowed with the 2-row pen resulted in more natural feeding behavior, maintenance of greater inter-cow distance while feeding, and greater avoidance of aggressive interactions at the feed bunk.

Amount of feed refusal and feed availability throughout 24 h influences DMI (Grant and Albright, 1995). French et al. (2005) compared dairy cattle fed TMR for 2.5% refusals at 18 h (clean bunk) with cattle fed a TMR for 5% refusals at 23 h post-feeding (full-fed). Although daily DMI and number of meals were unaffected by feed refusal amount, cattle fed to a clean bunk had shorter meal duration, less eating time, and faster eating rate compared with full-fed cows. Substantial limitations on feed accessibility (as little as 8 h/d) reduce DMI and milk production compared with free-

choice access to feed (Martinsson and Burstedt, 1990). Research is limited, but it appears that feed availability and periods of essentially empty bunks need to be incorporated into nutritional models to accurately predict feeding behavior and DMI.

Importance of TMR Feeding Frequency

Feeding strategy includes frequency of TMR delivery and frequency of feed push-ups to ensure access to feed. DeVries and von Keyserlingk (2005) concluded that delivery of fresh feed was the most important stimulus for dairy cows to eat compared with feed push-up and return of cows from the milking parlor. Consequently, frequency of feed delivery should be a primary factor to consider for improving prediction of DMI. Delivery of fresh TMR stimulates eating activity (DeVries and von Keyserlingk, 2005; DeVries et al., 2005). Feed push-up is secondarily important, and pushing up feed is more important during the day rather than at night (DeVries et al., 2005). The management goal is to ensure adequate feed accessibility throughout the day because limited feed access often encourages more aggressive interactions at the feed bunk, greater eating rate, and may limit DMI (Grant and Albright, 2001).

Greater feeding frequency is expected to improve ruminal fermentation conditions and DMI (Grant and Albright, 1995). For instance, Dhiman et al. (2002) observed a 19% decrease in ruminal NDF digestibility when TMR was fed once daily compared with 4 times daily which was associated with greater diurnal variability in ruminal pH. Additionally, Acatincai et al. (2009) found that twice daily feeding of TMR resulted in 10% less rumination activity compared with 3 times daily feeding. However, Mantysaari et al. (2006) compared once versus 5 times daily feeding of TMR and observed an increase in eating time, but a reduction in DMI with greater feeding frequency. Energy-corrected milk yield was unaffected, so gross efficiency of milk production was improved. However, lying time was reduced by nearly 15% with greater feeding frequency.

We summarized the results from several studies that have evaluated the influence of greater TMR feeding frequency on feeding behavior, DMI, and resting time. Although greater feeding frequency of TMR often increases eating time, the effect on DMI has been variable and often negative. Interestingly, in most studies where resting time was negatively impacted by greater feeding frequency, improvements in eating time did not result in greater DMI. It may be that increased feeding frequency improves DMI only if it does not negatively affect lying behavior. The data suggest that, with greater feeding frequency, at some point DMI and resting time may be compromised. This potential relationship between TMR feeding frequency, resting time, feeding activity, and DMI has been overlooked but may well be an important component of accurately modeling the influence of frequency of feeding on DMI. It is likely related to weather conditions and the effect on feed quality in the bunk. Recent research on the feeding environment suggests that priority should be placed on incorporating feeding frequency and feed access or empty bunk time into DMI prediction equations and nutritional models for dairy cattle.

IMPORTANCE OF TIME BUDGET BEHAVIORS: RESTING AND EATING REQUIREMENTS?

Grant (2004) defined the 24-h time budget as representing the net behavioral response of a cow to her social and physical environment. Deviations from benchmarked behavioral routines reflect departures from natural behavior and may serve as a basis for estimating DMI, performance, health, and economic loss due to inadequate management strategies. Dairy cows at 100% stocking density in free-stall housing spend 3 to 5 h/d eating, consuming 9 to 14 meals per day. In addition, they ruminate 7 to 10 h/d, spend approximately 30 min/d drinking, 2 to 3 h/d outside the pen for milking and other management practices, and require approximately 12 h/d of lying time (Grant and Albright, 2001). More recently, Gomez and Cook (2010) have shown how time outside the pen during milking, free-stall base, and lameness affect the cow's daily time budget.

Resting and Eating Behavior

The dairy cow appears to have a strong behavioral need for adequate rest. Dairy cattle are highly motivated to lie down for approximately 12 to 13 h/d (Jensen et al., 2005; Munksgaard et al., 2005). In fact, lying activity takes precedence over eating and social behavior when opportunities to perform these behaviors are restricted (Munksgaard et al., 2005). Physiological function, health, and productivity are impaired when the resting requirement is not met. Cows with restricted lying time have greater serum cortisol and lower growth hormone concentrations, impaired hoof health and locomotion, and sometimes lower milk yield (Munksgaard and Lovendahl, 1993; Singh et al., 1993; Grant, 2004; Cooper et al., 2007; Calamari et al., 2009).

The requirement for resting appears to be approximately 12 to 13 h/d based on results of numerous studies (11.5 h/d for low milk yield cows and 13.5 h/d for high milk yield cows, Grant, 2004; 12 to 13 h/d, Munksgaard et al., 2005; 11.4 to 13.7 h/d, Cook et al., 2005 and Drissler et al., 2005; 12.9 h/d, Fregonesi et al., 2007; 11.9 h/d, Gomez and Cook, 2010). Additionally, Jensen et al. (2005) found an inelastic demand for rest of 12 to 13 h/d for dairy heifers approximately 3 months pregnant. The measured range in resting time for lactating Holstein cows of varying milk yield, DIM, and BCS was 4.1 to 17.1 h/d (Bewley et al., 2010).

Eating time in non-competitive, tie-stall environments has been measured at 4.7 h/d for primiparous dairy cows and 5.2 h/d for multiparous cows with an average for all cows of 5.0 h/d (Dado and Allen, 1994). Albright (1993) summarized several studies that found an average eating time of 5.5 h/d (range 4.1 to 6.5 h/d). Eating in a free-stall environment at 100% or less feed bunk stocking density has been measured to be 5.2 h/d (range 3.3 to 7.0 h/d) for lactating dairy cattle fed TMR with a post-and-rail feed barrier (Grant, 2004) and approximately 4.7 to 5.0 h/d for dairy cattle fed a TMR with either a post-and-rail or headlock feed barrier (Huzzey et al., 2006). Variation in daily eating time and associated feeding behavior and DMI could be due to differing production levels and DIM, dietary chemical and physical attributes, body condition,

age, management environment, and even how eating behavior itself is measured. Although natural variation exists in resting and feeding time, approximately 12 and 5 h/d, respectively, may be a reasonable baseline for managing cattle fed TMR in a free-stall environment.

Resting and Feeding Behavior are Linked

Lying behavior has a high priority for cattle after even relatively short periods of lying deprivation (Munksgaard et al., 2005). Cows will sacrifice feeding in an effort to recoup lost resting time. Consequently, environmental factors that interfere with resting may also reduce feeding behavior. Metz (1985) evaluated cow response when access to either resting stalls or the feed manger was prohibited. Cows attempted to maintain a fixed amount of lying time, and their well-being was impaired when lying time was restricted for several hours daily. An additional 1.5 h/d standing time was associated with a 45-min reduction in feeding time. A similar relationship was observed by Batchelder (2000) where cows experiencing a stocking density of 130% of stalls and headlocks preferred lying in free stalls rather than eating post-milking and spent more time in the alley waiting to lie down rather than eating.

A review of published studies indicates that, for rest deprivation ranging between 2 and 4 h/d, there was a 30 to 58% compensation following the rest deprivation. The associated reduction in eating time has ranged between 32 and 45 min/d (Metz, 1985; Hopster et al., 2002; Cooper et al., 2007). Lying-deprived cows had reduced time spent eating during the actual period of lying deprivation as well as after the deprivation. From the data in these papers, it appears that cows sacrifice approximately 1 min of eating time for each 3.5 min of lost rest. If this relationship represents a long-term, chronic behavioral adaptation to environments that restrict resting time, then we need to adjust expected eating time and its predicted effect on DMI. For future nutritional models, it is suggested that time budget analysis of eating and resting activity will ensure that adequate time is available for cows to achieve their predicted daily DMI. Time budgeting (with a focus on the eating-resting interaction) may become an initial, important, and routine first step in ration formulation.

IMPLEMENTATION OF IMPROVED DRY MATTER INTAKE PREDICTION IN NUTRITIONAL MODELS

Model Approaches

The data set previously was used to develop two models: 1) a simple decision-support tool, implemented as a spreadsheet, that allows a nutritionist to evaluate the impact of stocking density on feed intake, and 2) a theoretical dynamic model illustrating the potential linkages between management, feeding behavior, DMI, rumen function, and cow health. For both modeling approaches we hypothesized that dairy cows have a minimum resting time requirement (min/d). Then, based on feed-bunk stocking rate (which is actually a mix of bunk and (or) stall stocking density; m/cow) and feeding

frequency, we varied eating time (min/d), number of meals, meal size (kg of DM / meal), and eating rate (g of DM / min) to maintain the minimum resting time.

Time budgeting was introduced into the model using the new term “cow management time.” Cow management time is inelastic as it is the sum of time spent away from pens for: milking, treatments, reproductive examinations, drinking, and other standing or social interactions performed on or by the cow. Initial time requirements for model inputs were either calculated (see next section) or based on data as described by Grant (2004).

Decision Support Tool

Initial evaluations of the data set were conducted using mixed step-wise regression for variable screening (JMP ver. 7.0.2). JMP Standard Least Squares methodology was used to parameterize the decision-support tool with parameters assumed significant at $P \leq 0.05$. Parameter screening was conducted to verify our hypothesized logic. Given the resting time requirement hypothesis, the logic followed that eating time (min/d) is a function of number of meals per day and eating rate (kg of DM/min) and there is a direct relationship between resting and eating time. Spearman ρ correlation coefficients supported a stepwise approach because eating rate, meal size, and number of meals were strongly correlated ($r = -0.65, 0.84, \text{ and } -0.79$, respectively). Based on this series of stepwise screening, the modeling approach implemented was to predict (in this order): resting time, meal size, number of meals, eating time, and DMI. Feeding frequency (times TMR fed per day) was implemented to adjust resting time.

Resting time (RT; min/d) was predicted with an r^2 of 0.94 from feed-bunk stocking rate (m/cow) and a base DMI (kg/d; Table 1). The base DMI was calculated using the lactating dairy cow DMI prediction of CNCPS ver. 6 (Tylutki et al., 2008). This equation contains BW (kg) and 4% FCM (kg/d) as parameters adjusted for DIM, temperature, and relative humidity. In our decision-support tool, DMI can be inputted if DMI of pens fed with adequate feed-bunk space is known (i.e. measured on the farm). Number of meals per day was adequately predicted (NM; $r^2 = 0.82$; RMSE = 0.6 n/d) from feed-bunk stocking rate and resting time. As would be anticipated, number of meals increased as feed-bunk space per cow increased. In contrast, as resting time was compromised the number of daily meals decreased.

Meal size (MS; kg of DM/meal) was predicted from resting time, number of meals per day, and an interaction between the two ($r^2 = 0.99$; RMSE = 0.1 kg of DM/meal). When evaluating number of meals and meal size a pattern emerged that, as number of meals per day decreased, the meal size increased. This relationship suggests that rumen health (driven by pH) would be compromised under overstocked conditions. Eating time (min/d) was predicted from meal size and feed-bunk stocking rate ($r^2 = 0.77$; RMSE = 32.2 min/d). The lower accuracy and low correlation with resting time suggested that other cow activities are also important in determining eating time. Most likely, standing time is increased as feed-bunk stocking rate increases. Currently, data limitations prevent exploring these potential relationships. Eating rate (ER; g of DM/min) was

predicted from meal size, feed-bunk stocking rate, and eating time ($r^2 = 0.83$; RMSE = 11.6 g of DM/min). Feed-bunk stocking rate greatly impacts eating rate; cows fed in overcrowded environments adjust their behavior to eat faster. Higher feed intake rates, coupled with fewer numbers of larger meals, results in greater rumen pH fluctuations and elevated risk of sub-clinical rumen acidosis (Stone, 2004; Shaver, 2005). Integrating this derived equation set highlights the potential environmental impacts on rumen and cow health mediated via feeding behavior. Dry matter intake can be predicted for this dataset with a high degree of accuracy ($r^2 = 0.91$; RMSE = 1.3 kg/d). Given that meal size and number of meals were included in the prediction, a simpler approach was implemented in the decision-support tool. The decision-support tool simply multiplied meal size times the number of meals to estimate DMI. Importantly, the predicted DMI is only for this dataset and could result in erroneous results if extrapolated beyond these data ranges. But, this approach serves as a starting point for future development of improved DMI prediction equations. A resting time adjustment (%) is predicted from feeding frequency (FF; $r^2 = 0.99$; RMSE = 0.2%). Data are limited comparing once versus multiple daily feedings or delivery of fresh TMR. Each feeding of TMR reduces resting time by 3.8%, thus recommendations to producers to feed more frequently may actually reduce resting time and DMI, a result many nutritionists would not expect. It would also be expected that this negative relationship between feeding frequency of TMR and DMI and resting time would plateau. Clearly, more research is needed to explore the relationships among TMR feeding frequency, feeding time, resting time, DMI, and efficiency of milk production.

The decision-support tool integrates the equations found in Table 1. Calculations include a resting time balance and an adjusted DMI based on feed-bunk stocking density. These results can be used to aid in ration formulation, on-farm troubleshooting, and management discussions with dairy producers. Insufficient data limit this analysis and additional data related to mixed parity groupings, stage of lactation, headlocks versus post-and-rail feeding systems, and other variables are needed.

Table 1. Equations developed for the decision support system.

$$\text{Resting Time (min/d)} = 148.7 + \text{FSR} \times 275.4 + \text{BPD} \times 17.6$$

$$\text{Number of Meals/d} = 13.8 + 3.7 \times \text{FSR} - 0.012 \times \text{RT}$$

$$\text{Meal Size (kg DM/meal)} = 4.4 + 0.003 \times \text{RT} - 0.5 \times \text{NM} - 0.003 \times (\text{NM} - 8.3) \times (\text{RT} - 591.2)$$

$$\text{Eating Time (min/d)} = 243.2 - 48.1 \times \text{MS} + 192.3 \times \text{FSR} - 1,494.1 \times (\text{FSR} - 0.4)^2 + 49.1 \times (\text{MS} - 1.8)^2$$

$$\text{Eating Rate (g DM/min)} = 190.7 + 7.0 \times \text{MS} - 44.4 \times \text{FSR} - 0.4 \times \text{ET}$$

$$\text{DMI (kg/d)} = -18.8 + 5.5 \times \text{MS} + 1.9 \times \text{NM} + 0.04 \times \text{ET}$$

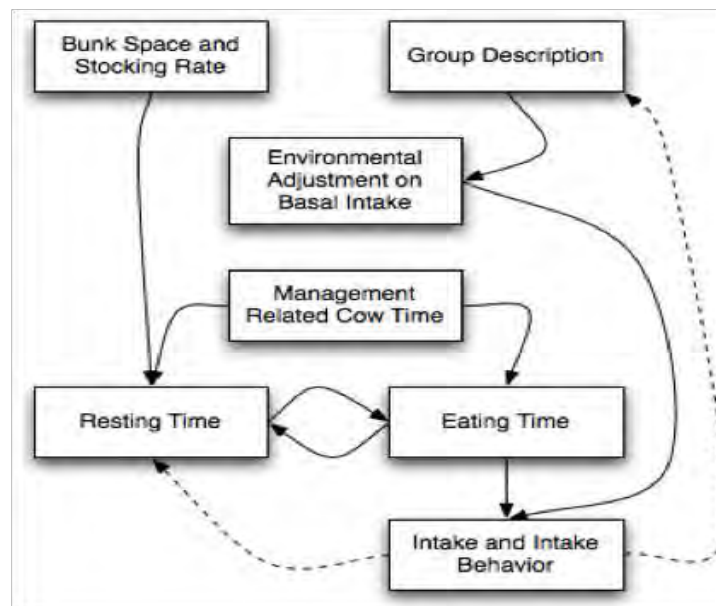
$$\text{Resting Time Adjustment (\%)} = 6.7 - 3.8 \times \text{FF}$$

Theoretical Dynamic Model

The objective of the dynamic model (developed using Vensim ver. 5.0.9) was to provide a structure relating resting time, eating time, and rumen fermentation with feedback via cattle health. Figure 2 illustrates the generic layout of the model with each box representing a sub-model. Initial parameter values for resting time, number of meals, meal size, eating time, and eating rate use the equations described for the decision-support tool (Table 1). Cattle group descriptions and adjustment of base DMI based on the physical environment are a combination of inputs and calculations from CNCPS ver. 6.1. The initial DMI is calculated as a weighted average for a group of mixed parity lactating dairy cows. In the future, the model can be expanded to address social and competitive issues due to mixed parity groups as more data become available.

The full model introduces the interactions among resting time, eating behavior, rumen function, and feedback to cattle health and time budgeting (cow management time). We have hypothesized that resting time disruptions, coupled with changes in feed intake behavior, introduce slug feeding and subacute ruminal acidosis. The acidotic condition increases lameness, health-treatment rates, hours standing, and other negative behavioral changes. These changes then increase management-related cow time which subsequently impinges on resting time. This scenario can lead to a cascading effect as eating behavior will be further altered resulting in even greater shifts in ruminal pH.

Figure 2. Dynamic model layout.



Further development of the dynamic model requires a sub-model to predict fermentation end-products. The crux of the problem involves the ability to predict meal patterns including a competition function between animals. Other factors such as headlocks versus post-and-rail, flooring composition at the feed bunk, parity, stage of lactation, and other physical and social environmental factors are required. An important example of management restrictions that need to be modeled include underfeeding (i.e. the pen running out of feed). This restriction in feed availability would impact total daily DMI, milk production, resting time, eating time, rate of intake, rumen health, and so forth. As for other components of the model, more research data are needed to effectively assess the importance of these factors in predicting DMI

CONCLUSIONS AND PERSPECTIVES

The data summarized and the models presented are only an initial attempt to incorporate the social environment and other management inputs into a nutritional model to improve the accuracy of DMI prediction in lactating dairy cows. Clearly, the need also exists to improve DMI prediction in non-lactating mature and growing dairy cattle. Our primary objective in describing these modeling approaches is to stimulate research and further development of nutrition models. The feeding environment is comprised of both physical and social components that we know modulate feeding behavior and feed intake in dairy cattle. In the future, nutritional models need to incorporate these important management inputs to better predict feeding and other behaviors, DMI, and cow performance and health responses. Finally, we propose that time budget analysis could become a routine and important component of DMI prediction and ration formulation.

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FORMULATING DAIRY RATIONS WITH NON-FORAGE FIBER SOURCES: WHERE TO BEGIN?

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INTRODUCTION

The last decade has brought increased pressure for land use, tighter commodity supplies, and higher cereal grain prices, which have resulted in significantly higher feed costs for dairies. These trends, however, have been accompanied by an increasing supply of high fiber byproduct feeds, many derived from biofuel production. Indeed, an estimated 40% of the corn grain harvested in the United States in 2010 was utilized by the dry milling industry (World Agricultural Outlook Board, 2011).

Other crops are also processed to recover particular fractions of the plant, and in many cases, the fiber component of the crop is of little value for manufacturing. As a result, many byproducts of industrial processing are relatively high in fiber content, making them particularly suitable as feedstuffs for ruminants. Some of the more common non-forage fiber sources (NFFS; $\geq 30\%$ neutral detergent fiber) fed in the United States are wet corn gluten feed (WCGF), distillers grains (DGS), soy hulls, and beet pulp. This article will highlight literature related to feeding NFFS, with the goal of providing nutritionists practical strategies for incorporating these feedstuffs into diets of lactating cows without compromising health or productivity.

FORMULATION STRATEGIES

Traditionally, many nutritionists have emphasized forage: concentrate ratio as a starting point for formulating dairy cattle rations. Unfortunately, this metric is quite imprecise for meeting the nutritional needs of a lactating cow; for example, both corn silage and wheat straw are considered forages, yet they have vastly different chemical and physical properties. These problems become even more obvious when including NFFS, which are high in fiber (like forages) but are rapidly digested and passed from the rumen (like concentrates). In recent decades, most nutritionists have shifted to relying on targeted concentrations of energy, neutral detergent fiber (NDF), protein, and micronutrients. Implicit in either the forage:concentrate or NDF/energy targets is the recognition that productivity of lactating cows is often limited by energy supply (Allen, 2000), yet adequate physically effective fiber is also required to maintain rumen health and milk fat yield.

When incorporating a novel ingredient into a TMR, it is tempting to directly replace an existing component of the diet. Studies have shown it is possible to successfully replace corn grain with soybean hulls (Ipharraguerre et al., 2002) or a combination of soybean hulls and cottonseed hulls (Beckman and Weiss, 2005). In both of these studies, milk fat concentration significantly increased, with few effects on other production parameters. However, it is rare that direct substitution represents the optimal use of such ingredients. This is evident from other trials in which soybean hulls or beet pulp replaced corn grain and decreased milk production (Nakamura and

Owen, 1989; Pantoja et al., 1994) or milk protein production (Mansfield and Stern, 1994; Mansfield et al., 1994).

Many NFFS provide valuable nutrients in addition to digestible fiber, and most often that nutrient is protein. Therefore, it is common that NFFS replace a combination of both cereal grains and oilseed meals in rations (Armentano and Dentine, 1988; Clark and Armentano, 1997; Younker et al., 1998). However, even this more balanced approach to formulating with NFFS can sacrifice productivity because of a decrease in digestible energy supply. Although NDF from NFFS is relatively digestible compared to forage NDF, replacing highly digestible non-fiber carbohydrate (NFC) with NDF can depress feed intake, decrease diet digestibility, and limit milk production (Anderson et al., 2006; MacLeod et al., 1985; Schingoethe et al., 1999; Staples et al., 1984).

More recent experience with NFFS suggests that these ingredients can be utilized most effectively when traditional carbohydrate targets are abandoned, and nonforage NDF is used to replace a combination of forage NDF and starch. These highly digestible NDF sources can supply substantial amounts of ruminally-fermentable organic matter with more constant acid production in comparison to high starch concentrates (Fellner and Belyea, 1991; Stock et al., 2000). They can also replace portions of forage fiber if the physical characteristics of the ration remain sufficient to stimulate rumination (Allen and Grant, 2000).

A series of 3 experiments reported by Boddugari et al. (2001) nicely demonstrates typical responses to these different approaches to NFFS utilization. First, a milling product similar to WCGF was used to replace 0, 50, 75, or 100% of the concentrates in a lactation diet. As indicated above, this replacement of NFC with NDF decreased dry matter intake, although in this case milk yield was maintained, resulting in improved feed efficiency (Boddugari et al., 2001). A second experiment then evaluated partial replacement of forage in addition to the complete replacement of concentrates by the milling product; these 4 diets contained 45, 53, 62, and 70% NFFS (DM basis), with as little as 30% forage in the most extreme diet. As the NFFS inclusion rate increased in this experiment, milk production increased, although without an increase in fat yield (Boddugari et al., 2001). Finally, a third study was conducted to compare a control diet to one with 40% milling product, replacing portions of both the forages and concentrates. This approach to NFFS utilization resulted in a 6 kg/d increase in fat-corrected milk yield, driven by a 20% increase in production efficiency (Boddugari et al., 2001). Indeed, a plethora of information indicates that optimal feeding of NFFS can not only reduce feed costs, but also improve productivity of dairy cattle (Aliyu and Bala, 2011; Ipharraguerre and Clark, 2003; Nadeem and Sufyan, 2005; Schingoethe et al., 2009).

Energy

Rather than focus on specific nutrients as energy sources, many nutritionists simply formulate for a target predicted energy density. However, this approach has shortcomings. Model predictions of energy supply are notoriously imprecise, and such predictions are even less likely to be accurate for NFFS. There are several reasons for this. First, models on which these energy predictions are based were derived from data which generally did not include diets with high inclusion rates of

NFFS. Another problem is that models do not attempt to account for associative effects within diets, which is likely to be a major factor when substantial amount of NFC are replaced by non-forage NDF (Beckman and Weiss, 2005). Finally, one of the more consistent responses to partial replacement of forage with NFFS is an increase in DMI (Kononoff et al., 2006; Mullins et al., 2010; Sullivan et al., 2011), which is not accounted for in models, making energy density predictions less relevant. Therefore, instead of formulating for energy density or starch targets, utilization of large amounts of NFFS requires a more flexible, iterative process.

Experience suggests that the following is an effective approach to formulating diets with high NFFS inclusion rates:

- 1) Determine a minimum effective fiber concentration to maintain rumen health and milk fat yield. Include forages necessary to meet this requirement, with an adequate safety margin.
- 2) Incorporate a combination of NFFS and concentrates to provide at least 34% NFC, letting total NDF rise with increasing NFFS incorporation.
- 3) Evaluate ruminally-available unsaturated fatty acid supply and adjust inclusion rates to limit the risk of milk fat depression (Lock, 2010).
- 4) Evaluate protein supply, including rumen undegraded protein, metabolizable lysine, and metabolizable methionine supply predictions. Adjust ingredient proportions or add bypass amino acids sources to balance protein supplies.
- 5) Re-evaluate targets for steps 1-3, then balance for micronutrients.

Using this approach, NDF concentrations may be much higher than in a typical diet, yet because of the high digestibility of the non-forage NDF, such diets can provide adequate ruminally-fermentable organic matter to support high production of microbial protein and volatile fatty acids (Hristov, 2006), and in turn, milk yield (Dann and Grant, 2009). Diets that incorporate more than 20% NFFS can support milk yields in excess of 50 kg/d with less than 22% starch and as much as 37% NDF (Boguhn et al., 2010; Ferraretto et al., 2011; Gencoglu et al., 2010). Many other NFFS-based diets have supported production levels above 35 kg/d with just 25-36% NFC (Batajoo and Shaver, 1994; Boddugari et al., 2001; Kononoff et al., 2006; Miron et al., 2003; VanBaale et al., 2001; Voelker and Allen, 2003).

One significant difference in this approach is that sources of fat will not be formulated into diets because of the lack of focus on energy density. However, this does not negate the utility of dietary fat in some NFFS-based rations. In cases where the ruminal acid load is already high, but more energy is needed to support milk production, adding fat can be a useful way to provide additional energy. In one study, cows fed high-NFFS diets in early lactation outperformed cows fed a traditional diet, but the addition of 2.25% hydrogenated fatty acids further improved productivity (Weiss and Pinos-Rodriguez, 2009). Inclusion of a fat source with limited ruminal availability may allow for further decreases in NFC content of NFFS-based diets, with possible improvements in productivity.

Physically effective fiber

Even though forage:concentrate ratio has little utility, the physical characteristics of the TMR cannot be ignored. Physical characteristics of the TMR have a major impact on chewing activity, which impacts rumen health, DMI, milk fat production,

and digestibility (Allen and Grant, 2000). Substituting NFFS for grain will likely have a minimal effect on particle size, but a substitution for forage can greatly reduce mean particle size of the diet. For this reason, nutritionists need to consider physically effective NDF (peNDF) when formulating diets.

There are multiple ways to calculate peNDF, but accepted definitions account for the ability to stimulate chewing, the ability to maintain milk fat concentration and production, or both (Grant, 1997). Thus, peNDF combines information on particle length and chemical content of the diet. Non-forage fiber sources have a small mean particle size, and are typically low in lignin and high in digestible fiber, so including NFFS in diets will decrease the physical effectiveness of NDF. This can be advantageous if ruminal distention is restricting DMI (Allen, 2000) as long as the level of fermentable carbohydrate does not exceed the rumen's capacity for neutralization and outflow of volatile fatty acids.

Despite the theoretical value of peNDF, a field-applicable method for estimating peNDF of a diet has remained elusive. One meta-analysis (Zebeli et al., 2008) demonstrated reasonably strong associations between $\text{peNDF}_{>1.18}$ with ruminal pH and milk fat yield. The $\text{peNDF}_{>1.18}$ variable is derived by determining the proportion of TMR particles retained on a 1.18-mm screen and multiplying by the total NDF concentration of the diet (Mertens, 1997). Although the meta-analysis suggested that $\text{peNDF}_{>1.18}$ is a valuable metric for typical dairy TMR, the database used to evaluate it was not focused on high-NFFS diets. In fact, the mean forage NDF concentration in the database was 21.9% of DM (Zebeli et al., 2008), and NFFS-based diets can contain as little as 12% forage NDF (Harvatine et al., 2002; Miron et al., 2003; Mullins et al., 2010). With such a small proportion of NDF coming from forage sources, using total dietary NDF as a factor in $\text{peNDF}_{>1.18}$ calculations is unlikely to result in a useful metric.

A comparison of recent results with low and high NFFS inclusion rates demonstrates this point. Yang and Beauchemin (2007) used primarily traditional forages and concentrates at different proportions and cut lengths to generate diets with a range of peNDF values. One finding from the study was that $\text{peNDF}_{>8.0}$ (the proportion of particles retained by a 8-mm sieve multiplied by dietary NDF content) was a far better predictor of ruminal pH dynamics than $\text{peNDF}_{>1.18}$ (Yang and Beauchemin, 2007). However, despite having one diet with a $\text{peNDF}_{>8.0}$ of just 9.6% of DM, milk fat yield was maintained across all treatments. In contrast, another recent study evaluated 3 diets with WCGF inclusion rates ranging from 33 – 56% of DM, with forage NDF concentrations decreasing from 15.3 to 9.3% of DM (Rezac et al., 2010). Although $\text{peNDF}_{>8.0}$ concentrations in these diets remained above 10.7% of DM, the lowest forage diet decreased milk fat yield by nearly 20% and caused clinical acidosis. In this experiment, $\text{peNDF}_{>1.18}$ values were even less predictive; $\text{peNDF}_{>1.18}$ was greater in the diet that induced milk fat depression than in the control diet (Rezac et al., 2010). Based on these comparisons, it seems clear that peNDF thresholds determined to be safe in traditional rations may not apply to high-NFFS diets. In these examples, milk fat was maintained when forage NDF was 16.0% of DM (Yang and Beauchemin, 2007) or 12.9% of DM, but not when it dropped to 9.3% of DM (Rezac et al., 2010), suggesting that forage NDF should not be ignored in NFFS-based diets.

Unfortunately, there is still no single tool for quantifying fiber adequacy in dairy rations that uniformly predicts rumen health responses to diets. For NFFS-based diets, we advocate an approach similar to that proposed by NRC (2001), using a sliding scale of forage NDF and total NDF concentrations. For example, a minimum of 18% forage NDF is recommended if total NDF content of the diet is just 27%, but only 15% forage NDF is considered necessary if total NDF is 33% of DM. This approach has been successfully extended to 12-13% forage NDF with 31-35% total NDF without inducing milk fat depression (Miron et al., 2003; Mullins et al., 2010; Rezac et al., 2010). This approach reflects the concept that non-forage NDF is approximately half as effective as forage NDF at maintaining ruminal function and milk fat yield (Swain and Armentano, 1994). If these guidelines are followed and diets are prepared such that >35% of particles are retained on an 8-mm sieve (Kononoff et al., 2003), then NFFS diets should support normal rumen function. Wet NFFS can be advantageous for meeting this fiber requirement because they tend to bind diet components together and prevent cows from sorting against longer forage particles (Sullivan et al., 2011).

Despite the importance of effective fiber for dairy cattle, it cannot be forgotten that milk fat depression is a multi-factorial problem. For example, ruminally-degradable starch supply may be an independent risk factor for both decreased ruminal pH (Zebeli et al., 2008) and milk fat depression (Maia et al., 2009). In fact, it's likely that one of the key reasons it is safe to feed high levels of NFFS in low-forage diets is because such diets are typically quite low in starch; we have fed diets as low as 14% starch (Rezac et al., 2010). Secondly, degradability of the forage NDF fraction must be considered as well. Even if recommended forage NDF concentrations are met, NFFS-based diets with very degradable forage NDF (i.e. from brown midrib corn silage) can still result in milk fat depression (Holt et al., 2010). Finally, some NFFS (especially DGS) can provide a substantial load of rumen available unsaturated fatty acids, which is another key risk factor that promotes milk fat depression (Hippen et al., 2010). All of these factors must be considered to formulate a diet that will support acceptable component production.

Protein

Use of NFFS can have a significant impact on protein fractions in a diet. Some NFFS, such as WCGF, provide a highly degradable source of protein, whereas others, such as DGS, tend to provide more rumen undegradable protein, especially if a dried product is fed (Kononoff et al., 2007). These factors can have a considerable effect on diet formulation. For example, if rumen undegraded protein from corn DGS is used to displace a bypass soybean meal product (thereby attempting to maintain metabolizable protein supply), the amino acid composition of metabolizable protein can shift substantially. In such a scenario, it is possible that the first-limiting amino acid can change from methionine to lysine, and supplementing with sources of limiting amino acids can support increased milk protein production (Nichols et al., 1998). Although model predictions of metabolizable amino acid supply are likely imprecise for high-NFFS diets, nutritionists should nonetheless consider adjusting sources of bypass protein if predicted supplies of methionine and/or lysine vary considerably from requirements.

LIMITATIONS AND PRACTICAL CONCERNS

Despite vast differences in the nutrient profiles across individual NFFS, similar nutrition concepts need to be considered as nutritionists incorporate these ingredients into diets. The first and most important thing to consider when incorporating a novel ingredient is the derivation of the feedstuff. Because some byproducts are treated like a waste stream during industrial processing, anti-nutritional factors can easily be introduced. Nutritionists should therefore be knowledgeable of the derivation process to aid in monitoring for potential problems.

Variability

The chemical and physical composition of feedstuffs can dramatically vary across batches. For example, the NRC (2001) reported a high standard deviations for the crude protein ($23.8 \pm 5.7\%$) and NDF ($35.5 \pm 6.8\%$) concentrations of WCGF. In a Canadian study, Droppo et al. (1985) tested the DM and nutrient composition of 4 samples from each of 14 truckloads of WCGF that had been delivered from a single starch plant. While the range of DM values was wide (40-48%), more concerning was the variability of protein and mineral content between loads; the coefficients of variation ranged from 12 to 35%. Not surprisingly, similar variability has been observed across suppliers for other NFFS (Kleinschmit et al., 2007). The variation in nutrient content likely reflects differences in sources of processing material, or differences in the processing technique for that particular batch. Thus, nutritionists must be conscious of this variation when incorporating these ingredients into diets.

There are approaches to decreasing the risks associated with variable ingredient composition. One approach is to work with a sole supplier that can demonstrate superior product consistency. Although such products often command a premium price, the resulting consistency in the TMR may make the added cost worthwhile. Another common strategy is to minimize the risk associated with any individual ingredient by using a mix of different NFFS sources. For example, Leiva et al. (2000) fed a diet containing 46% NFFS, but this was supplied by 4 different ingredients. The appropriate strategy for a given dairy depends largely on the number and types of NFFS that are cost-effective to purchase in the local area, as well as on the size of the dairy (see below).

Stability

One factor that limits the value of low-inclusion rate NFFS on small dairies is the limited shelf life of wet feedstuffs. Given that dairies often need to accept delivery of a full load of feed to acquire it at a reasonable cost, the farm needs to be able to utilize that load within 4-10 days, especially in warm climates. To increase shelf life, most NFFS can be dried, but this adds substantial cost and largely negates the value of being in close proximity to a source plant. In addition, wet products may be more digestible and support greater productivity in some cases (Anderson et al., 2006).

Although dry products are often the best option for maintaining product stability, other feed preservation strategies exist. For example, ensiling WCGF in a plastic silo bag sustained its quality, as determined by pH, temperature, and organic acid concentrations (Jaster et al., 1984). However, the small particle size of wet NFFS

can cause bags to stretch excessively and tear, and the poor flowability of these products can cause problems for upright silos. A potential solution is to blend the NFFS with some other forage and ensile the mixture (Schroeder, 2010). Another approach to preserving wet NFFS is to apply an anti-microbial agent such as propionic acid, which has been successful for short-term preservation (Geetha et al., 2009).

CONCLUSION

Incorporating NFFS into lactating dairy cattle diets provides an opportunity to improve farm profitability through decreased feed costs and possibly increased milk production. Several factors will need to be considered when adding these ingredients to lactation diets, and conventional rules of thumb may not apply when feeding these ingredients in large quantities. Diets formulated to complement the characteristics of any NFFS, rather than a single substitution for an ingredient, will enhance the likelihood of optimizing its use.

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POTENTIAL OF DEFATTED ALGAL MEAL DERIVED FROM BIOFUEL PRODUCTION AS A NEW GENERATION OF FEED PROTEIN SUPPLEMENT

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Marine microalgae have recently emerged as a promising source of biofuel production (Goldemberg, 2000). Biofuels, or energy derived from biological raw materials, have received heightened attention for reducing greenhouse gas emissions fostered by fossil fuels. The broad range of studies conducted on microalgae over the years only serves to emphasize their great potential as a useful, largely untapped natural resource of today. Since the 1950's, researchers have recognized microalgae for their versatility, such as for making fuels and for use in animal feed. The use of marine microalgae in animal agriculture supports the world's search for more renewable fuels. It was proposed that solar energy may be transformed into methane, a usable energy source when burned, by means of anaerobic algae digestion (Oswald and Gouleke, 1960). Others pushed the idea of using algae as an animal feed supplement after acknowledging its capacity to thrive in shallow bodies of water. In the early- and mid-1950's, studies developed processes to culture algae in ponds (Gotaas and Oswald, 1954; Oswald and Gotaas, 1955). Interest in algae as a food source arose with the realization that many algal species are extremely protein-rich. Reported protein values vary with respect to algal species, but may range anywhere from 28-39% protein such as in *Porphyridium cruentum*, to 60-70% protein as in *Spirulina maxima* (Becker, 1994). A study published in 1957 provided chicks with sewage-grown algae as a viable protein source; the dried algal mass contained at least 40% protein, in addition to an abundance of carotenoids (Grau and Klein). Growing-finishing pigs fed a barley-based diet including sewage-grown algae showed adequate growth, while pigs fed a naturally iodine-rich algal diet showed 10% increased daily body weight gain (Hintz and Heitmann, 1967; He et al., 2002).

The world's current growth rate is at 1.2% and is projected to nearly double in 56 years from approximately 6.9 billion to 13 billion (PRB, 2008). With a rising human population, the availability of food resources is at serious odds with its growing demand. Of particular importance are sources of protein. In animal diets, soybean meal is the most widespread source of high-quality protein incorporated to meet animal protein nutrient requirements as determined by the National Resource Council. Soybean meal may provide 44-86% crude protein and is also responsible for a significant percentage of the feed costs (NRC, 1998). Our current extensive animal agriculture industry heavily depends on soybean meal for meeting protein requirement of animals. According to the Food and Agriculture Organization, there are 1 billion cattle, 40 billion poultry, 1 billion swine, and 2 billion sheep and goat populations globally (Steinfeld et al., 2006). Since soybean meal is also a staple food for human consumption, its strong prevalence as a protein source in animal diets directly competes with its application in the human food market. When considering the world food crisis and our growing population, it is evident

that our current infrastructure of animal feed and human food sources is unsustainable. Thus, developing alternatives to using soybean meal in animal feed not only demands our immediate attention, but also is a necessary path.

