Proceedings of the 2006

Tri-State Dairy Nutrition Conference

"Celebrating 15 years of excellence in dairy nutrition"

1992 - 2006

- Nutrition and Animal Health
- Ration Formulation and Forages
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Tri-State Dairy Nutrition Conference

Planning Committee

Sustaining Members

Dr. Maurice L. Eastridge
Committee Chair
The Ohio State University
Department of Animal Sciences
221B Animal Science Building
2029 Fyffe Court
Columbus, OH 43210
(614) 688-3059
(614) 292-1515 FAX
eastridge.1@osu.edu

Dr. Herbert Bucholtz
Michigan State University
Department of Animal Science
2265H Anthony Hall
East Lansing, MI 48824
(517) 355-8432
(517) 353-1699 FAX
bucholtz@msu.edu

Dr. Timothy Johnson
Purdue University
Department of Animal Sciences
3-118 Lilly Hall
West Lafayette, IN 47906-1151
(765) 494-4810
(765) 494-9346 FAX
tjohnso2@purdue.edu

Advisory Members

Mr. Dean Andres
Akey, Inc.
112 Sylvanus St.
Archbold, OH 43502
(419) 445-2979
Email: dandres@akey.com

Mr. Dan Kurtz
Renaissance Nutrition, Inc.
6051 Roller Road
Salem, OH 44460
(330) 533-2560
(330) 533-8992 FAX
Email: dkurtz1@aol.com

Mr. Stacy Nichols
Vita Plus Corp.
3836 N 215 E St.
Winamac, IN 46996
(574) 946-7567 (Office/FAX)
(574) 595-0901 Cell
Email: snichols@vitaplus.com

Dr. Bob Baugher
Countryside Vet Service/Large Animal
4680 Mahoning Avenue
Warren, OH 44483
(330) 847-7337
Email: drbob@onecom.com

Mr. Fred Martsolf
Cargill, Inc.
17563 Kenmore Road
Lake Milton, OH 44429
(412) 999-4806 Cell
Email: fred_martsolf@cargill.com

Ms. Jennifer Swim
J.E. Swim Dairy Consulting
302 W. Front Street
Cambridge City, IN 47327
(765) 478-3345; FAX - same
Email: swimpom@infocom.com

Mr. Bob Krieger
Land O' Lakes-Purina Mills, Inc.
7907 50th Avenue
Remus, MI 49340
(989) 967-8351
(989) 330-1302 cell
(989) 967-3011 FAX
Email: bob_krieger@purina-mills.com

Mr. Mike McFadden
MSU-Extension
200 N. Main Street
Mt. Pleasant, MI 48858
(989) 772-0911, Ext. 302
(989) 773-1622 FAX
Email: mcfadd10@msu.edu

Conference Assistant

Ms. Laurie Winkelman
The Ohio State University
Department of Animal Sciences
222 C Animal Science Building
2029 Fyffe Court
Columbus, OH 43210
(614) 688-3143
(614) 292-1515 FAX
Email: winkelman.6@osu.edu

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M. L. Eastridge, Editor

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The Conference Planning Committee extends appreciation to Ms. Laurie Winkelman for her assistance in organizing the Tri-State Dairy Nutrition Conference and acknowledges Mrs. Michelle Milligan for assistance with preparation of the Proceedings.

Proceedings orders: Contact Ms. Laurie Winkelman at The Ohio State University at (614) 688-3143; email: winkelman.6@osu.edu

Abbreviations that may be found in this publication include:

AA = amino acids
ADF = acid detergent fiber
BCS = body condition score
BW = body weight
CP = crude protein
CV = coefficient of variation
DE = digestible energy
DIM = days in milk
DHI = Dairy Herd Improvement
DM = dry matter
DMI = dry matter intake
ECM = energy corrected milk
FA = fatty acids
FCM = fat corrected milk
ME = metabolizable energy
MCP = microbial crude protein
MP = metabolizable protein
NEFA = non-esterified fatty acids
NE_g = net energy for gain
NE_m = net energy for maintenance
NE_L = net energy for lactation
NDF = neutral detergent fiber
NFC = nonfiber carbohydrates
NRC = National Research Council
NSC = nonstructural carbohydrates
OM = organic matter
r = correlation coefficient
R^2 = coefficient of determination
RDP = rumen degradable protein
RFV = relative feed value
RMSE = root mean square error
RUP = rumen undegradable protein
SCC = somatic cell count
SD = standard deviation
SE = standard error
SEM = standard error of mean
TDN = total digestible nutrients
TMR = total mixed ration
VFA = volatile fatty acids

Note: Most of the units of measure in this publication are expressed in US equivalents; however, in some cases, metric units are used. Use the following to make conversions:

1.0 lb = 0.454 kg = 454 g
1.0 ft = 0.3 m = 30 cm
°F = (°C x 1.8) + 32
1 U.S. ton = 2000 lb = 909 kg
1 metric ton = 1000 kg = 1.1 U.S. ton (2200 lb)

Abbreviations for metric units are:

ppm = parts per million
mg = milligrams
g = grams
kg = kilograms
cm = centimeters
mm = millimeters
m = meters
km = kilometers

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Celebrating 15 years of excellence in dairy nutrition

Tri-State Dairy Nutrition Conference

1992 - 2006

T.R. Johnson[1], H.F. Bucholtz[2], and M.L. Eastridge[3]; [1]*Purdue University*, [2]*Michigan State University*, and [3]*The Ohio State University.*

The Tri-State Dairy Nutrition Conference, Fort Wayne, IN is a yearly conference for feed industry professionals who have direct contact with dairy producers. The conference began in 1992 as a spin-off from the 1991 Ohio Dairy Nutrition Conference. In September 1991, a meeting to discuss planning a tri-state conference was held with faculty and Extension staff from Purdue, Michigan State, and The Ohio State Universities. The first Conference was held May 20 - 21, 1992 on the Purdue campus in Fort Wayne, IN. A planning committee was formed after the 1992 conference. The present committee consists of five feed industry personnel, one consultant, one veterinarian, one Extension staff, and a faculty member from each of the three universities. An ad hoc member who represents the company hosting the pre-conference, and an OSU conference assistant meet with the planning committee. The faculty have continuous committee membership, but the other eight members serve 3-year staggered terms. The committee meets twice each year. Presentations and proceedings papers are oriented to timely, in-depth, and practical dairy nutrition topics to meet on-farm nutritionists' needs. Participation and support by industry have been very important to the success of the Conference. Due to a continuous expansion of attendance, the conference moved in 1996 from the Purdue campus to the Grand Wayne Convention Center that is located downtown. Because of renovation of the Grand Wayne Center, the Conference was held for one year (2005) at the Allen County War Memorial Coliseum in Ft. Wayne. A tradeshow and rotating industry-sponsored pre-conference are a part of the annual Conference. A conference web page http://tristatedairy.osu.edu was launched in 1997. Continuing education credit is offered to veterinarians and members of the American Registry of Professional Animal Scientists (ARPAS). The success of the Conference is demonstrated by attendance and citation or reprinting of proceedings manuscripts in the scientific, international, and popular press. Multi-state programs similar to the Tri-State Dairy Nutrition Conference can serve a vital role in bringing research and Extension faculty from different universities and allied-industry professionals together to meet the educational needs of a rapidly changing dairy industry. [see J. Dairy Sci. 82 (Suppl. 1):56, 1999; J. Dairy Sci. 89:1121-1368, 2006].

Table 1. Attendance at the Tri-State Dairy Nutrition Conference

Total Attendance	2005	2004	2003	2002	2001	2000	1999	1998	1997	1996	1995	1994	1993	1992
	469	493	513	535	464	399	436	450	350	358	337	275	265	152
Distribution (%) of attendance by state														
Ohio	22.0	25.0	22.8	28.9	28.5	28.7	28.9	30.2	32.6	41.6	37.4	42.4	36.9	45.6
Michigan	23.0	25.5	24.6	23.9	23.7	23.0	25.2	24.9	30.0	22.9	24.0	26.9	25.8	27.2
Indiana	14.0	15.2	16.3	16.6	14.7	17.5	16.6	19.2	16.9	20.1	16.6	17.0	21.3	19.9
Illinois	3.0	4.9	4.2	2.7	2.1	1.7	4.3	3.1	4.0	3.4	4.2	2.7	2.3	
Kentucky	2.0	2.0	2.5	1.3	2.4	1.7	2.9	5.0	4.0	2.8	1.5	1.9	2.3	
New York	3.0	4.7	4.6	4.3	7.1	6.6	6.6	4.1	5.1	1.1	3.0			
Pennsylvania	7.0	5.4	11.3	8.8	9.0	6.0	4.6	4.3	2.0	2.5	3.3	2.7	6.1	
Wisconsin	3.0	3.7	3.0	2.0	2.6	2.9	2.3	3.9	1.4	2.5	3.6	1.5	1.9	
Canada	4.0	4.7	4.4	2.8	3.3	3.7	1.3							
Japan	1.0													
Other states	18.0	8.9	6.3	9.6	6.2									
Distribution (%) of attendance by job affiliations														
Feed industry - sales/nutrition advisor	60.0	44.0	61.0	58.0	51.0	56.4	65.7	61.0	53.1	57.8	56.1	60.8	50.0	47.7
Feed industry - management/research	12.0	14.0	9.0	11.0	11.0	10.0	8.9	8.5	8.8	7.5	11.1	8.4	10.5	9.1
Private nutrition consultant	6.0	7.0	9.0	7.0	7.0	7.9	5.3	4.3	8.2	6.8	6.6	7.8	3.2	12.5
Veterinarian	5.0	8.0	9.0	3.0	8.0	5.7	9.5	6.4	8.8	8.0	7.6	4.8	16.9	8.0
Government agency - e.g. regulatory	0	0	0	0	1.0	0.7	0	0	0	0	0.5	0	0.8	0
Dairy producer	5.0	6.0	5.0	12.0	8.0	4.3	4.7	5.9	5.4	7.5	2.0	3.6	2.4	0
University - campus	5.0	15.0	5.0	3.0	8.0	8.6	3.6	10.7	12.3	6.8	11.1	9.6	11.3	14.8
University - county/district	7.0	6.0	2.0	6.0	5.0	6.4	2.4	3.2	3.4	5.6	5.1	4.8	4.8	8.0

The Meeting Within the Meeting

By Jeffrey Bewley, Purdue University[1]

Having always been fairly academically oriented, I've always enjoyed attending professional and technical conferences, such as the annual American Dairy Science Association meetings or regional nutrition conferences. I view them as an opportunity to keep abreast of the latest scientific knowledge and to continue my education. So, I was a bit confused a couple years ago when a colleague and friend of mine told me that when he attended meetings, he was more interested in "the meeting within the meeting" than the technical content of the program. This gentleman has been a successful salesperson and nutritionist for nearly 30 years; thus, I decided it would be in my best interest to understand better what he was referring to. According to my friend, "the meeting within the meeting" refers to everything that occurs outside of the presentation rooms during technical conferences. This may include conversations in the hallway before or after presentations; pre-arranged dinner meetings with customers, suppliers, or colleagues; or even discussions outside of the meeting rooms during presentations.

Being young and naïve, initially I thought this mentor of mine was really missing the point of attending conferences and seminars. However, it only took a couple of meetings for me to recognize that his emphasis on "the meeting within the meeting" was a major contributor to his success as a salesperson and nutritionist. A tremendous amount of business is conducted in this manner. Customers may be more receptive to listening to your ideas when they no longer have "home court advantage," as they usually do when you call on them at their dairy farm. Suppliers provide market insights and new information with regard to customer and competitor activity. In addition, they may be helpful with introductions to potential new customers. Conversations with colleagues provide additional market insight and increase and strengthen your professional network. During my time in the industry, I was consistently in awe of how much my co-workers and I accomplished during "the meeting within the meeting." I am grateful for the lessons I learned from my more experienced colleagues. Recognizing that many people who read this are either currently students or early-career animal science professionals, I encourage you to re-think your approach to attending meetings and conferences. The importance of networking, particularly early in your career, can not be underestimated. Whether you are looking for your first job, content with your current job, or looking for a new professional challenge, formal and informal networking will improve your chances for success. I encourage you to seek out opportunities to broaden your professional network. One particularly effective way to do this is to identify a few professional conferences to attend each year. If you are of an academic mindset, step out of your comfort zone a bit and introduce yourself to people in the hallways, hotel lobbies, and restaurants. Make it a priority to pre-arrange dinner meetings with customers, suppliers, or colleagues. A business professor of mine always suggested that "the best conversations always occur around meals." If you're a student, before you attend a conference, print some business cards with your contact information to hand out at the meeting. You can purchase perforated business cards at office supply stores and print these out on your home printer or you can order professionally-produced business cards. These will help people you meet remember you. Jeffrey Gitomer, a sales training guru, suggests that it's not "who you know" that's important, but rather "who knows you." By no means am I suggesting that you attend conferences without ever listening to a presentation. In the animal sciences, the science behind how we feed, breed, and manage the animals we work with is continuously and rapidly evolving. To be successful, we all must be committed to lifelong learning. That being said, there may be times at conferences when continuing a conversation in the hallway is more beneficial than going back into the meeting room. Your goal should be to establish a reasonable balance between the networking and technical aspects of a meeting. The next time you attend a meeting or conference, don't forget that the "meeting within the meeting" is just as important as the actual meeting. Attending professional conferences can be beneficial for your career in more ways than one!