The diversity of microalgae's relatively rich amino acid profiles and high mineral composition make them a promising candidate for incorporation into animal feed. In a study on the nutrient composition of *Spirulina maxima*, over 60% of the dried alga was composed of crude protein, which consisted of all the essential amino acids at a more than acceptable concentration, except for low sulphur amino acids (Clément et al., 1967). The alga supplement was also high in several vitamins, including B₁, B₂, and especially β-carotene (pro-vitamin A), and had adequate digestibility when fed to rats. In this case, low amino acid concentrations may be compensated for by providing animals with additional feed ingredients that may already be used, such as cereals that contain these proteins.

As such, the concept of using microalgae as an alternative source of protein supplementation in animal feed presents an extremely exciting opportunity to improve global food security by increasing soybean meal availability for human consumption. Expanding research on microalgae feed supplementation is of great value due to its potential both near and far to establish a more harmonious and sustainable relationship among humans, animals of agriculture, and food sources. Additionally, incorporating algal biomass into animal feed would alleviate its management as a general waste product.

Recently, we have tested replacements of soybean meal (containing 48% crude protein) with up to 15% defatted algal meal in a corn-soybean basal diet for weanling pigs. All swine diets met National Resource Council standards. Directly replacing soybean meal with either 6.6% whole algal meal or 7.2% defatted algal meal did not cause adverse effects on the growth performance, animal health, or protein metabolism of the pigs. Several plasma biochemical indicators, including alanine aminotransferase activity, alkaline phosphatase activity, and plasma urea nitrogen, were tested for differences across diets. The algal supplement showed no adverse effect on these biochemical measures. We will continue to investigate multiple alternative strains of algae and determine the most viable algal strains for optimum protein supplementation.

Despite the potential of incorporating microalgae in animal diets, present-day limitations emphasize the need for a group initiative, ultimately to develop a working protocol for refining algae biomass that is economically viable. Currently, harvesting algae is a relatively energy-intensive four-step process that does not support low-cost mass production. Algal fuel production involves large-scale monoseptic algae cultivation, dilution of algal broth to recover the biomass, removal of metabolites from the biomass, and purification of the crude powdery product that is relatively protein-rich (Hankamer et al., 2007; Gouveia and Oliveira, 2009). Today, replacing soybean meal with algae in animal diets is not cost-effective. As such, refining production techniques must be improved in such a way that the operational costs do not exceed the convenience of not replacing soybean meal with algal biomass.

The novel information gathered from our research could help develop technology that effectively cultivates algae and refines algal biomass at a feasible and economic level. Incorporating defatted algal biomass into the diets of different animal species, including aquaculture, would encourage a long-standing initiative to develop a more sustainable agricultural industry. This would further improve the human food market by freeing up more soybean meal for human consumption. Furthermore, the removal of algal byproducts from sources of water would help downgrade its contributions to environmental pollution. We are still at the forefront of research with microalgal supplementation in animal feed, the implications of which have beneficial impacts on the animal agriculture industry, human food security, and environmental stability. We have a compelling opportunity to revolutionize our use of algae to improve our sustainability.

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UNRAVELING THE SPECIFICS OF NUTRIENT PARTITIONING A HISTORICAL PERSPECTIVE OF DALE E. BAUMAN AND THE CONCEPT OF HOMEORHESIS



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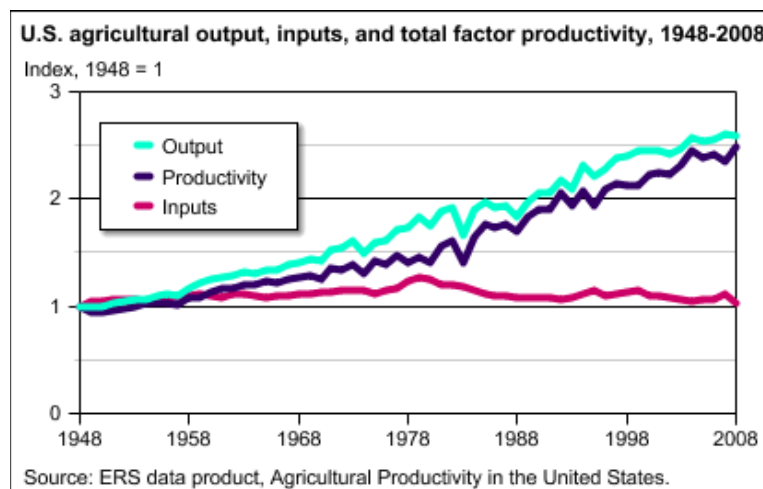
INTRODUCTION

The year 2011 marks the completion of the professorial career of Dale E. Bauman, Liberty Hyde Bailey Professor of Animal Science at Cornell University and beginning of his Emeritus and retirement years which we have every expectation will be both long and productive. Dale has had a remarkable career and one of great import to the animal industries of the world in general and the United States in particular. It is an appropriate time to describe some of the impact of his research and writings on our understanding of one of the great mysteries of biology, nutrient partitioning. Although this article will address nutrient partitioning, it in no way addresses the full extent or depth of Dale's many other contributions to science or his future contributions as an emeritus professor nor does it delineate the impact of the many graduates from his program, which is a large enough group to be characterized as a "School" or the role he played at the National Academy of Sciences/National Research Council and in the public and political arenas leading the debate on the safety of new technologies in agriculture. Suffice to say these topics require much more treatment than is available here.

HISTORICAL BACKGROUND

Although our primary farm animals were domesticated some 10,000 years ago it was not until the 1800's that any serious attention was paid to breed identification and the systematic use of records to establish performance differences between animals which could be used for selection decisions. This century also saw the birth of investigations into metabolism and factors which might control it. Scientists began to recognize that energy utilization was an important component of evolutionary success. Boltzman, 1886 pointed out in a lecture to the Imperial Academy of Science in Vienna that "Available energy was the main object at stake in the struggle for existence and the evolution of the world". Lotka, 1922 in an address to the National Academy of Science took this fact to the next logical step by pointing out that "Where the supply of available energy is limited, the advantage will go to that organism which is most efficient, most economical, in applying to preservative uses such energy as it captures." By the mid-1900's it was quite apparent that large differences existed between species and breeds of domestic animals and within animals of the same breed and species in efficiency of production as measured by feed required per unit of output. However, little was known regarding the causative factors for these differences. Additionally, real progress in improving production was being made with advanced reproductive techniques such as artificial insemination and improved genetic selection information, Figure 1. However, we did not understand what biological controls were being changed as we increased productivity of domestic animals. This was the "scientific milieu" Dale entered as he began his graduate studies at Michigan State for his Masters of Science which he received in 1968. He then went to the University of Illinois for his Doctoral Studies which he completed in September of 1969. Dale went on to make major contributions to our understanding of how animals partition nutrients and the mechanisms involved in controlling this process. First, at The University of Illinois from 1969-1978 and then at Cornell from 1979 to the present. While at Cornell, he and Bruce Currie crystallized the concept of Homeorhesis as the primary process controlling the flow of nutrients for specific physiological states. He and a series of fortunate co-authors further contributed to establishing the importance of nutrient partitioning in productivity and profitability of domestic animal operations and most recently with Jude Capper demonstrated how this impacts the carbon footprint of animal industries

Figure 1. Productivity Continues to Be the Engine of Growth in Agriculture

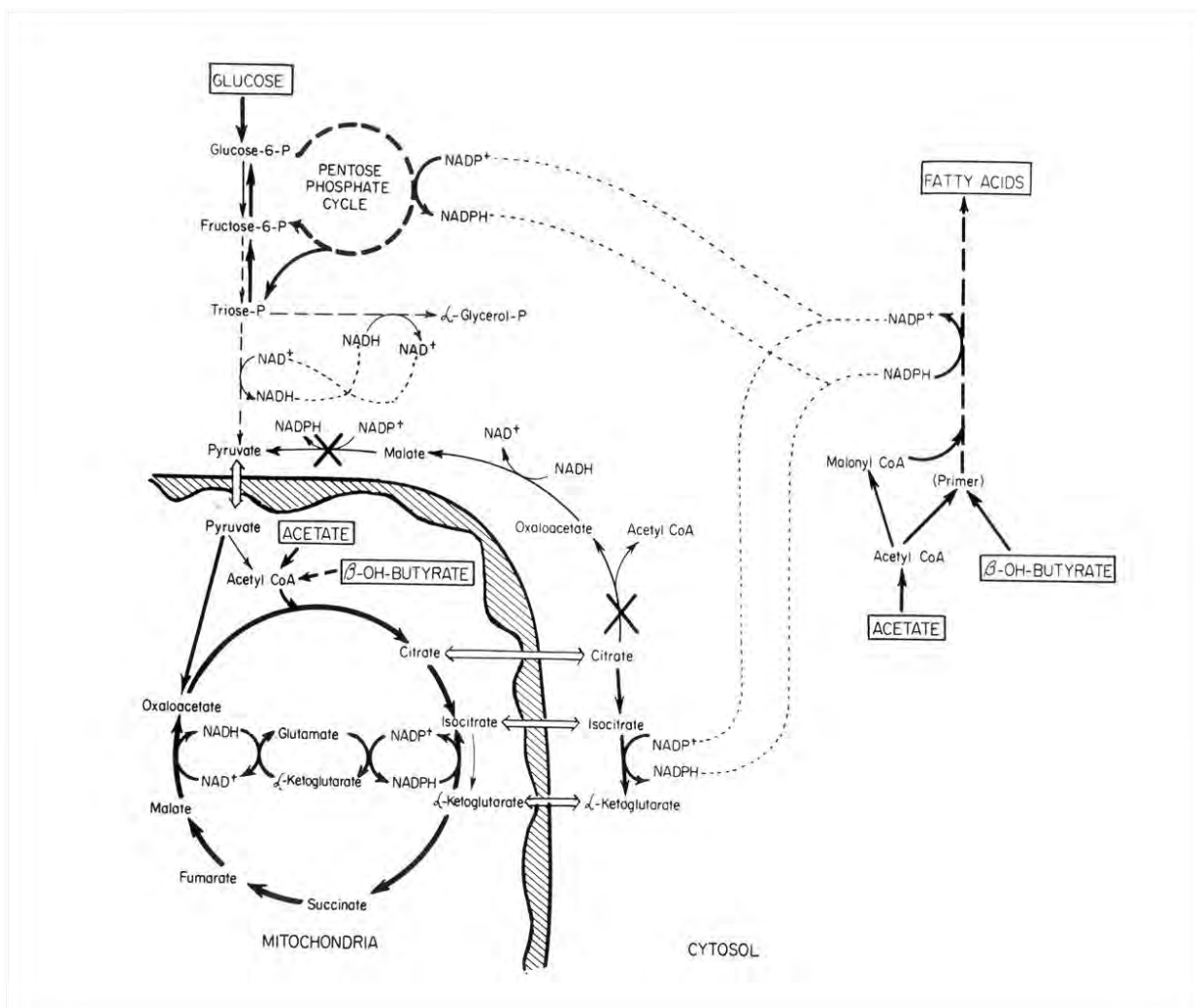


IN THE BEGINNING

Fatty Acid Synthesis in Ruminants

Dale began his career-long work in regulation of nutrient partitioning by examining genetically based adaptations in ruminants which were associated with fatty acid metabolism in general and the mammary gland in particular. Dale began these studies under the direction of Richard (Dick) Brown and Carl Davis at the nutrition field lab at the University of Illinois. In a remarkably concise 60 page dissertation he describes his research results outlining the biochemistry of fatty acid synthesis in ruminant and non-ruminant mammary tissue, the reason glucose is not used for fatty acid synthesis in the ruminant, the reason butyrate is utilized only for the synthesis of the initial four carbons of milk fatty acids in the bovine, the source of reducing equivalents for fatty acid synthesis in ruminant and non-ruminant mammary tissue and the cofactors required for fatty acid synthesis in ruminant mammary tissue. This research established the “glucose-sparing” concept for milk fatty acid synthesis in ruminants which shunts glucose away from fatty acid synthesis and spares it for lactose synthesis. Since glucose does not escape ruminal fermentation all systemic glucose in the ruminant arises from gluconeogenesis which can be quite challenging in high producing dairy cows. A key discovery of Dale’s Doctoral research is demonstrated in Figure 2 from his Dissertation (Bauman, 1969). In this figure, the pathways of fatty acid synthesis by ruminant mammary gland are shown demonstrating the absence of ATP-citrate lyase which catalyzes the cleavage of citrate to oxalacetate and NADP-malate dehydrogenase which catalyzes the conversion of malate to pyruvate. Since the citrate-cleavage pathway is non-functional in the ruminant mammary gland this produces an elevated intra-mitochondrial acetyl CoA concentration and acetyl Co cannot contribute to the cytosol acetyl CoA pool. This prevents glucose from furnishing carbons for fatty acid synthesis. In addition, β -hydroxybutyrate also cannot furnish carbons for fatty acid synthesis other than as the initial four carbon unit. Collectively, these results demonstrated for the first time how “glucose sparing”, which is a component of nutrient partitioning takes place in the ruminant mammary gland and this is a true genetic adaptation of ruminants to address the chronic shortage of glucose which is essential for lactose synthesis. Since lactose is the osmotic determinant of milk a reduction in lactose output would automatically lead to a reduction in milk yield. This work and other papers later (Bauman et al 1970, 1972, 1973; Ingle et al. 1972, 1973; Leung and Bauman, 1976; Scott et al. 1976; Etherton et al., 1977) explained some of the key differences between ruminants and non-ruminants in energy partitioning but did not address the phenotypic changes which must take place in the life cycle of a female mammal as she transitions from pregnancy to lactation. These changes are not genetic and occur only when the demands of lactation on the metabolism of the mother must be addressed. However, Dale also recognized there were other physiological states such as pregnancy, growth, under nutrition as examples which also required a coordination of metabolism. He had an intense interest in understanding how these changes take place while maintaining maintenance requirements of the animal. He also maintained a career-long interest in the regulation of fatty acid synthesis in the mammary gland and low milk fat syndrome in particular, (Bauman and Davis, 1974, 1975). He would come back to this area and eventually solve the problem of low milk fat syndrome but that topic is beyond the scope of this paper.

Figure 2. Pathways of fatty acid synthesis by the ruminant mammary gland. From Bauman,1969



EARLY CAREER

Metabolic Adaptations

After completing his doctoral work and accepting a position at the University of Illinois as Assistant Professor of Nutrition in the Dairy Science Department Dale began a series of studies on “metabolic adaptations” which occur with differing physiological states. This work spanned several degree programs and species and produced several highly cited publications., (Bauman et al. 1972,1973,1974; Ingle et al. 1973; Mellenberger et al. 1973,1974.) In particular, his papers with Roger Mellenberger and others on metabolic adaptations with onset of lactation were cited as landmark studies in lactation biology by Margaret Neville, Editor of the Journal of Mammary Biology and Neoplasia. Figure 3. In these series of papers they outlined the extent of the “metabolic adjustments” which had to be made to meet the demands of the new physiological

state. These changes were dramatic and as Dale often pointed out had to be “exquisitely coordinated” to avoid metabolic disease states from developing. A partial list of these changes is shown in Table 1.

Figure 3. Cover of Journal of Mammary Gland Biology and Neoplasia

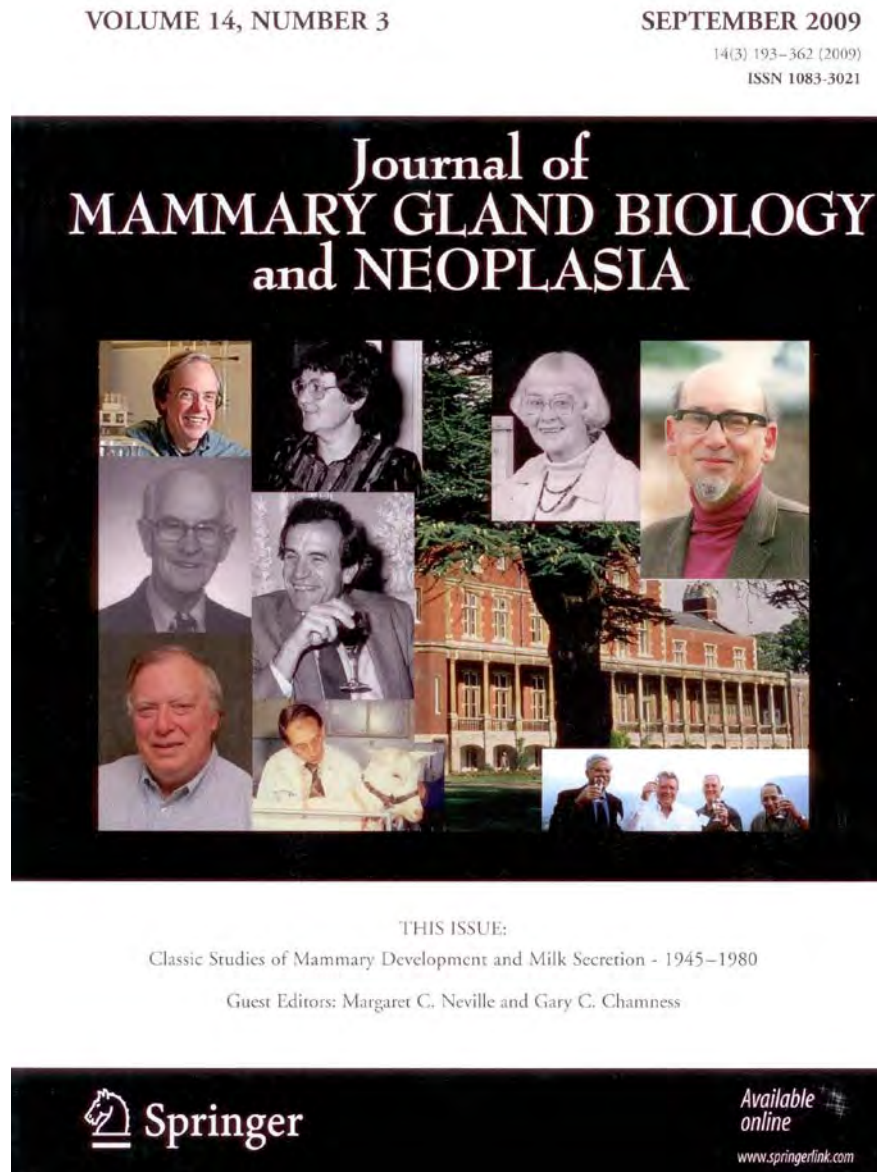


Table 1. Partial List of Physiological Adaptations That Occur In Lactating Dairy Cows

<i>Process or tissue</i>	<i>Response</i>
Mammary tissue	Increased number of secretory cells Increased nutrient use Increased blood supply
Food intake	Increased quantity
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
Skeletal muscle	Decreased glucose utilization Decreased protein synthesis Increased protein degradation
Bone	Increased Ca and P mobilization
Heart	Increased cardiac output
Plasma hormones	Decreased insulin Increased somatotropin Increased prolactin Increased glucorticoids Decreased thyroid hormones Decreased IGF-1

Adapted from Bauman and Currie, 1980

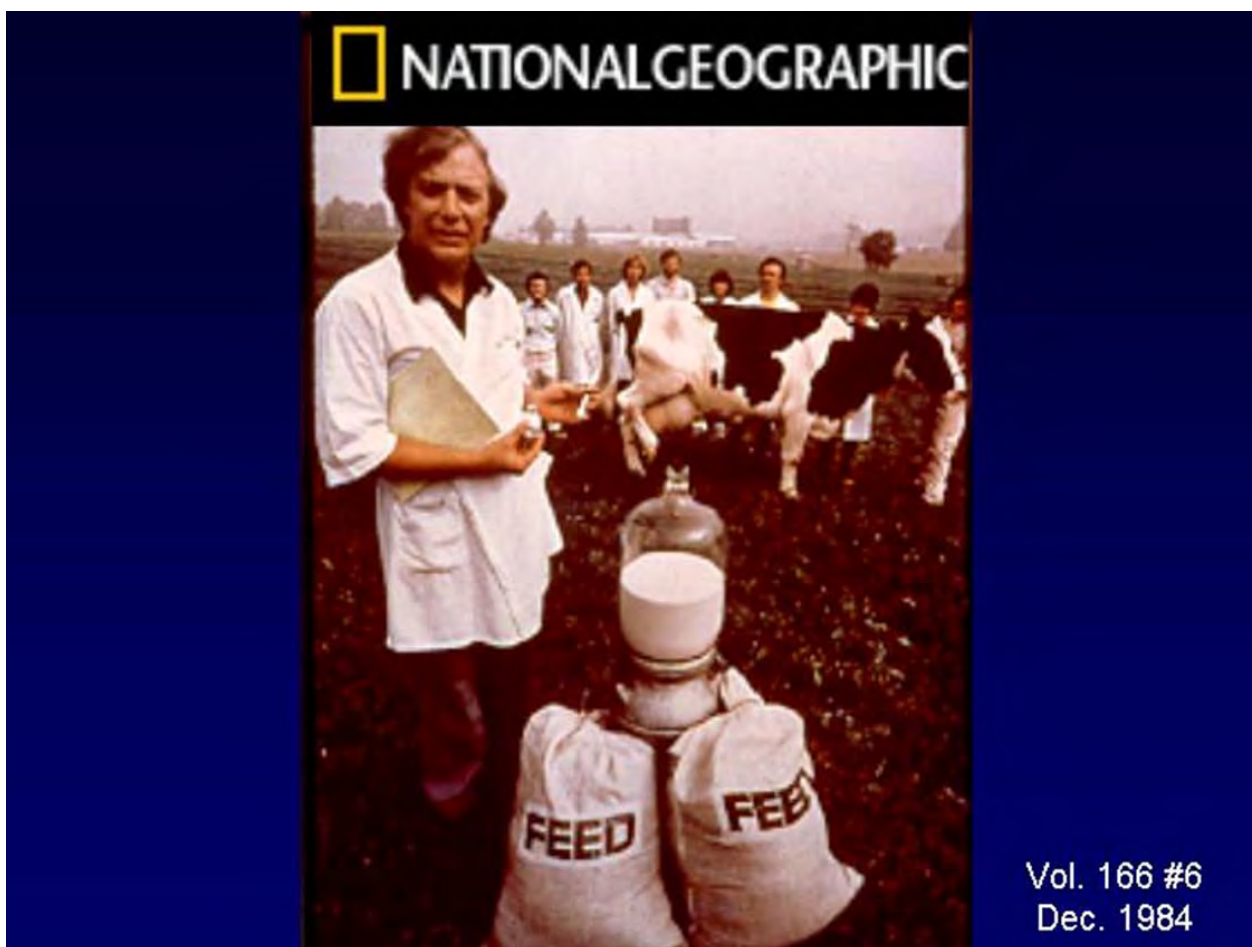
Endocrine Regulation

Of interest to Dale was the regulation and coordination of this process and he began working on the endocrine regulation of lactation. His first series of papers coauthored with Robert Collier, Jim Croom, Mike Akers, Allen Tucker and Ron Kensinger involved the hormones required for lactogenesis in the dairy cow and the role of prolactin in particular (Bauman et al., 1977; Bauman and Collier, 1978; Collier et al., 1976, 1977a, 1977b; Croom et al., 1976; Kensinger et al., 1979). It was also during this time (early 1970's) period Dale pointed out that somatotropin was probably a homeorhetic control and was potentially the next big opportunity for the dairy industry following the publication of the first Monsanto studies on bovine somatotropin (Machlin et al., 1973).

Dale's studies on metabolic adaptations and the hormonal regulation of lactation led him to the realization of the over-arching coordination of metabolism which was occurring during this process. At this point he was at Cornell University and began a series of studies involving bovine somatotropin. Initial studies were funded by NSF and used pituitary-derived bST provided by NIH. Subsequently his group collaborated with all of the companies developing recombinant bovine somatotropin for use in the dairy industry as well as support from USDA grants on the biology of somatotropin. These studies involved a number of graduate students (Colin Peel, Kris Sejrsen, Suzanne Sechen, Phil Eppard, Stuart McCutcheon, Joan Eisemann, Wendie Cohick, Karen

Plaut, Frank Dunshea, Mark McGuire, Yves Boisclair to name a few) and proved key to establishing the safety and efficacy of somatotropin for use in the dairy industry. His leadership role in this field was recognized by National Geographic magazine which featured the work by Dale's group in an issue on Biotechnology in December of 1984. He was also recognized by his peer scientific community with several awards.

Figure 4. Cover of National Geographic Magazine, December, 1984



Dales studies on the biology of somatotropin are too numerous to discuss but included all aspects of metabolism, production and animal health. A few of the key papers and reviews which Dale and his group published during this period are (Peel et al. 1981, Bauman et al. 1982, Bauman and Elliot, 1983, Bauman et al 1984). During this period Dale and Bruce Currie also published their landmark paper "Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis", (Bauman and Currie, 1980). Although this paper dealt with lactation and pregnancy, the concept was much larger than lactation and also took into account a host of physiological states where the process of homeorhesis permits the alteration of metabolism to support the demands of a new physiological state. A partial list of these states is shown in Table 2.

Table 2. APPLICATION OF HOMEORHESIS CONCEPT

Lactation	Hibernation	Incubation Anorexia
Pregnancy	Growth	Puberty
Growth	Egg Laying	Chronic Under nutrition
Aging	Seasonal Cycles	Exercise
Chronic Illness	Stress	Estivation

Partial List From. Collier, R.J., L. H. Baumgard ,A. L. Lock and D.E. Bauman. 2004.

The impact of this work and his ongoing description of somatotropin biology as well as contributions in several other fields led to Dale’s election to the National Academy of Sciences in 1988 along with several additional awards.

Dale spent a lot of time and energy developing ways to communicate his work on homeorhesis, biotechnology and animal agriculture to the lay public and to other scientists not familiar with the science of this area. One example he often used was Ranger Rick, a comic strip wildlife biologist who brought factual information on animal biology to children and adults. He would use actual stories from Ranger Rick and then explain how homeorhesis was involved in the biology described in the article. Another example lay in his response to a producer who listened to his talk on somatotropin biology and said “I don’t believe one hormone can do all those things.” Dale response “Have you ever had a daughter go through puberty” not only brought the house down but made the concept crystal clear to everyone. A final example Dale often used was the Amish farmer explaining to his peers why high milk production itself was not a stress on cows. The farmer said “A tight rope walker does not fall because the rope is too high. It is because he lost his balance.” Dale’s excellence in communication to his peers and the public was recognized by reception of the Charles A. Black Award from Council for Agricultural Science and Technology in 1995.

The concept of homeorhesis describes the regulatory process involved in coordination of metabolism to support a new physiological state from a metabolic point of view. Others have used various synonyms to describe this process from other points of view, Table 3, but none of them captures the coordination of metabolism that is central to homeorhesis. Nor do they directly address the process of nutrient partitioning as an outcome of the coordination of metabolism.

Table 3. Synonyms For Homeorhesis

TERM	DEFINITION	SOURCE
<i>Allostasis</i> :	process of achieving stability, or homeostasis, through physiological or behavioral change.	McEwen, 1988
Canalization	measure of the ability of a <u>population</u> to produce the same <u>phenotype</u> regardless of variability of its environment or genotype	Waddington, 1942
Rheostasis	biochemical and physiological processes that, through graduated quantitative regulation serve the adaptive needs of an organism facing internal or external environmental challenges.	Mrosovsky, 1990
Teleophoresis	Direction of nutrients towards productive functions	Chilliard et al. 1983
Homeorhesis	Coordinated changes to support a specific Physiologic state	Bauman & Currie, 1980

This paper resulted in a whole new field of studies on homeorhetic control mechanisms in biology. Thus, homeorhesis as a concept has been firmly established and there will be many more years of work ahead for a whole group of biologists to fully describe the control mechanisms involved. When the public debate on global warming and the role of animals in contributing to greenhouse gas production developed, Dale, Jude Capper and collaborators (Capper et al 2008), took a central role in explaining how modern technology and genetic selection has improved homeorhetic control mechanisms in domestic animals thereby reducing the environmental impact of animal agriculture on this process. Their paper published in Proceedings of the National Academy of Sciences received great attention by both the scientific community and the lay public. Looking back at the progress made since Dale began his career it is clear that Dale has advanced our understanding of the coordination of metabolism in domestic animals light years from where the field lay at the beginning. In the process he has made major contributions to our understanding of the regulation of metabolism not only in domestic animals but also the entire animal kingdom.

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MILK FAT AND HUMAN HEALTH – SEPARATING FATS FROM FICTION

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INTRODUCTION

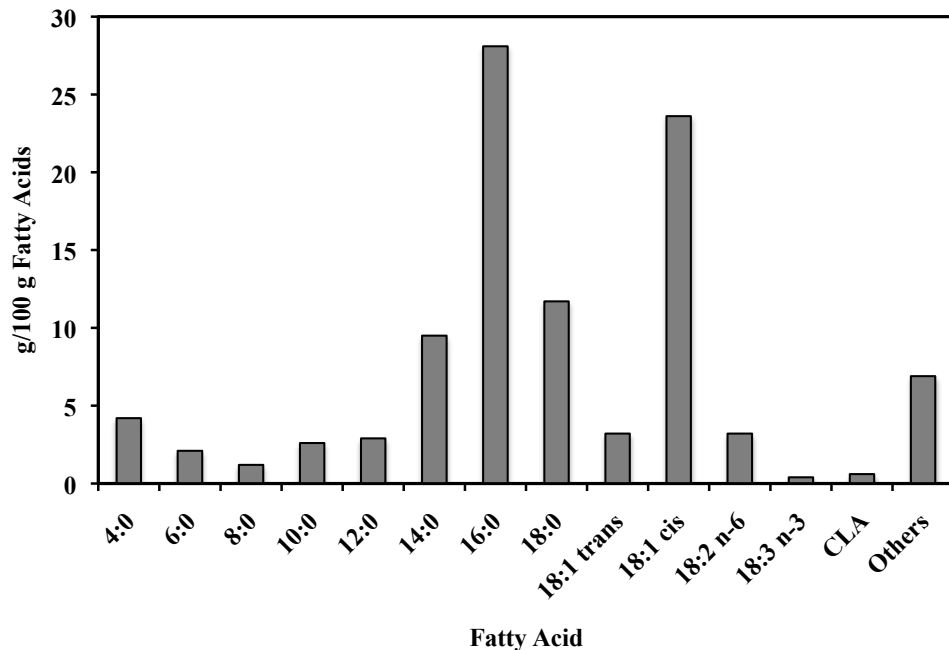
Milk and dairy products have been recognized as important foods for humans since 4,000 B.C. when Egyptian hieroglyphics were first used and when many now-common farm animals were first domesticated, including cows. The dairy sector has made continuous advancement over the years and today there are a wide variety of milks and dairy products readily available to the consumer. The importance of animal-derived foods in meeting the food security needs of the global population is well recognized (Demment and Allen, 2003; Randolph et al., 2007). Dairy products are an important source for many key nutrients including high quality protein, energy, and many essential minerals and vitamins. The recent “Dietary Guidelines for Americans” emphasized that dairy products provide critical amounts of 3 of the 4 essential “nutrients of concern” that are likely to be deficient in the diets of many adults and children: calcium, vitamin D and potassium (USDA, 2010). Fat is the most variable component of milk and accounts for many of the physical properties, manufacturing characteristics, and organoleptic qualities of dairy products. Milk fat content and fatty acid (FA) composition can be significantly altered through nutrition of the dairy cow, and this has been extensively reviewed elsewhere (e.g. Chilliard et al., 2000; Lock and Shingfield, 2004). This research has often involved studies designed to achieve shifts in the ratio of saturated FA (SFA) to polyunsaturated FA (PUFA). While modest changes have been achieved, this can often negatively affect cow performance and lead to challenges relating to the quality and stability of dairy products.

For over half a century, the concept of eating healthy has become synonymous with avoiding dietary fat and cholesterol, especially saturated fat, and on a population basis, a diet low in saturated fat remains at the heart of nutritional advice in many countries for lowering plasma cholesterol and reducing the risk of cardiovascular disease (CVD). In the case of dairy products, there has been a general perception that a food containing saturated fat is unlikely to be beneficial to health. Research, however, continues to unravel the complexities associated with individual FA and fats from different sources and it is becoming increasingly apparent that not all FA, or SFA, have the same biological effects. As will be highlighted in this review, from this research it is clear that broad generalizations about fats can be misleading and often inaccurate; rather one must consider biological effects and nutritional value on the basis of individual FA and within a whole diet context (Lock et al., 2008; Parodi, 2009).

SATURATED FATTY ACIDS

SFA typically comprise about 60-65% of milk FA (Figure 1) and thus milk fat is considered a saturated fat. The 2009 American Heart Association Pediatrics and Adult Nutrition Guidelines re-affirmed a target of reducing saturated fat intake to <7% of total energy intake (Gidding et al., 2009). One means to achieve this is by selecting fat-free (skim) and 1% fat milk and low-fat dairy products, and this has been a central public health recommendation in many countries. Recent estimates indicate that approximately 30% of our dietary intake of saturated fat comes from dairy products with cheese being the major source (Ervin et al., 2004).

Figure 1. Fatty acid composition of retail milk fat in the United States. Constructed using data from O'Donnell-Megaró et al. (2011).

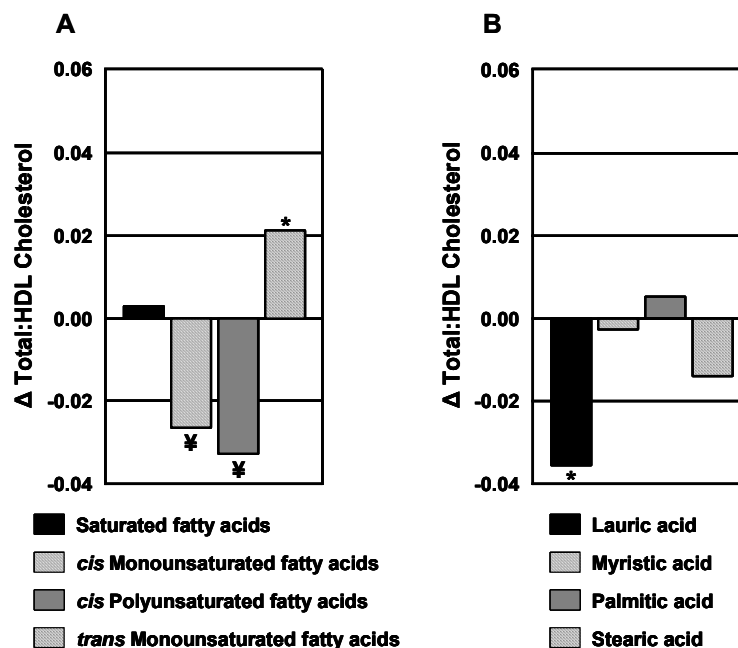


Initial research indicated that SFA intake was the major determinant of circulating cholesterol. However, the relationship of fats, cholesterol and health is far more complex than initially thought and today many dietary, genetic and secondary factors causing hypercholesterolemia have been identified (Grundy and Vega, 1990). In reviewing the history and politics behind the diet-heart hypothesis, Taubes (2001) concluded that after 50 years of research, there was little evidence that a diet low in saturated fat prolongs life. This conclusion is reinforced by recent results from several large-scale investigations. For example, the Women's Health Initiative, an 8-year randomized dietary modification trial involving ~50,000 women, represents the largest dietary intervention ever undertaken; results demonstrated no differences in risk of CHD, stroke or cardiovascular disease (CVD) for the group in which the dietary intervention reduced total fat intake and increased intakes of vegetables, fruits and grains (Howard et al., 2006). Likewise, a recent meta-analysis of 21 prospective

epidemiologic studies concluded, “there is no significant evidence for concluding that dietary saturated fat is associated with an increased risk of CVD” (Sira-Tarino et al., 2010). Clearly, the relationship of fats including saturated fats, cholesterol and CVD is more complex than initially thought and the risk of CVD is multifaceted (Parodi, 2009).

The Nutrition Committee of the American Heart Association has emphasized the diversity of the biological effects of individual FA and the need to evaluate specific FA with respect to a range of variables related to the risk of CHD (Kris-Etherton et al., 2001). The SFA in milk vary in structure and most have no effect on circulating cholesterol and no negative implications with regards to human health. Of the SFA in milk fat, only lauric (12:0), myristic (14:0) and palmitic (16:0) acids have been shown to increase blood levels of total cholesterol and LDL-cholesterol when compared to an isoenergetic substitution with carbohydrate (Figure 2), but these represent only 30-40% of total milk FA (see Figure 1). Further advances in this area have established that lauric, myristic, and palmitic acids also result in increases in circulating HDL-cholesterol, a change that is associated with a reduced risk of CHD (Mensink et al., 2003). Thus, the pattern of changes of circulating cholesterol in different lipid fractions is an important consideration, and several recent investigations suggest that comparisons of the ratio of total cholesterol:HDL-cholesterol is among the best indicators of atherogenic risk.

Figure 2. Predicted changes in the ratio of serum total to HDL cholesterol when carbohydrates constituting 1% of energy are replaced isoenergetically with fatty acids; A meta-analysis of 60 Trials. Panel A: saturated, cis monounsaturated, cis polyunsaturated, or trans monounsaturated (* = P < 0.05; ¥ = P < 0.001). Panel B: lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), or stearic acid (18:0) (* = P < 0.001; Mensink et al., 2003).



Despite the large number of studies on the effects of changing dietary lipid type it is worth noting that only a very limited number of intervention studies have examined the benefits of reduced-SFA dairy products on CVD risk factors in humans. Those that have been done were of fairly short duration and relied almost entirely on effects on plasma cholesterol (see review by Givens and Minihane, 2009). It is also important to recognize that individuals do not consume SFA as a dietary entity, but rather as fats in food, and this will be discussed further in the final section of this review.

TRANS FATTY ACIDS

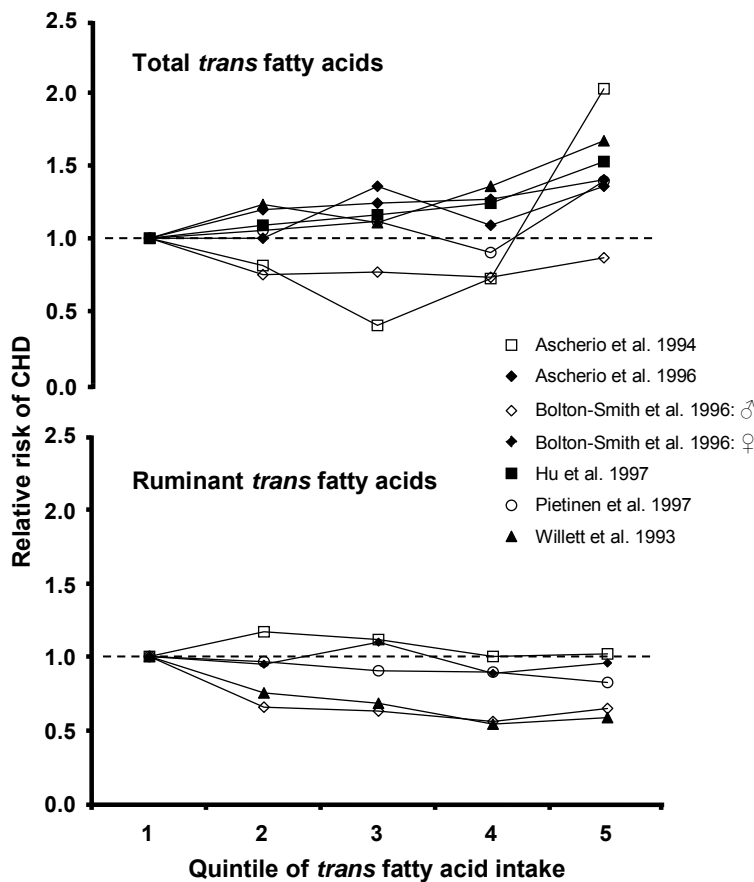
Milk fat contains about 2-4% TFA, mainly *trans* 18:1 with minor amounts of *trans*-PUFA such as CLA. The double bond in UFA present in foods is typically of a *cis* configuration, but TFA have been of considerable interest in recent years due to their association with increased risk of CVD and other chronic diseases. *Trans* double bonds are introduced into FA by one of two means, chemical processes during the formation of partially hydrogenated vegetable oils (PHVO; industrial sources) or as FA intermediates formed during rumen biohydrogenation (ruminant sources). PHVO have been used extensively in many prepared foods such as bakery products, cooking fats, margarine and fried products. In the last few years a number of countries have established policies aimed at reducing TFA intake in the human diet (Lock et al., 2005b).

Estimates for worldwide consumption of TFA have recently been reviewed (Craig-Schmidt and Rong, 2009). Over the last decade, available data from the US indicates that mean or median adult intake of *trans* fat is approximately 2 to 3 g/d or 1 to 2% of daily calories as estimated from semi-quantitative food frequency questionnaires, and 4 to 8 g/d or 2 to 3% of daily calories based on diet recalls and diet records; these estimates are lower than those published in the mid 1990's (Craig-Schmidt and Rong, 2009). In response to the various labelling requirements for TFA, the food industry has been transitioning to alternative practices that allow for a marked reduction in the use of PHVO in processed food products. As a consequence the TFA intake from industrial sources is declining, whereas the intake of TFA from ruminant sources has remained more or less constant; the net result is a reduction in the dietary intake of TFA but the proportion of total TFA intake from ruminant sources is gradually increasing. As Craig-Schmidt and Rong (2009) highlighted, within a few years the TFA in the food supply will be mostly limited to the „natural“ supply present in ruminant-derived fats in meat and dairy products. Thus, understanding the biological effects of TFA found in dairy products and differences among TFA isomers are of great importance.

PHVO generally contain about 40-60% TFA and the isomer profile is a Gaussian distribution that centers on *trans*-9, *trans*-10, *trans*-11 and *trans*-12 18:1. In contrast, the major TFA isomer in ruminant fat (milk and meat) is vaccenic acid (*trans*-11 18:1; VA). Differences in isomer profile are of importance because the position of the *trans*-double bond can influence both physiological properties and the rate of biochemical reactions (Lock et al., 2005b).

Over the past 50 years the relationship between dietary TFA intake and plasma lipid levels and human health, particularly CHD, has been extensively investigated. Data from controlled human intervention studies have consistently demonstrated that diets containing TFA result in increased serum total-cholesterol and LDL-cholesterol, and decreased HDL-cholesterol (see Figure 2, Panel A). Prospective epidemiological studies have consistently supported findings from intervention studies further indicating that higher intakes of TFA are associated with increased risk of CVD. These results have been broadly extrapolated to imply that high consumption of all sources of TFA is associated with an increased risk of CHD. However, further examination of the epidemiological investigations reveals that the positive association with risk of CVD can be explained entirely by the intake of industrial TFA (Figure 3). In contrast, the relationship between intake of ruminant-derived TFA and risk of CVD observed for these studies is a significant negative association, an inverse non-significant association, or no association (Figure 3).

Figure 3. Relative risk of coronary heart disease with increasing relative intake (quintiles) of total and ruminant-derived TFA. Risks are relative to the risk in the lowest quintile of TFA intake; the fully adjusted model is presented for each study. Adapted from Lock et al. (2005b).



The difference between TFA sources and the risk of CHD probably relates to differences in the TFA isomer profile as discussed earlier. In addition, an important related aspect is that the VA in milk fat can be converted to RA via $\Delta 9$ -desaturase. Consistent with this, biomedical studies with the hamster model demonstrated that a TFA-enriched milk fat had positive effects on plasma lipoprotein biomarkers (Lock et al., 2005a). Several studies have established that humans are capable of this conversion, with approximately 20% of VA converted to RA in humans, thereby doubling the CLA supply (Palmquist et al., 2005). Thus, this enzyme system may be key in differentiating VA from other trans 18:1 fatty acid isomers.

Finally, the impact of milk fat naturally enriched in TFA has been recently examined in human clinical studies (Chardigny et al., 2008; Motard-Bélanger et al., 2008). It is important to note that the *trans* fats found in dairy products are consumed in low amounts and results from these clinical studies indicate that current levels of intake have no significant impact on CVD risk factors. For additional information, we recently published a comprehensive review of epidemiological, clinical, and mechanistic studies examining the effects of ruminant TFA on CVD and cancer (Gebauer et al., 2011).

DAIRY-DERIVED FATS IN FOODS & HUMAN HEALTH

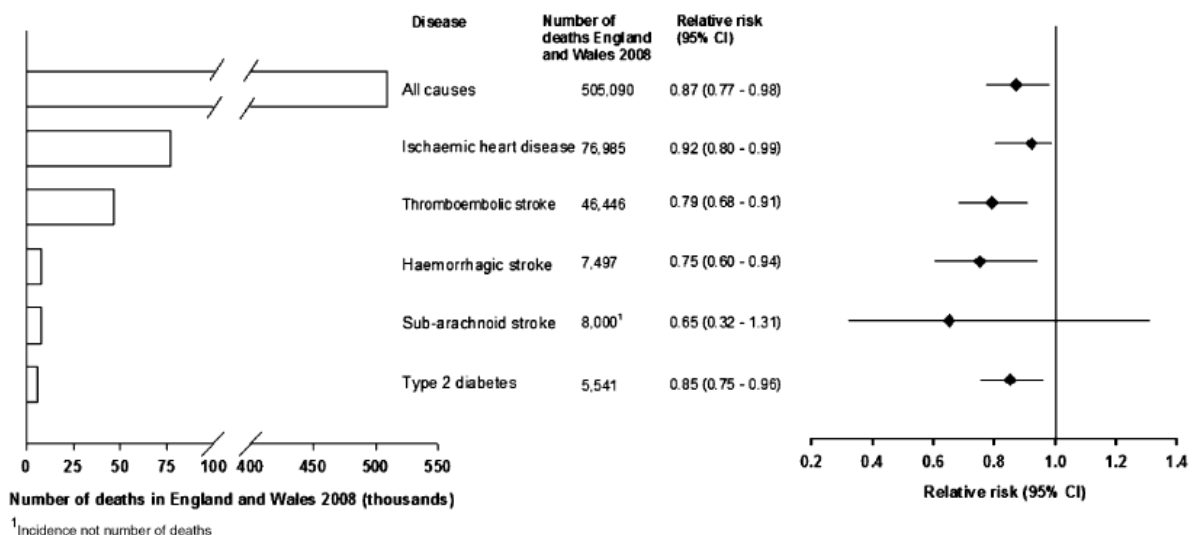
As mentioned previously, over the last decade, evidence has been accumulated that the composition and quantities of dietary fat is very important in determining the relative risk to diseases such as CVD and cancer, and that milk-derived fat may offer significant health benefits compared to some common sources of dietary fats. It is particularly important to recognize that individuals consuming dairy fats do not just consume SFA and the other groups of FA discussed in this review, but rather as fats in the whole dairy food which is highly complex and may contain beneficial ingredients.

The appropriateness of recommendations regarding the intake of dairy products (particularly in relation to reducing saturated fat intake by reducing dairy consumption) has been challenged by conclusions from a number of recent meta-analysis and data summaries including those by German et al. (2009) and Elwood et al. (2008, 2010). The long-term effects of milk and dairy product consumption on health would ideally be tested in adequately powered randomized control intervention studies with disease/death events as the key outcomes. So far no adequate studies of this type have been reported. As a result, Elwood et al. (2008; 2010) concluded that the most valuable evidence on associations between milk and dairy products and health and survival would be provided by long term prospective cohort studies. These avoid the weaknesses of case-control studies and of using markers of risk such as plasma cholesterol. The cholesterol weakness is important; while some FA in milk affect circulating cholesterol, a sole focus on cholesterol as a risk for CVD would fail to consider risk-reducing properties in milk as a whole.

Elwood et al. (2008) reported on meta-analyses that examined the associations between milk and dairy products and health and survival. Their results provided convincing evidence that a high intake of milk can provide long-term reductions in the

risk of CVD. The relative risk (RR) of stroke and ischemic heart disease in subjects with high milk or dairy consumption was shown to be 0.79 (95% CI 0.75 to 0.82) and 0.84 (95% CI 0.76 to 0.93) respectively, relative to the risk in those with low consumption. Figure 4 shows the numbers of deaths in England and Wales in 2008 from various causes, and the risks for these causes in subjects with the highest milk/dairy consumption, relative to subjects with the lowest milk/dairy consumption. This work has been extended to examine the evidence for differential effects of milk, cheese, and butter on incidence of vascular disease (Elwood et al., 2010). They found that there were very few prospective cohort studies available for cheese (five) and butter (six). For butter only three studies were suitable for meta-analysis yielding a non-significant RR for high vs. low consumption (0.93, 95% CI 0.84 to 1.02). For cheese only two studies were suitable for meta-analysis. While there is some additional evidence related to butter and cheese from retrospective case-control studies, this is weaker evidence than from cohort studies and overall this highlights a large gap in knowledge. Similarly, there are few studies that report disease rates in subjects who consume „regular-fat“ dairy foods, and in those who consume reduced-fat dairy foods. Subjects on low-fat milk, however, hopelessly confound the limited data available, due to the adoption of other health-related behaviors. The appropriate question to ask therefore is “do fat-reduced milks and dairy foods provide any additional advantage to human health, or does the reduction in fat reduce the benefits of whole milk and dairy products?”

Figure 4. The numbers of deaths in England and Wales in 2008 from various causes and the risks for these causes in subjects with the highest milk/dairy consumption, relative to subjects with the lowest milk/dairy consumption. Adapted from Elwood et al. (2010).



These results provide the best evidence available that those who consume large quantities of milk are at no greater risk of CVD than those who consume little and indeed there appears to be a small but valuable reduction in risk of CVD from increased consumption. As noted above, the evidence for cheese and butter is inconclusive.

These findings are in broad agreement with the recently reported outcome of a remarkable 61-year follow up of the Boyd-Orr cohort. This study involved the recruitment of 4,999 children in England and Scotland in 1937-39 with causes of death recorded from 1948 (van der Pols et al., 2009). Results demonstrated that a family diet in childhood, which was high in dairy products, did not give rise to a greater risk of CVD or stroke mortality. Indeed all-cause mortality was lowest in those with the highest dairy product and milk intake (basic Hazard Ratio for both, 0.69; 95% CI 0.57 to 0.84; P for trend <0.002). These findings are therefore suggestive that despite milk fat being rich in SFA, milk has properties that are beneficial in reducing the risk of CVD.

Overall, the available evidence does not support the concept that consumption of dairy products adversely affects the risk of CVD and indeed linking the benefits of milk consumption with deaths from key chronic diseases led Elwood et al. (2010) to conclude that high milk consumers have an “overall survival advantage” (Figure 4). It is unfortunate that due to a focus on the small rise in blood cholesterol with milk consumption, the debate on milk has never achieved a reasonable balance in the evaluation of risks and benefits. Several bioactive FA found in milk fat, milk proteins, and other components have potential benefits for health maintenance and the reduction of chronic disease risk, and this reinforces the need for the dietetic community to reconsider current recommendations on dairy products and human health. Continued recommendations to reduce milk fat intake may result in inadequate intakes of key nutrients in certain population groups. For additional information on the scientific evidence related to the impact of dairy product consumption and milk fat in human diets on overall health the reader is directed to the recent symposium review papers published in the *Journal of the American College of Nutrition* (Lock et al., 2008) as well as a review by German et al. (2009) which also provides future suggestions for milk fat-human health research.

CONCLUSION

In summary, despite the contribution of dairy products to the saturated fat intake of the diet, there is no clear evidence that dairy food consumption is consistently associated with a higher risk of CVD. Indeed, there appears to be an enormous disconnect between the evidence from long-term prospective studies and perceptions of harm from the consumption of dairy products (Elwood et al., 2010). Given the diversity of available dairy foods of widely differing composition and their contribution to nutrient intake within the population, recommendations to reduce dairy food consumption irrespective of the nature of the dairy product should be made with caution.

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SUSTAINABILITY AND DAIRY PRODUCTION: CHALLENGES AND OPPORTUNITIES¹

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INTRODUCTION

The productivity of the American farmer has increased dramatically over the last one hundred years. At the start of the 20th century a farmer produced enough food to feed less than 15 people and over 40% of the U.S. population was involved in agriculture-related businesses. By the 1940's farms were still diversified and productivity had increased to where a farmer produced sufficient food to feed about 20 people. Today the food produced by an average farmer feeds 155 people and farmers represent less than 2% of the U.S. population. Over the last 6 decades, the yield of grain crops and productivity of domestic animal species has more than doubled. As a consequence U.S. agriculture produces nearly one-fifth of the world's milk, eggs, and grain and about one-fourth of the world's beef. Increases in agricultural productivity over the last century mean that when compared to other global regions, American consumers also spend the lowest percent of their annual income on food - around 10%. This provides the opportunity for our population to pursue the wide range of lifestyles that we enjoy today.

Sustainability has historically been considered to be an economic issue; a sustainable system was one that produced food at a price that consumers could afford while providing sufficient income for the producer. More recently sustainable production has taken on a broader context and represents a system that balances economic viability, environmental impact and social acceptability. Thus, a sustainable agriculture system includes an economic dimension represented by an industry that is productive, efficient and profitable; an environmental dimension characterized by an industry that makes the most effective use of resources, maintains air and water quality and preserves wildlife habitat and rural landscape; and a social dimension demonstrated by an industry that cares for and takes into consideration the community, employees and animal welfare. In short, a sustainable agricultural system is one that provides for basic human food and fiber needs, is economically viable and enhances the quality of life of producers and society as a whole while preserving the resource base and environmental quality on which the future of agriculture depends.

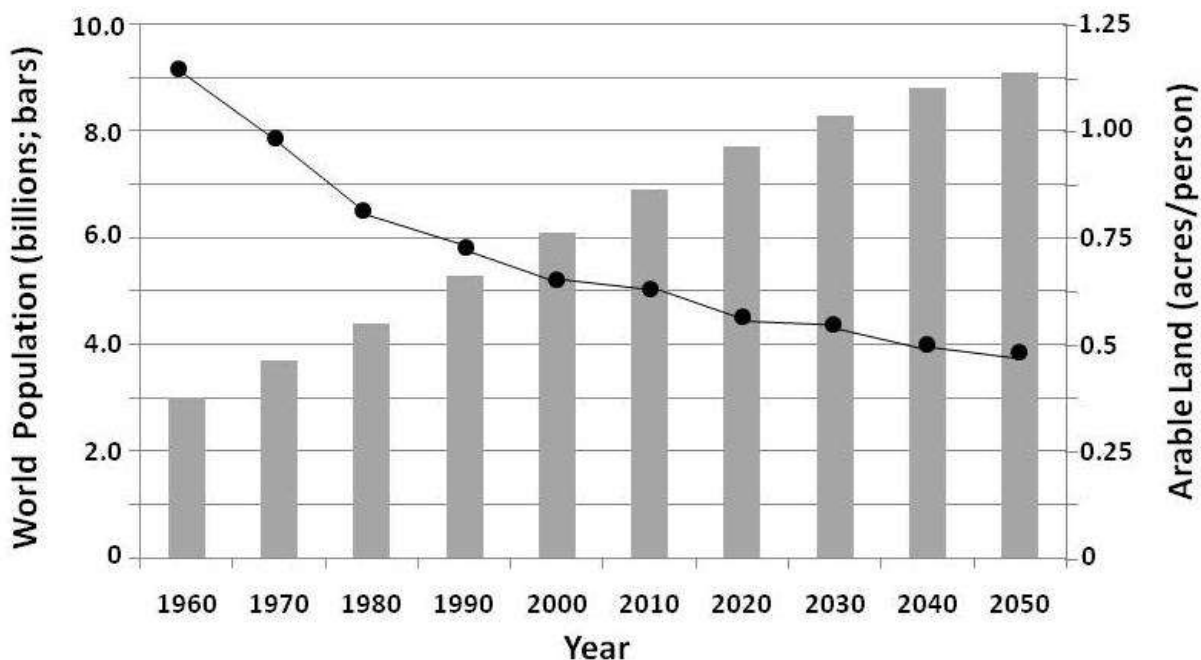
The IFPRI (1995) has eloquently articulated the global vision for a sustainable agriculture system as follows:

¹Portions of this paper are from an article by Bauman and Capper (2011) and Capper (2011).

“a world where every person has economic and physical access to sufficient food to sustain a healthy and productive life, where malnutrition is absent, and where food originates from efficient, effective, low-cost food and agricultural systems that are compatible with sustainable use and management of natural resources.”

Food security is a major challenge; on a global basis an estimated 925 million people are undernourished and 16,000 children die from malnutrition each day. In the U.S. more than 17 million American children are at risk of hunger and one in five families are food insecure (USDA/ERS, 2009). Thus, in spite of the remarkable growth in food production, many people do not have an adequate dietary intake of energy and protein, and even more suffer from some form of micronutrient malnourishment (UN/FAO, 2009). As we look to the future, achieving global food security will be an even greater challenge (Figure 1). The world population is estimated to increase to over 9 billion by the year 2050 (U.S. Census Bureau, 2008) and the arable land per person will continue to decrease (Figure 1). The UN/FAO (2009) concluded that 70% more food will be needed by 2050 and that limitations in land, water and other resources mean that 80% of this additional food supply must come from improved productivity. This essential improvement in productivity has been referred to as “sustainable intensification” (Godfray et al., 2010) and illustrates the key role that technology and improved efficiency will play in meeting future food needs.

Figure 1. Comparison of the global population with available arable land per person from 1960 to 2050. Figure constructed by authors using World population estimates from the U.S. Census Bureau (2008) and arable land/person estimates from Bruinsma (2009).



The objective of our presentation is a broad consideration of several key issues related to sustainability of animal agriculture, particularly the dairy industry. We have focused on the challenges and opportunities in three areas – productive efficiency, environmental issues and dairy products as foods.

THE IMPORTANCE OF PRODUCTIVE EFFICIENCY

In 1944, the U.S. dairy herd peaked at 26.5 million cows; the typical dairy farm was diversified with a herd size of about 6 cows and an average daily milk production of less than 7 kg/cow (Capper et al., 2009a). This contrasts sharply with the specialization of the modern dairy industry where cows have an average milk production over 30 kg/d and about 60% of the U.S. milk supply comes from dairy farms with over 500 cows (USDA, 2007). These impressive gains in daily milk production per cow over the last 6 decades reflect a better understanding of the biology of the dairy cow and the application of this knowledge to improve genetic techniques to select the most productive cows and the application of management practices and new technologies to support a high level of milk production. Genetic gains are estimated to represent about two-thirds of the improvement in milk yield per cow over the last 6 decades. The implementation of artificial insemination (AI) and genetic selection programs has been complimented by advances in feed analysis and diet formulation; improvements in milking systems; developments in cow comfort and facility design; and progress in disease treatment and the implementation of herd health programs. Thus, the dairy industry has utilized AI and genetic selection to increase the milk production potential of dairy cows and at the same time implemented management practices and technologies which provide an opportunity for cows to achieve their genetic potential.

So what is the biological basis for the high milk yield in genetically superior cows? Differences in nutrient partitioning provide the major biological basis for milk yield differences between high and low-yielding dairy cows. High-yielding dairy cows partition a greater portion of nutrient intake to support a higher milk yield and this is accompanied by an increased voluntary feed intake (Bauman et al., 1985; Reynolds, 2004). If a low-producing cow has a high nutrient intake she simply partitions the extra nutrients to body fat rather than to produce milk and milk components.

The increase in milk yield per cow is important to the dairy industry because it affects “productive efficiency”. We define productive efficiency as “milk output per unit of resource input”, and the advantage from improved productive efficiency relates to what is referred to as the “dilution of maintenance” effect (Bauman et al., 1985; VandeHaar and St-Pierre, 2006). Each day, the lactating dairy cow requires nutrients for maintenance and for milk synthesis. The maintenance requirement does not change with production level and can, therefore, be thought of as a fixed cost needed to maintain vital functions. Assuming milk composition remains constant, the nutrient requirement per unit of milk does not change, but the total energy cost for lactation increases as a function of milk yield. The lactation energy requirement can, therefore, be considered a “variable cost” of dairy production. This is illustrated in Figure 2 where the ME requirements of an average cow for 1944 and 2007 are compared (Capper et

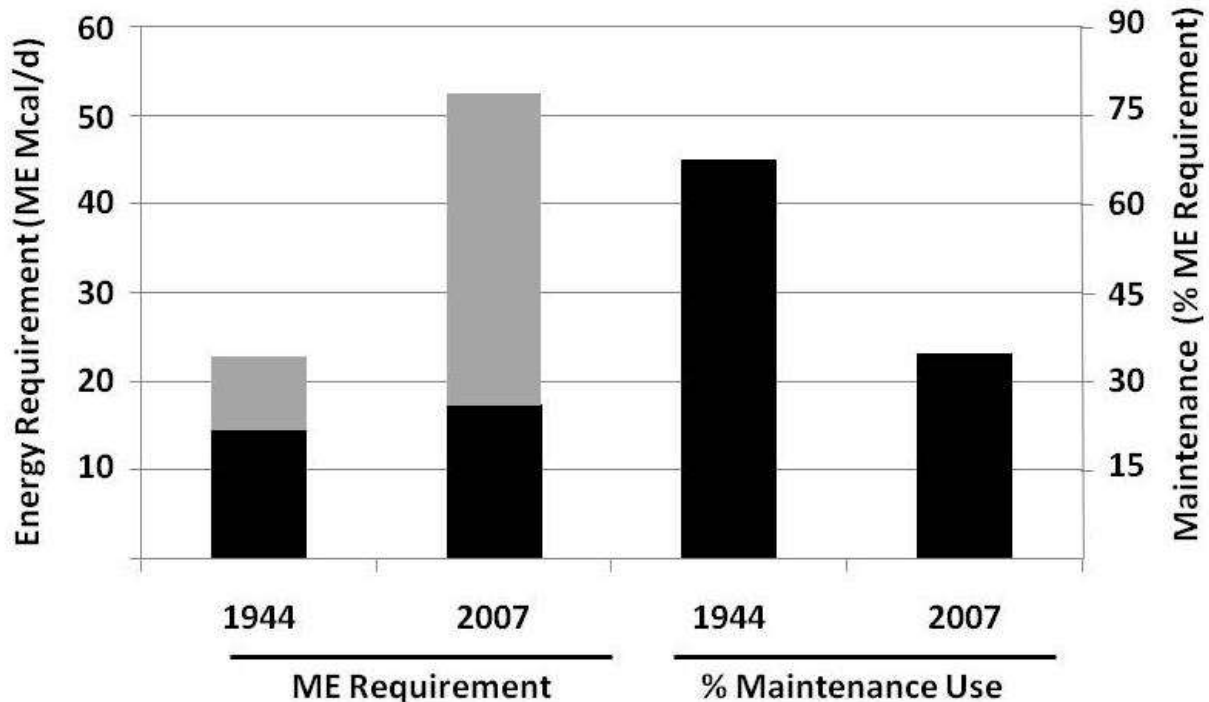
al., 2009a). In 2007, the average performance of a dairy cow resulted in a daily ME requirement over twice that of the average cow in 1944. This was mainly due to a higher milk production, although maintenance was marginally greater in 2007 because of the industry shift to a higher proportion of large-breed cows. The key comparison is the difference in nutrient use; in 1944 the average cow utilized 65% of ME intake for maintenance and only 35% for milk synthesis. These numbers are essentially reversed in 2007 where the average cow utilized only 33% of the ME requirement for maintenance and 67% of ME for the production of milk. As milk production increases, the total nutrient requirement also increases but productive efficiency is improved because the fixed cost (maintenance) is diluted out over more units of milk production. The net result is the energy requirement per unit of milk output is reduced and a unit of milk can be produced using fewer nutrients with less animal waste (Bauman et al., 1985; Capper et al., 2009a).

Productive efficiency gains for today's dairy industry notwithstanding, some consumers perceive that historical methods of food production are more environmentally friendly and better for cow welfare and well-being. This perception is frequently reinforced by an idyllic vision of the "good ole days" where cows grazed peacefully on a lush green hillside with the red hip-roof barn off in the distance. Modern dairy farms are often referred to as "factory farms" where it is claimed that cows are maintained in "filthy and disease ridden conditions"², and the milk they produce is "awash in fat, cholesterol, antibiotics, bacteria and pus"³. These claims, which show a disappointing ignorance of the dairy industry on many levels, are oft-repeated, especially by animal rights groups, and they are reinforced by videos and media accounts of animal abuse that are presented as typical of modern animal agriculture. Participants at the Cornell Nutrition Conference are well aware of management practices on commercial dairy farms, but we need to remind the public that dairy managers take pride in their operation. We need to emphasize that economic viability and welfare of the dairy herd are irrevocably connected and modern dairy producers strive to follow best management practices that benefit the productivity and welfare of their herd. Diets are balanced according to the latest computer-based ration formulation programs to meet the cow's nutrients requirements and they are fed as total mixed rations to maximize nutrient use and minimize losses. Commercial dairy operations house cows in a facility where temperature and ventilation are controlled and continuous access to water, feed and a dry bedded area is provided. The milking operation uses best practices for proper udder sanitation, milk let-down and milk removal. Modern dairies follow a rigorous herd health program throughout the life cycle of the dairy animals and if a calf or cow becomes ill they are treated by the herd veterinarian following the latest procedures and using efficacious treatments.

²Nierenberg, D. (Animal Agriculture and Climate Change Specialist, Humane Society of the United States). Comment at the Hudson Institutes Conference on Food for the 21st Century: Challenging the Conventional Wisdom, Washington DC, September 10, 2008.

³Heimlick, J. (author of many health and nutrition books). Comment from Heimlicks' Forward in the book Milk the Deadly Poison by R. Cohen, Argus Publishing, Inc., 1997.

Figure 2: Illustration of the “dilution of maintenance” effect. Bars for metabolizable energy (ME) requirement depict the portion of maintenance (dark and the portion for milk synthesis (shaded). Bars for % maintenance requirements for an average cow in the 1944 and 2077 dairy production systems. Adapted from Capper et al. (2009a).



Nevertheless, some scientists question whether high milk production and improved productive efficiency are contrary to the health and well-being of dairy cows (Rauw et al., 1998; Broom, 2001; Knaus, 2009). For example, Broom (2001) suggested that it may be necessary to stop “using genetic selection and some feeding methods that increase milk yield” because cows become stressed and “their normal biological controls are overtaxed”. Does the science support claims that cows on modern large farms are stressed and of poor health, and that high production and increases in productive efficiency are pushing cows too far?

Over the last 60 years gains in knowledge relating to the regulation of nutrient partitioning have provided an understanding of how essential biological processes are coordinated to maintain well-being in lactating dairy cows (Bauman and Currie, 1980; Bauman, 2000; Collier et al., 2005). This coordination operates on an acute basis (homeostatic regulation) to ensure constant conditions are maintained, and on a longer-term basis (homeorhetic regulation) to ensure adequate nutrient partitioning to support essential physiological functions and mammary synthesis of milk. The claims that high producing dairy cows are stressed and their welfare is compromised have been raised at regular intervals over the last half century. In each instance, scientists have found no support for these claims and concluded that they are based on a failure to understand

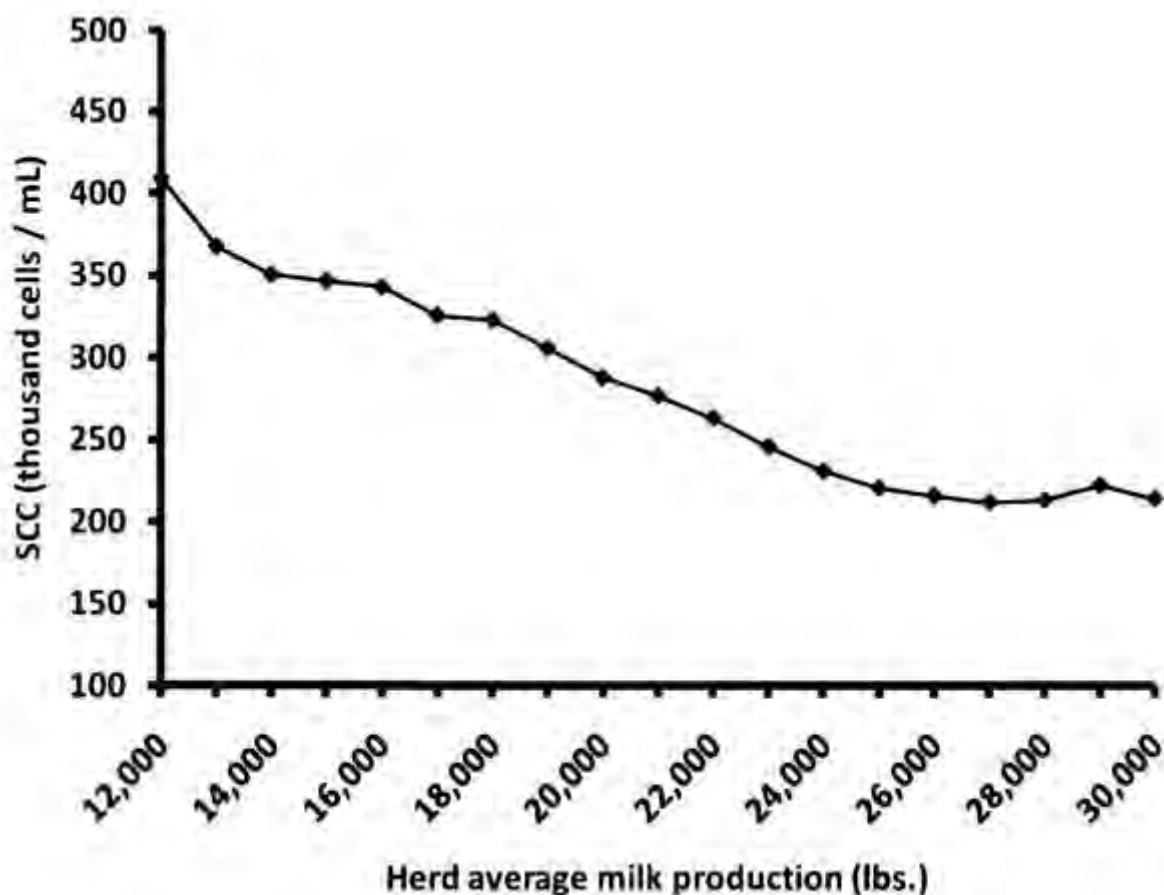
the biology of lactation (for examples, see Hammond, 1952; Bauman et al., 1985; Reynolds, 2004; Collier et al., 2005). Dairy herd managers, veterinarians and dairy consultants know that the performance of a dairy cow provides an irrefutable indication of her health and well-being. High-producing cows are not stressed and sick because they have an increased milk output; rather they achieve high milk production because they are healthy and have minimal stress. A clear example of this is provided by examining somatic cell counts (SCC). SCC is associated with mastitis and thus it represents an important milk measure that reflects mammary health, milking management and milk quality. When the major factors causing mastitis are accounted for, there remains a positive genotypic and phenotypic correlation between mastitis incidence (cases per cow) and milk yield (Wilton et al., 1972). Nevertheless the effect is very small, and mastitis is primarily associated with the quality of management and environmental factors. The importance of quality of management is evident by the fact that on a herd basis, the average milk SCC declines as average milk yield per cow increases (Figure 3), providing a clear refutation of the previously discussed perceptions that the health and welfare of high producing cows are compromised. It bears repeating that modern dairy cows achieve a high milk production because they are healthy and have minimal stress.

There must be an upper limit where the biological controls regulating nutrient utilization for milk synthesis are maximized, but that maximum plateau is not obvious. Today, top herds have an annual production average over 14,000 kg/cow with record cows producing over 30,000 kg. It is interesting to note the dilution of maintenance effect in these cows: in herds with an annual milk average of 14,000 kg, cows are utilizing about 75% of their ME requirement for the synthesis of milk, and for the current world record cow the value approaches 85%. Performance is the best indicator of a dairy cow's well-being and we know from the records set by top cows that the biological control systems will allow for increases in milk yield to at least these current record levels.

ENVIRONMENTAL ISSUES

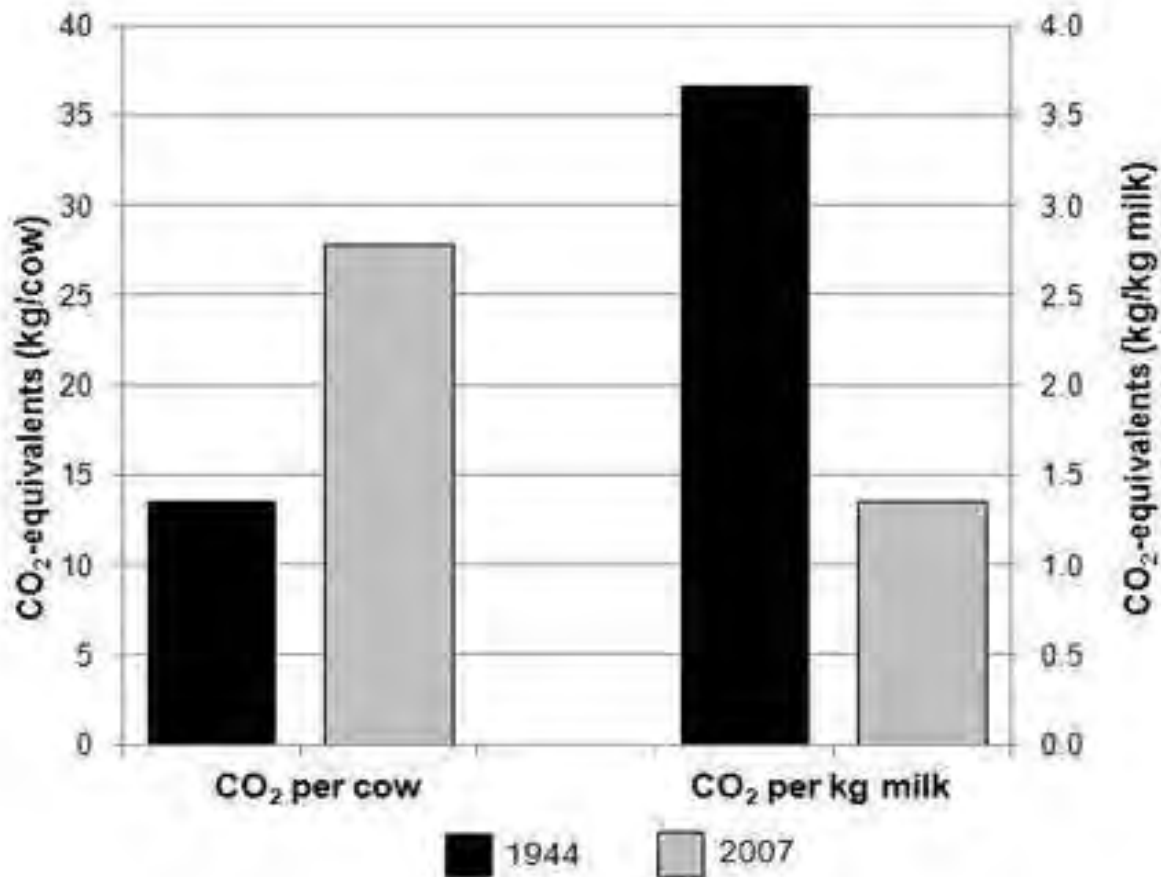
“Green” has become the color of the decade, and surveys indicate that environmental considerations are of increasing importance in consumer choices, including food purchases. All food production has an environmental effect and the environmental impact of dairy production is of particular significance. In December 2009 USDA and the Innovation Center for U.S. Dairy signed a Memorandum of Understanding to work jointly in support of the goal to reduce the dairy industries greenhouse gas (GHG) emissions by 25% over the next decade (Innovation Center for U.S. Dairy, 2010). However, some consumers romanticize older, inefficient production methods and perceive that dairy sustainability can best be achieved by extensive, low-input systems. It's an image that has emotional and philosophical appeal, but is it supported by science?

Figure 3. Comparison of milk somatic cell counts (SCC) and annual milk production per cow. Data are for 16,768 herds over the period of November 2009 to October 2010, with each point representing all herds at that annual level of milk production. From Bauman and Capper (2011) with data compiled and figure constructed by H.D. Norman and J.R. Wright (USDA-ARS-AIPL).



Feed and milk production comprise about 80% of the total environmental impact of dairy foods in industrialized countries, and an even greater percent in developing world regions (UN/FAO 2010). We recently used a whole-system model to quantify the environmental impact of milk production on U.S. dairy farms circa 1944 as compared to 2007 (Capper et al., 2009a). On an individual cow basis, the average 1944 cow had less than one-half of the daily carbon footprint (CO₂-equivalents) of modern high-producing dairy cows (Figure 4). This is consistent with the lower nutrient and resource requirement of the low-producing 1944 cow (Figure 2), so at first glance this appears to support the concept that the “good ole days” were more environmentally friendly. However, the dairy industry exists to produce milk rather than cows. When results are expressed per unit of milk, the advantages gained from improvements in productive efficiency on modern dairy farms are striking. The carbon footprint of a unit of milk produced in 2007 is only 37% of that in 1944 (Figure 4). The reduction in the carbon footprint of milk production over the last half-century represents a remarkable success

Figure 4. Comparison of the carbon footprint on a per cow basis and on a kg milk basis for 1944 and 2007 dairy production systems. Adapted from Capper et al. (2009a).