[1]*Jeffrey Bewley is currently pursuing a Ph.D. in Dairy Management at Purdue University with Dr. Mike Schutz. His research involves intervention technologies.*

Proceedings of the 2006 Tri-State Dairy Nutrition Conference

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Ration Formulation and Forages

Nutrition and the Environment

Concepts in Lipid Digestion and Metabolism in Dairy Cows

Dale E. Bauman and Adam L. Lock[1]
Department of Animal Science
Cornell University

Introduction

Fat and fatty acid metabolism and digestion in the dairy cow are of considerable interest, both to scientists and the dairy industry. This renewed interest is based on several reasons; first, the use of dietary fat supplements has increased, and will continue to do so, as nutritionists strive to increase the energy density of diets to meet requirements of the high producing dairy cow; second, we now recognize that fatty acids, both of dietary and rumen origin, can have specific and potent effects on ruminant metabolism and human health; and third, we now recognize that specific fatty acids produced in the rumen are potent regulators of milk fat synthesis in the mammary gland. Our objective in this review is to provide an overview of lipid metabolism in the dairy cow. Our focus will include the biological processes and quantitative changes occurring during the metabolism of fatty acids in the rumen and their subsequent absorption in the small intestine. In addition, we will discuss the interrelationship between rumen lipid metabolism and milk fat synthesis, and dietary factors that result in milk fat depression.

Lipid Metabolism in the Rumen

Extensive metabolism of lipids occurs in the rumen and this has a major impact on the profile of fatty acids available for absorption and tissue utilization. The two major processes that occur are hydrolysis of ester linkages in lipids found in feedstuffs and the biohydrogenation of unsaturated fatty acids (Figure 1). Hydrolysis of dietary lipids is predominantly due to rumen bacteria, and although the extent of hydrolysis is generally high (>85%), a number of factors that affect the rate and extent of hydrolysis have been identified. For example, the extent of hydrolysis is reduced as the dietary level of fat is increased or when factors such as low rumen pH and ionophores inhibit the activity and growth of bacteria (see reviews by Doreau et al., 1997; Harfoot and Hazlewood, 1997). Unsaturated fatty acids are toxic to many rumen bacteria, so the second major transformation that dietary lipids undergo in the rumen is biohydrogenation of polyunsaturated fatty acids **(PUFA)**. Biohydrogenation requires a free fatty acid to proceed; as a consequence, rates are always less than those of hydrolysis, and factors that affect hydrolysis also impact biohydrogenation. Classical pathways of ruminal biohydrogenation were established using pure cultures of rumen organisms and the bacteria involved in biohydrogenation have been classified into two groups, A and B, based on their metabolic pathways (Kemp and Lander, 1984). To obtain complete biohydrogenation of PUFA, bacteria from both groups are generally required. Although Group A contains many bacteria that can hydrogenate PUFA to *trans* 18:1 fatty acids, only a few species characterized as Group B can carry out the last step and hydrogenate the *trans* 18:1 fatty acid to stearic acid (Harfoot and Hazlewood, 1997). This feature of biohydrogenation explains why increased feeding

[1]Contact at: 262 Morrison Hall, Cornell University, Ithaca NY 14850, (607) 255-2262, FAX: (607) 255-9829, Email: deb6@cornell.edu and all36@cornell.edu; web site: http://www.ansci.cornell.edu/bauman/

of PUFA simultaneously causes an increase in the rumen concentration of monounsaturated fatty acids and a decrease in the concentration of saturated fatty acids.

The rates of rumen biohydrogenation of fatty acids are typically faster with increasing unsaturation, and based on available data from lactating dairy cows, linoleic and linolenic acids are hydrogenated to the extent of 70 to 95% and 85 to 100%, respectively (Lock et al., 2005; 2006). These averages are in agreement with values by Doreau and Ferlay (1994) who reviewed data from all ruminant species. Therefore, the extensive metabolism of dietary unsaturated fatty acids in the rumen results in stearic acid being the major fatty acid entering the duodenum. Figure 2 illustrates this based on linoleic acid intake (separated into tertiles) and compares changes in intake and duodenal flow (i.e. rumen output) of linoleic and stearic acids. Linoleic acid is generally the most common fatty acid present in diets for U.S. dairy cows and the intake varies widely; however, only a fraction of the linoleic acid consumed is actually available for absorption. On the other hand, typically very little stearic acid (18:0) is consumed, but we see a reciprocal increase in stearic acid flow to the duodenum (Figure 2) as a result of it being the end product of biohydrogenation of all 18-carbon unsaturated fatty acids (oleic, linoleic, and linolenic). Despite the dramatic changes that occur in the rumen, outflow of total fatty acids is very similar to dietary intake of fatty acids and this is true across a wide range of diets with different fatty acid intakes (Doreau and Ferlay, 1994; Lock et al., 2005). Therefore, an accurate determination of fatty acid intake will allow for a reasonable approximation of duodenal flow of total fatty acids, although the profile of the fatty acids will be vastly different (Figure 2). While simple in principle, an accurate determination of fatty acid intake can present some challenges, often due to the overestimation of total fatty acid content of forages and the difficulty in obtaining complete lipid extraction of highly saturated fat supplements (see review by Palmquist and Jenkins, 2003).

Fat supplements are used as a means to increase the energy density of the diet and many of these are referred to as inert. In this case, inertness simply means that the fat or fatty acid supplement has minimal affects on rumen fermentation. Although deemed inert at the level used, they can still be hydrolyzed if a triglyceride, or hydrogenated if unsaturated (Figure 1). Often, calcium soaps of palm fatty acids or canola are referred to as 'protected'. However, these are not protected from ruminal biohydrogenation, but they are rather ruminally inert with regard to their effects on the microbial population (Palmquist, 2006). Factors such as low rumen pH and increased unsaturation of the fatty acid can lead to dissociation of the Ca-fatty acid complex, allowing biohydrogenation to occur (Demeyer and Doreau, 1999). Thus, the feeding of a Ca-salt of unsaturated fatty acids will "protect" against adverse effects on microbial fermentation, but in most cases, it will not increase either the bypass of these fatty acids to the duodenum (Lundy et al., 2004) or their content in milk fat (Castañeda-Gutiérrez et al., 2005) compared with the feeding of the parent oil.

Improvements in analytical techniques have revealed an impressive complexity in the pattern of fatty acids that are produced during rumen biohydrogenation and subsequently incorporated into milk fat. Table 1 summarizes published data for lactating dairy cows and illustrates the range of positional and geometric isomers of *trans* 18:1 and conjugated linoleic acids **(CLA)** identified in the lipid material leaving the rumen. The established major pathways of biohydrogenation describe the formation of *trans*-11 18:1 and *cis*-9, *trans*-11 CLA but do not account for the fatty acid intermediates arising from minor pathways of rumen biohydrogenation. This is an area of increasing interest because of the recognition that some of these biohydrogenation intermediates have specific and

potent effects on ruminant metabolism and human health. For example, both *trans*-11 18:1 and *cis*-9, *trans*-11 CLA present in milk fat have been shown to have anticarcinogenic and antiatherogenic properties in animal models of human health (Bauman et al., 2006), while the role of *trans*-10, *cis*-12 CLA as a regulator of milk fat synthesis in dairy cows will be discussed later in this review. For further information on the biology of hydrolysis and biohydrogenation, and the effects of diet on these processes, the reader is referred to our recent review (Palmquist et al., 2005), as well as the classic review by Harfoot and Hazlewood (1997).

Fatty Acid Absorption in the Small Intestine

Since there is no significant absorption or modification of long and medium chain fatty acids in the omasum or abomasum, the lipid material available for absorption in the small intestine is similar to that leaving the rumen (Moore and Christie, 1984). This lipid material consists of approximately 80 to 90% free fatty acids attached to feed particles; the remaining lipid components are microbial phospholipids plus small amounts of triglycerides and glycolipids from residual feed material, which are hydrolyzed by intestinal and pancreatic lipases (Doreau and Ferlay, 1994). Before fatty acid absorption can occur, it is necessary for the fatty acids adsorbed on feed particles to be solubilized into the aqueous milieu. In all species, micelle formation is the key to this solubilization process, and therefore, key to efficient fatty acid absorption. In ruminants, both bile and pancreatic secretions are required for this process, and these are added to the digesta in the duodenum. Bile supplies bile salts and lecithin, and pancreatic juice provides the phospholipase enzymes to convert lecithin to lysolecithin and the bicarbonate to raise the pH. Lysolecithin, together with bile salts, desorb the fatty acids from feed particles and bacteria, allowing the formation of the micelle (Figure 3). The critical role of lysolecithin and bile salts in this process is illustrated in studies with sheep where fatty acid absorption was virtually abolished when bile secretion into the duodenum was blocked (Moore and Christie, 1984). Once micelles are formed, they facilitate transfer of water-insoluble lipids across the unstirred water layer of intestinal epithelial cells of the jejunum, where the fatty acids and lysolecithin are absorbed. Within the intestinal epithelial cells, the fatty acids are re-esterified into triglycerides and then packaged into chylomicrons for transport in lymph to the blood.

To allow for efficient intestinal absorption, ruminants have evolved a number of key differences and features in fatty acid absorption compared with non-ruminants. First, ruminant bile is characterized by an excess of taurine-conjugated bile acids. In the majority of herbivores, glycine-conjugated bile acids predominate, but in the mature ruminant, taurine-conjugates exceed glycine-conjugates approximately 3:1 (Noble, 1981). This is of significance because under the acidic conditions of the ruminant upper-small intestine, taurine-conjugated bile acids remain in a partially ionized condition and in the micellar phase where they are able to effect solubilization of fatty acids (Noble, 1981). Even at pH 2.5, taurine-conjugated bile acids remain soluble and partly ionized, while glycine-conjugated bile acids are insoluble in much less acidic conditions (pH 4.5) and unable to effect solubilization (Moore and Christie, 1984). Second, there are significant differences between ruminants and non-ruminants in the source of amphiphile or 'swelling agent', which promotes micelle formation. In ruminants, lysolecithin is the amphiphile involved in micelle formation, whereas monoglycerides plus bile salts interact with the fatty acids to form the micelle in non-ruminants (Davis, 1990). Freeman (1969) examined the amphiphilic properties of polar lipid solutes and found that lysolecithin had a pronounced effect on the micellar solubility of stearic acid (Table 2). In fact, lysolecithin's ability to increase the solubility of stearic acid is ~2-fold greater than that of other amphiphiles, including oleic acid which has been quoted recently as having

important amphiphilic properties when fed as a Ca-salt to ruminants (Moate et al., 2004; Block et al., 2005). Lysolecithin was, furthermore, the only amphiphile examined which was shown to significantly increase the distribution of stearic acid into the micellar phase and away from the particulate phase (Table 2). Considering that most fatty acids leaving the rumen are saturated and the predominant fatty acid is stearic acid, perhaps it is not surprising that the ruminant has evolved such an efficient system involving lysolecithin for solubilizing this fatty acid.

Our review of the available data from lactating dairy cows indicates that fatty acid absorption is relatively constant with no significant decline when fatty acid duodenal flow was high (Lock et al., 2005). Total fatty acid digestibility averaged 74% with a range (95% confidence interval) of 58 to 86%. These data are in agreement with Doreau and Ferlay (1994), who carried out an extensive review of the literature for all ruminant species and reported values for fatty acid digestibility ranging from 55 to 92%; again, this range was not related to fatty acid intake. One consideration is whether differences exist in the digestibility of individual fatty acids, with the digestibility of stearic acid in dairy cows in relation to the digestibility of other fatty acids being of particular interest. In general, the ability of ruminants to absorb fatty acids is much higher than that of non-ruminants (Noble, 1981). In non-ruminants, there is a wide divergence in the digestibility of fatty acids (Freeman, 1984), with the digestibility of individual fatty acids decreasing when chain length increases and increasing as the number of double bonds increases (Lessire et al., 1992). In particular, free palmitic and stearic acids are poorly absorbed in non-ruminants (Noble, 1981). However, as illustrated in Figure 4, although similar patterns are observed in ruminants, relative differences in the digestibility of individual fatty acids are modest; mean digestibilities for 16:0, 18:0, 18:1, 18:2, and 18:3 were 75, 72, 80, 78, and 77% (Lock et al., 2005). These data are in agreement with the review of Doreau and Ferley (1994), which reported that mean digestibilities were 77, 85, 83 and 76% for 18 carbon fatty acids with zero, one, two, and three double bonds, respectively.

Recent improvements in analytical techniques will allow the digestibility of individual fatty acids to be more thoroughly examined. However, application in feeding systems still requires accurate information on the profile of fatty acids leaving the rumen. Figure 4 also illustrates the considerable variation in the digestibility of individual fatty acids across studies. The overall conclusion is that differences in digestibility among individual fatty acids contribute very little to the extensive variation reported in the literature (range ~60 to 90%). Rather, the majority of this variation reflects differences among individual experiments, and thus relates to experimental approaches and analytical techniques as well as differences in diets and specific feed components. As emphasized earlier, stearic acid is the predominant fatty acid in the digesta and consequently is the major contributor to total absorbed fatty acids. Therefore, any discrimination against the absorption of stearic acid relative to the other fatty acids may be hardly noticeable since this is the predominant component in the digesta and more is absorbed than of any other fatty acid (Noble, 1981). Consequently, the composition of absorbed fatty acids is close to the composition of fatty acids entering the duodenum.

Milk Fat Depression

Nutrition is the predominant environmental factor affecting milk fat and represents a practical tool to alter its yield and composition. One of the most striking examples of nutritional effects on milk fat is the low fat milk syndrome, typically referred to as milk fat depression **(MFD)**, and our understanding of its etiology has advanced significantly in recent years. The MFD has been observed over a range of feeding situations, including

diets supplemented with fish oils or plant oils, and diets high in concentrates and low in fiber **(HC/LF)** (Bauman and Griinari, 2001). The fat content of milk can also be affected by the physical characteristics of the roughage (e.g. grinding or pelleting) or use of ionophores such as Rumensin® (Elanco, Greenfield, IN).