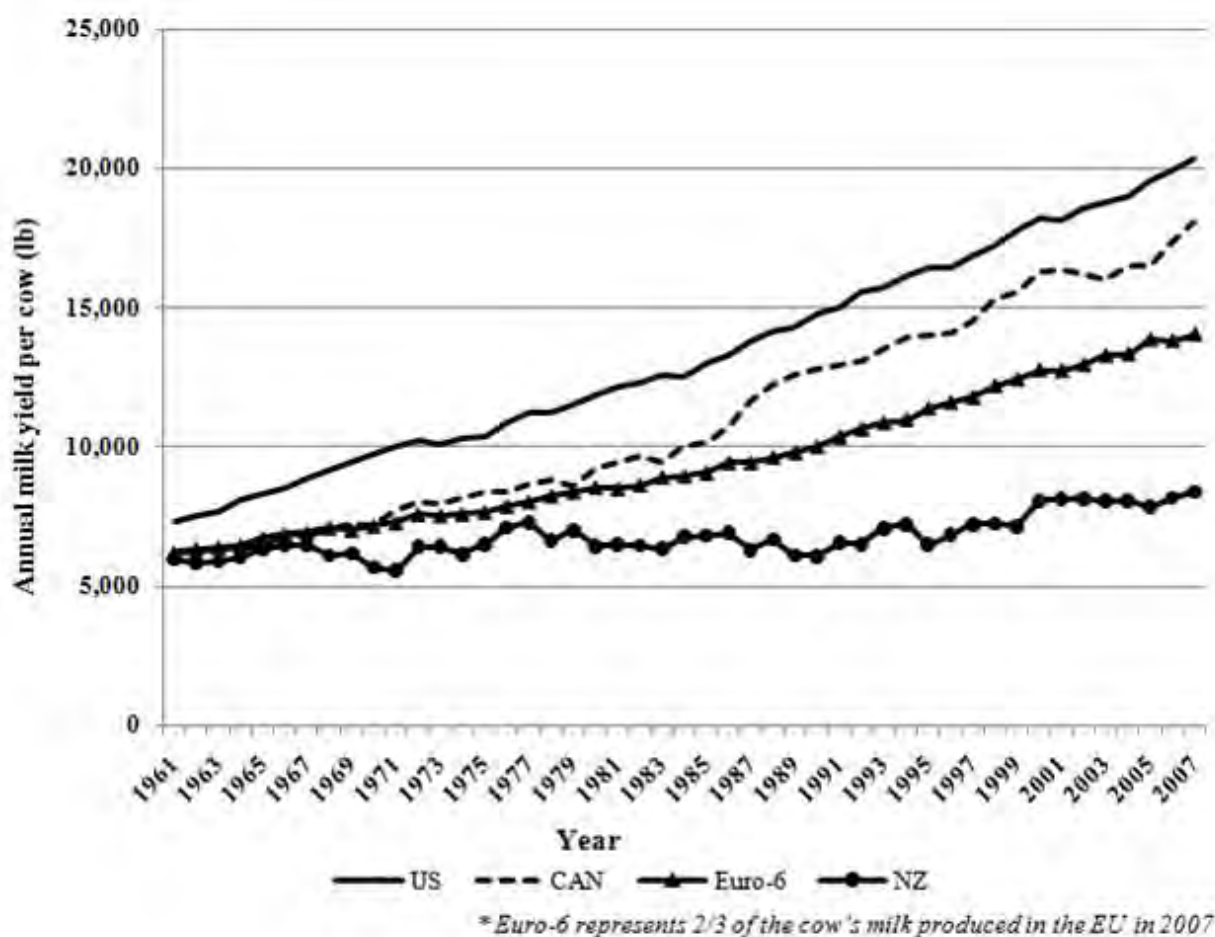
story for the environmental impact and sustainability of the U.S. dairy industry. As a consequence of productive efficiency gains between 1944 and 2007, the production of an equal quantity of milk in 2007 requires only 21% of the animals, 23% as much feed, 35% of the water, 10% of the land area and produces only 24% as much animal waste (Capper et al., 2009a). Particularly impressive is a comparison of the total dairy industry. In spite of reductions in cow numbers (9 million in 2007 vs. 25.6 million in 1944), the 2007 dairy industry produced 59% more milk with a total carbon footprint that was 41% less than the 1944 industry (Capper et al., 2009a).

If we examine international trends, increased milk production has a mitigating effect upon carbon emissions on a global basis. The environmental effects of regional variations in productivity are exemplified by the results of a recent UN/FAO (2010) report that modeled GHG emissions from dairy production using life cycle analysis (LCA). As intensity of production declines and the average milk yield shifts from approximately 9,000 kg/cow for North America to ~250 kg/cow for Sub-Saharan Africa, the carbon footprint increases from 1.3 kg CO₂-eq/kg milk to 7.6 kg CO₂-eq/kg milk

(Figure 5). However, assessing dairy system sustainability should not be limited to the environmental impact of dairying within a specific region, but must also consider economic and social implications. While the UN/FAO data could provoke the conclusion that all regions should adopt North American and Western European-style production systems, or that dairying should be focused in these areas and be discouraged in less productive regions such as Sub-Saharan Africa and South Asia, the significant social (both status and nutritional) and economic value of dairying in less-developed regions must not be underestimated. The challenge for global dairy production is to optimize sustainability within each region rather than prescribing the best “one-size-fits-all” global system.

The variation in carbon footprint among regions of the world reflects a wide range of system efficiencies. The efficiency of production systems also differs among industrialized regions. Figure 6 shows trends in milk production per cow from 1960 to 2007 for the U.S., Canada, an aggregate of the top-6 milk producing countries in Europe (Netherlands, UK, Germany, France, Italy, Poland), and New Zealand. Although milk yields were similar among regions in 1960, the lines have diverged markedly over time. The U.S. has shown the fastest rate of improvement, Canada and Europe are intermediate and New Zealand production has remained relatively static. Thus, in 2007 the average U.S. dairy cow produced over 9,100 kg milk per year in highly efficient U.S. dairy production systems as compared to annual production values of about 8,400 kg/cow for Canada, 6,400 kg/cow for the top 6 milk-producing countries in Europe, and 3,800 kg/cow for New Zealand (UN/FAO 2010). As discussed earlier, improvements in productivity for the U.S., Canada and Europe were made possible by advances in genetics, nutrition, management and animal health. An example of the effect of technology on environmental impact is the adoption of recombinant bovine somatotropin, which reduces GHG emissions per unit of milk by ~9% (Capper et al., 2008). Differences in the rate of improvement may, therefore, be partially explained by the attitude towards and the adoption of technology and innovative management practices within various regions. The U.S. is generally pro-technology whereas Europe is less receptive (Moses, 1999; Wilcock et al., 2004). The New Zealand dairy system a number of similarities to many U.S. organic dairy systems, and it represents some special challenges. First, it is a pasture-based system with an average lactation length of only 252 days. Second, the daily maintenance requirement is greater due to grazing activity, and milk production is lower due to an inadequate supply and balance of dietary nutrients (Kolver, 2003). Regardless of system specifics, higher productivity of milk with equal composition reduces the environmental impact of dairy production because fewer animals are required to produce the same amount of milk. On a herd basis, producing the same amount of milk with fewer resources (or more milk from the same quantity of resources) reduces the demand for non-renewable or energy-intensive inputs (e.g. land, water, fossil fuels and fertilizers) and promotes environmental stewardship.

Figure 5. Average annual milk yield and carbon footprint per kilogram of milk for selected global regions (Capper 2011). Based on data from UN/FAO (2010).



DAIRY PRODUCTS AS FOODS

Health and wellness are of foremost importance to consumers and diet plays a critical role in health maintenance and the prevention of disease. The value of dairy products and other animal source foods in meeting the food security and nutritional needs of the global population is well recognized (Murphy and Allen, 2003; Randolph et al., 2007) and these are included in dietary recommendations to promote health by governments and public health organizations around the world. The 2010 Dietary Guidelines for Americans recommends low-fat or fat-free milk or milk products at two to three servings/day depending on age (USDA, 2010). Dairy products are nutrient-dense foods and represent the best source for many essential dietary nutrients. At current U.S. intakes, dairy products are a major source of the daily requirements for protein and 9 other essential minerals and vitamins, yet supply only 10% of total calorie intake (Figure 7). Furthermore, the protein in dairy products and other animal source foods is of higher nutritional quality than plant protein sources because of its ideal balance of essential amino acids (Hegsted and Chang, 1965; Murphy and Allen, 2003).

Figure 6. Annual milk yield per cow for four major dairy-producing regions. Adapted from Capper et al. (2009b)

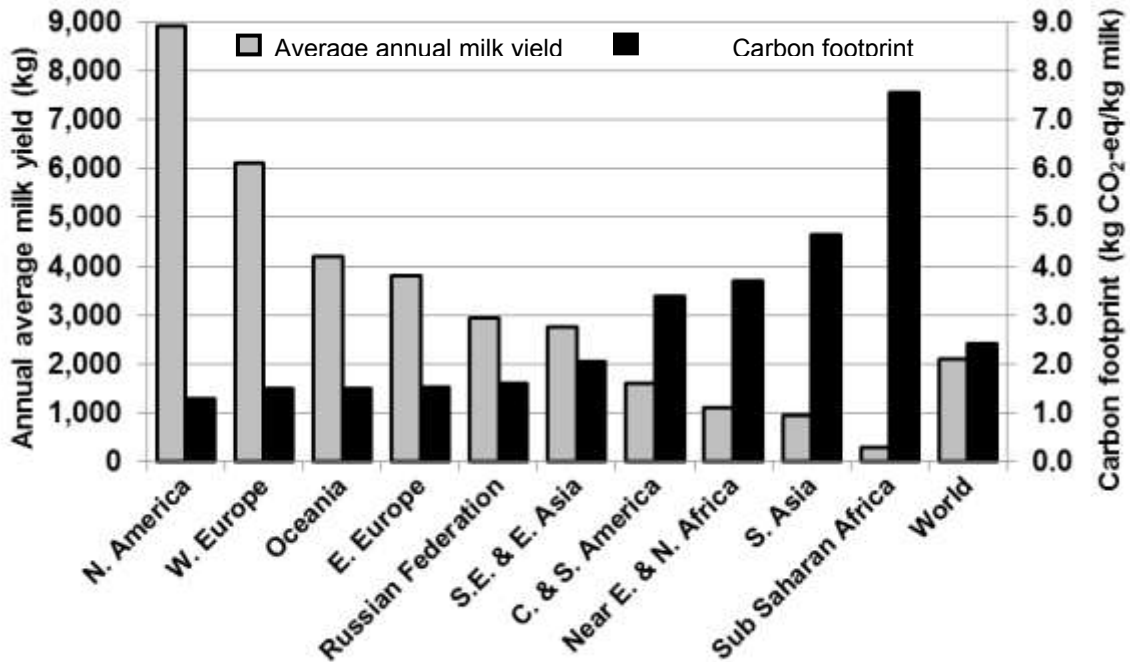
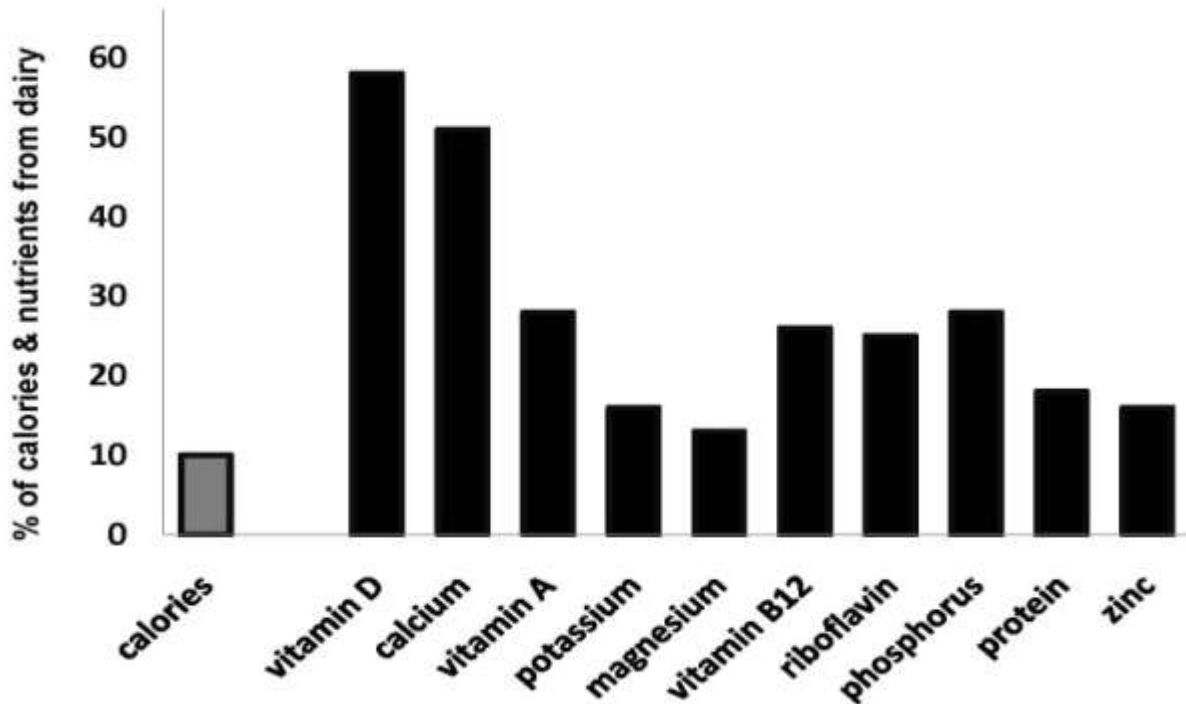


Figure 7. Contribution of dairy products to the daily requirement of key essential nutrients in the U.S.. Figure constructed from NHANES data for 2003-2006 (> 2 yr age) and is available at: <http://tinyurl.com/DairyResearchInstitute>



The nutrient composition of foods is an essential consideration in developing a sustainable agricultural system. Some have overlooked this and advocated policies and practices to alter food consumption by extensively replacing animal-source foods with plant-based foods. One reason often cited by animal rights and vegan groups is the claim that the consumption of dairy products and other animal source foods is harmful and unhealthy. These organizations have mounted media campaigns claiming dairy products and meat are the cause of cancer, cardiovascular disease (CVD), diabetes, and other chronic human diseases. Foods differ in their nutrient content, but does the science support the claim that animal source foods are responsible for adverse health effects and chronic disease?

A clear indication of the importance of animal source foods comes from multidisciplinary studies of children in developing countries. When the diets of schoolchildren had little or no animal source foods, the intake of essential micronutrients was inadequate resulting in negative health outcomes including severe problems such as poor growth, impaired cognitive performance, neuromuscular deficits, psychiatric disorders and even death (Nuemann et al., 2002; Murphy and Allen, 2003). Even in the U.S., milk and dairy products are generally the most economical source of limiting essential nutrients, and Weaver (2009) highlighted the health benefits of including dairy products in vegetarian diets.

Evaluation of the long-term health effects of specific foods would be best determined in randomized controlled trials; however, these studies are nearly impossible, in large part because of the long latency period associated with the development of chronic diseases. Thus, the best evidence comes from prospective cohort studies that have disease events and death as the outcomes - many such studies have involved animal-source foods. Results from individual investigations and meta-analysis of these studies provide convincing evidence that consumption of milk and dairy products is associated with beneficial effects in long-term health maintenance and the prevention of chronic diseases including diabetes, CVD, and many types of cancer (e.g. World Cancer Research Fund/AICR, 2007; Elwood et al., 2008; 2010; German et al., 2010). Overall, the science clearly demonstrates the importance of milk and dairy products in childhood development, health maintenance, and the prevention of chronic diseases.

The report “Livestocks Long Shadow” (Steinfeld et al., 2006) has fueled claims that animal agriculture has a devastating effect on the environment. However, a more recent scientific review of that report revealed inaccuracies in assumptions and methodology and challenged its global extrapolations (Pitesky et al., 2009). Nevertheless, some authors have suggested that policy measures are needed to radically reduce the consumption of animal source foods as a means to reduce global GHG (Garret, 2009; Carlsson-Kanyama and Gonzalez, 2009) and that a global shift towards a vegan diet is vital to save the world (UN/EP, 2010). It’s important to reiterate that the production of all foods has an environmental impact, but does science support the claim that among foods, those produced by animal agriculture have catastrophic effects on our environment?

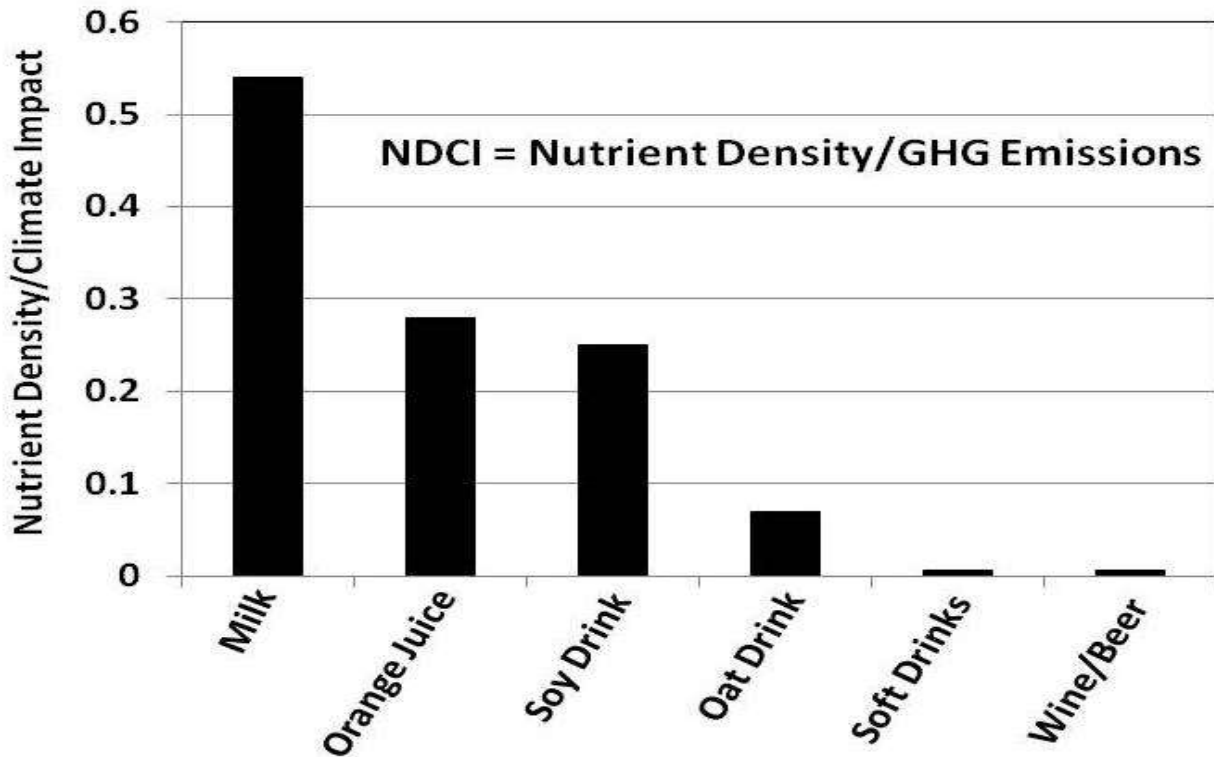
Several studies have used LCA methodology and compared the environmental impact of different food products. In some cases the comparisons were on the basis of mass or dietary energy, and these results indicate that per unit of mass or energy, vegetables and grain production have a lower carbon footprint than the production of dairy products or meat (Carlsson-Kanyama, 1998; Kramer et al., 1999; Dutilh and Kramer, 2000; Weber and Matthews, 2008). However, recommendations to achieve a balanced diet are based on much more than the mass or energy concentration of foods, and such simplistic assessments of environmental impact are meaningless. Rather it is essential that environmental impact be based on the nutritional value of alternative food choices. A few studies have used a more functional nutritional unit by expressing comparisons on the basis of selected macro-nutrients such as energy, protein and fat and these studies also conclude that it is beneficial to replace dairy products and meat with plant foods (e.g. Pimental and Pimental, 2003; Baroni et al., 2007; Carlsson-Kanyama and Gonzalez, 2009; Davis et al., 2010). However, these comparisons did not consider protein quality or bioavailability; plant proteins are typically deficient in one or more essential amino acids, whereas animal source proteins have a near ideal balance of essential amino acids. Furthermore, these studies failed to consider that foods differ in their composition of other essential macro- and micro-nutrients, and it is critical these also be considered in comparisons of the environmental impact of alternative food choices.

Nutrient density index, also referred to as nutrient profiling, is a system that allows comparison of foods based on their content of essential nutrients in relation to the daily recommended values for these nutrients (Fulgoni et al., 2009; Drewnowski, 2010). There are 21 essential nutrients which vary widely in foods, and comparison of the environmental impact of foods requires a functional unit that is relevant from both a nutritional and environmental perspective. Smedman et al. (2010) were first to do this when they compared beverages using an index based on a food's provision of required nutrients (nutrient density; ND) in relation to GHG emissions in production of the food (climate impact; CI). As shown in Figure 8, the advantage was to milk with a substantially higher NDCI index. Thus, when a functional unit that considers both nutritional and environmental perspective is utilized, orange juice, soy drink, and oat drink all have a greater environmental impact than cow's milk. Clearly future considerations of sustainable diets need to utilize a NDCI approach to evaluate both the provision of essential nutrients and the GHG emissions of a particular food item.

CONCLUSIONS

A sustainable agricultural system has the overall goal of producing sufficient nutritious and safe foods that are accessible and affordable for the population. Thus the use of sustainable agriculture practices that maximize efficiency and produce food with fewer resources is critical to balance present and future needs. The U.S. dairy industry has a remarkable record of advances in productive efficiency and environmental stewardship over the last half-century with annual milk/cow increasing over 400% and a two-thirds reduction in the carbon footprint for producing a unit of milk. Furthermore

Figure 8. Nutrient density in relation to climate impact (NDCI) index for various beverages. NDCI index is based on a food's provision of required nutrients (nutrient density) in relation to greenhouse gas emissions in production of the food (climate impact). Foods with higher NDCI index better supply essential nutrients with minimal carbon footprint. Figure constructed by authors using data from Smedman et al. (2010).



research has continued to demonstrate the importance of dairy products as a source of essential nutrients for the health and prevention of chronic diseases. There are detractors who make negative claims relating to dairy production and animal agriculture in the areas of productive efficiency and cow well-being, environmental impact of milk production, and dairy products as foods; yet examination of the science shows no support for these negative claims. Future improvements in productive efficiency and environmental stewardship will need to continue if we are to produce sufficient food for the predicted global growth in population. Overall, the advances in dairy production conferred by more efficient and environmentally friendly methods, and the nutritional and health value of dairy foods represent a “good news story” for the dairy industry – one that is often not recognized by the public, and sometimes not even by those associated with the dairy industry. The facts are clear and it’s important we communicate them to consumers and policy makers.

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ALTERNATIVE MODELS OF DIGESTION AND PASSAGE: DESCRIPTIONS AND PRACTICAL IMPLICATIONS

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INTRODUCTION

Nutrient digestibility is a function of both digestion and passage processes. The actual processes of digestion and passage are very complex and any models of them are simplifications that allow mathematical descriptions. Two of the central tenets of modeling are: (1) models are always simplifications of reality and (2) models only need to be as complex as necessary to meet their intended purpose. These tenets follow the principle of logic of Occam's Razor, which states "All other things being equal, the simplest solution is best." For modelers, it may be more appropriate to paraphrase a principle about theories that is attributed to Albert Einstein, "Models (theories) should be a simple as possible, but no simpler." Most of nutritional models that are used in the field, currently, are very simple representations of reality. They typically describe digestion processes as single compartment systems with first-order kinetics of digestion and passage that are assumed to be in steady-state. There is little doubt that these models are the simplest descriptions of reality, but are they adequate to meet their intended purpose?

The objective of this treatise is to describe the assumptions, logic and mathematics of current models for describing digestion and passage, propose more complicated models that address the limitations of current approaches, and discuss the practical implications of adding different types of complexity to current nutritional models.

APPROACH

Current and proposed models will be described as box and arrow diagrams and as their basic mathematical equations. But most importantly, the biological rationale and implications of the models will be described. It is the experience of the author that describing the crucial biological rationale is often more limiting to model creation than is mathematical acuity. Simulation languages such as Stella, PowerSim, or Vensim can easily do the numerical integration necessary to solve complex models. Even spreadsheets can be developed to solve the numerical integration of a complex series of model equations.

Whether we like it or not, some basic mathematical knowledge is needed to understand the direct connection between the biological rationale and its mathematical description. Also knowledge about the mathematical manipulation of equations is needed to understand the derivations of the simple equations we use in nutritional models. Models will be described in terms of their derivatives, which is quite easy.

Integral solutions for models will be derived when they help to demonstrate implications or consequences of model structure. When models become complex, analytical integral solutions can be difficult or impossible to derive. In these situations, numerical solutions using simulation software can be used. When possible and appropriate, the steady-state solutions of models will be derived using algebra. The purpose of mathematical descriptions and derivations is not to overwhelm the reader with obtuse detail or emphasize the mathematical complexity inherent in nutritional models, but is to describe exactly each model in mathematical terms. Hopefully, the mathematical descriptions and solutions will provide insight and inspire the next generation of modelers to generate new nutritional models that improve their utility for practical nutrition. For those less interested in model mathematics, it is hoped that the verbal descriptions, flow diagrams, and graphs of results of the models will provide a basic understanding of each model's purpose and implications.

Types of Compartmental Models

Most current nutrition models are based on first-order, compartmental models; therefore other types of models will not be discussed. Most nutritional processes can be described or closely mimicked by first-order, compartmental models. These models have two major characteristics: structure and flows or fluxes. Model structure refers to the number of compartments in a model and the unique way in which they are interconnected. Flows or fluxes are the inputs and outputs of each compartment in the model structure. Changing fluxes or flows in a model is relatively easy and is the most common way we change models, e.g., changing a fractional rate (k) changes a flux or flow in a model. Changing the structure of a model is much more difficult and has consequences (many times totally unpredictable) to any part of the model that is "down stream" from the change. The focus of this treatise will be on the structure of models and their implications.

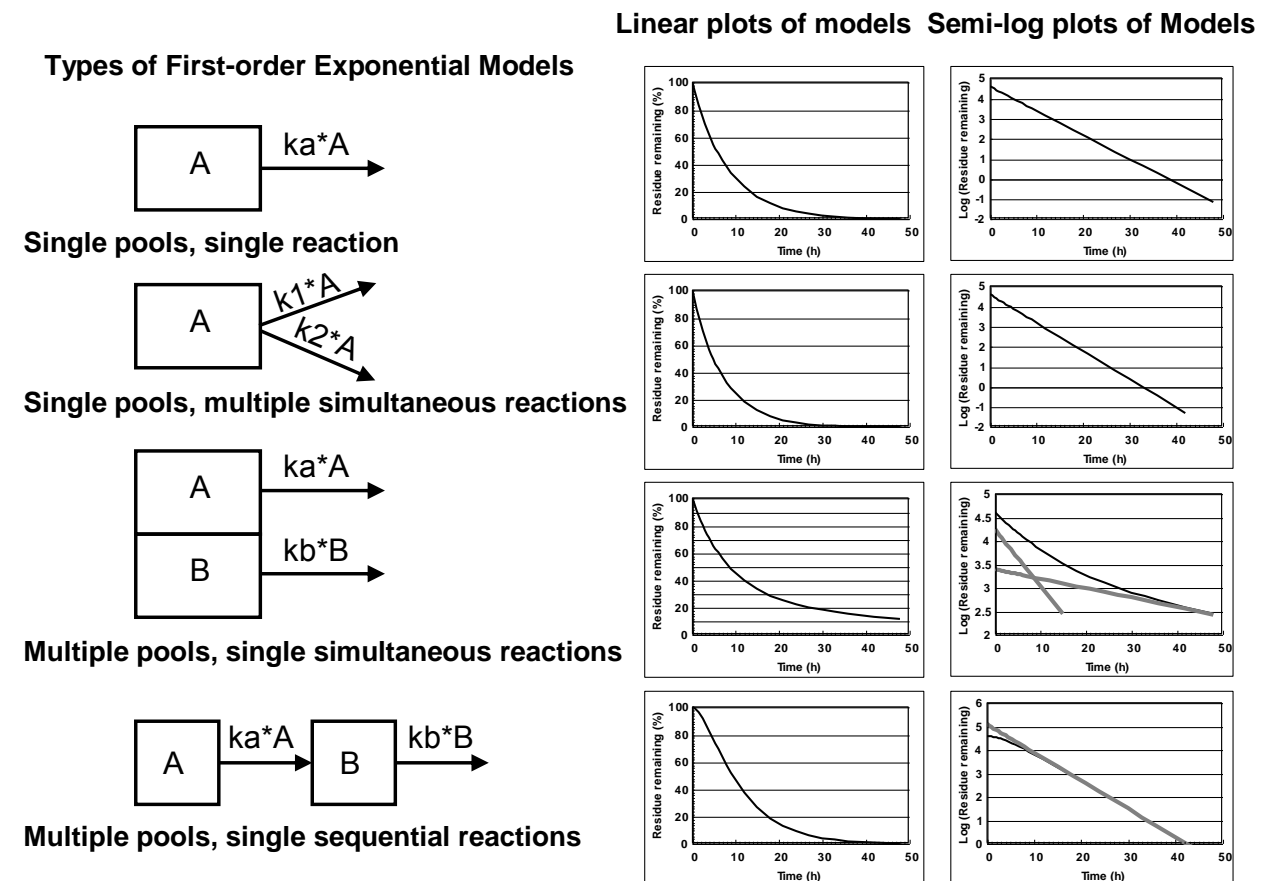
Before proceeding, it is important to define the format for describing models:

1. Pools or compartments will be identified by boxes in illustrations and by capital letters inside of brackets, e.g. [A], within the text.
2. First-order, fractional rates will be identified as lower case "k" with a letter or subscript indicating the pool on which it operates, e.g., k_a or k_a , within the text.
3. Fluxes or flows will be identified as arrows into or out of pools:
 - a. If the flux is a constant, absolute rate, it will be identified as an upper case letter, e.g. "I", for an input flux or flow,
 - b. If a constant flux is divided into fractions for input to multiple pools, the fraction will be identified as a split arrow in illustrations and as a lower case "r" in the text times the constant flux that it partitions, e.g., (I^*r) , and
 - c. If the flux is a proportion of a pool (first-order), it will be identified as an arrow in illustrations and with its equation in figures and the text, e.g., $(-k_a*[A])$.
4. Note that both fluxes and fractional rates are "rates." It is crucial for understanding that these two "rates" be distinguished from each other.

- Fluxes or flows are “absolute rates” that have the units of amount/time, e.g., kg/d or kg d⁻¹ and
- Fractional rates are “relative rates” that only have units of fraction/time, e.g., /h or h⁻¹.
- There is no advantage or utility in converting fractional rate constants into percentages/h (% h⁻¹) because mathematically they are fractions and have to be converted from percentages back to fractions before they can be used in models.
- The units for a flux with a first-order, fractional rate are determined by the units of the pool, e.g., flux = $k_a \cdot A$ and if A has the units of mg and k_a has units of /h then the flux, or absolute rate, is mg/h. Data does not have to be converted to percentages to calculate first-order fractional rates.

Mertens (2005) described four basic types of first-order, compartmental models and they will be reviewed briefly to establish the building blocks for the alternative digestion models to be described (Figure 1). Note that the single-compartment, single-reaction

Figure 1. Types of first-order, or exponential, models with their linear and semi-logarithmic plots to illustrate how they can be detected from measurements in systems.



and single-compartment, multiple-reaction models have similar linear and logarithmic (sometimes called semi-log) graphs. The difference in these two models cannot be detected if only the disappearance of compartment or pool [A] is measured. The two rates of the multiple-reaction model can be detected and measured only if the accumulation of one of the output pools is measured. The practical implication of this is that when we measure a k_d based on the disappearance from one pool, we do not know if the k_d is a single rate operating on a single homogeneous pool or the sum of multiple rates simultaneously operating on a single pool.

The multiple-compartment models (Figure 1) generate distinctly different graphs from each other and from the single-compartment models. In the multiple pool, simultaneous reaction model, we obtain a curvilinear semi-log graph when pools [A] and [B] are measured as a single residue, but we can “peel” the curves into two linear lines if rates k_a and k_b differ in magnitude such that after some time, the effect of one rate has diminished to the point that the other rate is the only one being measured. The slowest rate can be quantified and subtracted from the total curve to obtain the faster rate.

The multiple-compartment, sequential reaction model (Figure 1) generates a curve for the combined compartments of A and B that is very similar to the single compartment model; however, there is a distinct deviation at early fermentation times. Forcing materials through multiple sequential compartments essentially creates a “lag effect” and these types of models are sometimes called time-dependency models. By fitting data to the latter part of the semi-logarithmic plot and extrapolating to zero time, we can also describe this model as a discrete lag model by solving for the time when the extrapolated line equals the logarithm of the starting value of the residue.

Finally, it is helpful to explain why logarithms and logarithmic transformations are used in describing first-order models. The equations for the single-compartment, single-reaction model in Figure 1 are as follows.

1. The change in the amount of [A] for any interval of time is the derivative of [A], which is $d[A]/dt$. For a first-order model:
 - a. $d[A]/dt = -k_a[A]$,
 - b. If you want to know the amount of pool [A] at any time you have to integrate $d[A]/dt$ over all time, which gives the result
 - c. $[A]_t = [A]_i e^{-k_a t}$, where $[A]_t$ is the amount in [A] at any time = t, $[A]_i$ is the initial amount of [A] at time = 0, and k_a is the fractional rate constant.

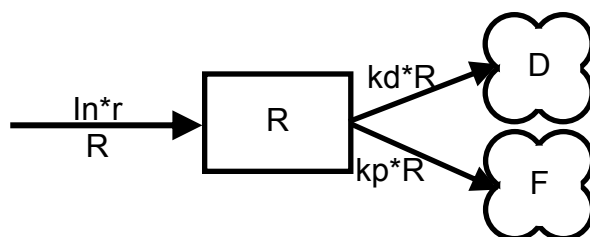
2. We rarely measure the amount that disappears per unit of time, i.e. the derivative equation, $d[A]/dt$, but we typically measure the amount remaining after a period of time, which is the integral of the derivative, i.e., $[A]_t = [A]_i e^{-k_a t}$.
 - a. The integral equation contains the exponential function as a mathematical consequence of defining the derivative, $d[A]/dt$, as a proportion of [A].
 - b. Another mathematical consequence of this simple exponential function is that it can be converted into a linear function of time using logarithms:

- i. Taking the natural logarithm ($\ln = \text{base } e$) of each side of the equation $[A]_t = [A_i] * e^{-k_a * t}$ gives
 - ii. $\ln[A]_t = \ln[A_i] - k_a * t$ because the \ln of $(e^{-k_a * t})$ is $(-k_a * t)$ and adding logarithms is the same as multiplying their anti-logarithms.
 - iii. The logarithm transformation of an exponential equation has the form of $\ln[Y] = a + b * t$, so by regressing (or plotting) the natural logarithm of the pool remaining at any time = t , the slope of the regression is the fractional rate constant “ k_a ” and the intercept is the logarithm of the initial amount of A or $[A_i]$.
- c. In addition, the logarithmic transformation can be used to calculate a fractional rate constant from two data points using the equation $k_a = (\ln[A_2] - \ln[A_1]) / (t_2 - t_1)$.

CURRENT NUTRITIONAL MODELS

Current nutritional models are based on single or multiple pools with single or simultaneous reactions. The most common model used for ruminal flows is the single pool, simultaneous rate model shown in Figure 2, where the simultaneous rates are for digestion (k_d) and passage (k_p). In the animal, there is a flow into the simple rumen model associated with feed intake of a specific feed component ($\ln * rR$).

Figure 2. Flow diagram and differential equations for the most common model of ruminal digestion and passage that is used in current nutritional models.



$$\begin{aligned}
 d[R]/dt &= + \ln * rR - (k_d + k_p) * [R] \\
 D\{D\}/dt &= + k_d * [R] \\
 D\{F\}/dt &= + k_p * [R]
 \end{aligned}$$

\ln is the intake flow (kg/h), rR is the fraction of intake that is R
 $[R]$ is the ruminal pool of any feed component or nutrient
 k_d is rate of digestion and k_p is rate of passage
 Cloud $\{D\}$ is the accumulation of digested feed component
 Cloud $\{F\}$ is the accumulation of feed component excreted in feces

The equations for the model in Figure 2 can be solved by assuming the animal is in steady-state, which means that the pool [R] in the rumen is not changing, and then solving for [R]:

$$d[R]/dt = 0 = I_n \cdot R - (k_d + k_p) \cdot [R]; \text{ therefore,} \\ I_n \cdot R / (k_d + k_p) = [R].$$

By substituting [R] into the $d\{D\}/dt$ equation

$$d\{D\}/dt = + k_d \cdot I_n \cdot R / (k_d + k_p),$$

we define the fractional digestion coefficient, or digestibility (Dig) as proportion of the total intake flow that is digested:

$$\text{Dig} = (d\{D\}/dt) / I_n; \text{ by substitution}$$

$$\text{Dig} = [k_d \cdot I_n \cdot R / (k_d + k_p)] / I_n \cdot R, \text{ which simplifies to}$$

$$\text{Dig} = k_d / (k_d + k_p).$$

This shows how the equation we use to calculate ruminal digestibility is derived and demonstrates how to use of the steady-state assumption to derive digestibility in more complicated models. It is important to note that the equation for (Dig) is only for feed components that are potentially digestible. Some feed components, such as NDF, have a potentially digestible fraction (f_d = potentially digestible NDF / total NDF) and an indigestible fraction (f_i) for which $k_d = 0$. For these feed components the equation for (Dig) is the sum of the two fractions:

$$\text{Dig of NDF} = f_d \cdot k_d / (k_d + k_p) + f_i \cdot 0 / (0 + k_p), \text{ which simplifies to}$$

$$\text{Dig of NDF} = f_d \cdot k_d / (k_d + k_p).$$

Even though they use fractional rate constants, models using the $[k_d / (k_d + k_p)]$ solution are not dynamic kinetic models, but are, in fact, steady-state solutions of dynamic models. The steady-state solution for the simple compartmental model has served nutrition well by accounting for the effects of passage and digestion kinetics on digestibility. It is the simplest model that can be developed, but perhaps it is too simple to explain some of the digestion and passage processes that can now be measured and are important factors that affect intake and digestion.

Current models do not account for digestion lag effects, may not partition fiber into its essential pools, do not model adequately the physiology of particle size reduction and passage, and do not account for differences in rates of passage or digestion that are related to particle size. Alternative models for each of these processes need to be developed, evaluated and implemented to improve our ability to mimic the essential aspects of the real digestion and passage processes in ruminants.

PROPOSED NUTRITIONAL MODEL MODIFICATIONS

Digestion Lag

Models describing the lag phenomena are rudimentary and not incorporated into current nutrition models. Part of the resistance to measuring lag and using it in nutrition

models has been the uncertainty of its source and its relevance. It is conceivable that part of the lag effect is a function of the techniques used to measure digestion kinetics. It is evident that technique differences in collection and preparation of inoculum, in maintaining minimum temperatures and in anaerobicity of inoculum and media can affect the initial fermentation of in vitro methods. However, it is also evident that samples within the same in vitro run vary significantly in lag time. It can also be postulated that feeds may vary in their hydrophobicity and rate of hydration, in their particle size (and size of openings in feed particles or surface area) that provide access to bacteria, and in their active sites for attachment. In addition, Mourino et al. (2001) demonstrated that initial low pH may inhibit the attachment of bacteria and initiation of fermentation. The observation that lag time varies among feeds within in vitro and in situ runs or batches suggests that assuming it is a constant associated with a specific laboratory and technique for the calculation of single point rate estimates may be suspect.

Mertens (1973, 1977) proposed that a discrete lag time could be used to describe the lag phenomenon associated with in vitro digestion. Mertens and Loften (1980) described a method for calculating discrete lag time from logarithmic transformation of digestion data. Although discrete lag time provides a quantitative measure of the lag phenomenon, it fails to adequately describe biological reality.

Biologically, a discrete lag time assumes that no digestion occurs prior to the lag time and then digestion begins instantaneously. This is illogical based on biological concepts and disagrees with observations that digestion gradually begins during the lag phenomena. Although it is biologically unsatisfying, Mertens (1977) demonstrated that discrete lag time could be used to adjust the traditional method of calculating digestibility by making one assumption. If we assume that passage begins immediately, but that digestion does not begin until after the discrete lag time, we can calculate the amount of material remaining at the end of the lag time.

Before discrete lag ends:

$$d[D]/dt = -kp*[D]$$

At the end of lag time:

$$D_L = D_i * e^{-kp*L}; \text{ where } D_L \text{ is the digestible pool size at the end of lag, } D_i \text{ is the initial digestible pool, } kp \text{ is passage rate constant, and } L \text{ is discrete lag time.}$$

After discrete lag time:

$$d[D]/dt = -(kd + kp)*D_L \text{ and by substitution;}$$

$$d[D]/dt = -(kd + kp)* D_i * e^{-kp*L}, \text{ and then}$$

$$Dig = [kd / (kd + kp)] * e^{-kp*L}.$$

This equation is valid only if digestion has a lag time and passage does not.

Allen and Mertens (1988) proposed that the lag phenomenon could be modeled as a sequential process in which the first pool was unavailable [U] for microbial fermentation and that this pool was converted to a pool that was available [A] for microbial fermentation (like the multiple compartment model with single sequential reactions in Figure 1). In an in vitro system with no rate of passage, the equations for each pool are:

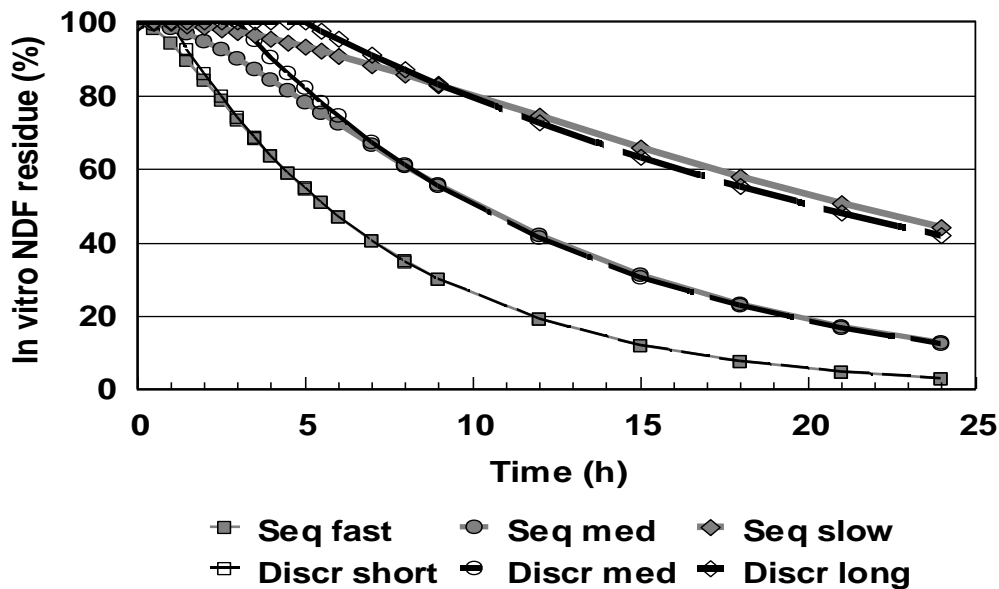
$$\begin{aligned} d[U]/dt &= -kL*[U], \\ d[A]/dt &= +kL*[U] - kd*[A], \text{ and} \\ d[D]/dt &= +kd*[A]. \end{aligned}$$

These derivatives can be analytically integrated to obtain the amount of each pool and the amount of total undigested residue [R_t] at any time:

$$\begin{aligned} [U_t] &= [U_i]*e^{-kL*t}, \\ [A_t] &= ([U_i]*kL / (kd - kL))*(e^{-kL*t} - e^{-kd*t}), \\ [R_t] &= [U_t] + [A_t], \\ [R_t] &= ([U_i] / (kd - kL))*(kd*e^{-kL*t} - kL*e^{-kd*t}), \text{ and} \\ [D_t] &= [U_i]*\{1 - \{kd/(kd - kL)\}*e^{-kL*t} - \{kL/(kd - kL)\}*e^{-kd*t}\}. \end{aligned}$$

The equation for [R_t] can be used to demonstrate the effects of kL and kd on the shape of the curve for residue remaining during in vitro or in situ fermentations (Figure 3).

Figure 3. Comparison of sequential (Seq) and discrete (Discr) lag models when the sequential lag and digestion rates are fast, medium and slow, which results in short, medium, and long times for the discrete lag parameter estimated from the data generated from the sequential lag model equations.



The equations for the sequential model can be written for an animal, which has both an intake flux and rate of passage, and by assuming that both the unavailable and available fractions of the feed can pass out of the rumen at the same rate. By assuming

the digestion process is in steady-state (which means that none of the pool sizes are changing $d[U]/dt = d[A]/dt = d[D]/dt = 0$) we can derive the equation for digestibility:

$$d[U]/dt = 0 = + \text{In} - k_L * [U] - k_p * [U]; \text{ where In} = \text{absolute rate of intake (d[Intake]/dt),}$$

$$d[A]/dt = 0 = + k_L * [U] - k_d * [A] - k_p * [A], \text{ and}$$

$$d[D]/dt = 0 = + k_d * [A]; \text{ where d[D]/dt is the absolute rate of digestion.}$$

The fractional digestion coefficient, or digestibility, is:

$$\text{Dig} = (d[D]/dt) / (d[\text{Intake}]/dt) = (k_d * [A]) / \text{In}.$$

By solving the preceding equations for their pool sizes, i.e., [U], [A], and [D] we find that:

$$[A] = (\text{In} * k_d) / \{(k_d + k_p) * (k_L + k_p)\}, \text{ thus}$$

$$\text{Dig} = [(\text{In} * k_d) / \{(k_d + k_p) * (k_L + k_p)\}] / \text{In}, \text{ which simplifies to}$$

$$\text{Dig} = (k_d * k_L) / \{(k_d + k_p) * (k_L + k_p)\} = \{k_d / (k_d + k_p)\} * \{k_L / (k_L + k_p)\}.$$

Comparing the digestibility solution for the discrete lag model to this sequential lag model shows that the “lag adjustment” term ($e^{-k_p * L}$) in the former is replaced by $\{k_L / (k_L + k_p)\}$ in the latter model. Interestingly, the ratios of ruminal digestibility of both lag adjustments when compared to a no-lag model agree quite well when calculated on the same data (Table 1).

Table 1. Comparison of sequential lag model simulations with those of the discrete lag model on prediction of ruminal NDF digestibility.

Sequential lag model ^a		Discrete lag model ^b		Ruminal digestibility of potentially digestible NDF with rate of passage ($k_p = .02/h$)				
kL	kd	Lag	Rate	No lag ^c	Sequential ^d	Ratio ^e	Discrete ^f	Ratio ^e
1.070	0.150	1.00	0.1500	0.882	0.866	0.982	0.865	0.980
0.363	0.150	3.00	0.1476	0.881	0.836	0.950	0.829	0.942
0.138	0.150	5.00	0.1099	0.846	0.771	0.911	0.766	0.905
1.050	0.100	1.00	0.1000	0.833	0.818	0.981	0.817	0.980
0.340	0.100	3.00	0.0986	0.831	0.787	0.947	0.783	0.942
0.155	0.100	5.00	0.0869	0.813	0.738	0.908	0.736	0.905
1.020	0.050	1.00	0.0500	0.714	0.701	0.981	0.700	0.980
0.318	0.050	3.00	0.0494	0.712	0.672	0.944	0.670	0.942
0.147	0.050	5.00	0.0454	0.694	0.628	0.905	0.628	0.905

^a $U \xrightarrow{k_L} A \xrightarrow{k_d} D$; where $U \Rightarrow A$ and $A \Rightarrow D$ are first-order processes.

^b $U \xrightarrow{\text{X}} A \xrightarrow{k_d} D$; where no digestion occurs during discrete lag $U \Rightarrow A$ and $A \Rightarrow D$ is first-order processes.

^cCalculated assuming no lag: Rum NDF Dig = $k_d / (k_d + k_p)$.

^dCalculated assuming sequential lag: Rum NDF Dig = $\{k_d / (k_d + k_p)\} * \{k_L / (k_L + k_p)\}$.

^eRatio of Rum NDF Dig of the lag model divided by the no lag model.

^fCalculated assuming sequential lag: Rum NDF Dig = $[k_d / (k_d + k_p)] * e^{-k_p * L}$.

Given that the lag phenomena is, at least partially, a feed characteristic, it would appear that adding lag adjustments to current nutritional models should be considered. Additional research is needed to document true feed differences in lag phenomena and to develop means of removing or eliminating any lag affect associated with techniques among laboratories.

Multiple Pools for Feed Components

Many chemical components in feeds do not have homogeneous kinetic characteristics. Most notably fiber, but also protein and starch, can have an indigestible fraction. Digestion kinetics of fiber digestion was established when Waldo (1970, 1972) first suggested that the undigested residues after long fermentation times should be considered as indigestible and subtracted from the total to obtain a potentially digestible fraction that followed first-order kinetics. Early kinetic analysis of forage NDF by Smith et al (1972) used a 72 h fermentation to measure the indigestible NDF (iNDF) and observed that the remaining digestible fraction followed first-order kinetics as indicated by linear semi-logarithmic plots. Most nutritionists accept the concept that fiber and some proteins contain digestible and indigestible fractions.

Mertens (1977) observed that when in vitro fermentations were extended to 96 h and longer the resulting digestible fraction generated curvilinear logarithmic plots, which would indicate either non-first-order kinetics or multiple first-order pools (see Figure 1). More recently, Raffrenato and Van Amburgh (2010) have extended in vitro fermentations to 240 h to measure the iNDF and confirmed the existence and the magnitudes of fast and slow digesting pools of NDF. Their research clearly confirms the concept that there are fast and slow digesting pools of NDF. The practical question is, “Do we need to model this level of complexity into the structure of nutritional models that are used in the field to evaluate and formulate ruminant diets?” All models are simplifications of reality, but what level of modeling detail is “simple enough to accomplish the goal of the model, but no simpler?” The answers to these questions are crucial for feed evaluation laboratories because it determines what iNDF needs to be measured or estimated for “acceptable” description of NDF digestion kinetics in applied nutritional models. To address this issue, it is necessary to compare prediction of ruminal digestibility of NDF by three-pool models to those of two-pool models, which is the model used in most applied nutritional models.

To remove the noise associated with the measurement of serial NDF residues from 0 to 240 h, a simulated data set was developed using the sequential lag model described in the last section with a fast, slow and indigestible pool. Using this approach, the exact rates of the input data are known. Twenty-four data sets were generated for both grasses and legumes that included two levels of iNDF, three amounts of slow-pool NDF, two fractional rates for the slow, and two for the fast pool ($24 = 2 \times 3 \times 2 \times 2$) that encompassed a wide range of kinetic characteristics for each forage (Table 2).

Table 2. Kinetic characteristics of twenty-four simulated NDF residues for grasses and legumes.

Kinetic Parameter	Grass			Legume		
	Low	Medium	High	Low	Medium	High
iNDF (% NDF)	10		20	30		40
pdNDFs (Slow pool, % NDF)	10	20	30	10	15	20
pdNDFf (Fast pool, % NDF)	50	60-70	80	35	40-55	60
Slow kds rate (h ⁻¹)	.008		.012	.004		.008
Fast kdf rate (h ⁻¹)	.08		.12	.12		.18
Lag rate (h ⁻¹)	.25	.25	.25	.25	.25	.25

For each of the data sets, residue amounts were generated for 0, 3, 6, 9, 12, 18, 24, 36, 48, 60 and 72h and fitted to the two-pool model using NLIN (SAS 9.1.3):

$$\text{NDFRes}_{(t)} = \text{pdNDF} * e^{(-kd*[t-L])} + \text{iNDF}.$$

Residue amounts were also generated for 0, 6, 12, 24, 48, 72, 96, 120, 144, 192 and 240h and fitted to the three-pool model using NLIN (SAS 9.1.3):

$$\text{NDFRes}_{(t)} = \text{pdNDFf} * e^{(-kdf*[t-L])} + \text{pdNDFs} * e^{(-kds*[t-L])} + \text{iNDF}.$$

Ruminal NDF digestibilities were calculated using the equations:

2-pool digestibility = $\text{pdNDF} * (\text{kd} / (\text{kd} + \text{kp}))$; where $\text{kp} = .02$ and other coefficients were 2-pool NLIN parameter estimates (Table 2) and

3-pool digestibility = $\text{pdNDFf} * (\text{kdf} / (\text{kdf} + \text{kp})) + \text{pdNDFs} * (\text{kds} / (\text{kds} + \text{kp}))$; where $\text{kp} = .02$ and other coefficients were 3-pool NLIN parameter estimates (Table 2).

The accuracy of the two- and three-pool models in predicting ruminal fiber digestibility was determined by comparing them to the true digestibility determined from the simulation inputs:

True digestibility = $\text{pdNDFf} * (\text{kdf} / (\text{kdf} + \text{kp})) + \text{pdNDFs} * (\text{kds} / (\text{kds} + \text{kp}))$; where $\text{kp} = .02$ and other coefficients were known simulation parameters.

The results of this simulation experiment suggest that measuring in vitro residues at >72 h and estimating a slow digesting pool and rate may not improve predictions of ruminal NDF digestibility enough to warrant the additional time and expense (Tables 3 & 4).

Although there may be advantages to measuring NDF digestion kinetics more accurately for research or forage improvement projects, it is less convincing that adding a third pool will improve applied nutrition models, given the variability in measuring in vitro residues and the difficulties in fitting data to models with multiple exponential pools. It appears that fitting three-pool data to a two-pool model generates “acceptable” parameter estimates that predict ruminal NDF digestibilities within 0.94 to 0.97 of the true value for legumes (Table 3) and within 0.94 to 0.96 of the true value for grasses

(Table 4). It was unexpected to observe that three-pool parameter estimates generated using non-linear regression were in some cases so different from the simulation input data as to be “unacceptable” and they generated predictions of ruminal NDF digestibility that were only 0.96 to 0.98 of the true values. It is especially surprising that these estimates were so poor given that the simulated data had no measurement variation or noise. It is doubtful that methods for estimating three-pool parameters from fewer observations would generate better results.

Models for Passage

Perhaps the greatest limitation of most applied nutritional models is the overly simplistic way in which passage of feed through the rumen is described. It has been known for a long time that long particles in the rumen do not have the same escape rate as small particles (if they can escape at all). It has also been known for a long time that marker excretion curves (no matter how faithfully they track feed particles) do not match the excretion curves that would be generated by most of the nutritional models in practical use. It is granted that rate of passage is difficult to measure because of marker instability and the complex mathematics of the physiological processes associated with selective retention of large particles, sequestering of small particles in the large particle pool, reduction in particle size during rumination, differences or correlations between liquid and particle flows, and the biphasic nature of a long-particle top layer over a more liquid bottom layer in the rumen. However, it is unlikely that the process of passage is any more complex than that of digestion if we were to delve into the myriad relationships of specific bacteria digesting specific feed tissues or the multitude of tissue types that comprise NDF and may differ in digestibility.

It appears that we have been satisfied with the simple concept that all fiber, protein, starch, etc, acts as one single compartment in the rumen because the mathematics is easy to understand and use, although we know that this is not even a close approximation to reality. It is amazing that we nutritionists spend money to obtain more information about smaller chemical and digestion fractions in feeds (to paraphrase, are we spending more and more to learn less and less until we spend everything to learn nothing?). However, with the exception of peNDF or corn silage processing score, we spend almost nothing to measure the physical properties of feeds. Much of the rationale for this situation is that we do not have ways to use particle size measurements because our current nutritional models describe passage too simply. However, passage models and rate of passage data are available, but they have not been put into practice.

Table 3. Selected comparisons of two- and three-pool models parameter estimates for ruminant NDF digestibility of legume forages compared to the true ruminant NDF digestibility generated from simulation inputs.

Parameter	HHHL ^a	HHLH	HMLH	LHHL	LHLH	LMLH	LLLH
Simulation inputs							
Indig Pool	40	40	40	30	30	30	30
Slow Pool	20	20	15	20	20	15	10
Fast Pool	40	40	45	50	50	55	60
Fast kd	0.18	0.12	0.12	0.18	0.12	0.12	0.12
Slow kd	0.004	0.008	0.008	0.004	0.008	0.008	0.008
Two-pool parameter estimates							
2-Pool I	55.9	52.0	48.9	45.8	41.	38.8	35.6
2-pool kd	0.1052	0.0726	0.0779	0.1084	0.0757	0.0800	0.0838
2-Pool Lag	1.88	2.02	2.09	1.91	2.06	2.11	2.15
True Rum Dig	39.33	40.00	42.86	48.33	48.57	51.43	54.29
2-P Rum Dig	37.05	37.65	40.68	45.72	45.94	48.97	52.01
Ratio	0.942	0.941	0.949	0.946	0.946	0.952	0.958
Three-pool parameter estimates							
3-Pool I	39.3	39.0	35.7	32.1	28.7	25.4	17.5
3-Pool Slow	20.1	10.2	18.7	17.0	10.3	23.9	21.7
3-Pool Slow kd	0.0072	0.0059	0.0026	0.0010	0.0055	0.0028	0.0012
3-Pool Fast kd	0.1325	0.1331	0.1341	0.0966	0.1331	0.1339	0.1343
True Rum Dig	41.71	47.86	43.00	44.52	56.86	48.33	55.67
3-P Rum Dig	40.58	46.55	41.84	42.92	55.31	47.02	54.16
Ratio	0.973	0.973	0.973	0.964	0.973	0.973	0.973

^aLetters represent in order: high or low indigestible pool; high, medium or low slow pool; high or low fast rate; and high or low slow rate.

Mertens and Ely (1979, 1982) developed a model of ruminal digestion and passage that incorporated three compartments for particle size in the rumen to simulate selective retention, particle size reduction, and excretion of variable fecal particle size as major processes of passage. Interestingly, this model also contained fast and slow digesting fiber fractions; however, it did not contain the digestion lag phenomenon as described by Allen and Mertens (1988). Mertens and Ely (1979) modeled the rumen to contain three first-order mixing pools that sequentially moved particles from large to medium to small particle pools (Figure 4), and modeled the small and large intestines as a single subsequent compartment. The intestines should probably be described as a plug-flow system, but only their ruminal model will be used for the remaining discussion.

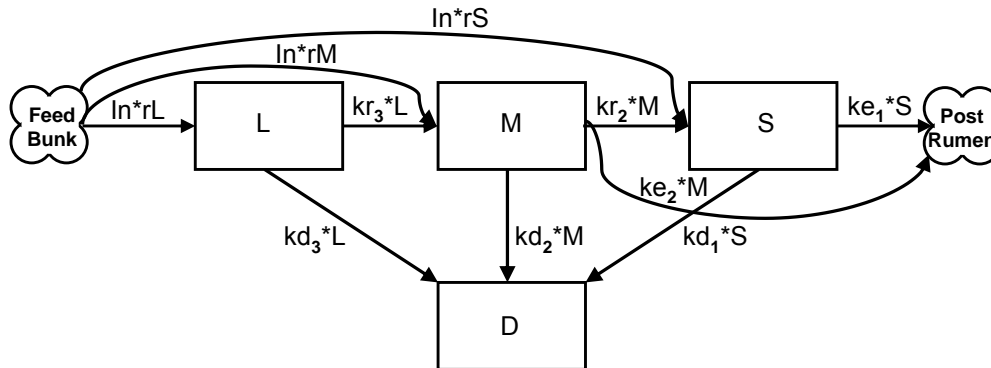
Table 4. Selected comparison of two- and three-pool models parameter estimates for ruminal NDF digestibility of grass forages to the true ruminal NDF digestibility generated from simulation inputs.

Parameter	HHHL ^a	HMHL	LHHL	HLLL	LMHL	LMLH	LLLH
Simulation inputs							
Indig Pool	20	20	10	20	10	10	10
Slow Pool	30	20	30	10	20	20	10
Fast Pool	50	60	60	70	70	70	80
Fast kd	0.12	0.12	0.12	0.08	0.12	0.08	0.08
Slow kd	0.008	0.008	0.008	0.008	0.008	0.012	0.012
Two-pool parameter estimates							
2-Pool I	38.0	31.8	28.0	24.6	21.7	17.4	13.0
2-pool kd	0.0697	0.0779	0.0726	0.0610	0.0795	0.0562	0.0607
2-Pool Lag	1.98	2.09	2.02	2.35	2.10	2.30	2.35
True Rum Dig	51.43	57.14	60.00	58.86	65.71	63.50	67.75
2-P Rum Dig	48.19	54.24	56.48	56.81	62.53	60.89	65.46
Ratio	0.937	0.949	0.941	0.965	0.952	0.959	0.966
Three-pool parameter estimates							
3-Pool I	19.5	19.0	18.2	13.6	8.8	7.8	0.0
3-Pool Slow	28.7	17.1	29.4	13.6	16.8	29.4	17.0
3-Pool Slow kd	0.0108	0.0090	0.0066	0.0024	0.0085	0.0063	0.0017
3-Pool Fast kd	0.0941	0.0661	0.0668	0.0678	0.0662	0.0670	0.0680
True Rum Dig	54.11	55.50	48.57	58.86	63.50	56.57	66.86
3-P Rum Dig	52.81	54.32	47.55	57.62	62.15	55.38	65.45
Ratio	0.976	0.979	0.979	0.979	0.979	0.979	0.979

^aLetters represent in order: high or low indigestible pool; high, medium or low slow pool; high or low fast rate; and high or low slow rate.

One of the concerns about a complex model of passage through the rumen is the ability to generate the particle size reduction and particle escape parameters required by these models. However, Mertens et al. (1984) reported that the model of Mertens and Ely (1979) could generate a large population of fecal marker excretion curves by simply changing the particle size distribution of the feed that was fed and swallowed while keeping the rates of particle size reduction and ruminal escape constant (Figure 5). Changes in the distribution of particles entering each ruminal particle pool resulted in different patterns of fecal marker excretion similar to those observed in rate of passage experiments. If fed or swallowed particle size is the main contributor to the differences in overall rates of passage, then measuring feed particle size distributions would provide the relevant inputs for passage in a multi-compartment model of the rumen.

Figure 4. The ruminal digestion and sequential passage model for a digestible nutrient from the model of Mertens and Ely (1979).



$$\begin{aligned}
 d[L]/dt &= + In*rL - (kd_3 + kr_3)*[L] \\
 d[M]/dt &= + In*rM + kr_3*[L] - (kd_2 + kr_2 + ke_2)*[M] \\
 d[S]/dt &= + In*rS + kr_2*[M] - (kd_1 + ke_1)*[S] \\
 d[D]/dt &= + kd_3*[L] + kd_2*[M] + kd_1*[S]
 \end{aligned}$$

In = Absolute Intake rate (kg/h)
 rL , rM , & rS are fractions of large, medium & small particles in Intake
 kr_3 & kr_2 are particle size reduction rates for large and medium particles
 ke_1 & ke_2 are ruminal escape rates for small & medium particles
 kd_1 , kd_2 & kd_3 are digestion rates for small, medium & large particles

As with models for lag and multiple digestible pools, it is important to evaluate the impact of a sequential multi-compartment passage model of the rumen on ruminal digestibility. Using the steady-state assumption, the equation for calculating ruminal digestibility of the digestible NDF fraction can be derived:

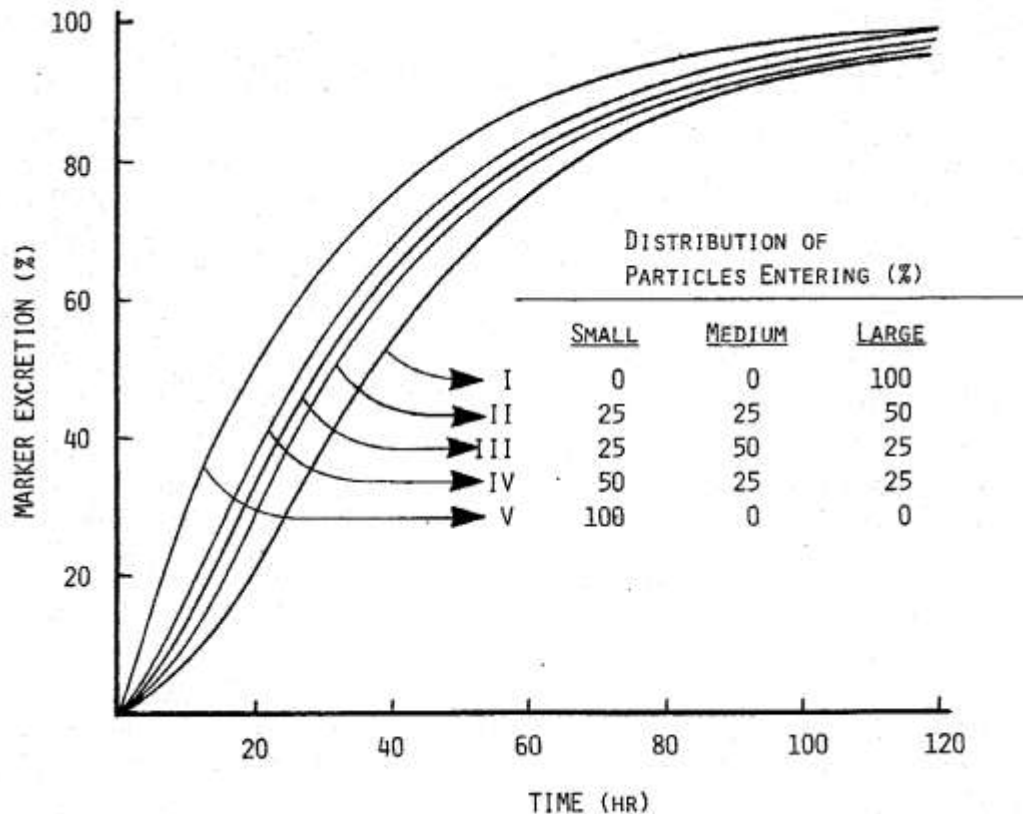
$$\begin{aligned}
 Dig = & [kd_3*rL / (kd_3 + kr_3)] + \\
 & \{kd_2*[rM*(kd_3 + kr_3) + kr_3*rL]\} / [(kd_2 + kr_2 + ke_2)*(kd_3 + kr_3)] + \\
 & \{kd_1*[rS*(kd_2 + kr_2 + ke_2)*(kd_3 + kr_3) + kr_2*(rM*(kd_3 + kr_3) + kr_3*rL)]\} / \\
 & [(kd_1 + ke_1)*(kd_2 + kr_2 + ke_2)*(kd_3 + kr_3)].
 \end{aligned}$$

Although this equation seems incredibly complex, it can be easily included in a practical steady-state nutritional model. Perhaps it could be simplified, but the order of similar terms in the equation shows the symmetry of the solution. The difficulty of using the equation is in obtaining input parameters. This process can be simplified by assuming that digestion rates of all particles are the same ($kd_1 = kd_2 = kd_3$) and that rates of particle reduction and escape are constant as described in by Mertens and Ely (1979), i.e., ($kr_3 = .07/h$; $kr_2 = .14/h$; $ke_2 = .006/h$ and $ke_1 = .035/h$).

The steady-state assumption for the model in Figure 4 can also be solved for the apparent passage rate (App kp) for a comparable single compartmental model:

$$App\ kp = [kr_3*(kr_2 + ke_2)*ke_1] / \{rL*(kr_2 + ke_2)*ke_1 + (rM + rL)*kr_3*ke_1 + kr_3*[rS*(kr_2 + ke_2) + kr_2*(rM + rL)]\}.$$

Figure 5. Simulated cumulative marker excretion from selected distributions using the model of Mertens and Ely (1979). Copy of Figure 2, page 139, in Techniques in Particle Size Analysis of Feed and Digesta in Ruminants (P.M. Kennedy, ed.), Can. Soc. Anim. Sci. Occ. Pub. No.1.



The solution for the passage rate constant of a single compartment ruminal model from the turnover of pools in the sequential model can be used to generate an apparent k_p that can be directly compared to the sequential passage model of Mertens and Ely (1979) when the particle size distribution of the input feed is varied.

The sequential passage model of Mertens and Ely (1979) predicts ruminal NDF digestibilities that range from 1.11 times that of the single compartmental model using a comparable apparent rate of passage for forages with large relative particle size to 1.04 times that of the single compartmental model using a comparable apparent rate of passage for rations with small relative particle size (Table 5). Corresponding ratios using a single compartmental model with a constant $k_p = .02/h$ are 1.07 for forages with large relative particle size to 0.90 for rations with small relative particle size (Table 5). These relatively large changes in ruminal NDF digestibility due to differences in passage of fiber through the rumen that are only related to the particle size distribution of the input feed, suggest that improvement in the passage kinetics of most current nutrition models is warranted.

These differences in ruminal NDF digestibility were predicted by the sequential passage model without changing the rates of particle size reduction and escape from the rumen. It is postulated rates of particle size reduction and escape are more a function of the physiological state of the animal (level of milk production or stage of lactation, or both). Using this logic would allow a sequential passage model to account for the independent effects of both the diet and the animal on the process of passage. By monitoring the size of the large and medium particle pools predicted by the sequential passage model while in steady-state, it may also be possible to change the escape rate of small particles to mimic the sequestering effect of the large and medium particle pools.

Table 5. Comparison of ruminal NDF digestibility (NDF dig) using a sequential multi-compartment or a single compartment ruminal model that uses either an apparent rate of passage calculated from the sequential model or a constant $k_p = .02/h$.

Parameter or result	Rapid digestion rate Forage			Medium digestion rate Normal Ration			Slow digestion rate Finely chopped ration		
fi ^a	0.45	0.45	0.45	0.35	0.35	0.35	0.25	0.25	0.25
kd/h ^b	0.15	0.15	0.15	0.10	0.10	0.10	0.05	0.05	0.05
rL ^c	0.75	0.25	0.05	0.75	0.25	0.05	0.75	0.25	0.05
rM ^c	0.15	0.50	0.55	0.15	0.50	0.55	0.15	0.50	0.55
rS ^c	0.10	0.25	0.40	0.10	0.25	0.40	0.10	0.25	0.40
Seq NDF dig ^d	0.516	0.489	0.474	0.582	0.540	0.519	0.576	0.516	0.485
Apparent k_p ^e	0.022	0.028	0.031	0.022	0.028	0.031	0.022	0.028	0.031
App NDF dig ^f	0.478	0.465	0.457	0.531	0.510	0.498	0.517	0.484	0.465
App k_p Ratio ^g	1.079	1.052	1.038	1.097	1.060	1.042	1.114	1.065	1.043
Con NDF dig ^h	0.485	0.485	0.485	0.542	0.542	0.542	0.536	0.536	0.536
Con k_p Ratio ^g	1.064	1.008	0.977	1.074	0.998	0.958	1.075	0.962	0.906

^aFraction of NDF that is indigestible.

^bDigestion rate NDF for all pools.

^cProportion of feed intake that is large (rL), medium (rM) or small (rS) particle size.

^dRuminal NDF digestibility calculated using the equation for the Mertens and Ely (1979) model (see text).

^eApparent rate of passage for a single compartment model that corresponds to the pool turnovers of the sequential model of Mertens and Ely (1979) (see text).

^fRuminal NDF digestibility calculated using the apparent k_p in a single compartment model.

^gRatio of the ruminal NDF digestibility of the sequential model to that of the apparent or constant k_p in the single compartment model.

Currently, most nutritional models use estimates of digestion rate that are determined by in vitro or in situ techniques using samples that are ground to various sizes (the most common size being through a 1-mm screen). Although it is logical to use digestion rates of 1-mm samples for the small particle pool in the rumen, it is less

convincing that these rates should apply to the large and medium particle pools. Although it may be possible to also calculate an apparent digestion rate constant for a single compartment ruminal model, it would be easier and more rigorous to use the sequential model and adjust the digestion rates of the large and medium particles in relation to those determined directly on small particles (model inputs for kd_3 , kd_2 , and kd_1 are available, see Figure 4).

The flexibility of a sequential passage model like that proposed by Mertens and Ely (1979) to changes not only in particle size distributions in feed inputs, but also in rates of particle size reduction and escape (influenced by both animal and feed characteristics), in rates of digestion of large, medium and small particles, and in the sequestering effects of large and medium particle pools on small particle escape indicate that the effects of changing the passage process in most current models would have greater impact than adding either sequential lag models or multiple digestion compartment models.

CONCLUSIONS

Current applied nutritional models are based on assumptions that allow a very simplified approach for the prediction of ruminal digestibility of fiber and other major feed components. The assumption that rumen dynamics can be adequately described by single digestion and passage fractional rate constants needs to be reviewed and revised. Both digestion and passage are more complex than described by models based on this simple assumption.

Recent observations suggest that digestion involves a lag phase, especially for fiber. Research also suggests that fiber digestion is most accurately described by a three-pool model containing fast, slow and indigestible pools. The value of adding a lag phase for digestion has not been established, but it appears that adding a third slow-digestion pool for fiber may not provide significant advantage in the prediction of ruminal NDF digestibility in relation to the cost and difficulties in generating the needed kinetic parameters.

It appears that the weakest link in current applied nutrition models is the assumption that the rumen is a single compartment for passage. Our knowledge of the physiological processes associated with the bi-phasic nature of ruminal particulate matter, selective retention of large particles, reduction in particle size during rumination, and differences between liquid and particle flows from the rumen indicates that a single compartment model is probably inadequate. Models are available that can use measurements of feed input particle size distribution to alter the flow of particles from the rumen. The use of these models and their steady-state solutions are recommended as the first priority for the improvement of ruminant models of digestion and passage. Sequential passage models can not only account for differences in feed particle size distribution, but also for differences in rates of size reduction and escape, in sequestering of small particles in the rumen, and in differences in digestion rates of large, medium and small particles in the rumen.

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AMMONIA EMISSIONS FROM DAIRY OPERATIONS – WHAT DO WE KNOW?

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Ammonia emissions from dairy farms are receiving attention due primarily to air quality concerns. At the same time, ammonia emissions represent losses of nitrogen (N) from the farm and can be an indication of a lower efficiency of N use from the feeding program. Ammonia emissions from animal agriculture represent about 55% of the total ammonia emissions in the U.S. (Aneja et. al., 2008). Total ammonia emissions from agriculture account for 81% of the U.S. ammonia emissions when both the animal and fertilizer emissions are considered. Table 1 contains the emissions of ammonia by various classes of animals for both the U.S. and New York. Nationally, the dairy sector represents 23.6% of the total ammonia emissions from animals while this figure is 83.6% in New York. It is interesting to note that EPA is predicting a decrease in ammonia emissions from dairy from 2010 to 2030. This is based mainly on a projected decrease in the number of dairy cows. In addition to ammonia, there is concern about the formation of PM_{2.5} particles (equal to or < 2.5 um). These form as atmospheric reactions between ammonia and acids in the air such as sulfuric and nitric. The result is fine particles including ammonium sulfate and ammonium nitrate. These are the PM_{2.5} particles that contribute to respiratory problems when inhaled.

The importance of ammonia emissions for the dairy and livestock industries are that they appear to be the next compound to be regulated by EPA. Currently, EPA is examining the data from the National Air Emissions Monitoring Study (NAEMS) and expects to finalize the methods to determine ammonia emission estimates in June, 2010 (USEPA, 2011). Initial results from the NAEMS study were presented at this conference last year (Gooch, 2010).

Table 1. Ammonia Emissions from Livestock, tons/year ^a

	U.S. 2010	U.S. 2030	NY 2002
Dairy cattle	565,892	546,666	34,443
Beef cattle	691,174	733,662	1,958
Poultry	648,200	869,348	527
Swine	484,223	518,082	2,348
Other animals			1,904
Total	2,390,489	2,667,758	41,173

^a USEPA, 2004

AMMONIA PRODUCTION IN DAIRY ANIMALS

Dairy animals, and other ruminants, produce very little ammonia directly. The primary source of ammonia emissions by ruminants is the result of the conversion of urea-N in the urine to ammonia. The following steps summarize this process:

- 30 – 70% of the total manure N excreted by dairy cattle is in the urine.
- 50 – 90% of the total N in the urine is present as urea.
- The fecal portion of the manure contains the enzyme urease.
- The urease enzyme rapidly converts urinary urea-N to ammonia.
- 1 mole of urinary urea-N is converted to 2 moles of ammonia.
- This enzymatic conversion is affected by both pH and temperature. The enzyme exhibits more activity at higher temperatures and a pH of 6.8 – 7.6 (Muck, 1982). Enzyme activity is reduced in colder temperatures or when pH is lower or higher than the optimum range.

HOW MUCH AMMONIA DO DAIRY COWS EMIT?

There are a wide range of ammonia emission factors for dairy cattle in the literature. This would be expected given the differences that exist in housing types, levels of milk production, ration nutrient composition, quantity of N consumed per day and many other factors. A review paper indicated that daily ammonia emissions averaged 59 g/cow with a range of 0.82 to 250 g/day (Hristov et. al., 2010). A daily ammonia emission of 130 g/cow was reported in a trial monitoring emissions in an Idaho open-lot dairy (Leytem et. al., 2011). Yearly ammonia emission factors used around the world for dairy cattle range from 45 to 83.8 lbs/cow/year (Aneja et, al., 2008). The yearly ammonia emission factors for the dairy herds in the NAEMS study ranged 28 to 36 lbs/cow (Gooch, 2010). This range excludes the California dairy herd in the study that had a much lower ammonia emission factor. A range in yearly ammonia emission factors of 7.9 to 46.2 lbs. per dairy cow are reported (USEPA, 2005). These factors vary depending on the type of housing and manure system used.