The MFD is properly diagnosed by an observed reduction in milk fat yield, as milk fat percentage can be influenced by a change in milk volume with no actual change in milk fat produced. Several general characteristics have been identified that provide insight into the biology of MFD (Bauman and Griinari, 2003). First, the changes that occur with diet-induced MFD are specific for milk fat; fat yield can be reduced by 50% or more with little or no change in milk yield or the yield of lactose or protein. Second, the yield of most of the different fatty acids in milk fat is reduced, but the decline is greatest for *de novo* synthesized fatty acids. As a result, milk fat composition shifts toward lower proportions of short chain and medium chain fatty acids (<16 carbons) and a greater concentration of longer chain fatty acids (>16 carbons). Third, changes in ruminal microbial processes are an essential component for the development of MFD. These changes in the rumen environment are often associated with a decrease in rumen pH and a shift in the acetate:propionate ratio. Fourth, for MFD to occur, the diet must contain unsaturated fatty acids and the pathways of their biohydrogenation in the rumen must be altered. Thus, the induction of MFD is centered on both an altered rumen environment and an alteration in the rumen pathways of PUFA biohydrogenation.

Davis and Brown (1970) were among the first to recognize that increases in the milk fat content of *trans* fatty acids **(TFA)** was associated with MFD caused by feeding HC/LF diets. As the database grew, it became evident that MFD was often related to an increase in the TFA content of milk fat across a wide range of diets (Griinari et al., 1998). However, there were also many situations where increases in milk fat content of TFA did not correspond to changes in milk fat production, and thus, the basis for MFD had to be more complex than a simple relationship to the ruminal production of TFA. A key development in understanding diet-induced MFD occurred when we utilized improved analytical techniques and discovered that it was the pattern of *trans* 18:1 isomers rather than total TFA that was correlated to MFD. Specifically, we demonstrated that MFD was associated with a marked increase in the milk fat content of *trans*-10 18:1 (Griinari et al., 1998). Thus, under certain dietary situations, a portion of the linoleic acid undergoes biohydrogenation via a pathway that produces *trans*-10 18:1 (Figure 5). *Trans*-10, *cis*-12 CLA is also an intermediate in this pathway, and we found that the milk fat content of this unique CLA isomer also increased in many dietary situations associated with MFD (Bauman and Griinari, 2001). Over the same interval, we were also conducting studies with pure CLA isomers and discovered that *trans*-10, *cis*-12 CLA was a potent inhibitor of milk fat synthesis (Baumgard et al., 2000). We established that the dose response relationship was curvilinear and found that as little as 2.5 g/day of *trans*-10, *cis*-12 CLA delivered post-ruminally was sufficient to cause a 25% reduction in milk fat (deVeth et al., 2004). Effects of *trans*-10, *cis*-12 CLA are specific for milk fat and its mechanism and that for diet-induced MFD involves coordinated reductions in key mammary enzymes involved in the regulation of milk fat synthesis (Griinari and Bauman, 2006).

As a result of these advances, Bauman and Griinari (2001) proposed the "biohydrogenation theory" to explain MFD and hypothesized that "under certain dietary conditions the pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates which are potent inhibitors of milk fat synthesis." Clearly, *trans*-10, *cis*-12 CLA represents one example, and results from several recent studies have led investigators

to suggest the existence of additional fatty acid intermediates that inhibit milk fat synthesis (Perfield and Bauman, 2005). Dietary situations causing MFD result in alterations in the biohydrogenation pathways, and as a consequence, changes in many fatty acid intermediates occur and most are correlated with MFD (Loor et al., 2005a; Shingfield et al., 2006). Since correlation does not imply causality, it is important to directly examine the biological activity of specific fatty acids. Of particular interest, milk concentrations of *trans*-10 18:1 are highly correlated with the extent of diet-induced MFD. However, the limited availability of *trans*-10 18:1 has precluded a direct examination of its effect, and its presence could simply be an indication of the change in rumen fermentation associated with diet-induced MFD, rather than a significant cause of the reduction in milk fat synthesis. We recently showed that *trans*-9, *cis*-11 CLA caused a reduction in milk fat synthesis (Perfield et al., 2005), and another report indicated that the *cis*-10, *trans*-12 CLA also reduced milk fat synthesis in lactating dairy cows (Sæbø et al., 2005). Therefore, three CLA isomers have been identified as regulators of milk fat synthesis, and the production of these is increased in different types of diet-induced MFD. Further identification and characterization of rumen-derived inhibitors of milk fat synthesis and the conditions which result in their formation will enable us to more effectively troubleshoot problems in low fat test on commercial farms.

We are seeing more problems with MFD in the last few years. This increased occurrence of MFD is likely due to a number of reasons; for example, changes in rumen biohydrogenation pathways may have been caused by poor silage making conditions the past several growing seasons, increased occurrence of sorting of TMR due to attempts to increase effective dietary fiber, the increased use of unsaturated fat sources in diets, and Rumensin supplementation of certain diets. In addition, higher DMI will increase passage rates from the rumen, potentially increasing washout of biohydrogenation intermediates, including those that could cause MFD (Overton and Bauman, 2003). Of particular interest is the increased use of by-products feeds and Rumensin in dairy cow diets. By-product feeds can contain a considerable amount of lipid, which is predominately linoleic acid. In particular, corn distillers' grains have relatively high lipid content which is highly variable (~9 to 18% of DM). Such variation can significantly alter the dietary supply of unsaturated fatty acids to the dairy cow, thereby increasing the risk of dietary-induced MFD. In addition, Rumensin supplementation of certain diets appears to impact rumen fermentation in such a manner that MFD is sometimes observed. Results are inconsistent and not well described, but they appear to be associated with the classical factors affecting MFD. Duffield et al. (2003) identified that herds fed a TMR low in fiber were more prone to Rumensin-related milk fat problems as compared to TMR-fed herds with adequate fiber or component-fed herds. As in other situations of diet-induced MFD, the associative effects of feed ingredients in the rumen ultimately affects the production of unique biohydrogenation intermediates. Table 3 lists a number of potential risk factors for reduced milk fat and areas to address when troubleshooting low milk fat tests. Further research is needed to fully evaluate these interrelationships and to develop nutritional strategies designed to avoid dietary-induced MFD problems in today's high producing dairy cows.

Summary

Digestion and metabolism of dietary lipids is complex, and in this paper, we have provided an overview of the biology of these processes in dairy cows. Dietary lipids undergo extensive hydrolysis and biohydrogenation in the rumen, resulting in the lipid material leaving the rumen consisting primarily of free fatty acids that are highly saturated. Although lipid hydrolysis and classical pathways of fatty acid biohydrogenation are well established, analytical

improvements have revealed the complexity of these processes. Clearly, several minor biohydrogenation pathways exist, and many factors related to diet and rumen environment affect these processes; as a consequence, there are numerous fatty acid intermediates produced during rumen biohydrogenation, and some of these affect biological processes in the cow, including rates of milk fat synthesis. The dairy cow has evolved a number of key differences in fatty acid absorption compared with non-ruminants; these allow for efficient absorption of fatty acids and include differences in both bile salt composition and the amphiphile involved in micelle formation, as well as the slow and continuous release of relatively small amounts of fatty acids into the duodenum. Consequently, in general, the ability of ruminants to absorb fatty acids, particularly saturated fatty acids, is much higher than that of non-ruminants. Available data from lactating dairy cows indicate that relative differences in the digestibility of individual fatty acids are modest and contribute little to the extensive variation reported in the literature. Rather, this variation likely reflects differences in diets, specific feed components, and methodology among individual experiments.

The problem of diet-induced MFD has challenged producers and scientists for over a century, and in the last few years, we are seeing many more problems with low milk fat tests. We now recognize that MFD involves the interrelationship between digestive processes in the rumen and the synthesis of milk fat by the mammary gland; specific biohydrogenation intermediates produced in the rumen under certain dietary situations are potent inhibitors of milk fat synthesis in the mammary gland. Consequently, our ability to predict and troubleshoot commercial problems related to milk fat is dependent on a complete understanding of the dynamic interactions in the fermentation of feedstuffs in the rumen and the biological activities of the fatty acid intermediates produced under these different conditions. Obviously, our knowledge of lipid digestion and metabolism is rapidly advancing, and the opportunity and challenge is to effectively apply this knowledge in the feeding and management of today's high producing dairy cows.

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Table 1. Range of positional and geometric isomers of *trans* 18:1 and conjugated linoleic acids **(CLA)** and their ruminal outflow (g/day) in lactating dairy cows.[1]

Trans 18:1			Conjugated Linoleic Acids		
	Ruminal Outflow			Ruminal Outflow	
Isomer	Min	Max	Isomer	Min	Max
trans-4	0.4	2.0	*trans*-7, *cis*-9	<0.01	0.01
trans-5	0.4	3.4	*trans*-7, *trans*-9	<0.01	0.02
trans-6-8	0.4	16.2	*trans*-8, *cis*-10	<0.01	0.3
trans-9	1.4	13.1	*trans*-8, *trans*-10	<0.01	0.10
trans-10	1.5	114.0	*cis*-9, *trans*-11	0.31	2.86
trans-11	17.0	148.0	*trans*-9, *trans*-11	0.14	0.29
trans-12	1.9	20.8	*trans*-10, *cis*-12	0.02	1.84
trans-13 + 14	4.2	60.3	*trans*-10, *trans*-12	0.05	0.23
trans-15	2.0	29.0	*cis*-10, *trans*-12	0.08	0.29
trans-16	2.3	18.2	*cis*-11, *trans*-13	0.01	0.33
			trans-11, *cis*-13	<0.01	0.46
			trans-11, *trans*-13	0.09	2.02
			cis-12, *trans*-14	0.12	0.85
			trans-12, *trans*-14	0.07	0.19

[1]Data derived from five studies where samples were collected from either the omasum or duodenum of lactating dairy cows (Piperova et al., 2002; Shingfield et al., 2003; Qiu et al., 2004; Loor et al. 2004; 2005b).

Table 2. Amphiphilic properties of some polar lipids. Adapted from Freeman (1969) and Freeman (1984).

Amphiphile	Amphiphilic Index[1]	Increase or decrease (%) in $K_{m/o}$ of stearic acid[2]
Oleic Acid	0.138	-11
Monoglyceride (1-Mono-olein)	0.138	+37
Linoleic Acid	0.154	---
Lauric Acid	0.164	---
Lysolecithin	0.280	+115

[1]The amphiphilic index is defined as the increase in stearic acid solubility in bile salt solution per unit increase in amphiphilic concentration.

[2]Distribution coefficient describing the distribution of stearic acid between the particulate oil phase and the micellar phase; a positive (+) value indicates that an amphiphile increases the distribution of stearic acid into the micellar phase, which would favor absorption.

Table 3. Partial list of potential risk factors for reduced milk fat and areas to address when developing nutritional strategies designed to avoid dietary-induced milk fat depression.[1]

Altered Rumen Environment	Supply of PUFA
• Low rumen pH/low peNDF	• Amount (esp. linoleic acid intake)
• Feed particle size	• Availability
• Fiber	• PUFA:SFA
• Starch (NSC)	• Feeding Pattern
• Rumensin®[2]	• Variation in fat content and FA composition of feed ingredients
• Feeding Pattern	

[1]FA = fatty acids, NSC = nonstructural carbohydrates, peNDF = physically effective neutral detergent fiber, PUFA = polyunsaturated fatty acids, and SFA = saturated fatty acids.
[2]Elanco, Greenfield, IN.

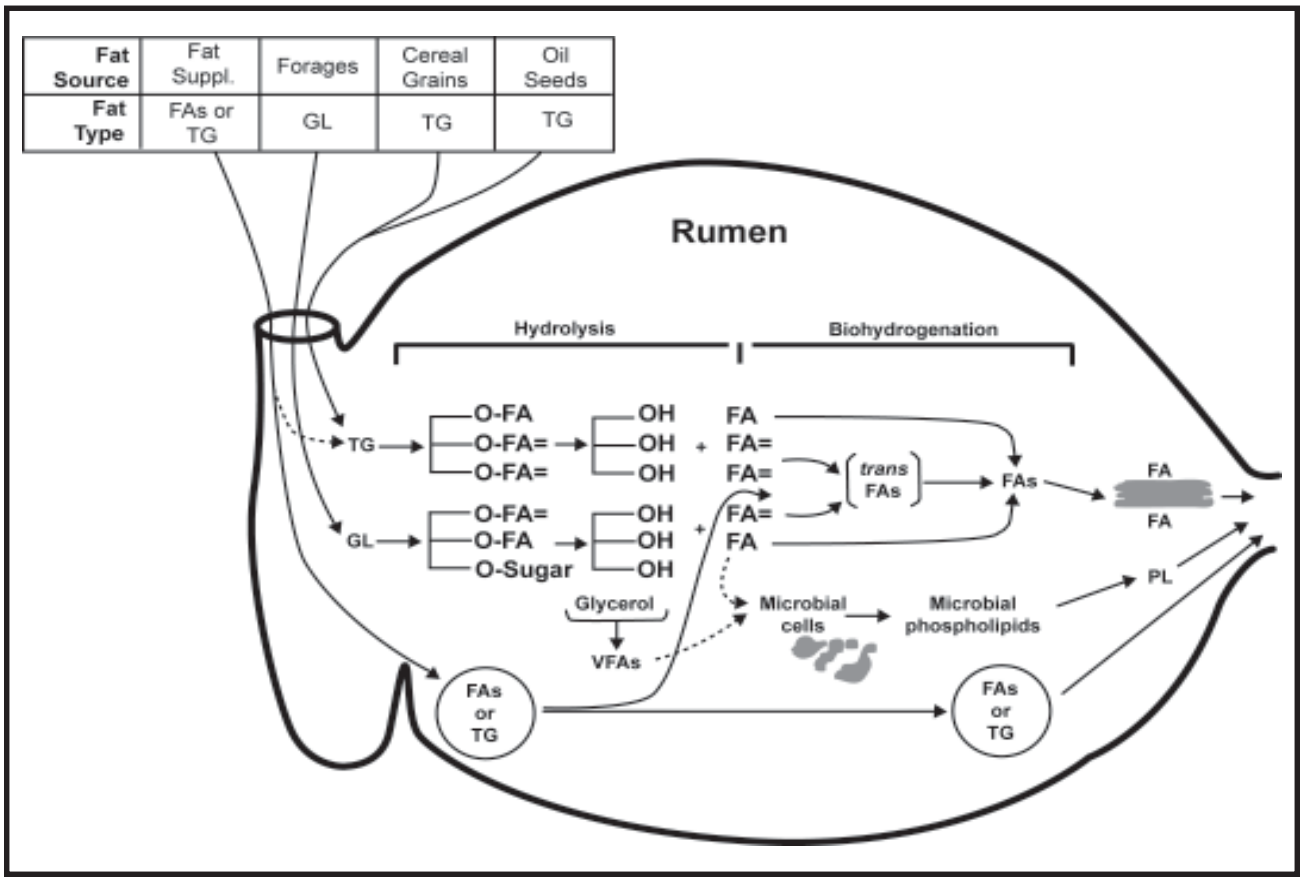

Figure 1. Lipid metabolism in the rumen. Also shown are the predominant fat types in common feedstuffs (TG = triglycerides, GL = glycolipids and FA = fatty acids). Adapted from Davis (1990).