DAIRY CATTLE RATIONS AND AMMONIA EMISSIONS

A large number of research studies have examined the relationships between ration crude protein, rumen degradable protein (RDP), N intake and ammonia emissions. A key factor related to ammonia emissions from manure is the quantity of urinary N and urinary urea N. Dairy cattle were fed 5 diets ranging from 13.3 to 19.4% CP (Olmos Colmenero and Broderick, 2006). The daily grams of urinary urea-N excreted increased from 63 to 208. Even though ammonia emissions weren't determined in this trial, the ammonia release potential increases as urinary urea-N excretion goes up. Dairy cows were fed diets containing 15.4, 13.4 or 12.9% CP (Agle et. al., 2010). Daily urinary-N excretion was significantly higher on the 15.4% CP diet (188 g/day) than the other diets (133 and 115 g/day). In this trial, the cumulative ammonia emission rate from manure was higher on the high CP diet compared with the other rations. An extensive trial used 15 diets varying in metabolizable protein from 8.8 to 12% of DM (Weiss et. al., 2009). Diet CP ranged from 14.4 to 17.7%. All of the diets contained 10.7% RDP calculated with the NRC Dairy model. These diets varied in type of forage and starch content. There was an increase in both fecal and urinary N as diet MP increased. However, the increase in urinary-N was 3.5 times higher than the increase in fecal N. Ammonia production from manure increased as diet MP increased.

The effects of varying the forage to concentrate ratio on manure NH₃-N emissions have been reported (Aguerre et. al., 2011). Rations ranging from 47 to 68% forage were used. These rations were all similar in CP (16.1 – 16.2%) and balanced for similar RDP and RUP levels. There was no effect of forage to concentrate ratio on manure NH₃-N emission (14.1 g/cow/day). Arriaga et. al. (2010) fed diets containing 16.9, 15.9 or 14.1% CP to cows in a tie-stall barn and measured ammonia concentration on the barn floor. There was a 36.5% decrease in ammonia concentration on the barn floor when the lower CP diet was fed compared with the high CP diet. In this study, a 1 unit decrease in diet CP reduced ammonia concentration on the floor by 13%. A trial was conducted using a commercial dairy herd fed rations averaging 18 or 16.5% CP (Aguerre et. al., 2010). The NH₃-N concentration on the floor in this free-stall herd was 27% lower when the lower CP diet was fed.

DAIRY REPLACEMENT HEIFERS

Replacement heifers accounted for 15% of the total dairy herd ammonia emissions in model simulation runs (Garnsworthy, 2004). A study conducted in California evaluated changes in ration CP levels, N excretions and ammonia emissions in growing dairy heifers (James et.al., 1999). Dairy heifers weighing 572 to 1074 pounds were fed rations with either 9.6 or 11% CP. Heifers fed the lower CP ration excreted 14% less N and had a 28% decrease in ammonia emissions. The daily quantity of urinary urea N excreted by Holstein heifers increased from 3.8 g/day to 95.8 g/day as ration crude protein levels increased from 9 to 21% (Marini and Van Amburgh, 2003). Urinary urea-N, as a % of the total urinary N excreted, increased from 17.5 to 79% from the low to high CP ration in the same study. Similar trends in both urinary urea-N excretion and urinary urea-N as percent of total urinary N have been reported by other workers (Gabler and Heinrichs, 2003; Hoffman et. al., 2001). These higher urinary urea-N values would be expected to increase ammonia emissions by the heifers.

POST EXCRETION CONSIDERATIONS

The effects of management or housing considerations on ammonia emissions were reviewed at this conference (Powell and Broderick, 2009). One housing alteration that can lower ammonia emissions in free-stall barns is the use of sloped floors with a urine collection tube. This decreases contact between the liquid and solid portion of the manure. The type of bedding used in barns can also have an effect on ammonia emissions. The lowest ammonia emissions were when pine shavings were used as bedding. Emissions increased when shredded newspaper, straw and recycled manure solids were used for bedding. Flushing the floor in housing facilities with either water or a formalin solution decreased emissions by 14 to 50% (Ogink and Kroodsma, 1996). A recent paper indicated that applying tannin directly on the barn floor lowered ammonia emissions by 19% (Powell et.al., 2011b).

WHOLE FARM CONSIDERATIONS

What are the opportunities to lower ammonia emissions on a whole farm basis? A paper at the 2002 Cornell Nutrition Conference provided an example (Jonker et.al., 2002). This example used a process based model for a dairy herd with 320 milking and dry cows, 290 replacement heifers with herd milk production of 26,000 lbs. of milk per cow per year. Table 2 contains the results of these model runs.

Table 2. Impact of different technologies on yearly nitrogen air emissions

Technology Used	N emissions, lbs./year	Change from baseline, %
Baseline	66,220	
Precision feeding	51,480	-22
Lagoon cover	55,220	-16
Soil incorporation	43,340	-35
Precision feeding + lagoon cover	43,120	-35
Precision feeding + soil incorporation	34,320	-48
Lagoon cover + soil incorporation	28,600	-57
Precision feeding + lagoon cover + soil incorporation	23,540	-65

Rotz and Onema (2006) used a whole farm simulation model to evaluate the impact of different management strategies on ammonia emissions from dairy farms. The dairy farm used had 100 cows, 85 heifers and 222 acres of crop land. Table 3 contains the ammonia loss data from these model simulation runs.

Table 3. Ammonia emissions from dairy farms, lbs. N/cow

Barn type	Tie Stall	Free Stall	Free Stall	Free Stall
Manure handling practice	Daily spread, surface	Slurry tank, surface	Slurry tank, injected	Earthen pond, irrigate
Loss in the barn	36.3	57.4	58.3	57
Manure storage	0	15.4	15.6	77.2
Field application	90	77	6.6	36.3
Grazing	10.3	10.1	10.3	9.9
Total/cow	136.6	159.9	90.8	180.4

USING MILK UREA NITROGEN TO MONITOR AMMONIA EMISSIONS

How can we monitor ammonia emissions from dairy cows? There have been a number of papers that have examined the relationships between ration CP, urinary urea-N and milk urea nitrogen (MUN). This concept is interesting since many dairy producers receive daily MUN values on milk shipped. Workers in the Netherlands fed

dairy cows diets with varying RDP balances and concluded that MUN was a good indicator of ammonia emissions (van Duinkerken et. al., 2005). MUN has also been indicated to be a good predictor of urinary-N excretion (Burgos et. al., 2007; Nousiainen et. al., 2004). Dairy cattle were fed diets containing 15, 17, 19 or 21% CP and both ammonia emissions from manure and MUN were measured (Burgos et. al., 2010). There was a linear increase in ammonia emission from manure as diet CP increased. Daily emissions of ammonia from manure increased from 57 to 149 g N. These workers reported a strong ($r^2 = 0.85$) between ammonia emissions and MUN. A recent paper summarized data from 9 trials that included 37 diets (Powell et. al., 2011c). Ammonia emissions per cow per day ranged between 9.9 and 95.4 g in these trials. MUN values ranged between 8 and 16. There was a strong positive relationship ($R^2 = 0.79$) between urinary urea-N (g/day/cow) and MUN. The relationship between diet CP% and MUN had an R^2 of 0.87.

As a second step, these workers developed relationships between lowering MUN and ammonia emissions. There were 3 studies conducted in tie-stall barns. As MUN decreased from 14 to 10 mg/dl, ammonia emission reductions of 10.5 to 37.3% were observed. Ammonia emissions were reduced 10.5 to 33.7% for cows in free-stall barns as MUN decreased from 14 to 10 mg/dl. The use of MUN as a monitor of ammonia emissions has been adopted in Wisconsin as a best management practice (WIDNR, 2010). This group is giving a reduction credit of 20% for ammonia emissions if the annual average MUN is 10 or less and 10% reduction if the MUN level is between 10 – 12%.

SUMMARY

1. Ammonia emission factors reported in the literature vary widely for dairy cattle and include nutritional, housing and environmental factors. Methods and process based models need to be developed that can be applied to farms to comply with future ammonia emission factors.
2. A primary way to lower ammonia emissions from dairy cattle is to balance rations to meet, but not exceed, animal MP and RDP requirements. It has been estimated that a 1 unit decrease in ration CP to 16% will lower ammonia emissions by 20% (Kebreab et. al., 2002).
3. A number of housing, manure storage and manure application practices exist that can lower post-excretion ammonia emissions.
4. Farm level ammonia emissions can be reduced up 50 – 70% by utilizing a combination of ration, housing, manure storage and manure application practices.
5. Milk urea nitrogen may be a practical and reliable tool to predict ammonia emissions on dairy farms.

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STATE, REGIONAL AND FARM-SCALE NUTRIENT BALANCES: TOOLS FOR ENHANCED EFFICIENCY OF WHOLE-FARM NUTRIENT USE

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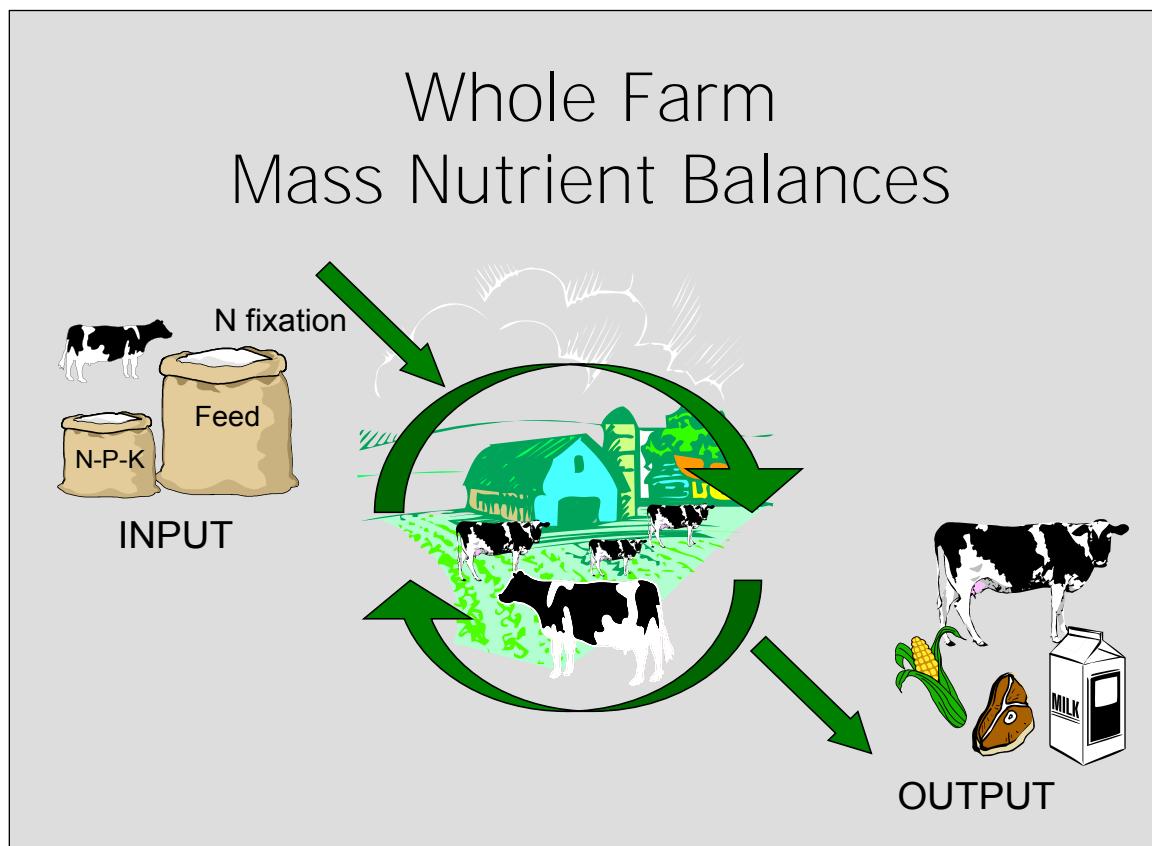
There are many concerns about nutrient use locally, regionally, nationally and in some cases, globally. Most of us are familiar with concerns relating to phosphorus (P) use and impacts based on freshwater quality impacts such as algal blooms and a general increase in plant growth and decay in inland waters. More recently, nitrogen (N) has been receiving attention by scientists. In contrast to fresh waters, coastal/salt water bodies are N limited so additions of N can increase algae and plant growth and decay in these water bodies. Further, the role of the N cycle at a national and global scale are increasingly recognized, as exhibited by a recent report from the Environmental Protection Agency (EPA) Science Advisory Board entitled “Reactive Nitrogen in the United States: an Analysis of Inputs, Flows, Consequences and Management Options” ([http://yosemite.epa.gov/sab/sabproduct.nsf/WebReportsLastMonthBOARD/67057225C780623852578F10059533D/\\$File/EPA-SAB-11-013-unsigned.pdf](http://yosemite.epa.gov/sab/sabproduct.nsf/WebReportsLastMonthBOARD/67057225C780623852578F10059533D/$File/EPA-SAB-11-013-unsigned.pdf)). This EPA report describes “reactive” N (N_r) as essentially all chemically and biologically reactive N that is in the air, or in and on the soil, distinct from inert N_2 gas that comprises about 78% of our atmosphere. Much of the N_r generated annually is for or a result of food production and much of this N is eventually released into the environment, where it may remain for years or decades in various forms contributing negatively to human health and the environment. Indeed, the National Academy of Engineering has identified management of N as one of the “grand challenges” facing this country.

In this paper we will look at trends in N and P balances in New York State at the farm, Chesapeake Bay watershed, and state levels, and suggest a way forward to assist farms to meet nutrient use efficiency expectations while remaining economically viable.

TRENDS IN FARM BALANCES

More efficient management of nutrients involves managing the nutrients that remain on the farm to the greatest degree possible. This will require a shift away from use of insurance applications/additions and book values to implementation of practices that include precision feed and forage management and a focus on **optimizing nutrient use efficiency**. The key solutions lie in practices that allow farms to safely and confidently manage nutrient use (both agronomic and purchased feedstuffs) and thereby increase farm nutrient use efficiency and reduce loadings to watersheds while finding value in remaining nutrients or carbon sources. Knowing a farm's nutrient mass balance is one

Figure 1: A farm nutrient mass balance is the difference between nutrient (N, P, and K) imports and exports expressed, for dairy farms, on a per cwt milk production or a per acre cropland basis.

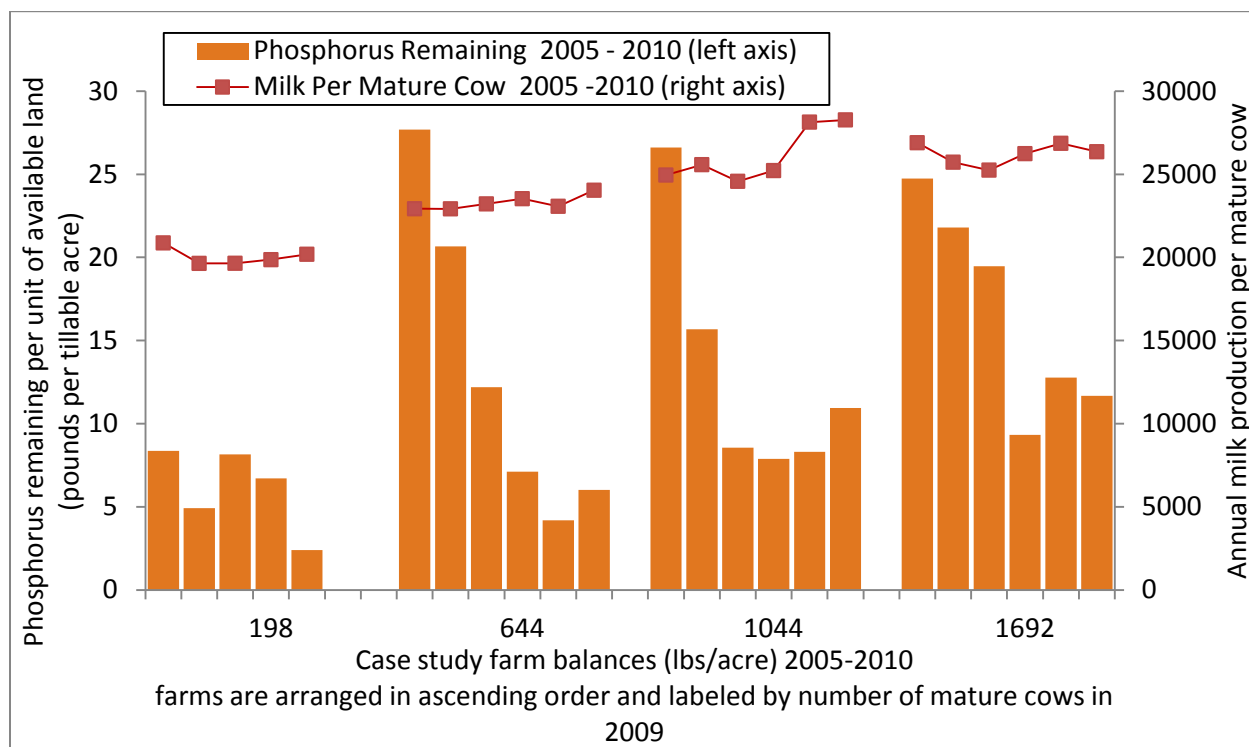


step toward improving our understanding and management of nutrient movement onto, within, and away from any particular farm.

The mass balance assessments require records be kept for purchase of feed, fertilizer, animals and imported bedding, and for exports of nutrients through sales of milk, crops, animals, and/or export of manure. Such balances, when done annually, can reveal trends that are important for longer-term decision making, and monitoring of the impact of management changes on potential of environmental loss. Not only can such N, P and K balances be reduced without a reduction in milk production, but some farms experience an increase in milk production, as is shown in P balances of the farms in Figure 2. The farms participating in the annual assessment shown in Figure 2 have demonstrated clearly that independent of size of operation, gains can be made to reduce annual nutrient excess without the loss of production.

These reductions reflect both the willingness of the producer to reduce balances over time and the potential for making changes that improve production efficiency and reduce risk of nutrient to the environment. Similar trends were seen in a database of 54 New York State dairy farms who participated in the mass

Figure 2: Phosphorus balance (P remaining per tillable acre) for four farms ranging in animal numbers from 198 to 1692 cows, over five to six years of participation in the Cornell Nutrient Management Spear Program annual mass balance project.



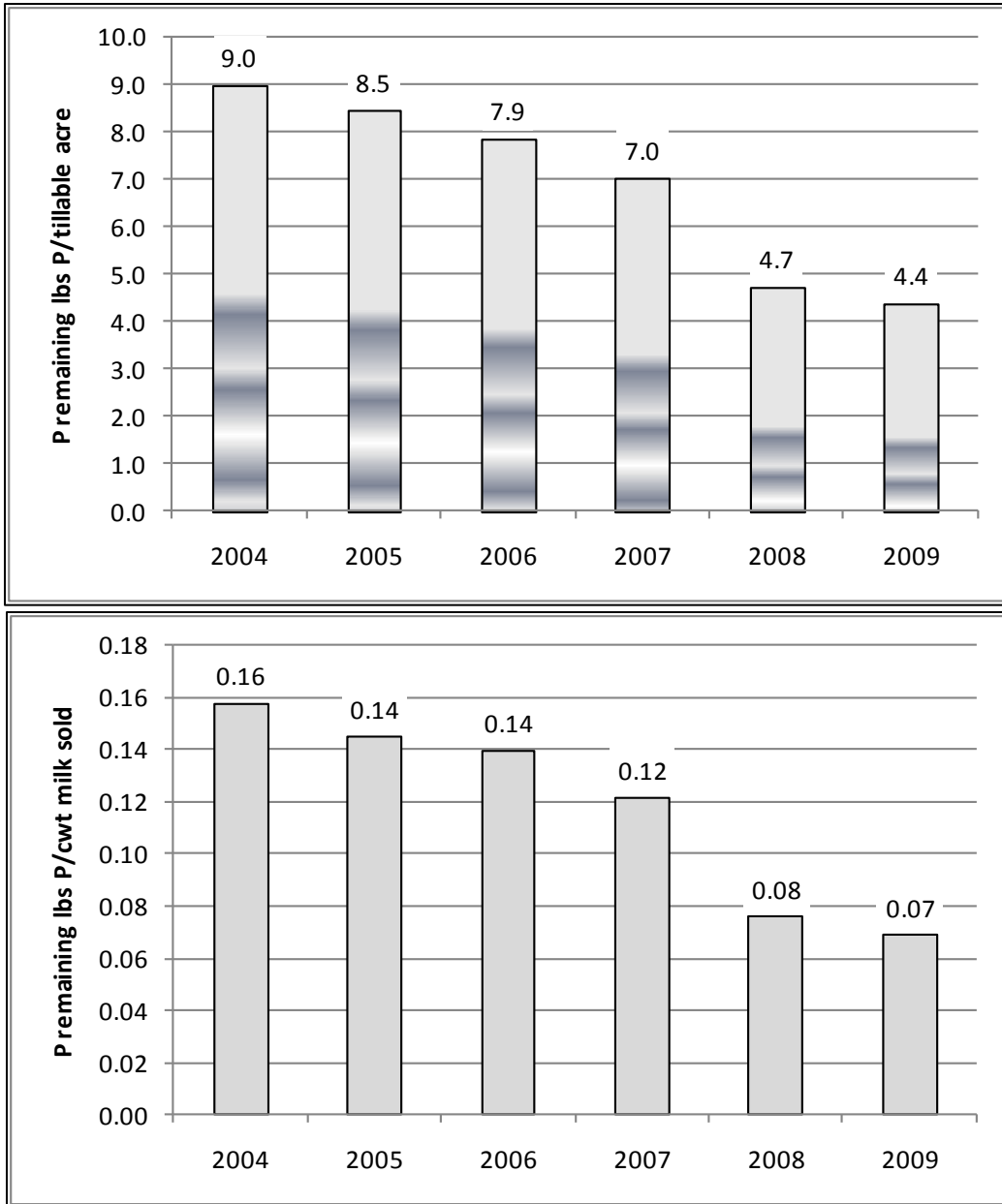
balance assessment project for 4 or more years. When contrasting the mass balances of the first two years in the project with those in the last two years in the project, reductions in nutrients ranged from 28 to 53%. The largest gains were made by farms that had large surpluses in their starting year (Table 1).

As shown in Table 1, mass balance trends over time can differ depending on the initial levels at which the farm was operating, with a tendency to larger reduction where initial balances were above levels achievable by 75% of all farms in the New York State dataset. Gains will be region specific, too. Assessment of balances in the Upper Susquehanna Watershed showed a 50% reduction in P balance of farms that participated 3 or more years (9 lbs P/acre in 2004 versus 4.4 lbs P/acre in 2009). Similarly, the P balance per cwt was reduced from 0.16 to 0.07 lbs P/cwt. (Figure 3). In this group the P imported as purchased feed decreased 29% from 2004 to 2009.

Table 1: Percent reduction in excess N, P and K for 54 dairy farms that participated in the Nutrient Management Spear Program mass balance assessment project. Farms are separated into two groups depending on their initial N, P and K balances.

	Average N remaining for farms with beginning N balance less than 105 lbs N/tillable acre.	Average N remaining for farms with beginning N balance greater than 104 lbs N/tillable acre.	Average N remaining for all 54 farms that participated for four years or more.
Average of first 2 yrs	40	174	67
Average of last 2 yrs	28	124	48
Percent reduction	30%	29%	28%
Number of farms	43	11	54
	Average P remaining for farms with beginning P balance less than 13 lbs P/tillable acre.	Average P remaining for farms with beginning P balance greater than 12 lbs P/tillable acre.	Average P remaining for all 54 farms that participated for four years or more.
Average of first 2 yrs	7	22	10
Average of last 2 yrs	5	11	6
Percent reduction	29%	50%	40%
Number of farms	43	11	54
	Average K remaining for farms with beginning K balance less than 39 lbs K/tillable acre.	Average K remaining for farms with beginning K balance greater than 38 lbs K/tillable acre.	Average K remaining for all 54 farms that participated for four years or more.
Average of first 2 yrs	16	53	22
Average of last 2 yrs	11	25	14
Percent reduction	31%	53%	36%
Number of farms	45	9	54

Figure 3: Phosphorus balance for dairy farms in the Upper Susquehanna Watershed dairy farms monitored from 2004-2009 (211 farm balances)

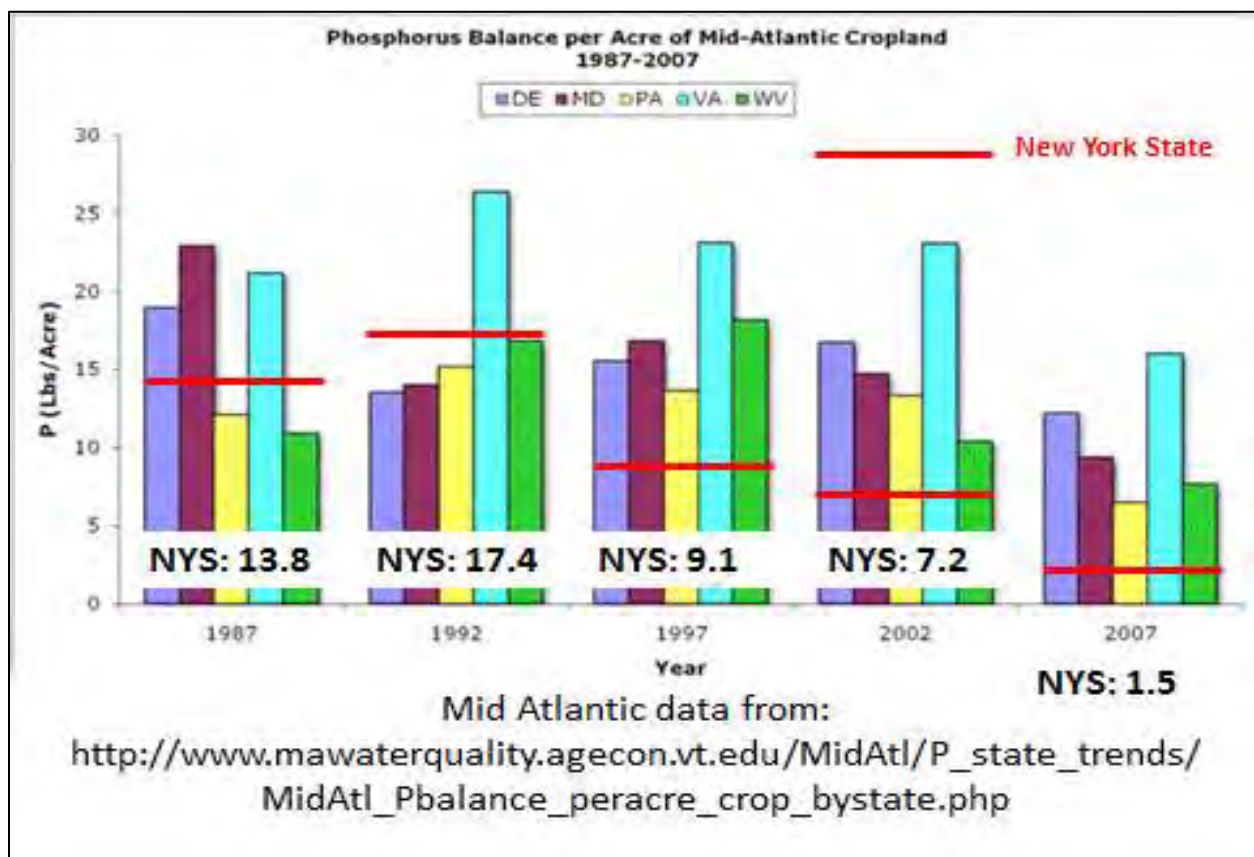


TRENDS IN STATEWIDE BALANCES

The improvements illustrated by the individual farm balances are reflected in the statewide P balance as well. In New York, the statewide P balance (manure P plus fertilizer P minus P in crop harvest), has shown a drastic reduction from 14 and 17 lbs P/acre in 1987 and 1992, respectively, to 1.5 lbs/acre in 2007 (Figure 4).

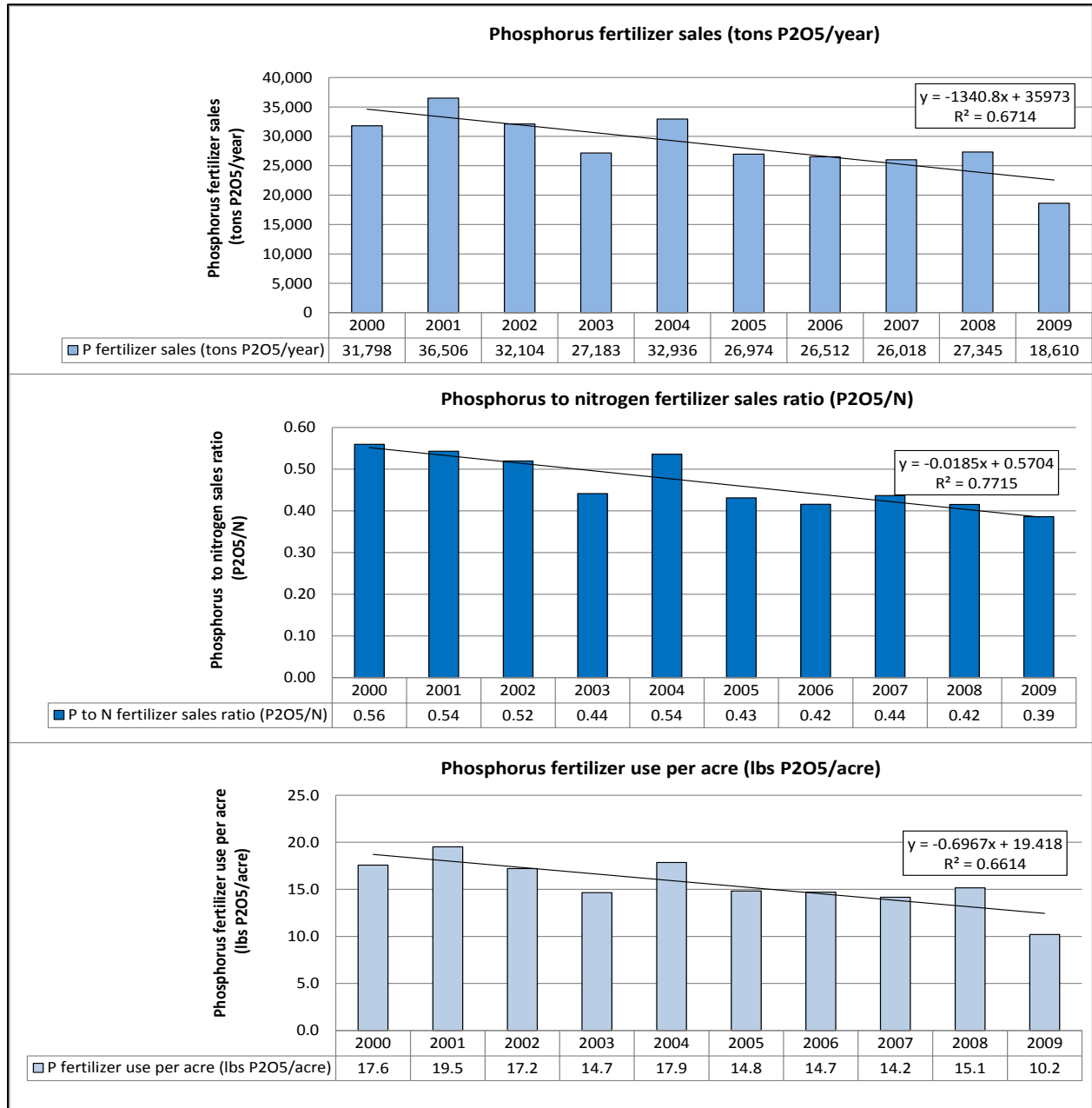
The trends in balance reflect a reduction in P fertilizer use (Figure 5) from 17-20 lbs P/acre in 2000-2003 to 10 lbs P/acre in 2009. This change was due to was an increased demand for fertilizer blends with less P, as reflected in a steady decline in the P₂O₅/N ratio since 2000 (figure 5). Similarly, a reduced use of mineral P for dairy cow rations and large improvement in precision feeding and home-grown forage production over these years contributed greatly to the lower P balance, illustrating the potential for changes across management units on the farm.

Figure 4: Phosphorus balance per acre (lbs P/acre) for New York and the mid-Atlantic States reflect the drastic changes implemented by New York State dairy farms.



The decline in balance was also apparent in an evaluation of the counties that make up the Upper Susquehanna Watershed. However, for the Upper Susquehanna Watershed, the P balance is now negative, with insufficient manure P and fertilizer P use to maintain current soil test levels - a trend that needs to be viewed with concern.

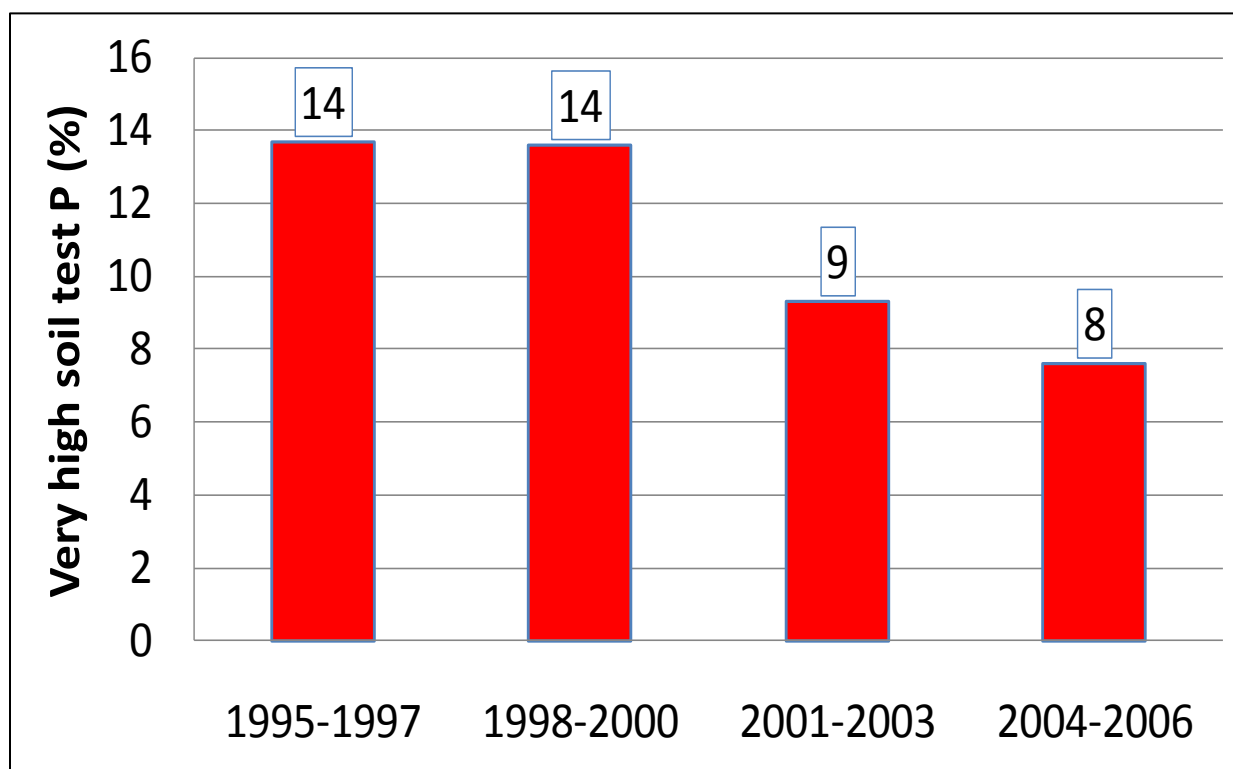
Figure 5: Changes in on-farm fertilizer P use in New York State since 2000 reflect greatly reduced P fertilizer use. Crop yields increased over this time period.



IMPLICATIONS

The negative P balance for the Upper Susquehanna Watershed is reflected in a change in soil test P levels, showing a decrease from 14% between 1995 and 2000 testing above the agronomic optimum soil test P for crops like corn to 8% in 2004-2006 (Figure 6). Although analysis of field-by-field distribution of P is needed, these state

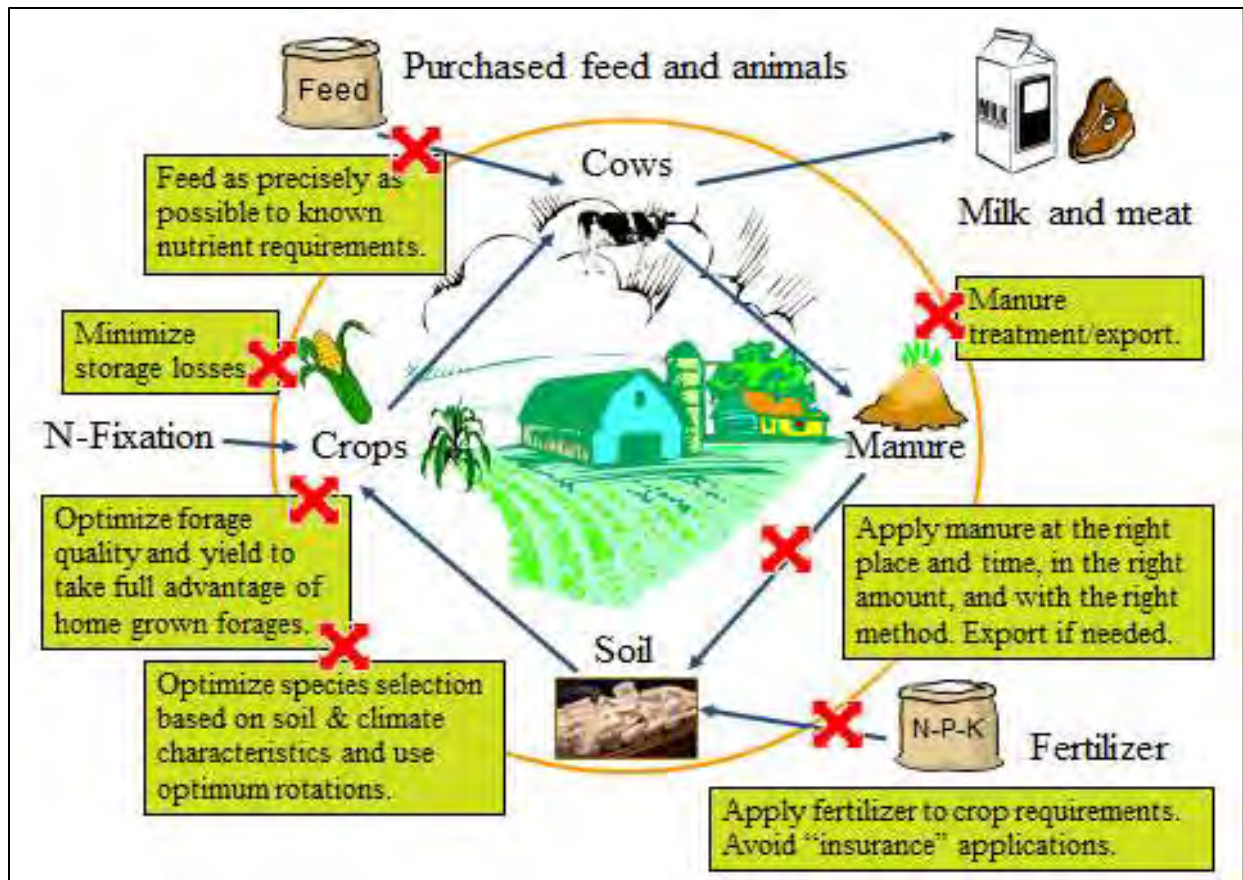
Figure 6: Soils Very High in agronomic soil test P (>40 lbs/acre Cornell Morgan test) for Upper Susquehanna Watershed fields.



and watershed trends raise concerns about impact of further reductions in P balances on the long-term sustainability of farming in low or negative P balance regions.