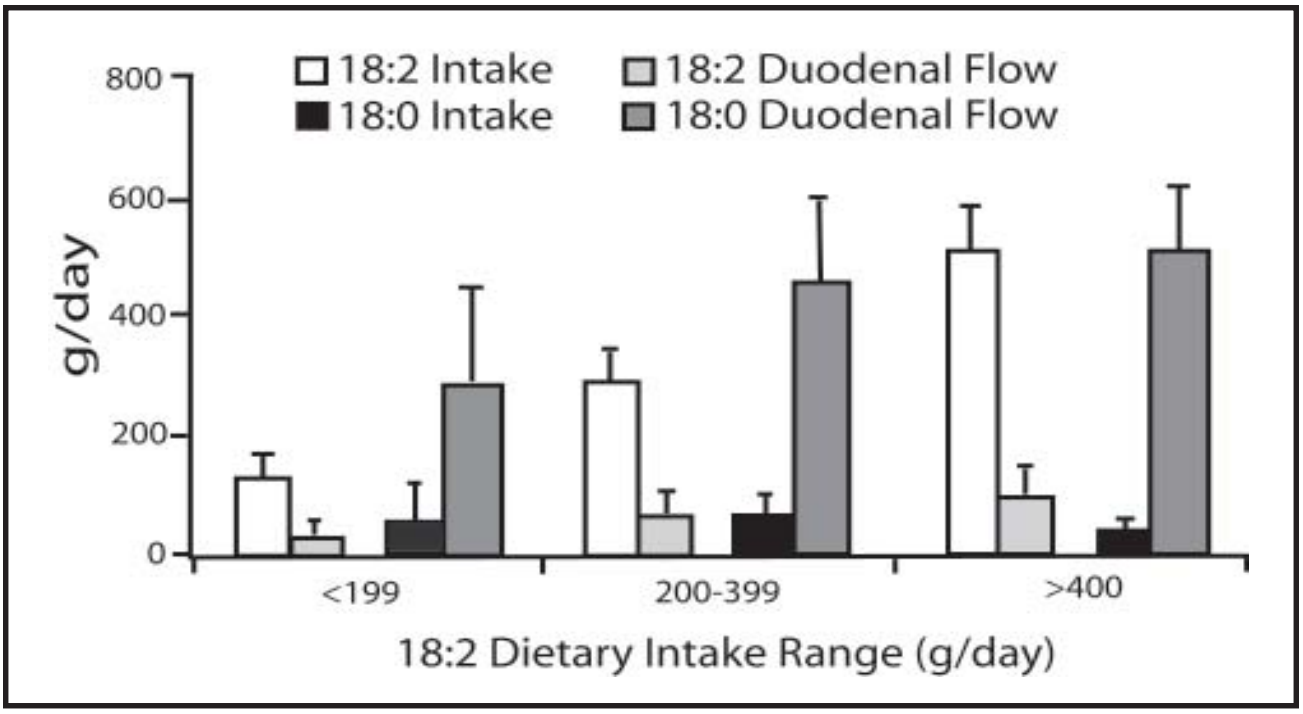

Figure 2. Relationship for linoleic acid (18:2) and stearic acid (18:0) intake and duodenal flow. Values represent means ± SD for the data obtained from 20 published studies involving 80 treatments reporting individual fatty acid intakes and duodenal flow; data are separated into tertiles based on the dietary intake of linoleic acid. Adapted from Lock et al. (2006).

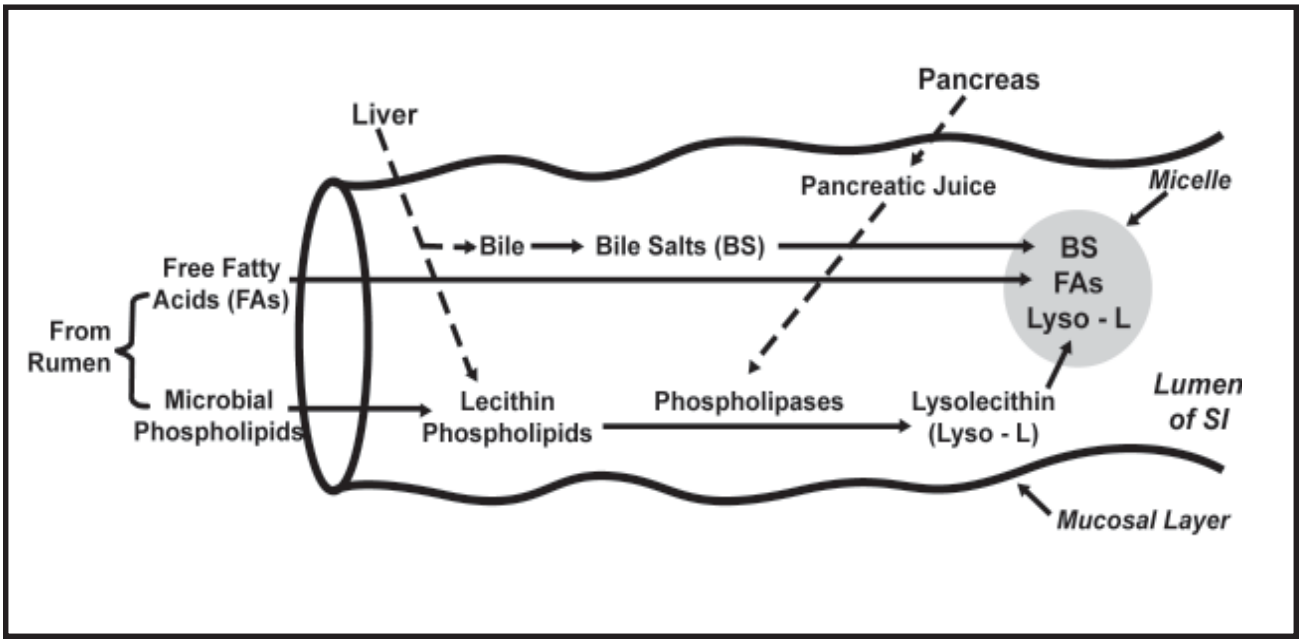

Figure 3. Fat digestion in the small intestine (SI) of the dairy cow. Adapted from Davis (1990).

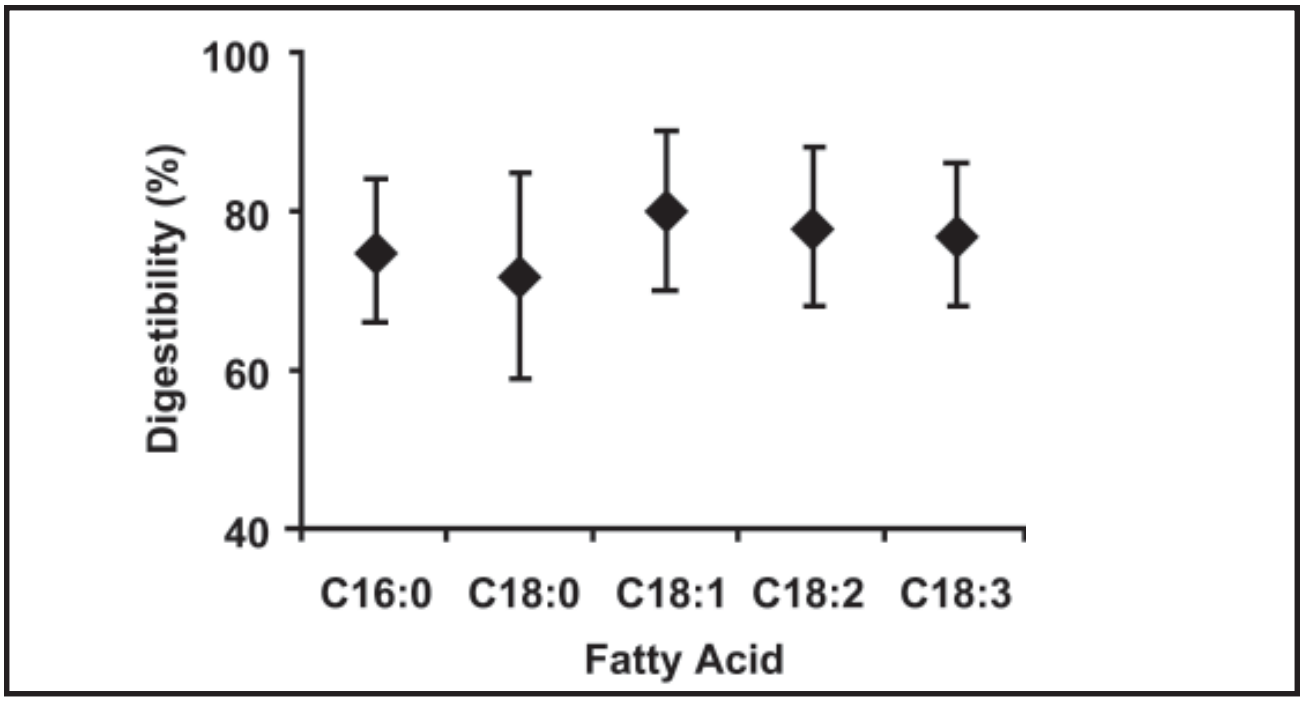

Figure 4. Comparison of individual fatty acid digestibilities in lactating dairy cows. Values represent means ± SD for the data obtained from 14 published studies involving 70 treatments. Digestibilities were calculated by differences between duodenal/omasal and ileal/fecal samples; total fatty acid digestibility averaged 74 ± 9%. Adapted from Lock et al. (2005).

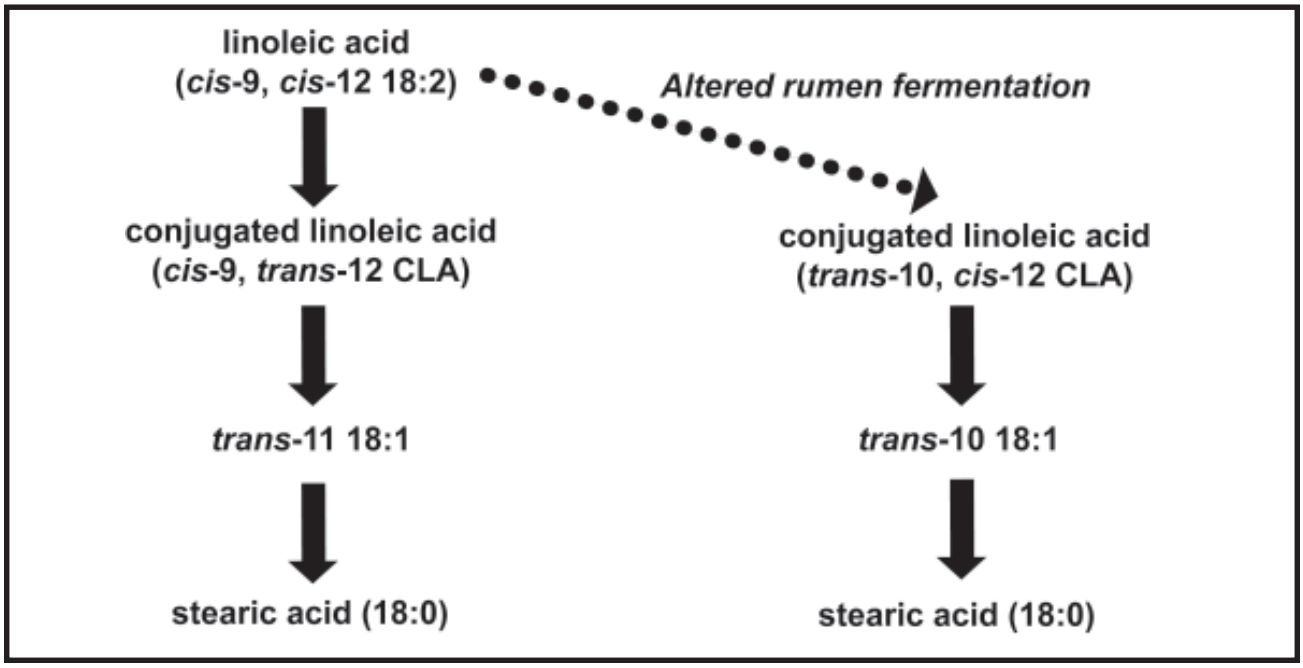

Figure 5. Generalized scheme of ruminal biohydrogenation of linoleic acid under normal conditions and during diet-induced milk fat depression (dotted line). Adapted from Griinari and Bauman (1999).

Positive and Negative Effects of High Energy Consumption on Reproduction in Lactating Dairy Cows

Milo Wiltbank[1], Hernando Lopez, Roberto Sartori, and Ahmet Gument
Department of Dairy Science
University of Wisconsin-Madison

Introduction

Seven years after graduating from the University of Wisconsin-Madison, John Smith (name changed by request) had found that the management headaches associated with running a 500 cow dairy were becoming overwhelming. He had built a newer freestall facility using a loan from his banker and was able to stock it with the 100 dairy cows purchased from his father's operation and many other cows purchased from surrounding operations. He now had the operation filled for the last 3 years. He had one problem with drainage into his lagoon that had resulted in one end of the barn being flooded on some occasions, but other than that, he was happy with the new facilities. The new double-8 parallel parlor was extremely efficient, and he had gone to 3-times per day milking to increase milk production and to utilize the parlor to greater capacity. His diet was balanced by a local nutrition professional, and he had been fairly pleased with the results. Milk production had climbed to where it now averaged over 85 lb/cow/day with 3.51% fat and 3.01% protein. His somatic cell count had dropped to 142,000 cells/ml and so he was pleased with his milk quality. His problem was that he continually had to buy springing heifers to keep the facility full. He had purchased 45 springing heifers during the last year, and it seemed that he would have to purchase about the same number during the coming year. Even though it had been over 3 years since he had first filled the facility with cows, he just could not generate enough heifers from his own operation to keep the facility full.

He knew he had reproduction problems but was not able to determine how to resolve them. He felt that part of the problem was the extremely high energy ration that his nutritionist had recommended about 11 months earlier. He had detected a lot of lame cows since that time. He did all of the artificial insemination **(AI)** in his herd and felt that he had a good technique; however, he did split straws (breed 2 cows with 1 straw of semen) in more than half of the breedings. Our group from the University of Wisconsin-Madison was asked to come in and do an evaluation of his operation as part of a class for our advanced undergraduate and graduate students. In this class, the students work with faculty members to diagnose any problems and help to design practical solutions for the participating dairy producers.

On the first visit, the students found out that the producer split straws of semen and felt that this was probably a primary problem. I asked them to continue to dig into the records to obtain data related to this potential problem or other problems. After 2 weeks of analysis of computer records and on-farm analysis, they had a different conclusion.

Although John said that he was using Ovsynch for timed AI of cows, he did not have any cows that were bred after Ovsynch before 150 days in milk. In other words, he only started using Ovsynch if he had not caught the cow in heat after more than 100 days of heat detection. In fact, some of these cows had not been bred for the first time

[1]Contact at: Dairy Science Department, 1675 Observatory Drive, Madison, WI 53705, (608) 263-9413, FAX: (608) 263-9412, wiltbank@wisc.edu

by 200 days in milk. The average days to first breeding were 169 days. The students were definitely finding the real problem of reproduction on John's dairy. We asked John what was happening to his heat detection program, and he felt that the lameness issue had really decreased his heat detection efficiency. However, the students evaluated his current lameness scores and did not find that it was abnormally high. John told us that he was responsible for the heat detection, but that as herd manager, he found many other things that took a lot of his time. He did try to do heat detection at least 2 times per day for about 15 minutes at each time but was not always consistent with this time. We asked why he did not use Ovsynch on cows earlier in lactation, but he said that he did not have the time to set up the hormonal injections. He was in charge of all aspects of reproduction, as well as overall management of the dairy and there were only so many hours in the day. In addition, he did not want to spend money on the hormones if he could possibly catch the cow in a natural heat.

The students evaluated the conception rate on the lactating dairy cows and found a 41% rate. This was higher than most of the farms that we evaluate, particularly given the high level of milk production. They also found no difference between the cows bred with a full straw of semen or cows bred with a half straw of semen. So much for straw splitting providing an explanation for poor reproduction.

What had changed the reproduction on John Smith's dairy? Probably a combination of factors had converged to cause "the perfect storm" in reproduction on his dairy. On his family's smaller dairy operation, he could do a fine job with reproduction because he could completely focus on this one job. Now, his many management responsibilities took precedence, and he did not have enough time to do the job the way it needed to be done. So, in other words, he had not begun to delegate out this critical responsibility to other people (perhaps an AI organization could be contracted to help with reproduction). Second, he had not been aggressive enough in using new reproductive management technology at an early enough time in lactation to receive maximum benefits from these new technologies. Third, the milk production in his cows had been rapidly increasing, and this increase had dramatically changed the reproduction in his dairy cows. He needed to make management changes to counter these changes in reproductive efficiency. Unfortunately, he was spending over $100,000 per year on buying springing heifers, and there was no end in sight because of too few replacement heifers being produced by his herd. He also had so many cows that were not pregnant in late lactation that he would have to cull many non-pregnant cows in the next few months. This was a problem that would not be solved overnight but could be solved in the next 2 to 3 years by immediately implementing some aggressive reproductive management strategies.

Is the problem on John Smith's dairy the genetics of the cows? Maybe partially, but the major reproductive problems become apparent in these cows only when milk production increases to higher levels. Is the problem nutritional? Maybe partially, but there is probably not a major problem with nutritional deficiencies as much as nutrition becoming focused on and succeeding in increasing milk production. Is the problem with John Smith's management? This is definitely an important part of the problem. He could hire a reproductive management specialist for a fairly large salary and still make a good return on the investment. He clearly must delegate reproductive management responsibilities to others. In particular, he should hire someone to help with heat detection and hormonal injections. He also needs to change his management strategy to incorporate timed AI programs into an earlier stage of lactation (probably before 100 days in milk). These 2 changes would help him more efficiently catch cows in heat and to breed any cows at an early stage of lactation if they

were not caught in heat. He also needs to design an early pregnancy diagnosis and resynchronization strategy into his management strategy.

Unfortunately, the reproductive problems on John Smith's farm are not unique. Each farm is challenged to efficiently use labor and control costs to improve reproduction. This review will discuss the major changes that are occurring in reproduction in high producing lactating dairy cows and some reproductive management strategies to deal with these changes.

Changes in Some Reproductive Measures in Lactating Dairy Cows

Time to first ovulation

Use of ultrasonography, combined with hormonal assays has allowed a greater understanding of ovarian function during the period from parturition to first ovulation. Following parturition, there is a surge in circulating follicle stimulating hormone **(FSH)** during the first week (Ginther et al., 1996), probably due to the decrease in circulating estradiol after calving. There is subsequent emergence of the first follicular wave on average at 4 (Ginther et al., 1996) to 12 (Savio et al., 1990; Haughian et al., 2002) days postpartum. Some cows ovulate this first follicular wave; however, first ovulation is delayed in many lactating dairy cows with time to first ovulation averaging 33.3 ± 2.1 days in Holstein cows in the U.S.A. (compilation of 10 studies reported in Ferguson, 1996). Pasture-fed dairy cattle had, on average, 4.2 waves of follicle growth before first ovulation with maximal size of the largest follicle increasing as first ovulation approached (McDougall et al., 1995). This delay in first ovulation is generally attributed to the period of negative energy balance during the early postpartum period in dairy cattle, and a reduction in the pulsatile luteinizing hormone **(LH)** secretion needed to stimulate the final stages of follicle growth and estradiol production (Savio et al., 1990; Staples et al., 1990; Beam and Butler, 1997; Lamming and Darwash, 1998; Roche et al., 2000; Butler, 2001).

Although delayed first ovulation is associated with negative energy balance during early lactation, it is not as clearly associated with level of milk production. Cattle that have been selected for high milk production compared to control lines (lower genetic merit for milk yield) have been reported to have a longer interval to first postpartum ovulation (+14 days reviewed by Lucy, 2001); +8 days [Gong et al., 2002]) and the first postpartum detected estrus (+4.5 days, [Hageman et al., 1991]). However, in our recent study, there was no relationship between level of milk production and percentage of cows that were "anovular" at 71 days in milk (Lopez et al., 2005a) with about 25% of cows being "anovular" irrespective of milk production (55 to 132 lb/day average production from 50 to 71 days postpartum). The surprisingly high rate of anovulation in this study is somewhat higher but not inconsistent with other recent studies (Moreira et al., 2001; Gumen et al., 2003; Santos et al., 2004). Unfortunately, these studies did not report any potential relationship between level of milk production and anovulation. Erb (1984) reviewed four North American studies in which they contrasted the levels of milk production, or genetic potential for milk production, between ovulatory and anovulatory cows. He concluded that high milk production did not cause anovulation in Holstein dairy cows, but anovulatory cows produced more milk than their herdmates (Erb, 1984).

Recently, we have suggested that anovulation be classified into physiological categories based on maximal size of the largest growing follicle and circulating estradiol (Wiltbank et al., 2002). Consistent with this classification, cows with lower body condition scores (BCS of 2.5 or lower out of 5) had a greater likelihood of anovulation and had smaller maximal size of anovulatory follicles. Nevertheless, the majority of

anovulatory cows (63%) had adequate BCS and average follicular sizes greater than ovulatory size (≥ 20 mm). For example, 20% of cows with a BCS of 3.25 were found to be anovulatory. Thus, negative energy balance and inadequate follicular growth can explain a portion of anovulatory dairy cows but does not seem to be an adequate explanation for all anovulatory dairy cows. Similarly, a simple relationship between anovulation and level of milk production does not appear to exist, and therefore, more complex physiological models are needed to fully explain anovulation in dairy cows (Gumen and Wiltbank, 2002; Wiltbank et al., 2002).

Conception rate

Most studies of reproduction in dairy cattle have focused on conception rate and pregnancy loss because of the economic implications for commercial dairy operations of these reproductive measures (for reviews see Lucy, 2001; Lopez-Gatius, 2003). Nevertheless, the relationship between various measures of fertility (conception rate) and level of milk production remains controversial. Washburn et al. (2002) analyzed the relationship of conception rate and milk production over more than a 20-year time period (1976 to 1999) in dairy herds in southeastern U.S.A. Conception rates decreased from ~ 55 to 35% during this time period as milk production dramatically increased. However, differences in recording of data could also be at least partially responsible for these changes (Lucy, 2001). Faust et al. (1988) showed a clear relationship between level of milk production and conception rate in primiparous Holstein dairy cattle. In contrast, Peters and Pursley (2002) reported that higher producing cows had higher conception rates following the use of Ovsynch protocols than lower producing cows. Most large data sets have demonstrated an antagonistic relationship between milk production and fertility, but the size of the effect has been questioned (Gröhn and Rajala-Schultz, 2000; Hansen, 2000; Lucy, 2001). Nevertheless, it seems clear that high producing, lactating dairy cows have much lower conception rates than heifers (Xu and Burton, 1999; Royal et al., 2000; Lucy, 2001; Peters and Pursley, 2002; Washburn et al., 2002; Gumen et al., 2003; Lopez-Gatius, 2003). For example, Pursley et al. (1997) reported much higher conception rates in heifers (74.4%) than in lactating cows (38.9%). In a recent study, we compared embryo quality on day 5 after ovulation from normally-ovulating, lactating dairy cows versus similar age and size non-lactating dairy cows (Sartori et al., 2002). Although fertilization rate was similar (88 to 90%), the percentage of embryos that were viable was much lower in lactating cows (52.8%) than in non-lactating cows (82.3%) (Sartori et al., 2002). This study was done during the cool time of the year so that heat stress was not a problem. The collection of embryos from heat-stressed, lactating dairy cows resulted in a reduced fertilization rate (55.3%) and even a greater reduction in percentage of viable embryos (33.3%) (Sartori et al., 2002). A major effect of milk production on fertility is found during heat stress and may not be present during cooler times of the year (Lopez-Gatius, 2003). This is probably due to a greater increase in body temperature in higher than lower producing dairy cows exposed to the same environmental temperatures (Sartori et al., 2002). However, the lower conception rates in lactating dairy cows, even during cool times of the year, suggest that not all the reduction in fertility can be explained by greater heat stress. Obviously, fertility is a complex trait and is likely to be related to numerous factors, including uterine infection, negative energy balance, urea concentrations in the blood, vitamins, fertility of sire, accuracy of estrous detection, insemination technique, etc. (Faust et al., 1988; Staples et al., 1990; Ferguson, 1996; Lamming and Darwash, 1998; Gröhn and Rajala-Schultz, 2000; Roche et al., 2000; Royal et al., 2000; Butler, 2001; Lucy, 2001; Moreira et al., 2001; Gong et al., 2002; Washburn et al., 2002; Lopez-Gatius, 2003; Santos et al., 2004a; Santos et al., 2004b). For example, an increase in double ovulation rate in high-

producing dairy cows (illustrated below) would increase the chances for pregnancy, even though possible negative effects of high milk production could decrease the percentage of ovulated oocytes that produce a pregnancy. Thus, a simple relationship between milk production and conception rates seems unlikely.

Duration of estrus

It is clear that low rates of estrous detection are reducing reproductive efficiency on commercial dairy farms. Indeed, Washburn et al. (2002) reported a decrease from 50.9% in 1985 to 41.5% in 1999 for estrous detection rates in Holstein dairy herds in southeastern U.S.A. However, studies have reported both a negative relationship between level of milk production (Harrison et al., 1989; Harrison et al., 1990) or no relationship (Fonseca et al., 1983; Van Eerdenburg et al., 2002) using visual observation twice daily to measure expression of estrus. We have recently completed a study in which we evaluated the duration of estrus in a group of lactating dairy cows using the HeatWatch system (Lopez et al., 2004). This system allowed continuous monitoring of all mounts 24 h per day and can be used to calculate the duration of estrus in individual dairy cows. Cows with milk production above the herd average (~ 88 lb/day) had shorter ($P < 0.001$) duration of estrus (6.2 ± 0.5 h) than cows with lower milk production (10.9 ± 0.7 h). This effect was not due to a parity effect because separate analysis of primiparous and multiparous cows showed a similar effect. Figure 1 shows the relationship between level of milk production and duration of estrus. In order to consistently observe this strong negative relationship between level of milk production and duration of estrus, it is critical that milk production data be collected close to the time of estrus, only data from ovulations after the first postpartum ovulation be utilized (first ovulation has low expression of estrus), all ovulations be consistently monitored throughout the observation period (to avoid false estrus or missing data from ovulations), and that duration of estrus be monitored on a continuous basis with an electronic heat monitoring system.