Statewide, regional and farm N balances have shown a decline over the past two decades as well, mostly driven by a reduction in the total pool of N excreted in manure. The current gross balances of 55 lbs N/acre (statewide) and 29 lbs N/acre (Upper Susquehanna Watershed) indicate many fields are not receiving adequate N to support optimum yields given that neither fertilizer N nor manure N has 100% uptake efficiency. This raises questions of sustainability for farms that routinely operate this way. Losses of ammonia in the barn, losses from storage, and losses from land application of manure present opportunities, indicating that current manure and fertilizer management could be improved to some extent, but the overall negative balances indicate such improvements need to go hand in hand with addition of N from other sources (cover crops, greater reliance on N fixation, shorter rotations, etc.) to optimize both crop production and nutrient use efficiency. The current status further illustrates the need to both document farm-level balances and to manage these balances for improvements in nutrient use efficiency (N, P, K and other nutrients), for profitability, and for a reduced environmental footprint (Figure 7). This requires the engagement of the farm managers and their advisors, including the nutritionist and the crop advisor.

Figure 7: Whole farm mass balances can be used as an indicator of nutrient use efficiency across all farm management units (herd, crop, bunk/storage, and manure management) and aid in implementation of changes in best management practices that help the farm's profitability and reduce its environmental footprint.



CALL TO ACTION

Experiences with farms that participated in mass balance assessments over the past 4-6 years have shown that improvements will be implemented where economically feasible and that the annual mass balance assessment is a great tool to guide and monitor such changes. A balance analysis helps farm managers to benchmark from year to year, to compare their performance to other like operations, and to determine nutrient management strengths and also where nutrient use inefficiencies occur. To help with on-farm assessments, a software program was developed to allow users to:

- Calculate the amount of nutrients being imported to the farm as purchased feeds (i.e., not homegrown), fertilizers, animals, and bedding material, and being exported from the farm as milk, animals, crops, and manure/compost.
- Generate reports that show farm N, P and K imports and exports in tons for the whole farm and in pounds per acre cropland, per pound of product sold, or per animal unit.

- Identify areas of concern and opportunities for more efficient nutrient use that, if addressed, could increase profitability and reduce environmental impact.

For more information on how to use the software and data collection necessary to use the program, see the Nutrient Mass Balance webpage of the Nutrient Management Spear Program: <http://nmsp.cals.cornell.edu/projects/massbalance.asp>.

CONCLUDING REMARKS

For the sustainability of the dairy sector in any state, it is important to find ways to enhance profitability while minimizing environmental loss of N and P. Farm nutrient mass balances can illustrate environmental and economic imbalances quickly, independent of location of the farm. Balance assessments are useful for livestock, dairy and crop farms alike; they can help identify management alternatives that enhance nutrient use efficiency and farm profitability. We urge farms to consider participating in the annual assessment, as case study farms have clearly illustrated the potential for large gains in nutrient use efficiency when monitoring of progress becomes part of the package of best management practices, and when producers have complete control of where to make changes in their individual operations. We also urge nutritionists to get involved as imported feed is for most dairies the single largest contributor to nutrient imports and hence farm balances of dairy farms.

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NEW INSIGHTS INTO THE PATHOGENESIS OF CLAW HORN DISRUPTION LESIONS

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RELEVANCE OF LAMENESS TO THE DAIRY INDUSTRY

A growing concern of the dairy industry is to increase dairy cattle wellbeing in anticipation of a demand from the general public of welfare certified dairy products. Lameness is one of the most important welfare issues of high producing dairy cows in North America (Vermunt, 2007). It is a debilitating condition that challenges sustainability of production systems used in North America because of the pain and subsequent animal welfare consequences (Vermunt, 2007) and also the significant economic losses (Warnick et al., 2001). A study conducted in England concluded that lameness was the second most costly disease in the dairy industry following only mastitis (Kossaibati and Esslemont, 1997).

Lameness results in earlier culling of animals as well as lower carcass weight, conformation class, and fat cover class and hence a lower carcass economic value (Booth et al., 2004; Bicalho et al., 2007c; Fjeldaas et al., 2007). It has also been reported that prevention or early identification and treatment of the problem can improve the value of the carcass and reduce culling rates (Fjeldaas et al., 2007). Several studies have also shown that lameness has a negative effect on the fertility of dairy cows (Sprecher et al., 1997; Hernandez et al., 2001; Garbarino et al., 2004). More recently it has been reported that cows detected with clinical lameness in the first 70 days in milk (**DIM**) were 25% less likely to become pregnant compared to non-lame cows (Bicalho et al., 2007c). The prevention of lameness is the most important step to reduce its welfare implications for cows and associated economic losses to the dairy farmers (Mill and Ward, 1994). Hence it is important to create a system that accurately predicts the occurrence of lameness, thus allowing farmers to target high risk animals with preventive strategies.

IMPORTANCE OF LAMENESS TO THE WELLBEING OF DAIRY COWS

Lameness is a crucial welfare issue in modern dairy production (Espejo and Endres, 2007; Vermunt, 2007). Lameness causes discomfort and pain of long duration (Green et al., 2002). Additionally, the observation of lameness has been classified as the most representative animal-based indicator of welfare in dairy cattle (Whay et al., 2003). There is an increasing societal concern about the moral and ethical treatment of food animals (Fulwider et al., 2008). Lameness is of welfare concern due to its debilitating effects and high prevalence in herds throughout the world (Cook, 2003; Bicalho et al., 2007c). Furthermore, dairy cattle mortality is a major cause of economic losses and is an important animal welfare issue (Thomsen and Houe, 2006). A large retrospective cohort study with over 900 dairy farms reported that dairy operations with high

prevalence of lameness ($\geq 16\%$) had 2.9 higher odds of on farm dairy cow mortality compared to dairy farms with low lameness incidence (McConnel et al., 2008); dairy cows that died on the farm because of lameness were usually euthanized by a farm employee or veterinarian. Lameness is perhaps the biggest challenge for dairy farmer to overcome as society becomes more concerned with the origin of their food and the welfare of farm animals.

Polls and surveys conducted within the United States show general agreement that there is public support for the protection of farm livestock and poultry (Swanson, 2008). The animal welfare assurance and audit programs developed by the private sector are an attempt to assure consumers that best practice measures and independent oversight result in a reasonable quality of life for food-producing animals. It is a possibility that milk processing plants will start to market and commercialize milk from welfare-certified herds in an attempt to anticipate the demand from welfare-oriented consumers. In fact, the commercialization of bST (bovine somatotropin) free milk is a reality; consumers perceive that welfare of the animals from bST-free herds is better than otherwise. As it happened to bST-free milk, the motivation for marketing welfare-certified milk will come from the concern of the general public (consumers) regarding the wellbeing of dairy cows. Some attempts to voluntarily achieve welfare certification are already in place; The New York State Cattle Health Assurance Program (NYSCHAP) is an example of such a program. The NYSCHAP welfare certification requires that at least 85% of each animal management group must have a locomotion score of two (using a five-point-scale visual locomotion score system). This benchmark would be at the very least a hard to achieve goal for most dairy farms given the reported prevalence of lameness throughout the United States (Cook, 2003; Espejo et al., 2006; Bicalho et al., 2007c).

Dairy farmers in North America are not regulated in regards to the welfare of their animals and production standards except in extreme cases of neglect and abuse. In contrast, regulation of food animal production has become part of mainstream life for European Union livestock and poultry producers (Swanson, 2008). The freedom that European producers once had to produce animals as they saw fit gradually vanished by public command. To enable the dairy industry in the United States to effectively anticipate and respond to societal concerns about ethical treatment of animals, there is a great need to identify opportunities to prevent the incidence of lameness in dairy cattle.

THE PATHOGENESIS OF NON-INFECTIOUS CAUSES OF LAMENESS

Despite the undeniable relevance of lameness resulting from non-infectious diseases, very little is known about its pathophysiology. Although severe cases of laminitis (inflammation of the laminar tissue of the digit) caused by abnormally high intake of readily available carbohydrates have been described in the literature (Bazeley and Pinsent, 1984), the link between subclinical laminitis and claw lesions has been recently challenged (Logue et al., 2004). To make matters worse, research knowledge on the pathogenesis of equine laminitis was uncritically generalized to the field of bovine lameness without taking into account the profound anatomical and physiological

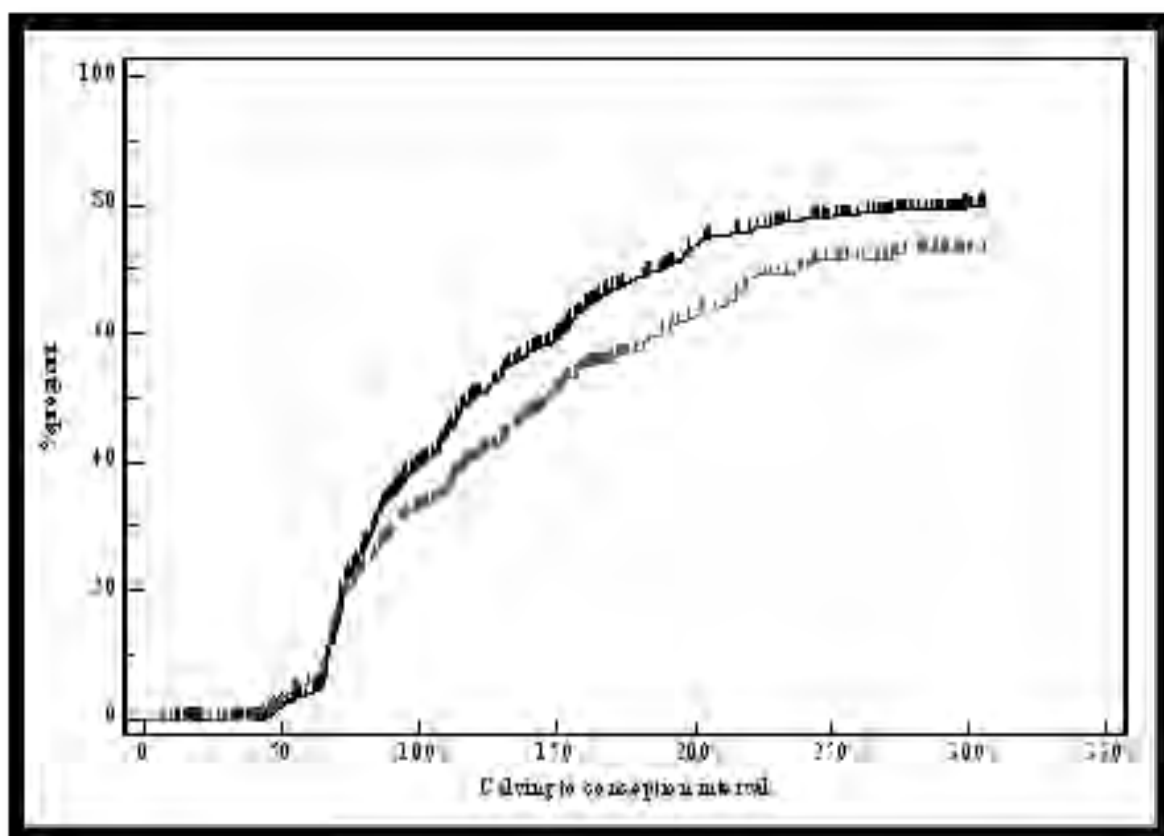
differences between the two species. Thus far, there is limited evidence that claw horn lesions in cattle are caused by subclinical laminitis (Logue et al., 2004; Thoefner et al., 2004; Lischer et al., 2002). Lately, the hypothesis that claw lesions are a consequence of contusions within the claw horn capsule has been suggested (Tarlton et al., 2002; Raber et al., 2004). Raber et al. (2004) reported that it is widely accepted by workers in the Northern Hemisphere that most bovine claw lesions (and thus lameness) originate from contused tissue within the claw horn capsule. While it has been reported that sole ulcers and white line lesions are caused by subclinical laminitis (Thoefner et al., 2004), there are others who clearly state that the evidence to support this is limited (Logue et al., 2004). The suspensory apparatus in cattle is less well developed than in the horse and the digital cushion must support a considerably higher proportion of the body weight (Raber et al., 2004). The digital cushion is a complex structure composed mostly of adipose tissue located underneath the distal phalanx; it plays an important function of dampening compression of the corium tissue beneath the cushion. The biomechanical importance of the digital cushion in alleviating compression under the tuberculum flexorum of the distal phalanx is well known (Raber et al., 2006; Raber et al., 2004; Logue et al., 2004).

RESEARCH SUMMARY

Research currently in progress or recently completed by key personal, has focused on the impact of lameness on production parameters, validation of lameness detection systems, pathophysiology of sole-ulcers and white-line-diseases, and evaluation of lameness prevention strategies. Our recent research has allowed us to explore a new pathogenesis theory for claw horn disruption lesions (**CHDL**) and consequently envision novel preventive strategies. Historically, lameness researchers and experts believed that CHDL were caused by sub-clinical rumen acidosis and that the poor body condition observed in affected cows was a consequence of lameness and not a cause of lameness. We currently demonstrated that cows with low BCS have significantly thinner digital cushions and therefore a lower capacity to protect the corium tissue from compression by the third phalanx. Details about our recently completed significant activities and its link to our proposed project are described below.

Previously, we estimated the detrimental effects of lameness on calving-to-conception interval and hazard of dying or being culled in lactating Holstein cows. Data were collected from 5 dairy farms located in upstate NY from November 2004 to June 2006. The study design was a prospective observational cohort study. Cows were assigned a visual locomotion score (VLS) using a 5-point scale ranging from 1 = normal, 2 = presence of a slightly asymmetric gait, 3 = the cow clearly favored 1 or more limbs (moderately lame), 4 = severely lame, to 5 = extremely lame (non-weight bearing lame). In total 1,799 cows were enrolled. In 2 alternative categorizations, cows were considered lame if at least 1 VLS was ≥ 3 during the first 70 DIM and secondly, if at least 1 VLS was ≥ 4 for the same period. Lameness (VLS ≥ 3) was detected at least once in 26.5%, 54.2%, 33.9%, 51.8%, and 39.3% of all cows in farms 1 to 5, respectively. The hazard ratio of being detected pregnant was 0.85 for lame cows (VLS ≥ 3) versus non-lame cows; hence, lame cows were at a 15% decreased risk of

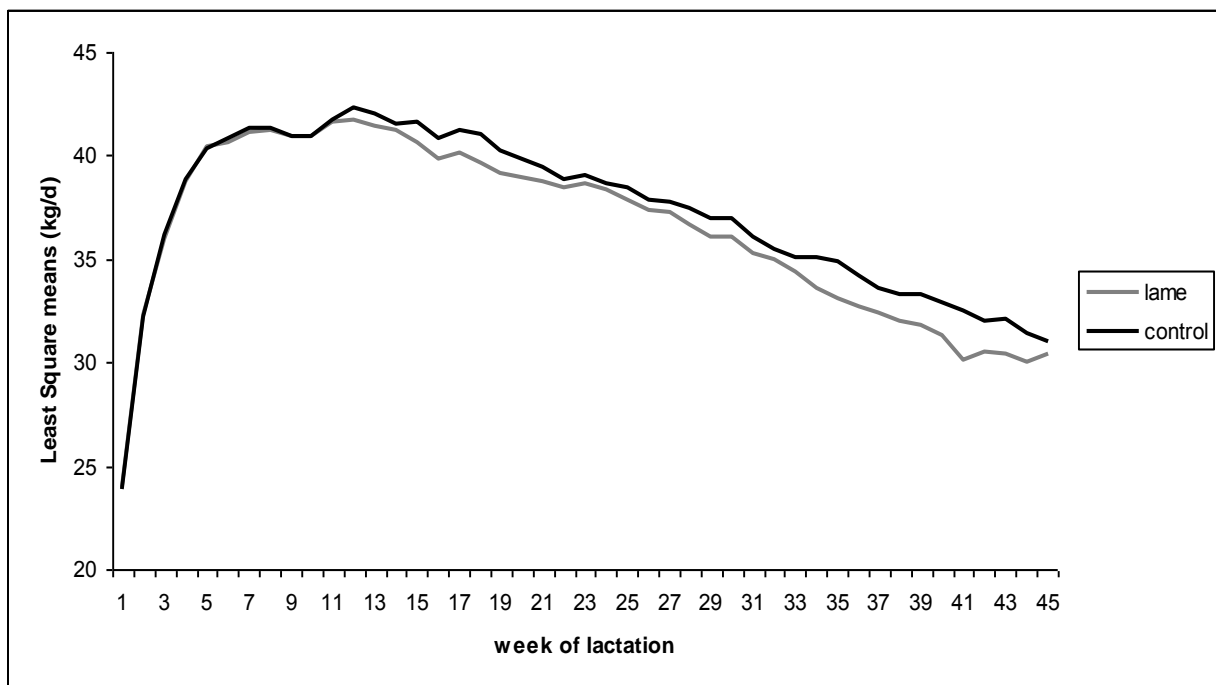
Figure 1: Impact of lameness on reproduction, survivability, and milk production of dairy cows (Bicalho et al., 2007b; Bicalho et al., 2008)



pregnancy than non-lame cows. When lameness was redefined as VLS ≥ 4 , the hazard ratio having been detected pregnant was 0.76 for lame cows versus cows with VLS < 4 (Figure 1). Lameness increased the hazard ratio of culling/death, 1.45 and 1.74 for VLS ≥ 3 and VLS ≥ 4 , respectively, versus cows with VLS < 3 and VLS < 4 , respectively. The detrimental effects were amplified when considering only severely lame and non-weight-bearing cows.

Recently, we have shown that high milk production in the beginning of the lactation is an important risk factor for CHDL; lame cows produced an excess of 3 kg/d more milk during the first three weeks of lactation compared to non-lame cows. However, when using an ANOVA that included the average milk production for the first 3 weeks of lactation as an independent variable, it was revealed that lameness incidence was associated with a milk production loss of up to 424 kg/cow per 305-day lactation (Figure 2). In summary, lameness significantly decreased the hazard of pregnancy, increased the hazard of culling/death, and was associated with significant milk loss.

Figure 2: The effect of lameness on milk production (Bicalho et al., 2008)

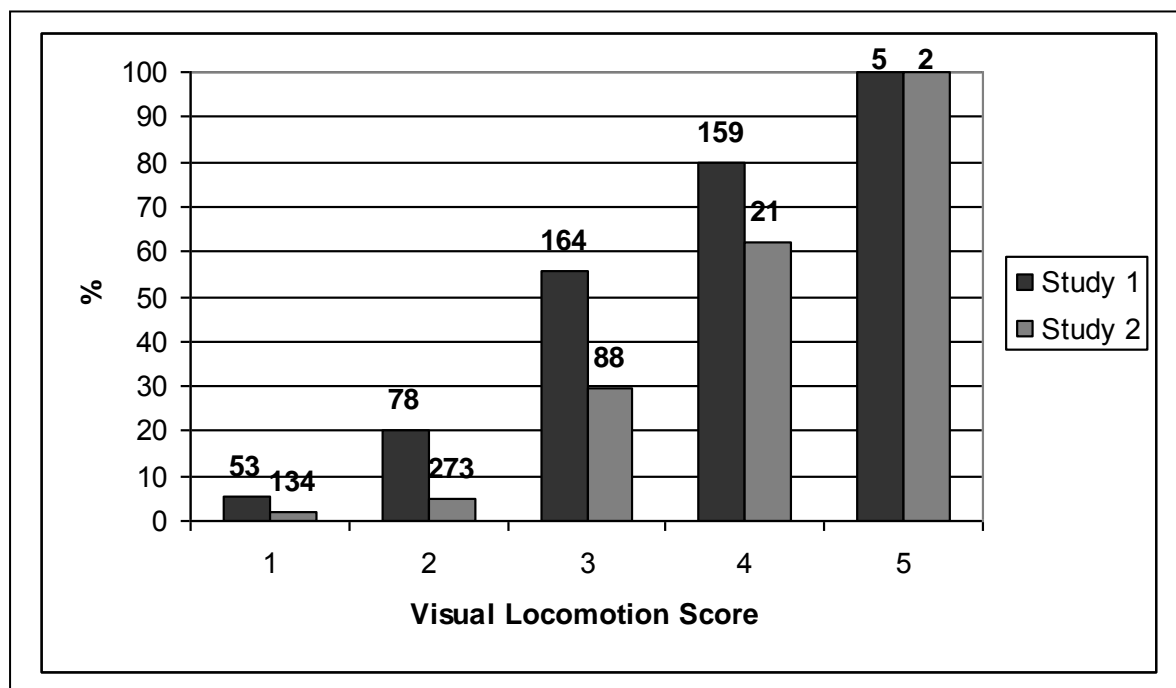


THE ACCURACY OF VISUAL LOCOMOTION SCORE (BICALHO ET AL., 2007A)

Visual locomotion scoring of cows is normally used in lameness research as a method to identify lameness. To define the accuracy of such system and also to define the best cut-off for lameness classification, we designed and conducted a large field trial on two commercial dairy farms. Of the cows diagnosed with foot lesions, 33% were detected with sole ulcer, 26% with white line disease, 14% with white line abscess, and 27 % with other diseases. A strong increasing trend in the proportion of cows with painful lesions was detected as VLS increased. The proportion of cows with painful lesions were 6% (n = 53), 20% (n = 78), 55% (n = 164), 80% (n = 159), and 100% (n = 5) for VLS 1 to 5, respectively (Figure 3). A receiver operating characteristic curve analysis was performed and the optimal sensitivity specificity relationship was determined when a cutoff point of $VLS \geq 3$ was used to detect PL. When the cut-off of $VLS \geq 3$ was used a sensitivity of 67% and a specificity of 86% was achieved for the identification of painful foot lesions. This study validated the use of VLS to diagnose painful foot lesions.

Sole ulcers and white line abscesses are ubiquitous diseases with a chronic nature that have the highest associated economic losses amongst all foot lesions. Their underlying causes are still not fully understood. The digital cushion is a complex structure composed mostly of adipose tissue located underneath the distal phalanx and plays an important function of dampening compression of the corium tissue beneath the cushion. The biomechanical importance of the digital cushion in alleviating compression under the tuberculum flexorum of the distal phalanx is well known (Raber et al., 2006; Raber et al., 2004; Logue et al., 2004).

Figure 3: The association of visual locomotion scores and incidence of painful foot lesions. (Bicalho et al., 2007)



ASSOCIATION OF DIGITAL CUSHION THICKNESS WITH LAMENESS AND BODY CONDITION SCORES (BICALHO ET AL. 2009)

We recently conducted an observational cross-sectional study to investigate the association between claw horn lesions and the thickness of the digital cushion. The thickness of the digital cushion was evaluated by ultrasonographic examination of the sole at the typical ulcer site (Figure 4). A total of 501 lactating Holstein dairy cows were enrolled in the study. The prevalence of sole ulcers was 4.2% and 27.8% (P-value <0.001) for parity 1 and parity greater than one, respectively. The prevalence of white line disease was 1.0 and 6.5% for parity 1 and parity greater than one, respectively. The prevalence of lameness (visual locomotion score ≥ 3) was 19.8% and 48.2% (P-value < 0.001) for parity 1 and greater than 1, respectively. The prevalence of sole ulcers and white line diseases was significantly associated with thickness of the digital cushion; cows in the upper quartile of digital cushion thickness had an adjusted prevalence of lameness that was 15 percentage points lower than the lower quartile (24.4% versus 8.6% prevalence). Body condition scores were positively associated with digital cushion thickness. The mean gray value of the sonographic image of the digital cushion had a negative linear association with digital cushion thickness ($R^2 = 0.14$) indicating that the composition of the digital cushion may change with its thickness. Furthermore, digital cushion thickness decreased steadily from the first month of lactation and reached a nadir 120 days after parturition (Figure 5). These results give support to the concept that sole ulcers and white line abscesses are related to contusions within the claw horn capsule and such contusions are a consequence of the lower capacity of the digital cushion to dampen the pressure exerted by the third phalanx on the soft tissue beneath.

Figure 4: Sagittal section of the bovine digit illustrating the site of ultrasonography. (Bicalho et al., 2009).

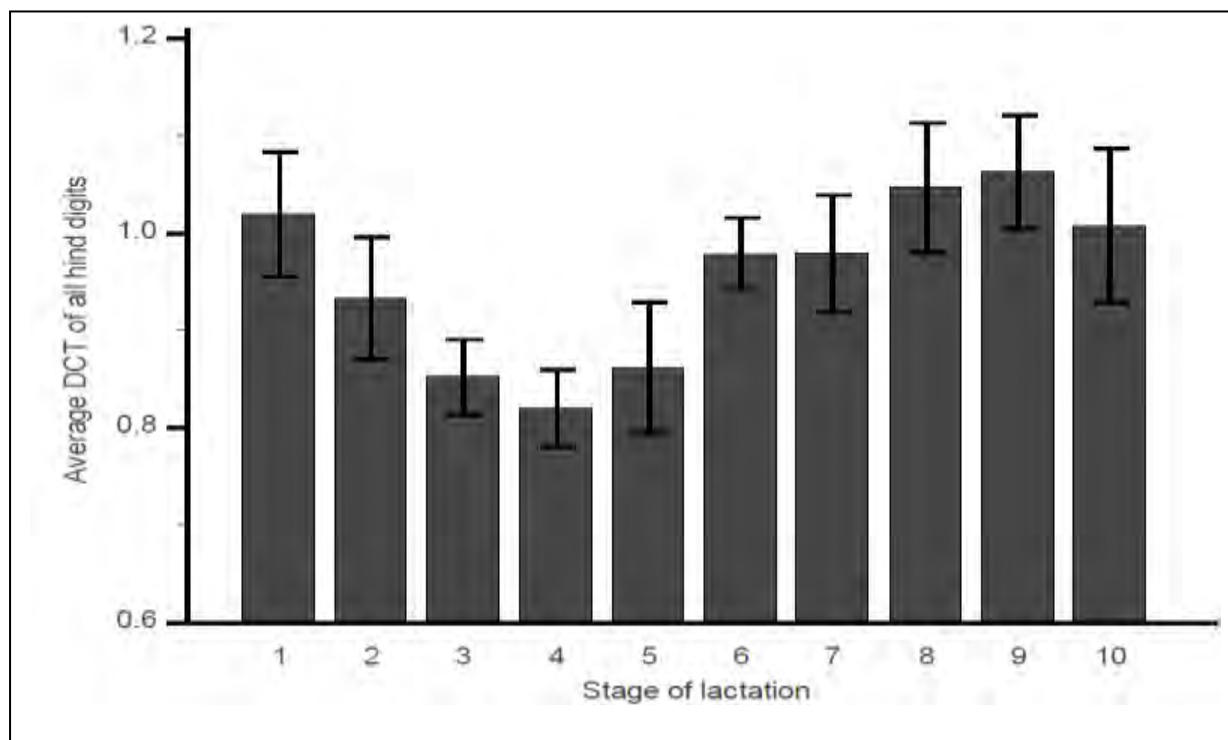


The objective of this study was to select the most parsimonious statistical model that could accurately predict the incidence of lameness in the subsequent lactation by using information available at the dry-off hoof trimming. Our hypothesis was that digital cushion thickness, body condition score, age, and the presence of CHDL at dry-off are associated with the incidence of foot lesion (sole ulcers and white-line-disease) in the subsequent lactation. Data were collected from a dairy farm located near Ithaca NY from September 11th of 2008 until January 15th of 2009. A prospective cohort study design was used. The data were collected at dry-off by the research team and throughout the subsequent lactation by trained farm employees. The following data were collected at dry-off: body condition score which ranged from one to five with a quarter point system as described by Edmonson (1989), cow height measurement which was assessed as the distance in centimeters from the floor to the dorsal aspect of the caudal sacral joint, and visual locomotion score as described by Bicalho (2007).

PREDICTING THE PROBABILITY OF LAMENESS IN THE SUBSEQUENT LACTATION USING A PARSIMONIOUS LOGISTIC REGRESSION MODEL WITH PREDICTING VARIABLES COLLECTED AT DRY-OFF

Additionally, all cows were hoof trimmed by one of the research team members and digital cushion thickness and digital lesions were recorded as described by Bicalho (2009). After the onset of lactation, cows were monitored on a daily basis for visual signs of lameness (presence of a limp) by trained farm employees. Cows that were limping were taken to the hoof trimming table for therapeutic hoof-trimming. Therapy was applied according with the diagnosed foot disorder and following a protocol designed by the Cornell Ambulatory and Production Medicine Clinic; data were recorded and entered into Dairy Comp 305. To predict the incidence of CHDL in the subsequent lactation logistic regression models were fitted to the data using Stata (StataCorp LP, Texas, USA). After variable selection steps the following variables were

Figure 5: Adjusted mean digital cushion thickness (MDCT) of all four hind digits by stage of lactation.(Bicalho et al., 2009)

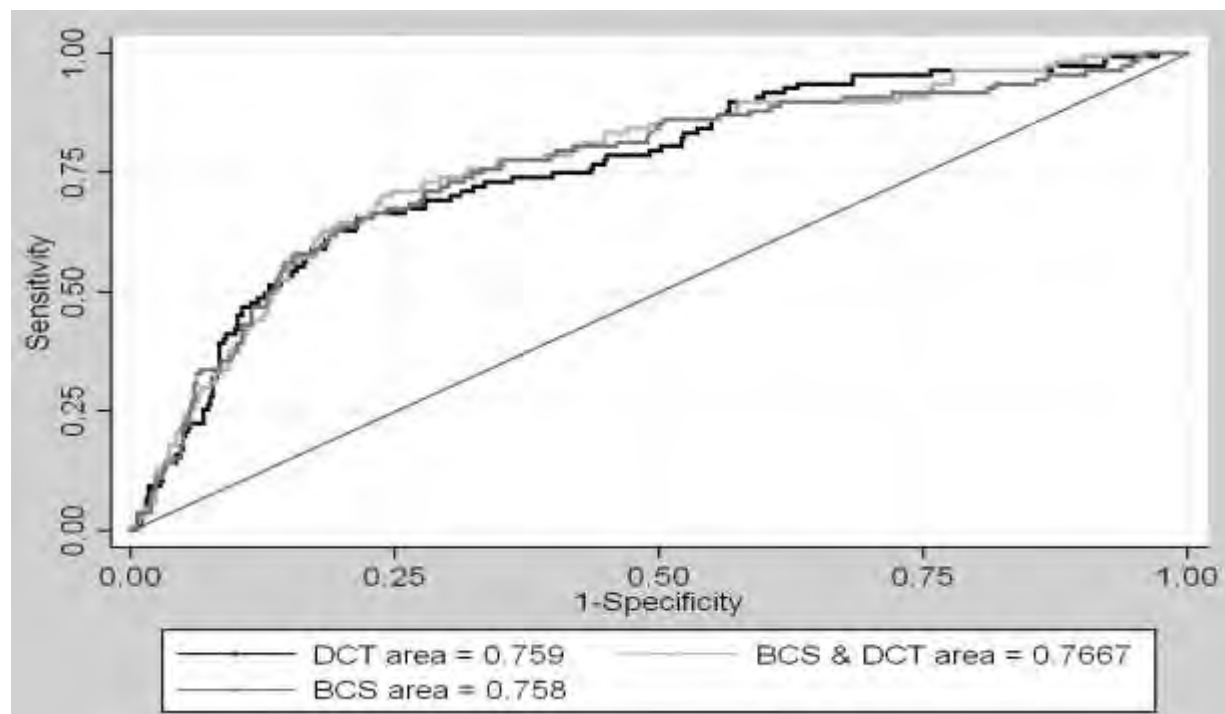


significant (P -value ≤ 0.10); digital cushion thickness (**DCT**), BCS, CHDL at dry-off, and age in days (**AGED**).

To select the most parsimonious logistic regression model with good predictability of CHDL in the subsequent lactation three different models were evaluated. All three logistic regression models predicted the incidence of CHDL in subsequent lactation with good accuracy; the area under the ROC curves were 0.76, 0.76, and 0.77 for the first, second and third logistic regression models, respectively (Figure 6). There was no significant difference between the areas under the ROC curves for the three models. When the recommended probability cut-offs were used to dichotomize cows into high risk and low risk for lameness in the sub-sequent lactation an overall accuracy of 0.74, 0.76, and 0.76 was estimated for models 1, 2, and 3 respectively.

To illustrate the dynamics of the sensitivity and specificity as the probability cut-off is gradually incremented from 0 until 1, a graphical analysis was performed for the third logistic regression model (Figure 7). The intersection of the sensitivity and specificity lines indicates the recommended cut-off probability for defining lameness. Further analysis and predictions were completed for the third logistic regression model. Predicted probabilities calculated with the probability equation described in Table 4 had a bimodal distribution, likely because of the effect of the binomial independent variable CHDL at dry-off (Figure 8). Older cows with low BCS at dry-off and a CHDL detected at dry-off hoof trimming had the highest probability of CHDL incidence in the subsequent

Figure 6: Receiver operating characteristic curves for all 3 logistic regression models.



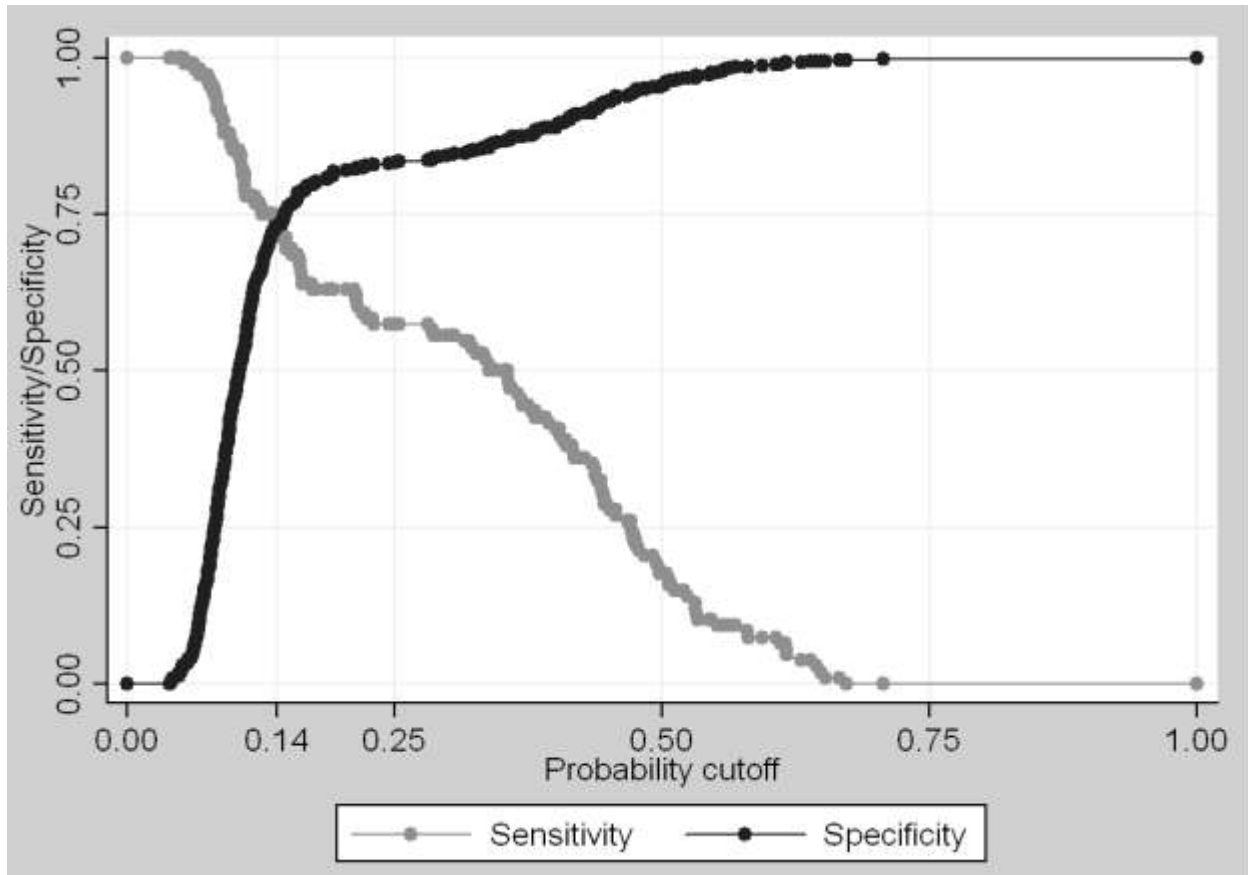
lactation (predicted probability = 0.65, 95% C.I. 0.49 – 0.78, Table 4). Whereas the lowest predicted probability of lameness was for a young cow with high BCS and without CHDL at dry-off (predicted probability = 0.03, 95% C.I. 0.01 – 0.08, Table 4).

In conclusion, we were able to predict lameness in the subsequent lactation with an overall accuracy of 0.76 using a the simple logistic regression equation described below:

$$P(\text{lesion}) = \frac{e^{-1.05 - 0.57 \cdot \text{BCS} + 0.0005 \cdot \text{AGED} + 1.64 \cdot \text{Lesiondry}}}{1 + e^{-1.05 - 0.57 \cdot \text{BCS} + 0.0005 \cdot \text{AGED} + 1.64 \cdot \text{Lesiondry}}}$$

We recently conducted a pilot study using a randomized clinical trial design to determine the effect of milking lame cows (VLS>2) twice daily versus thrice daily on milk production, culling, body condition score, and prevalence of lameness. The study was conducted on a large commercial dairy farm (3,000 milking cows) near Ithaca NY from January 1st until May 20th of 2009. Our hypothesis was that lame cows would benefit from a lower frequency milking schedule because they would spend less time standing on their feet, and consequently intra-claw corium concussions caused by the third phalanx would be decreased. Visual locomotion score and BCS of the entire milking herd were performed by two trained veterinarians. A total of 700 clinically lame cows were randomly assigned to one of two treatments: twice daily milking group and thrice daily milking group. Enrolled cows were VLS and BCS scored monthly for a total of 4 months. Additionally, daily milk production and culling information was recorded.