In a subset of these cows (n = 71), we analyzed maximal follicular size and circulating estradiol concentrations on the day of estrus (Lopez et al., 2004). High producing cows (103 lb/day) had larger follicles (18.6 ± 0.3 versus 17.4 ± 0.2 mm diameter; $P < 0.01$) but lower circulating estradiol (6.8 ± 0.5 versus 8.6 ± 0.5 pg/ml; $P < 0.01$) compared to lower producing cows (71 ± 1.3 lb/day). Correlations were evaluated between a number of different values. Surprisingly, there was no detectable relationship between maximal follicular size and peak estradiol concentrations ($r = -0.17$; $P = 0.15$). As expected, duration of estrus was positively correlated with peak estradiol concentrations ($r = 0.57$; $P < 0.0001$) and negatively with milk production ($r = -0.51$; $P < 0.0001$). Level of milk production was also negatively correlated with follicular size ($r = -0.45$; $P < 0.0001$). As discussed below, we theorize that high milk production leads to decreased circulating estradiol concentrations, resulting in decreased duration of estrus. Decreased estradiol could also cause increased follicular size by delaying the time to estradiol-induction of estrus, gonadotrophin releasing hormone **(GnRH)**/LH surge, and ovulation in high-producing cows.

Double ovulation rate

Another reproductive trait that has been directly linked to milk production is double ovulation rate (for a more complete review see Wiltbank et al., 2000; Lopez et al., 2005a). From a practical standpoint, double ovulation rate appears to be the underlying cause of increased twinning rate in lactating dairy cows, with 93% of twins being non-identical (Silvia Del Rio et al., 2004). Numerous factors have been recognized as possible regulators of twinning rates, including age of dam, season, genetics, use of reproductive hormones or

antibiotics, ovarian cysts, days open, and peak milk production [reviewed in Wiltbank et al., 2000]. In a large study on risk factors for twinning, Kinsel et al. (1998) concluded, "the single largest contributor (> 50%) to the recent increase in the rate of twinning is the increase in peak milk production". We performed a study in which we evaluated double ovulation rate in 240 dairy cows Fricke and Wiltbank, 1999) that had ovulation synchronized with the Ovsynch protocol (Pursley et al., 1995; Pursley et al., 1997). Ovulation was determined by transrectal ultrasonography at the time of the second GnRH injection and 48 h later. The mean milk production, determined 3 d before ovulation, was 80.5 ± 1.8 lb/day and cows were segregated by whether they were below or above the mean value. Double ovulation rate in cows that were above average production was 20.2% compared to 6.9% in those below average ($P < 0.05$) (Fricke and Wiltbank, 1999). This difference was similar regardless of lactation number. Recently, we reported results of a study (Lopez et al., 2005a) that evaluated naturally ovulating dairy cattle and found a similar relationship between milk production and double ovulation rate (Figure 2). Cows that produced less than 88 lb/day had a very low double ovulation rate, whereas, cows producing above 110 lb/day had more than a 50% double ovulation rate. It is surprising that there is such a dramatic inflection point in double ovulation rate as milk production increases above 88 lb/day, and it is still unclear what physiological changes occur as milk production increases above this critical value. This increase in double ovulation rate is likely to continue to increase twinning rate in dairy herds as milk production increases. It is also clear that this effect of milk production is most related to the level of production within the 2 weeks before the cow ovulates and not to total milk production during the entire lactation. This effect was also similar when a more extensive regression model was used for analysis, and when multiparous and primiparous cows were analyzed separately (Lopez et al., 2005a). As with duration of estrus, the first postpartum ovulation differed from other ovulations, showing a high double ovulation rate that was unrelated to milk production (Lopez et al., 2005a).

Circulating Steroids and Steroid Metabolism in Lactating Dairy Cows

A number of studies have evaluated circulating hormone concentrations in lactating dairy cows. As discussed above, cows with higher milk production ovulate larger follicles but have lower circulating estradiol concentrations (Lopez et al., 2004). In addition, higher producing dairy cows have a larger volume of luteal tissue but reduced circulating progesterone (Lopez et al., 2005a). Table 1 shows a comparison of dairy heifers and lactating dairy cows that were monitored by daily ovarian ultrasonography and hormonal analyses (Sartori et al., 2004). It is clear that cows ovulated larger follicles but had reduced circulating estradiol-17ß concentrations. This is somewhat surprising because it would be expected that cows with larger follicles would tend to have greater follicular estradiol-17ß production. Again paradoxically, lactating cows had a much larger volume of luteal tissue but reduced circulating progesterone. This study also shows the much higher multiple ovulation rate in lactating cows. Other studies have also reported changes in circulating hormones and size of ovarian structures in lactating cows (Ahmad et al., 1995; Inbar et al., 2001).

There appear to be two reasonable explanations for the disconnection between circulating steroid hormones and size of follicles and corpus luteum **(CL)**. The first possible explanation is that follicles and CL are less steroidogenically active in lactating dairy cows. This could be due to inadequate circulating stimulatory hormones, substrate for steroidogenesis, or intracellular steroidogenic pathways. There were more LH pulses in lactating than similar size non-lactating cows (Vasconcelos et al., 2003), suggesting that LH is not likely to be the cause of reduced

steroidogenic output. In addition, the primary substrate for bovine ovarian steroidogenesis is high-density lipoprotein, and this is particularly elevated in lactating dairy cows (Grummer and Carroll, 1988). There is a reduction in circulating insulin-like growth factor-1 in lactating dairy cows, and this could be related to reduced steroidogenic capacity (Lucy, 2000). Nevertheless, the hypothesis that ovarian structures in lactating dairy cows have reduced steroidogenic output has not yet been adequately investigated and therefore, cannot be disregarded or advocated at this time.

A more likely explanation is that lactating dairy cows have increased metabolism of steroid hormones as milk production increases. Circulating hormone concentrations are determined by rates of production and metabolism of the hormone. Increased feed consumption, such as during lactation, has been shown to alter circulating progesterone and excretion of progesterone metabolites during continuous delivery of progesterone (Parr et al., 1993a; Parr et al., 1993b; Rabiee et al., 2001a; Rabiee et al., 2001b). Increased steroid metabolism due to high feed consumption could alter the reproductive physiology of any species but may particularly alter reproduction in species with extreme increases in feed intake, such as lactating dairy cows. We propose that some of the reproductive changes in lactating dairy cows are caused by dramatic increases in steroid metabolism due to elevations in feed consumption and liver blood flow.

In recent experiments, we tested the hypothesis that increased liver blood flow as a result of elevated feed intake in lactating dairy cows would increase steroid metabolism (Sangsritavong et al., 2002). We found that prior to feeding, liver blood flow was greater in lactating (1561 ± 57 L/h) than similar size and age non-lactating (747 ± 47 L/h) cows. The liver blood flow and metabolism of progesterone and estrogen increased immediately after any amount of feed consumption in both lactating and non-lactating cows (Sangsritavong et al., 2002). The metabolism of estrogen and progesterone was much greater (2.3 X) in lactating than in non-lactating cows (Sangsritavong, 2002; Sangsritavong et al., 2002). Thus, the changes in metabolism of estrogen and progesterone in response to feeding are immediate and appear to be related to acute changes in liver blood flow. In lactating cows, a continuous high plane of nutrition appears to chronically elevate liver blood flow and metabolism of steroid hormones to approximately double the amount observed in similar size and age non-lactating cows. These results indicate that even with a similar level of hormone production, there would be lower circulating hormone concentrations in lactating dairy cows.

Can elevated steroid metabolism explain the paradox of reduced circulating steroids in spite of larger follicular and luteal sizes? If we use the data in Table 1 to calculate a rough index of circulating progesterone concentration divided by luteal volume, we find that heifers have roughly twice the value that is calculated for lactating cows (1.0 versus 0.5 ng/ml of progesterone per cm^3 of luteal volume). A similar calculation for circulating estradiol and follicular volumes also yields about a 2-fold greater value in heifers than cows (6.5 versus 3.2 pg/ml circulating estradiol per cm^3 of follicular volume). These values correspond closely to the roughly 2-fold elevation in metabolism of estrogen and progesterone that we have found in lactating vs non-lactating cows (Sangritavong, 2002; Sangritavong et al., 2002). A recent analysis of a larger group of individual lactating cows using this index showed a closer relationship of this index (circulating hormone/volume of tissue) to milk production ($R^2 = 0.44$ to 0.47; $P < 0.01$) than found when comparing either circulating hormones or follicular or luteal volume alone to milk production (Lopez et al., 2005a). Thus, although we cannot rule out the importance of changes in steroidogenic production by luteal and follicular tissue, it seems reasonable that the changes in circulating estradiol and progesterone can be

accounted for by increased rates of steroid metabolism in lactating cows.

We have synthesized this information into a simplified working model (Figure 3). Lactating cows have greater energy requirements than non-lactating cows (for example, a cow producing 110 lb/day of milk will require 53 Mcal/day of net energy vs 12.5 Mcal/day for a non-lactating cow; NRC, 2001). The high feed consumption required to meet these energy requirements leads to a dramatic increase in liver blood flow (Sangsritavong, 2002; Sangsritavong et al., 2002) which leads to elevated metabolism of both estrogen and progesterone. This would cause a reduction in circulating estrogen and progesterone concentrations, even in the midst of high production of steroid hormones by the follicle or CL.

This simple model could potentially explain some of the results described in the sections above. Figure 4 provides a model that focuses on changes in circulating hormones and follicular and luteal sizes that occur due to the elevated steroid metabolism in lactating cows with elevated milk production. In high-producing, lactating dairy cows, follicle growth rate may be similar to lower producing cows, but circulating estradiol would increase at a slower rate due to elevated steroid metabolism. Thus, estradiol would continue to rise until eventually circulating estradiol is sufficiently elevated for a sufficient length of time to induce a GnRH/LH surge. The LH surge is likely to be induced at a larger follicular size in high producing dairy cows and probably at a lower circulating estradiol concentration (based on our previous results). In addition to lower estradiol concentrations at the start of estrus, there is also likely to be a more rapid decrease in circulating estradiol after the LH surge due to elevated estradiol metabolism. Therefore, it makes sense that a higher producing cow would have a shorter duration of estrus because of increased steroid metabolism. Thus, this model provides a logical and likely explanation for the changes in duration of estrus, and for the paradox of lower circulating steroids but larger ovarian structures occurring in lactating dairy cows. In addition, it provides scenarios for how elevated steroid metabolism due to high milk production could reduce fertility. The preovulatory follicle and oocyte would be exposed to a longer period of elevated LH pulses that could lead to ovulation of an overstimulated oocyte and reduced fertility (Ahmad et al., 1995; Ahmad et al., 1996; Revah and Butler, 1996) . Alternatively, a reduced rate of progesterone rise following ovulation could also reduce fertility, as has been suggested by others (Folman et al., 1973; Ahmad et al., 1996; Dunne et al., 1999; Mann, 2001). Nevertheless, this model does not yet explain how very high milk production (> 88 lb/day) can produce the dramatic increase in double ovulation rate. Our recent intensive study of hormonal changes associated with selection of single, double, or triple dominant follicles in lactating dairy cows demonstrates that reduced circulating estradiol near follicle selection is not responsible for multiple dominant follicles (Lopez et al., 2005b), as we originally proposed (Wiltbank et al., 2000). Nevertheless, circulating progesterone is decreased and LH and FSH are increased near the time of selection, making it possible that changes in hormonal metabolism may still have a role in this process.

The critical involvement of estrogen and progesterone in almost every aspect of reproductive physiology makes changes in steroid metabolism an attractive explanation for the numerous changes in reproduction that have been observed in lactating dairy cows. The elevation in steroid metabolism is a logical extension of elevated metabolic activity in lactating dairy cows. Nevertheless, more definitive data are needed to link any particular reproductive change to elevated metabolism of a particular reproductive hormone. The physiological relevance of the models in Figures 3 and 4 can be tested by timely supplementation of estradiol and/or progesterone, as well as potentially decreasing activity of specific steroid-metabolizing liver

enzymes. Another review is needed to correlate these models and other physiological models with the numerous older, recent, and on-going scientific investigations of steroid hormone supplementation in cattle. Future practical manipulations of reproductive function in lactating dairy cows can be more rationally designed as the precise effects of elevated steroid metabolism on reproductive physiology in lactating dairy cows continue to be more fully defined.

Practical Reproductive Management Implications

The next section will briefly suggest some practical implications and reproductive management strategies for each of these areas.

Decreased duration of estrus due to high milk production

What does this practically mean for a dairy farm? We used the data on duration of estrus versus milk production to analyze what would happen to heat detection efficiency for cows with different levels of milk production. In Figure 5, the probability of detecting a cow in heat with different frequency of heat detection is shown. If a cow is producing about 70 lb/day, a 4-time per day heat detection program will detect about 90% of cows that are in estrus. However, this same program (4 times/day) will only detect about 50% of cows in heat if they are producing above 100 lb/day. This result gets even worse if heat detection is done only twice per day or once per day. It should be noted that all of the probabilities in this analysis were based on actual ovulation by the cows (detected by ultrasound). Some producers will say that the high producing cows are not cycling, but they are cycling normally. They do not detect them in heat because they have so short of a time that they are in heat. Increasing number of times that cows are checked for heat can help to solve this problem. Many producers are using heat detection aids, such as tail chalk, to help find cows that are in showing heat at a time that they are not present. This can be critical because high producing cows are showing heat for only 4 hours or less in many cases. Most dairy producers in the United States are incorporating timed AI programs, such as Ovsynch, into their reproductive management programs to allow high-producing cows to be bred in a timely manner.

Treating anovular cows

Although level of milk production is not normally associated with incidence of anovulation, dairy producers still need to design programs to treat anovular cows. Generally, 20% of dairy cows will not be cycling by 70 days after calving. This percentage will increase if there are a high percentage of cows with low BCS (2.5 or less). These cows need to be quickly assigned to a hormonal program (and possibly nutritional program if they have low BCS) that will start the cows cycling. An Ovsynch program alone is not the ideal treatment for anovular dairy cows. Use of a CIDR® (Pfizer, Inc., New York, NY) or estradiol should be incorporated into these programs to be optimal treatments for non-cycling dairy cows.

Increasing double ovulation rate (and twinning rate) with increasing milk production

From a practical standpoint, it appears that there may be little that we can do to change this trend. Using Ovsynch does not seem to increase or decrease double ovulation, with double ovulation related to milk production whether we look after a hormonal synchronization program or a natural estrus. Obviously, not all double ovulations result in twins, but increasing double ovulation rate will almost surely result in increased twinning rates on higher producing farms. It seems clear that the main increase occurs after cows are producing about 90 lb/day. Thus, we must anticipate that we will have a dramatic increase in double ovulation rate in cows producing over 90 lb/day, and this will result in an

increase in twinning rate in cows that conceive during this time of high milk production. We must align our management procedures to deal with this increasing twinning rate if we are increasing milk production into this range on our dairy farms. First, we must set a program to diagnose twins. Second, we should set up procedures to manage cows that are likely to have twin births. Twinning cows will calve earlier (10 to14 days on average) and are likely to have more problems during the calving process. These twin calving cows were, on average, our highest producing cows during the previous lactation; therefore, we must carefully design our calving and early lactation procedures with these twinning cows in mind.

Decreasing conception rate due to higher milk production

As discussed above, there are many different factors that impact conception rate in lactating dairy cows and higher milk production is just one of these factors, and on many farms, it may be a fairly minor factor. The effect of milk production on fertility is dramatically amplified during hotter times of the year. This is because there is a greater increase in body temperature as cows increase milk production. This increase in body temperature leads to decreased reproductive success, particularly death of the early embryo.

From a practical viewpoint, we have tried to utilize the information that many of the problems with fertility in dairy cows appear to occur during the first week after breeding. We hypothesized that we could improve reproduction just by transferring a good quality embryo at 7 days after expected time of AI. So in a fairly large experiment, we compared conception rate **(CR)** in our herd when cows were bred either by AI or by embryo transfer **(ET)**. During 365 days, 550 potential breedings were used from 243 lactating Holstein cows (77 lb/day of milk). Cows were synchronized (GnRH-7days-PGF_{2a}-3days-GnRH) and randomly assigned to receive AI immediately after the second GnRH injection (day 0) or to receive transfer of one embryo 7 days later. Circulating progesterone and follicular and luteal sizes were determined on days 0 and 7. Pregnancy diagnosis was performed on days 25 or 32, and pregnant cows were reevaluated on days 60 to 66. Synchronized cows with single ovulation had similar ($P > 0.30$) CR on days 25 to 32 with ET (n = 176; 40.3%) and AI (n = 160; 35.6%). Pregnancy loss between days 25 to 32 and 60 to 66 also did not differ ($P = 0.38$) between ET (26.2%) and AI (18.6%). When single (n = 34) and multiple (n = 57) ovulators were compared, independent of treatment, multiple ovulators had greater ($P < 0.01$) circulating progesterone on day 7 (2.7 versus 1.9 ng/ml), and there was a tendency ($P = 0.10$) for greater CR in multiple ovulators (50.9 versus 38.1%). However, there was no difference in CR between AI and ET cows with multiple ovulation (50.0 versus 51.7%). The CR tended to be lower for AI than ET in single-ovulatory cows ovulating smaller (≤15 mm; 23.7 vs. 42.3%; $P = 0.06$) but not average (16 to 19 mm; 41.2 versus 37.3%; $P = 0.81$) or larger (≥ 20 mm; 34.3 versus 51.0%; $P = 0.36$) follicles. Thus, ET did not improve overall CR in lactating cows but size and number of ovulating follicles may determine success with these procedures. We obviously have a large number of future experiments to do in order to resolve the problems with fertility in lactating dairy cows.

Many laboratories are currently experimenting with a number of changes in timed AI programs that may increase conception rates in high producing dairy cows. There are numerous intriguing possibilities, but they still lack sufficient data to allow recommendation at this time.

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Table 1. Comparisons (means ± SEM and percentages) between all (single- and multiple-ovulating) heifers (n = 27) and lactating cows (n = 14) with typical interovulatory intervals (Sartori et al., 2004).

	Heifers	Lactating Cows	P-value
Interovulatory interval (days)	22.0 ± 0.4	22.9 ± 0.7	0.28
Day of luteolysis	18.5 ± 0.4	18.9 ± 0.6	0.53
Cycles with two waves, % (no./no.)	55.6 (15/27)	78.6 (11/14)	0.15
Cycles with three waves, % (no./no.)	33.3 (9/27)	14.3 (2/14)	0.19
Cycles with four waves, % (no./no.)	11.1 (3/27)	7.1(1/14)	0.68
Day of emergence of second follicular wave	8.9 ± 0.3	11.1 ± 0.6	<0.01
Interval (days) from emergence of last wave and ovulation	10.1 ± 0.5	10.9 ± 0.5	0.29
Days from luteolysis to ovulation	4.6 ± 0.1	5.2 ± 0.2	<0.01
Incidence of co-dominant follicles during first wave, % (no./no.)	3.7 (1/27)	35.7 (5/14)	0.01
Multiple ovulation rate, % (no./no.)	1.9 (1/54)	17.9 (5/28)	0.02
Maximal size of largest ovulatory follicle (mm)	14.9 ± 0.2	16.8 ± 0.5	<0.01
Estradiol peak preceding ovulation (pg/ml)	11.3 ± 0.6	7.9 ± 0.8	<0.01
Maximal luteal tissue volume (mm^3)	7303 ± 308	11120 ± 678	<0.01
Progesterone peak (ng/ml)	7.3 ± 0.4	5.6 ± 0.5	0.01

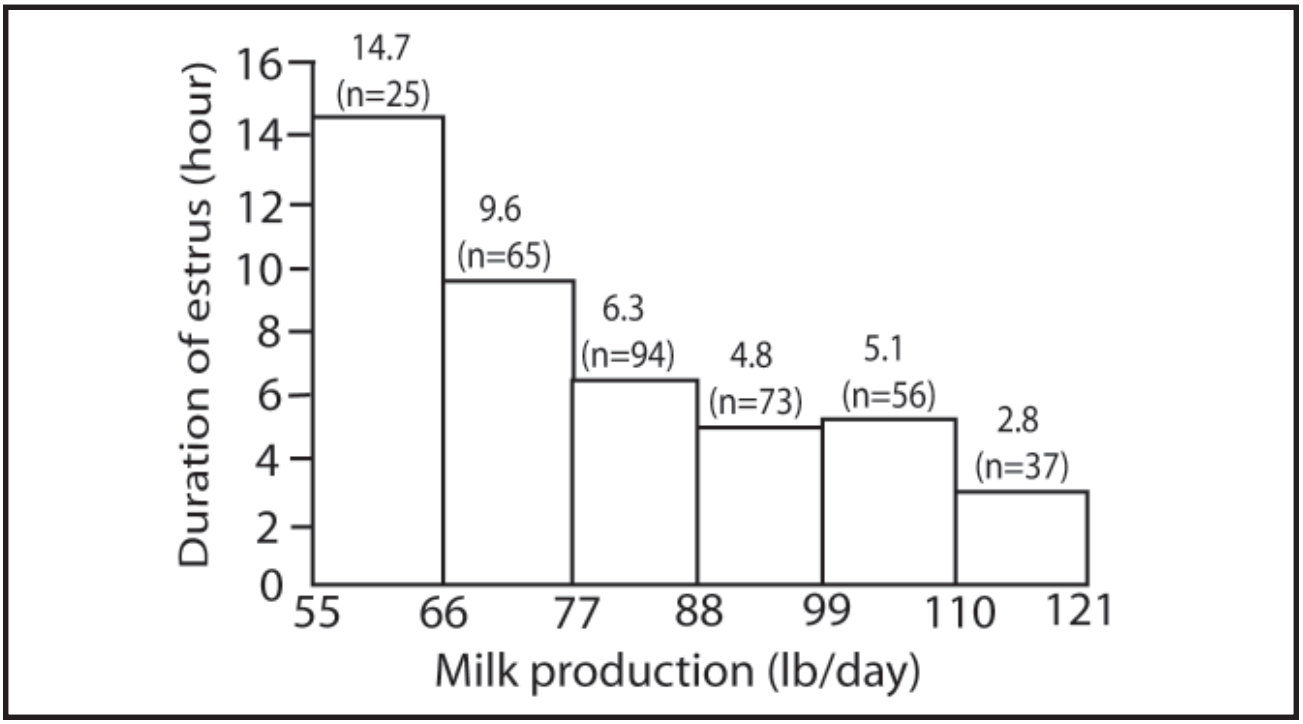

Figure 1. Relationship between level of milk production and duration of estrus. Analysis included all single ovulations (n = 350) except first post-partum ovulations. Average milk production is for the 10 days before estrus (Lopez et al., 2004).

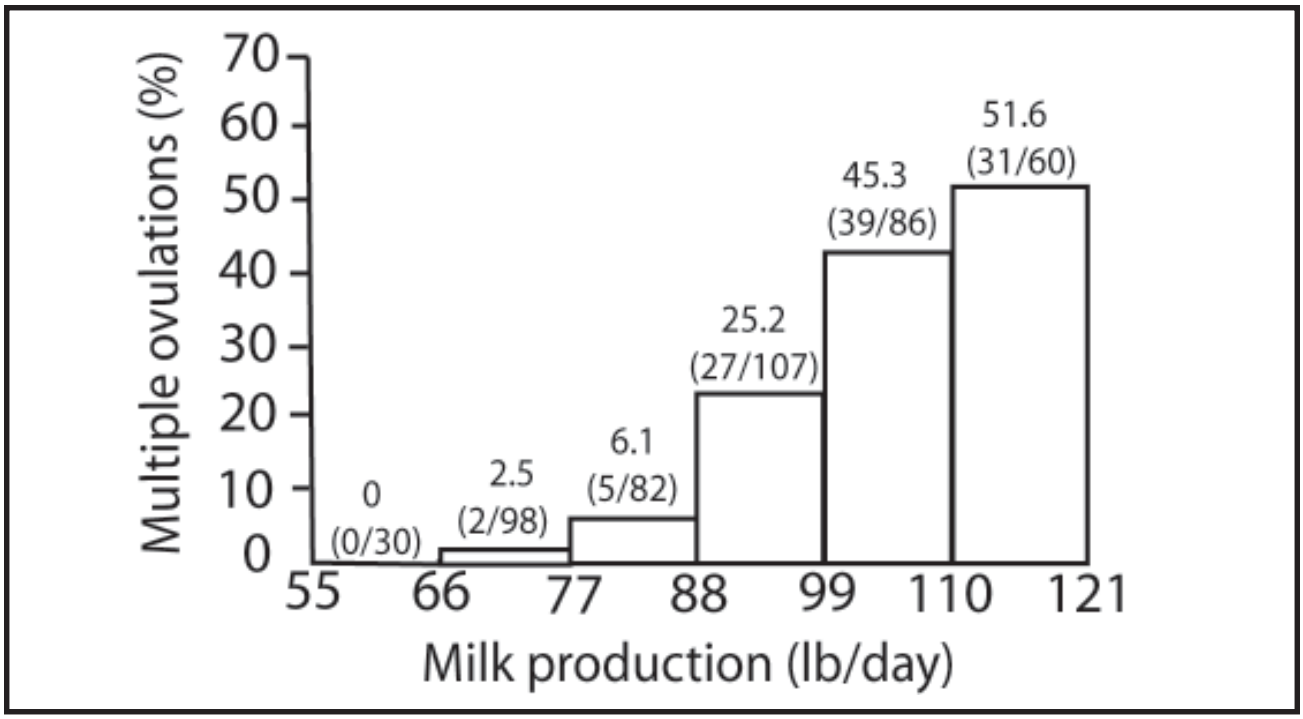

Figure 2. Relationship between incidence of multiple ovulation and milk production. Analysis included all ovulations (n = 463) except first post-partum ovulations. Average milk production was for the 14 days before estrus (Lopez et al., 2005a).