Figure 7: Sensitivity and specificity analysis for the third logistic regression model which included the variables BCS, AGED, and lesion at dry-off as independent variables.



DEMONSTRATION THAT A LOWER MILKING FREQUENCY (TWICE DAILY VERSUS THRICE DAILY) DECREASED THE PREVALENCE OF LAMENESS, AND IMPROVED BODY CONDITION SCORE OF LAME COWS

A mixed general linear model was used to assess the effect of milking frequency of lame cows on milk production. Lame cows that were milked twice daily produced a total of 3.5 lb/day more milk compared to the lame cows that were milked thrice daily. It is possible that the lower milking frequency allowed lame cows to spend time resting and eating which resulted in better milk production. Additionally, lame cows in the 2X milking group significantly improve BCS and had a lameness prevalence that was 14.4 percentage points lower than the controls by the end of the study period (Figure 9).

Figure 8: Frequency distribution plot of the predicted probabilities from the third logistic regression model.

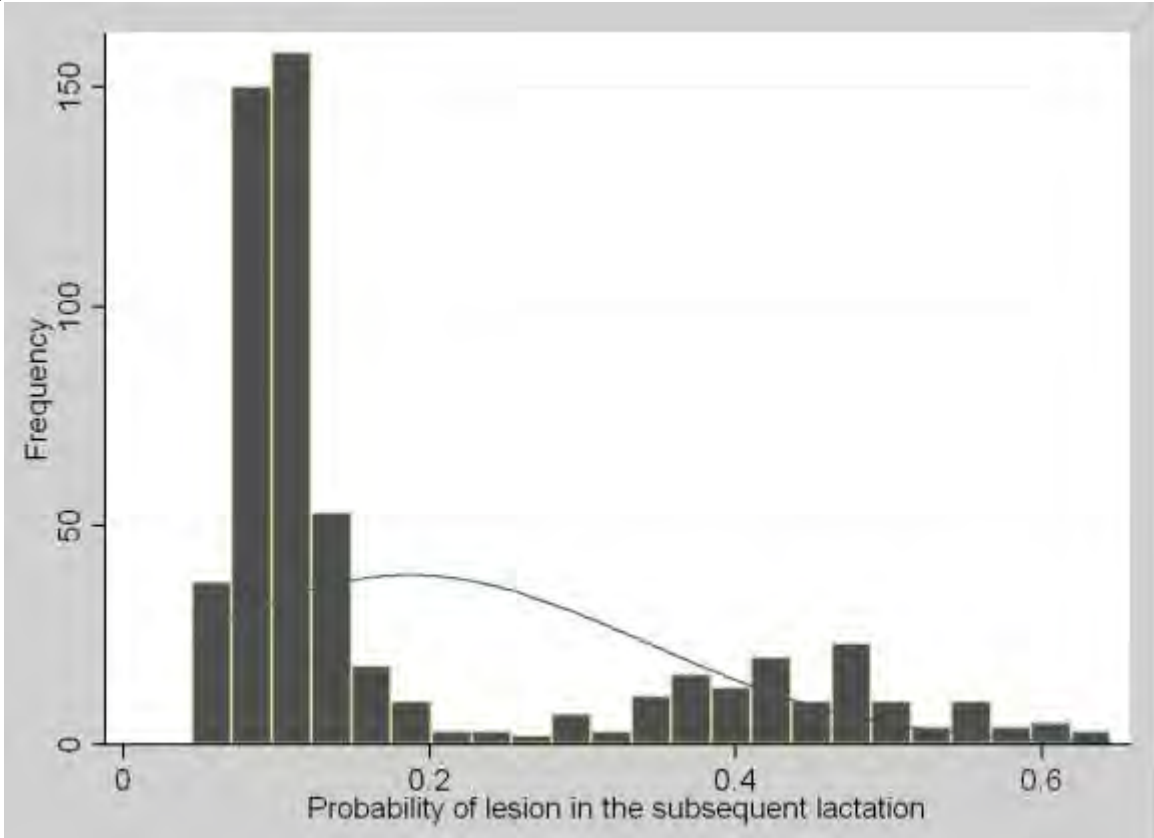
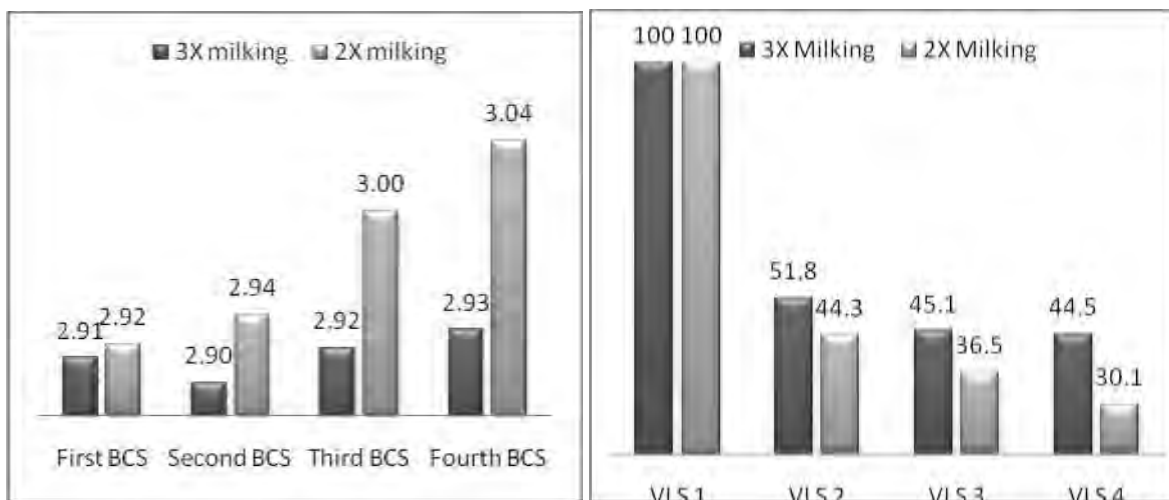


Figure 9: Lameness cows that were milked twice daily recovered from lameness and poor BCS better than lameness cows that were milked thrice daily. The left graph illustrates median BCS by milking frequency groups and the graph on the right illustrates the % of lame animals (VLS > 2) by milking frequency groups.



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MANAGING THE DYNAMICS OF FEED INTAKE AND BODY CONDITION SCORE DURING THE TRANSITION PERIOD AND EARLY LACTATION

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Achieving high dry matter intake (DMI) during early lactation is a major determinant of transition cow success, as energy balance is tightly linked with reproductive performance (Butler and Smith, 1989) and aspects of health and immunity (LeBlanc, 2010). Although a common notion is that milk yield is the major driver of negative energy balance, several data summaries (Santos et al., 2009; reviewed by Grummer et al., 2010) suggest that the relationship of negative energy balance is actually greater with DMI than with milk yield.

Clearly, nutritional and environmental management of dairy cattle during the dry and transition period have important carryover ramifications both for DMI and overall lactational and reproductive performance along with health in early lactation. The purpose of this paper is to briefly overview intake regulation in dairy cattle, describe key metabolic changes in transition cows as they integrate with intake regulation and then to review key nutritional factors during both the prepartum and postpartum period that impact peripartur DMI so that we can optimize energy and nutrient intake and subsequent performance and health outcomes.

INTAKE REGULATION IN DAIRY CATTLE

The first key concept to understand is that intake regulation in dairy cattle is complex. The various metabolic factors that influence DMI in dairy cattle were well-reviewed by Ingvarsen and Andersen (2000) and includes a variety of direct and indirect signals related to the environment, immune system, adipose tissue, signals from the gut and pancreas, and energy sensing of the liver relative to overall energy demand (Figure 1). It is likely that changes in these signals (and cow-to-cow variation in response to various environmental and metabolic stimuli) are responsible both for changes in overall average pen DMI but also variation in cow to cow DMI that likely is more associated with transition management challenges than average pen DMI per se.

More recently, Allen and coworkers (Allen et al., 2005; Allen et al., 2009) proposed that a major regulator of DMI in ruminants, and particularly dairy cattle, was hepatic energy status. This is largely driven by oxidation of fuels such as propionate derived from ruminal fermentation of rapidly fermentable carbohydrates and nonesterified fatty acids (NEFA), which are increased in the bloodstream during periods of negative energy balance and body fat mobilization (Figure 2). In periods when oxidative fuel metabolism by the liver exceeds liver energy requirements, the brain is signaled to decrease DMI. As will be discussed more in detail below, this theory is particularly attractive in explaining metabolic influences on DMI during the prepartum period. As will be described below, modulation of these pathways, particularly by propionate is less

likely during the immediate postpartum period because of the large increases in liver energy demands along with other reasons that will be discussed below.

Figure 1. “Simplified” diagram on intake regulation in dairy cattle. From Ingvartsen and Andersen, 2000.

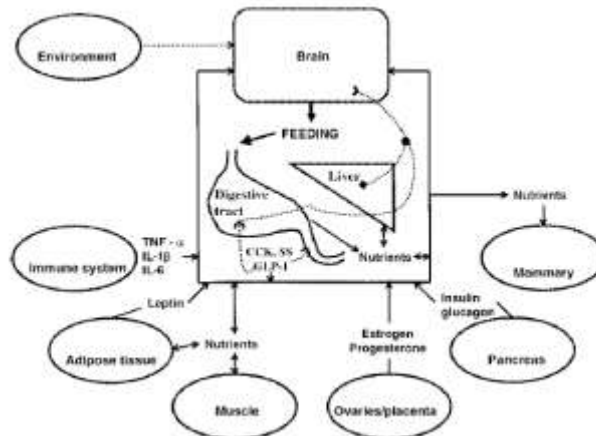
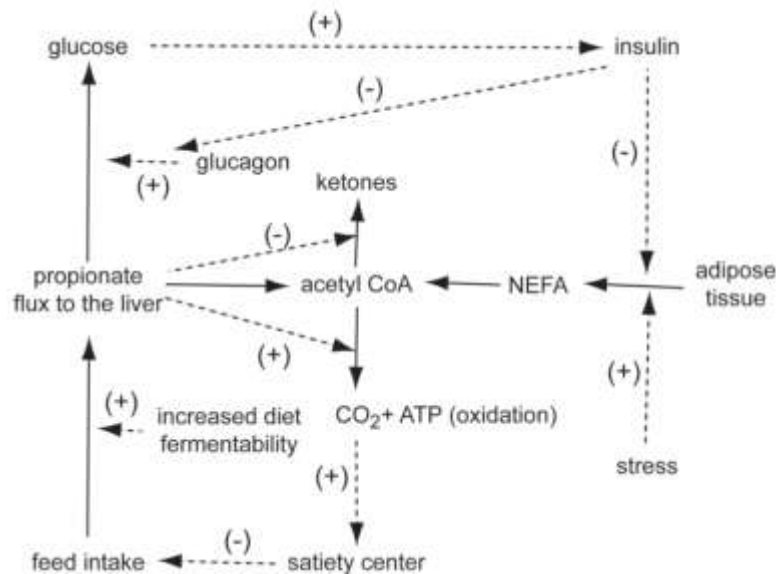


Figure 2. Mechanisms of intake regulation according to the hepatic oxidation theory. From Allen et al., 2009.



METABOLIC ADAPTATIONS IN THE TRANSITION COW

It is well-recognized that the dairy cows undergo important metabolic adaptations during late pregnancy to support fetal demands and at the onset of lactation to support milk production. These homeorhetic adaptations involved in the regulation of nutrient and energy partitioning during late pregnancy and early lactation occur in a variety of

target tissues, and typically involve changes in responses of tissues such as adipose tissue and muscle to homeostatic signals such as insulin and epinephrine (Bauman and Currie, 1980; Bell, 1995). As described above, one major adaptation includes a large increase in glucose demand by the mammary gland that is supported by dramatically increased glucose output by the liver (Reynolds et al., 2003). In addition, peripheral tissues (primarily skeletal muscle) decrease their use of glucose for fuel (Bauman and Elliot, 1983; Petterson et al., 1993), thereby sparing glucose for use by the gravid uterus and lactating mammary gland. Furthermore, increased mobilization of body fat stores facilitated by changes in adipose tissue metabolism contributes to meeting increased whole-body needs for energy at the onset of lactation (Petterson et al., 1994). The net result of these adaptations is coordinated support of fetal needs and subsequent high milk production in the face of decreasing and eventually insufficient DMI during late pregnancy and early lactation.

These changes in tissue metabolism that occur in dairy cows during the transition period are mediated largely by changes in responses to hormonal signals such as insulin. Decreased responses of these tissues to insulin are referred to in general terms as insulin resistance. As referenced above, some aspects of insulin resistance (such as those related to skeletal muscle) are very favorable for support of pregnancy and lactation because of glucose sparing for the fetus and lactating mammary gland (Bell, 1995). At the same time, we believe that insulin resistance in adipose tissue may contribute to the increasing circulating concentrations of NEFA and decreasing DMI as cows approach calving. Allen et al. (2005) suggested that the increased circulating concentrations of NEFA during late pregnancy and subsequent oxidation of these NEFA by the liver is the cause of the decreased DMI as cows approach calving. Increased resistance of adipose tissue to insulin would predispose to the cow to mobilize NEFA, hence potentially creating a vicious cycle of NEFA mobilization and DMI reduction during the late prepartum period. This would also help to explain metabolically why high body condition score (BCS) cows have lower DMI and more rapid decreases in DMI during the prepartum period than cows of moderate or low BCS (Grummer et al., 2004).

Several years ago, we became interested in further understanding the nature and timing of insulin resistance, with specific focus on determining whether the relationships of NEFA and DMI could be modulated during the transition period. Initial research conducted in our lab (Smith, 2004) suggested that adipose tissue in periparturient dairy cows actually may be more refractory to insulin during the prepartum period than during the postpartum period. Subsequent work also generally supported the concept that insulin resistance may be greater during the prepartum period than the postpartum period (Smith et al., 2006).

As a result of this work and other circumstantial evidence that accentuated insulin resistance during the prepartum period contributes to lower peripartal DMI, elevated NEFA concentrations, and increased body condition score (BCS) loss during early lactation, we wanted to determine whether specific modulation of insulin resistance in adipose tissue during the prepartum period would decrease NEFA mobilization and change the patterns of DMI and NEFA during the transition period. Using an

experimental approach, we administered compounds (thiazolidinediones; TZD) analogous to those used to treat Type II diabetes in humans to dairy cows during the prepartum period. In the first study, TZD administration tended to decrease circulating concentrations of NEFA and tended to increase DMI during the period from 7 days before calving until 7 days after calving (Smith et al., 2007). Importantly, TZD administration did not appear to interfere with the glucose sparing by peripheral tissues that is important for support of pregnancy and lactation.

In a second study (Smith et al., 2009) conducted using larger numbers of cows, we replicated the results of the first experiment in that TZD administration during the prepartum period decreased circulating NEFA concentrations and increased DMI during the immediate pre- and postpartum periods. In addition, TZD administration improved postpartum energy balance, decreased BCS loss, and decreased days to first ovulation in treated cows. These results suggested that specific modulation of insulin resistance in adipose tissue could have very positive effects on metabolic changes during the transition period and have substantial carryover effects on the dynamics of metabolism and performance during early lactation. It should be noted that this work was conducted as proof of concept relative to the mechanisms of metabolic regulation; TZD currently is not available in a form that can be used practically in the dairy industry and would require regulatory approval before such use.

PREPARTUM NUTRITIONAL MANAGEMENT AND RELATIONSHIPS WITH PERIPARTAL DRY MATTER INTAKE

Although modulation of insulin resistance using pharmaceutical approaches is intriguing, it causes us to ask questions regarding which aspects of nutritional management may influence insulin resistance. During the past few years, energy nutrition of cows during the dry period has received substantial renewed attention (Drackley and Janovick-Guretzky, 2007) and an increasing body of information suggests that energy nutrition may interact with insulin resistance during the late prepartum period.

For many years, the emphasis of researchers and industry professionals was to maximize DMI in order to ensure that cows consumed enough energy during the dry period. This strategy was supported in part by research that demonstrated that cows with lower NEFA concentrations during the last two weeks before calving on commercial dairy farms had decreased incidence of most postcalving metabolic disorders (displaced abomasum, ketosis, retained placenta, mastitis; Dyk, 1995). Given that higher DMI typically results in lower circulating NEFA, the association between higher DMI and improved health and performance was implied. Our experience would suggest that many farms indeed had improved health and performance when management changes were implemented that increased DMI of cows, particularly during the close-up period.

On the other hand, evidence suggests that plane of nutrition, in particular energy intake during the prepartum period, modulates the degree of insulin resistance and hence the relationships between NEFA and DMI during the immediate peripartal period.

Mashek and Grummer (2003) reported that cows that had larger decreases in DMI during the prepartum period, generally because of higher DMI during weeks 3 and 4 before calving, had higher concentrations of plasma NEFA and liver triglycerides during the postpartum period. More direct experimental evidence was provided by Douglas et al. (2006), who reported that cows fed at 80% of calculated energy requirements for the entire dry period had lower NEFA concentrations during the postpartum period, lower concentrations of both circulating glucose and insulin during the prepartum period, and higher DMI during the postpartum period than cows consuming 160% of predicted energy requirements throughout the dry period. Similarly, Holcomb et al. (2001) reported that cows subjected to feed restriction during the late prepartum period had blunted NEFA curves during the periparturient period. In addition, Holtenius et al. (2003) determined that cows that were dramatically overfed (178% of calculated energy requirements) for the last 8 weeks before calving had higher concentrations of insulin and glucose during the prepartum period, greater insulin responses to glucose challenge during the prepartum period, and higher concentrations of circulating NEFA during the postpartum period than cows fed for 75 or 110% of calculated energy requirements. Furthermore, Agenas et al. (2003) reported that the same cows fed for 178% of calculated energy requirements prepartum had lower DMI and prolonged negative energy balance during the postpartum period compared with cows assigned to the other two prepartum treatments. Dann et al. (2006) demonstrated that overfeeding (150% of calculated energy requirements) during the far-off period may have exacerbated insulin resistance as cows approached calving, resulting in higher NEFA and BHBA and lower DMI and energy balance during the first 10 days postcalving.

Recently, we compared responses to insulin through glucose tolerance tests conducted on dry cows fed a high energy, corn silage-based (~ 0.69 Mcal/lb of NEL; 170% of predicted energy requirements) ration versus a high straw, bulky diet (~ 0.61 Mcal/lb of NEL; 119% of predicted energy requirements; Schoenberg and Overton, 2011). Responses of NEFA to the glucose tolerance test were more refractory in the cows fed the high energy diet, suggesting that feeding the high energy diet to dry cows accentuated the insulin resistance expressed in adipose tissue. Collectively, these results support that overfeeding energy to dry cows results in changes in metabolism that in turn likely predispose cows to decreased DMI and higher NEFA during the immediate peripartal period.

This knowledge has supported the evolution in recommendations for energy nutrition of dairy cows during both the far-off and close-up periods during the past several years, with the goal of meeting, but not dramatically exceeding, energy requirements. My target range for both the far-off and close-up periods is between 110 and 120% of energy requirements. In practice, this can be achieved by formulating diets during the far-off period to contain no more than 0.59 to 0.63 Mcal/lb of NEL in order to achieve the target NEL intake of approximately 15 to 17 Mcal for Holsteins during this timeframe. During the close-up period, conventional recommendations as described above have been to maximize DMI, and hence energy intake. Although this still applies in many herd situations, we believe that some well-managed herds in which close-up cows consume large amounts of feed (> 31 to 32 lbs/day of dry matter in comingled

cow/springing heifer groups) have increased rates of metabolic disorders because of excessive energy intake during the close-up period. Accordingly, some of these herds have had success in moderating energy intake during the close-up period in group-feeding situations by incorporating straw or other low potassium, low energy forage to lower overall dietary energy concentration. Our recommendations would be to formulate the close-up diet at approximately 0.64 to 0.66 Mcal/lb of NEL if the group is a commingled cow/heifer group and approximately 0.61 to 0.63 Mcal/lb of NEL if the group is composed of mature animals and DMI is high. This lower energy diet also can be an acceptable one-group dry cow approach if overall herd management dictates such an approach. Diets formulated in these ranges will help to ensure adequate, but not excessive energy intake within the dynamics of group-feeding and competition among animals.

Diets formulated using a combination of corn silage and straw to form the forage component of the diet typically can have between 5 to 10 lbs of chopped straw, making feeding management a critical component of implementation of bulky, low energy dry cow diets. As described by Drackley (2007), the three key components of this implementation are 1) prevention of sorting, 2) ensuring continuous and non-crowded access to the TMR, and 3) careful monitoring of dry matter content and attention to detail. Most of these diets will contain added water in order to aid with prevention of sorting. A final point relative to these types of diets is that it is important to account for the metabolizable protein requirements of the cow during late pregnancy. These diets typically contain lower amounts of ruminally fermentable carbohydrate than those that have been typically fed for the last ten to fifteen years, and therefore will supply less metabolizable protein from ruminal bacteria. Inclusion of rumen-undegradable protein sources to result in total metabolizable protein supply in the range of 1,100 to 1,200 g/d is critical for early lactation performance and overall success. Furthermore, in anecdotal cases where these diets have been linked with lower milk yield during early lactation, I speculate that energy intake may have been pushed too low, especially during the close-up period.

POSTPARTUM NUTRITIONAL MANAGEMENT AND RELATIONSHIPS WITH DRY MATTER INTAKE AND METABOLISM IN EARLY LACTATION

As is described in another paper in the proceedings (Dann and Nelson, 2011), the amount of research specifically conducted to explore the relationships of postpartum nutritional management and the dynamics of DMI and BCS during early lactation has been very limited. Allen et al. (2009) would suggest that feeding highly fermentable diets to cows during early lactation would decrease DMI and overall energy status. I contend that modulation of DMI by propionate during very early lactation is less likely than at other phases of lactation for several reasons. First, NEFA likely are the predominant oxidative fuel for liver during this period and so any hypophagic effect of propionate would depend upon NEFA supply to the liver. Second, we demonstrated that there is a positive correlation between liver capacity to convert propionate to glucose and fat free NEL intake (proxy for carbohydrate intake) in cows at d 1 and 21 postcalving that does not exist either before calving or at peak lactation (Drackley et al.,

2001). This suggests that the liver has the capacity to direct additional propionate toward glucose. Third, hepatic energy requirements increase dramatically at the onset of lactation (Reynolds et al., 2003). The first point is supported by recent work (Stocks and Allen, 2011), in which they determined that the hypophagic effects of propionate increased when hepatic acetyl CoA concentrations are higher, as they would be if cows were mobilizing large amounts of adipose tissue with the corresponding proportionate uptake of NEFA by the liver.

The limited work in fresh cows (with the exception of the results from Dann and Nelson, 2011) suggests that feeding more fermentable diets during early lactation does not decrease DMI or negatively impact other aspects of performance and metabolic health. Andersen et al. (2002; 2003) fed cows either a low (25% concentrate) or high (75% concentrate) diet with whole crop barley silage as the forage base from calving through 8 wk postcalving. Feeding the high energy diet did not affect DMI, increased net energy intake and milk yield, and did not affect BCS change in early lactation. Cows fed the high energy diet had greater liver capacity to convert fatty acids to CO₂, lower capacity to convert fatty acids to triglycerides in liver, and lower blood ketones (Andersen et al., 2002).

Rabelo et al. (2003; 2005) fed cows and first calf heifers either low or high energy diets prepartum followed by either low or high energy diets postpartum until d 20 postcalving, then all cows were fed the high energy diet through d 70 postcalving. The postcalving diets were based upon alfalfa silage and corn silage – the “low” energy diet contained 29.9% NDF and 41.4% NFC; the “high” energy diet contained 24.9% NDF and 47.2% NFC. Cows fed the high energy diet postpartum tended to have higher DMI and had higher energy intake from d 1 to 30; overall effects of treatment from d 1 to 70 postcalving were not significant. Rates of increase of milk production were greater for cows fed high energy diets postcalving, and plasma concentrations of BHBA were substantially lower for cows fed the high energy diet on d 7 and 21 postcalving.

Although these studies (Andersen et al., 2002; Rabelo et al., 2003) suggest that higher energy diets are preferable during the postcalving period, the diets fed by Andersen represent the extremes and those fed by Rabelo are both higher energy diets by industry standards. This area certainly warrants active investigation.

SUMMARY AND CONCLUSIONS

Success in transition cow programs depends upon excellent management in a number of different areas to manage the dynamics of DMI and body condition mobilization along with optimize performance. Our understanding of the metabolic regulation underpinning the changes that occur in energy metabolism of cows during the transition period is increasing, and with this understanding has come new potential opportunities for enhancing transition cow health and performance. Controlling energy intake of cows during the prepartum period (both far-off and close-up) is an important factor that predisposes cows to smoother adaptations to lactation. Furthermore, available information suggests that feeding higher energy diets (or not feeding a diet

lower in energy than the high cow diet) promotes higher energy intake and milk yield along with better metabolic status during the postpartum period.

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CLA-INDUCED MILK FAT DEPRESSION IN LACTATING EWES IS ACCOMPANIED BY REDUCED EXPRESSION OF GENES INVOLVED IN MAMMARY LIPID SYNTHESIS*

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Conjugated linoleic acids (CLA) are produced during rumen biohydrogenation and exert a range of biological effects. The t10, c12 CLA isomer is a potent inhibitor of milk fat synthesis in dairy cows and some aspects of its mechanism have been established. CLA-induced milk fat depression (MFD) has also been observed in small ruminants and our objective was to examine the molecular mechanism in lactating ewes.

Multiparous lactating ewes (n = 16) were fed a basal ration (0.55:0.45 concentrates to forage; dry matter basis) and randomly allocated to 2 treatments. Treatments were zero CLA (Control) or 15 g/d of lipid-encapsulated CLA supplement containing c9, t11 and t10, c12 CLA isomers in equal proportions. Treatments were for 10 wk and CLA supplement provided 1.5 g/d of t10, c12.

Results demonstrated that there were no effects of treatment on milk yield or milk composition for protein or lactose at wk 10 of the study ($P > 0.1$). In contrast, CLA treatment decreased both milk fat percent ($P < 0.01$) and milk fat yield (g/d) ($P = 0.07$) by almost 22%. Major effects were on the de novo synthesized fatty acids (FA) (<C16) which decreased in proportion (15%) and daily yield (27%) due to CLA treatment ($P < 0.05$). In addition, the proportion of preformed FA (>C16) increased ($P < 0.05$) and there were numerical decreases in the yields of 16 carbon FA (15%) and >16 carbon FA (6%). Consistent with the FA pattern, mRNA abundance of fatty acid synthase (FASN), acetyl-CoA carboxylase (ACACA) and stearoyl-CoA desaturase (SCD1) decreased by 35 to 45% in the CLA-treated group ($P < 0.05$). Similarly, CLA treatment decreased mRNA abundance of GPAT (glycerol-3-phosphate acyltransferase; $P = 0.15$) and DGAT1 (diacylglycerol acyltransferase; $P = 0.09$), genes involved in fatty acid esterification, by almost 30%. The mRNA abundance for SREBP-1 and INSIG1, genes for proteins involved in regulation of transcription of lipogenic enzymes, was decreased by almost 60% with CLA treatment ($P < 0.05$). Furthermore, mRNA abundance of lipoprotein lipase (LPL), responsible for the hydrolysis of circulating triglycerides to allow mammary uptake of FA, decreased by almost 30% due to CLA treatment ($P = 0.06$).

In conclusion, the mechanism for CLA-induced MFD involved the SREBP transcription factor family and a coordinated down-regulation in transcript abundance for lipogenic enzymes involved in mammary lipid synthesis. A similar mechanism occurs in the dairy cow; thus the lactating ewe will be an effective model to further investigate the mechanism of MFD.

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USE OF A COMPETITION INDEX TO DESCRIBE DIFFERENCES IN PHYSIOLOGICAL PARAMETERS ASSOCIATED WITH ENERGY METABOLISM AND STRESS IN OVERSTOCKED HOLSTEIN DAIRY COWS

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When cows are crowded at the feed bunk (**FB**) aggressive displacements increase as cows vie to gain access to feed; it is likely that some cattle are more successful than others during these interactions. Previous work has shown that level of success in agonistic interactions may be an important determinant of an animal's ability to cope with an aversive environment. For example Mendl et al. (1992) showed that pigs that were aggressive but also displaced frequently during agonistic interactions (Low Success) had greater salivary cortisol concentrations and lower weight gains than individuals that were aggressive but successful at displacing others (High Success). This relationship between competitive success and physiological outcomes in overstocked cattle has never been explored. The objective of this study was to evaluate how stress physiology and energy metabolism are affected based on a cow's ability to compete for limited access to the FB.

Forty Holstein dairy cattle were housed in an overstocked pen (5 stalls/10 cows and 0.34m linear FB space/cow) in groups of 10 (4 heifers and 6 multiparous cows) for 14 d. Plasma NEFA and glucose were measured from blood sampled every 2 d and during a glucose tolerance test (**GTT**) performed on d 13. Feces, collected every 2 d, were analyzed for fecal cortisol metabolites (**FCORT**). Plasma cortisol response to an ACTH challenge was measured on d 14. Feeding behavior and displacements at the FB were recorded from d 7 to d 10 of the observation period. A competition index (**CI**) was calculated for each cow by dividing the number of displacements the animal initiated at the FB by the total number of displacements the animal was involved in, either as an initiator or receiver. Cows were then divided into 3 sub-groups based on their CI: High-Ranking (**HR**: $CI \geq 0.6$), Middle-Ranking (**MR**: $0.4 \leq CI < 0.6$), and Low-Ranking (**LR**: $CI < 0.4$).

Heifers accounted for 7%, 36% and 79% of the total number of animals in the HR (n=15), MR (n=11), and LR (n=14) groups, respectively. LR cows had greater NEFA and FCORT concentrations during the overstocked period compared to both MR and HR cows ($P \leq 0.05$) despite having no differences in average daily feeding time and proportion of total daily time spent feeding during the 3-h post fresh feed delivery. During the GTT, the glucose response curves of cows in the 3 CI groups were not different; however, LR had a greater insulin response ($P=0.03$) suggesting differences in tissue responses to insulin between 3 CI categories. Average cortisol response to ACTH was not different between the 3 CI categories ($P=0.53$). Cows that are less successful at competitive interactions may be at greater risk for health complications associated with negative energy balance, as there was evidence of possible insulin resistance and greater daily NEFA concentrations among these cows. LR cows may also experience a greater stress load during overstocking as evidenced by higher

FCORT concentrations. Heifers seem to make up the LR group when they are forced to compete with multiparous cows. To protect LR cattle overstocking must be avoided during periods of increased metabolic stress such as the transition period.

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EARLY LIFE MANAGEMENT AND LONG TERM PRODUCTIVITY OF DAIRY CALVES

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For many years, early life management of the calf has focused on survival rates and rumen development. However, recent studies suggest that colostrum status as well as nutritional status during the pre-weaning phase may have long term carry-over effects on milk yield potential (Foldager and Krohn, 1994; Bar-Peled et al, 1997; Shamay et al., 2005; Terré et al., 2009; Moallem et al. 2010).

The objective of this study was to investigate this relationship in the Cornell Dairy Herd using a Test Day Model (TDM) to evaluate the lactation response over eight years. The management objectives of the calf program have been to double the birth weight by weaning through increased milk replacer intake. The TDM was utilized to generate lactation residuals accounting for the effects of test day such as calving season, days carried calf, days in milk, and lactation number (Everett and Schmitz, 1994; Van Amburgh et al., 1997). Lactation residuals from the TDM were generated from 792 heifers with completed lactations and linear regressions were run on several measures of pre-weaning growth performance, management factors and TDM milk yield solutions. Significant correlations were found for pre-weaned average daily gain (ADG), weaning weight, year and month of birth. Pre-weaning ADG ranged from 0.13 kg to 1.23 kg and ADG had the greatest correlation with first lactation milk production. Using the TDM solutions, for every 1 kg of pre-weaning ADG, heifers produced 1,067 kg more milk during their first lactation ($P < 0.01$) and 235 kg more milk for every Mcal of ME intake above maintenance. Further, pre-weaning ADG accounted for 25 percent of the variation in first lactation milk yield. Other factors analyzed included age at first calving and birth weight but correlations with TDM lactation residuals were not significant.

Data from another farm confirmed these observations. In a commercial herd from northern NY state, for every 1 kg of pre-weaning ADG, milk yield increased by 1,113 kg in the first lactation and furthermore, every 1 kg of pre-pubertal ADG, from birth to breeding, was associated with a 3,281 kg increase in first lactation milk yield. These results suggest that increased growth rate prior to weaning results in some form of epigenetic programming that is yet to be understood, but has positive effects on lactation milk yield.

This analysis identifies nutrition and management of the pre-weaned calf as major environmental factors influencing the expression of the genetic capacity of the animal for milk yield. Furthermore, these data reinforce the observation that lifetime performance is influenced by early life development and dairy producers have the ability to manipulate this early life programming via nutrition. The length of time that heifer calves are responsive to the effects of nutrition warrants further investigation. However, we now know that this manipulation must start immediately after birth and continue for at least five weeks and must be in the form of liquid feed in order to have a positive influence on lifetime performance.

THE EFFECTS OF INCREASED MILKING FREQUENCY DURING EARLY LACTATION ON MILK YIELD AND MILK COMPOSITION ON COMMERCIAL DAIRY FARMS

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Increased milking frequency (IMF) during early lactation has the potential for carryover responses following the return to normal herd milking frequency. The objective of this experiment was to determine the consistency of response of cows in commercial dairy farms to IMF during early lactation. Cows ($n=398$) were assigned randomly at calving within each of the four participating farms to one of two treatments. The control group was milked twice-daily (2x) during the entire lactation. The IMF group was milked four-times daily (4x) starting on d 1 to 7, depending on farm, until d 21 postcalving and 2x thereafter. Cows in the IMF group were milked at the beginning and again at the end of the normal milking routine. These resulted in different milking intervals across the farms for the 4x cows with a minimum interval of 3.5, 4.0, 5.0, and 6 h for each of the four farms, respectively. On average, milk yield of cows subjected to IMF was increased by 2.2 kg/d during the first 7 months of lactation (34.6 vs. 32.4 kg/d; $P < 0.01$). Interactions of treatment with lactation group (primiparous vs. multiparous) were not significant. Although percentages of fat and protein in milk were decreased by early lactation IMF (3.69% fat and 3.05% true protein for control vs. 3.57% fat and 2.99% true protein for IMF; $P = 0.01$), overall yields of fat (1.18 vs. 1.21 kg/d; $P = 0.08$) tended to be increased and yields of protein (1.02 vs. 0.98 kg/d $P = 0.01$) were increased by IMF.

Early lactation IMF did not affect udder health as assessed by SCC linear score. There was a tendency for increased serum concentrations of nonesterified fatty acids and increased serum β -hydroxybutyrate for cows subjected to IMF. Cows subjected to IMF were 1.4 times more likely to be classified as subclinically ketotic as the control cows.

Although the direction of response was the same on all farms, within-farm analysis indicated that the magnitude of the milk yield response varied from 4 to 10% (3.1, 1.5, 1.8, and 1.8 kg/d for each farm). Differences in the magnitude of the response appears to be influenced by management practices specific to each farm, which included but were not limited to housing system, stocking density, nutrition, genetics and other covariates differing among farms.

In conclusion, early lactation IMF has the potential to increase yields of milk and milk components and has the potential for robust responses across dairy farms.

ANTIOXIDANT ACTIVITY OF CALF MILK REPLACERS

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A milk replacer (MR) is designed to mimic the nutritional benefits of milk in an effort to nourish a newborn calf, reduce calf mortality, strengthen immunity and increase animal life span and productivity. Antioxidants (AO) can enhance immune defense by reducing oxidative damage, but MR are traditionally not formulated for AO activity. The objective of this study was to compare total AO activities of bovine milk with six calf MR (Table 1), varying in amount and source of fat and protein. MR was donated by Milk Products, Inc. Milk was obtained from the Cornell Dairy Research Farm bulk tank, representing milk produced within 24 h by 455 cows. MR was mixed to 150 g/L with 40°C, purified water. Following hexane lipid extraction, both samples were extracted 5 times with ethyl acetate, then evaporated and reconstituted with 70% methanol/water. Samples were assessed for total AO activity using the peroxy radical scavenging capacity (PSC) assay (Adom and Liu, 2005).

In the case of MR A, type of protein (soy) had a positive effect ($P<0.01$) on AO activity, which is likely attributed to the isoflavones and cinnamic acid derivatives present in soy. With the exception of MR A, AO activity of natural bovine milk was higher than the MR. This may be due in part to its amino acid composition (Clausen et al., 2009) and its increased fat content (Chen et al., 2003) as well as its FA profile, which differs significantly from that of most MR by containing short, medium and long chain FA. Although fat content ($P=0.057$) tended to have an effect on AO activity, the PSC assay uses defatted milk/MR; thus, losses in lipid-bound AO are not accounted for (Lindmark-Mansson and Akesson, 2000). The two MR (B and D) with the commercial FA supplement had different ($P<0.01$) AO activity; however, this is likely due to the amount of vitamin and trace mineral premix. MR D had roughly half the amount of vitamin and trace mineral premix (and therefore half the levels of Vit A, C, D and E) of MR B because it is formulated to be fed at 1.02 kg/d, whereas MR B is to be fed at 0.57 kg/d.

Future research is warranted to compare MR with a broader range of FA profiles, fat sources and content as well as the effect of additional compounds in milk that may impact AO activity.

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Table 1. Antioxidant activity of milk and milk replacers¹

ID	Description	Protein Source	Animal Fat, %	Vegetable Fat, %	VCE, umol	SEM
A	21% CP, 20% fat	50% milk, 50% soy	100	0	86.0 ^a	1.92
Milk	Bovine milk; 27% CP, 29% fat	milk	100	0	52.7 ^b	1.92
B	FA supplement ² ; 22% CP, 20% fat	milk	98.4	1.56	44.3 ^c	1.92
C	20% CP, 20% fat	milk	100	0	16.1 ^d	2.35
D	FA supplement ² ; 28% CP, 18% fat	milk	98.6	1.39	14.9 ^d	1.92
E	28.5% CP, 15% fat	milk	100	0	12.1 ^d	1.92
F	5% plasma; 22% CP, 20% fat	animal	100	0	10.5 ^d	1.92

¹Results for total AO activity are expressed as umol of vitamin C equivalent (VCE)/mL of milk or reconstituted milk replacer

²FA supplement represents a commercial fatty acid supplement of specific short, medium and long polyunsaturated fatty acids

^{abcd}Means with different superscript differ, $P < 0.01$

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