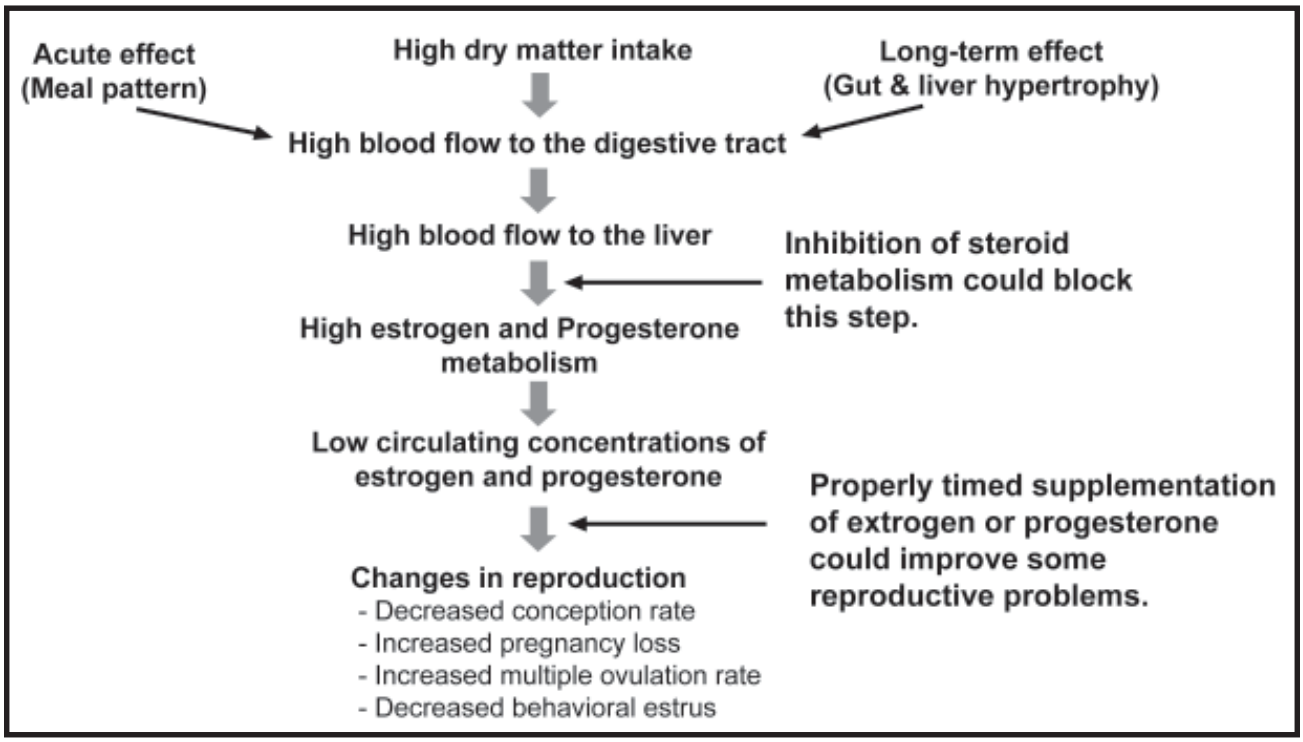

Figure 3. Schematic of the potential physiological pathway that may produce the changes in reproductive physiology observed in high-producing lactating dairy cows

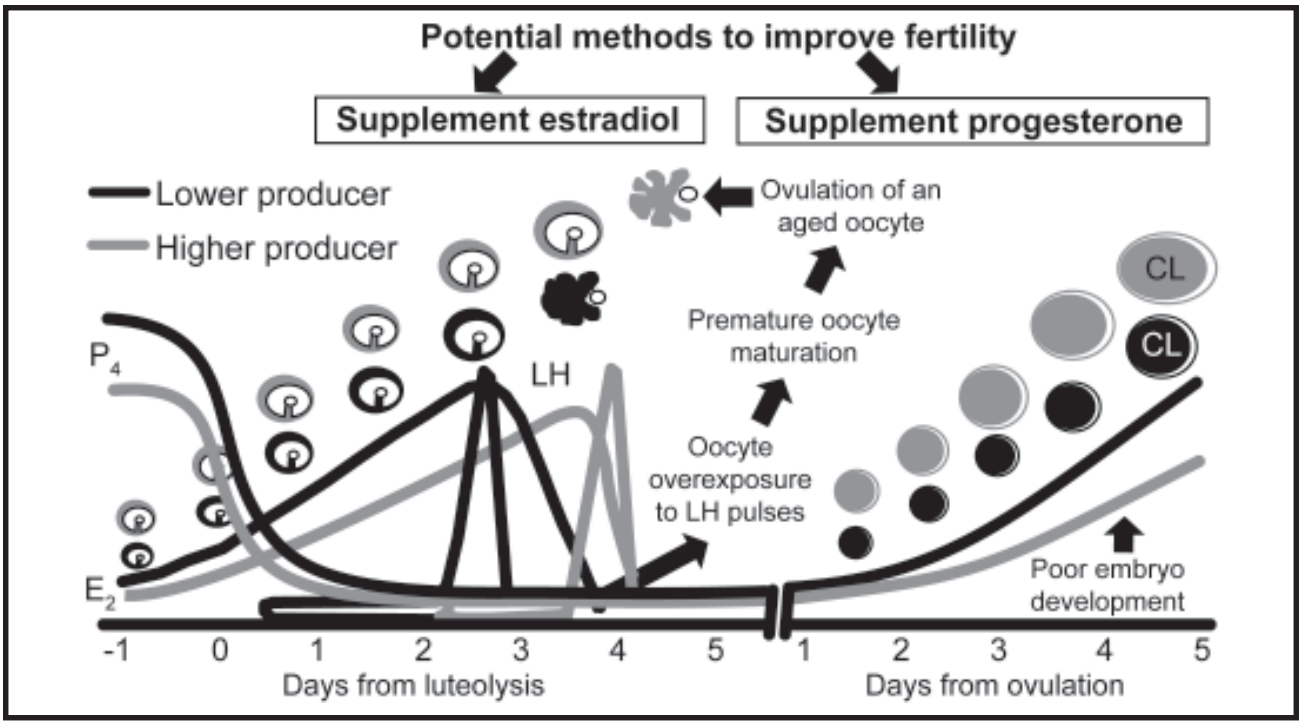

Figure 4. A physiological model showing the changes in circulating estradiol, luteinizing hormone (LH), and progesterone, as well as the growth patterns of the preovulatory follicle and corpus luteum (CL) in lactating dairy cows with higher or lower milk production. Possible reasons and potential treatments for lower fertility in higher producing lactating dairy cows, based on this model, are also shown.

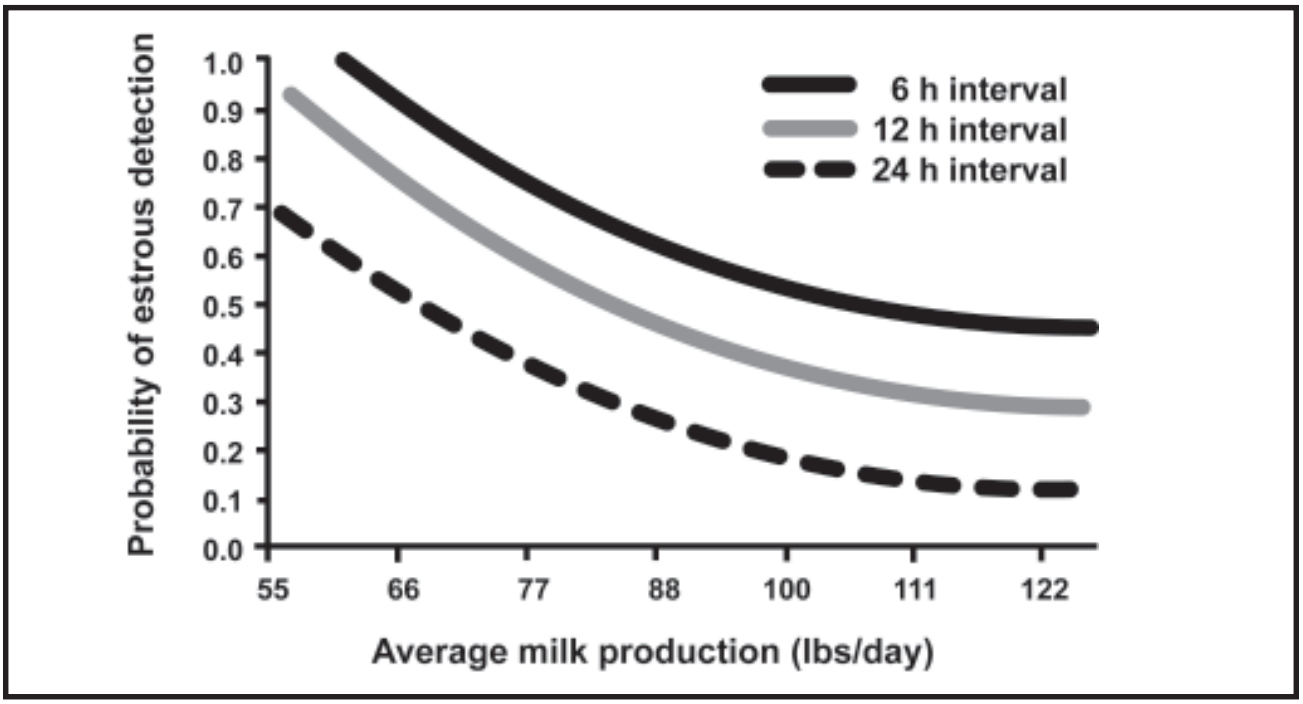

Figure 5. How the probability of heat detection changes with different frequencies of heat detection and different levels of milk production.

Effect of Photoperiod on Feed Intake and Animal Performance

Geoffrey E. Dahl[1]
Department of Animal Sciences
University of Illinois

Abstract

Exposure to extended periods of light is a commonly implemented management practice to improve overall yield and production efficiency in lactating dairy cattle, and recent studies support additional benefits of a reduced photoperiod during the dry period. Whereas the observation of greater milk yield indicates some effect of photoperiod manipulation at the mammary gland, support for the increase in milk must be provided also, suggesting involvement of other factors in the response. Feed intake increases with photoperiod manipulation, although the effect of light varies with the physiological state, i.e. lactating vs. dry. One consistency, however, is that increases in dry matter intake **(DMI)** are most consistently associated with lighting shifts that increase milk yield.

Introduction

Numerous studies across multiple locations support the concept that lactating cows exposed to 16 to 18 hours of light each day (i.e. long day photoperiod or **LDPP**) have greater milk yield relative to cows on a typical light schedule of natural photoperiod plus some additional light to accommodate milking on a 12:12 hour schedule (reviewed in Dahl et al., 2000). The increase in milk output appears to be a fixed response, with an average milk yield response of about 5.1 lb/day across production levels that range from less than 44 lb/day to over 88 lb/day (Dahl and Petitclerc, 2003). Exposure to LDPP can be effectively combined with other management approaches, such as bovine somatotrophin, to increase yield (Miller et al., 1999).

In contrast to the impact of LDPP on lactating cows, there is now substantial evidence that dry cows exposed to a reduced photoperiod (i.e. short days or **SDPP**) produce more milk in the subsequent lactation than contemporaries exposed to LDPP or even natural light conditions (Miller et al., 2000; Dahl and Petiticlerc, 2003; Auchtung et al., 2005), and those studies are buttressed by analysis of seasonal environmental influences of heat and light that indicate a negative effect of long days during the dry period on performance in the next lactation (Aharoni et al., 1999, 2000).

Photoperiod manipulation, therefore, is a useful tool to improve the lactational performance of cows, yet the physiological mechanisms that drive the response of dry versus lactating cows appears to differ. In addition, the impact of photoperiod on dry matter intake varies according to the physiological state of the cow. The remainder of this paper considers the difference between those mechanisms and their associated effects on intake.

Comparison of Physiological Responses to Photoperiod

One of the most consistent responses to photoperiod across species is a substantial increase

[1]Contact at: 230 Animal Sciences Laboratory, MC-630, 1207 W. Gregory Dr., Urbana, IL 61801, (217) 244-3152, FAX: (217) 333-7088, Email: gdahl@uiuc.edu

in circulating concentrations of prolactin **(PRL)**, and that response is well documented in cattle (Dahl et. al, 2000). In cattle, this increase in circulating PRL occurs regardless of gender, age, or stage of lactation, with the only exception being that at very low ambient temperatures, no increase in PRL occurs (Peters et al., 1981). Recently, we have noted an inverse relationship between PRL-receptor **(PRL-r)** mRNA expression and circulating PRL in cattle on different photoperiods, with SDPP animals expressing higher PRL-r relative to those on LDPP (Auchtung et al., 2003). This inverse relationship between PRL and PRL-r results from the shift in PRL secretion in response to photoperiod manipulation (Auchtung and Dahl, 2004). Higher PRL-r mRNA expression is associated with greater mammary growth during the dry period and improvements in immune function, both of which likely contribute to the higher milk yield in the subsequent lactation (Auchtung et al., 2004, 2005; Wall et al., 2005).

The effect of LDPP in lactating cows is not likely to result from the changes in PRL characteristically observed for at least two reasons. First, the previously mentioned failure of cows to respond with increases in PRL under cold ambient temperatures did not prevent the milk yield response to LDPP during lactation (Peters et al., 1981). Second, administration of exogenous PRL does not improve milk yield relative to placebo (Plaut et al., 1987). Although circulating growth hormone concentrations are unaffected by photoperiod manipulation in cattle, exposure to LDPP increases insulin-like growth factor-I in heifers, steers, and lactating cows (Spicer et al., 1994; Dahl et al., 1997; Kendall et al., 2003). Thus, an increase in IGF-I rather than PRL is more likely to be the endocrine mechanism of greater milk yield in lactating cows.

Photoperiodic Effects on Dry Matter Intake

Despite the differing mechanisms proposed for the responses, the milk yield increases observed following photoperiod manipulation during lactation, or the dry period, must be supported by additional energy partitioning to the mammary gland. Lactating cows exposed to LDPP have higher DMI compared with those without extended light exposure (Dahl et al., 2000; Dahl and Petitclerc, 2003). The increase in DMI does not appear to drive the higher yield of milk, however, as it lags the milk response (Dahl et al., 2000). As milk yield increases, the demand for energy to support that increment in milk stimulates intake, and producers should plan for an additional 2.2 lb/day of DMI in cows exposed to LDPP during lactation.

In contrast to lactating cows, dry cows on SDPP consume more feed than those on LDPP. The DMI increases an average of 2.2 lb/day in dry cows on SDPP, although this response is most apparent in the early to middle portion of the dry period. Because milk yield is not a factor, it follows that this response is directly associated with the reduced light exposure. We have not observed any carryover effect on intake in the next lactation, although the length of time that we typically track that response (i.e. 42 days) is likely insufficient to observe a response with the number of animals in our studies.

One possible explanation for altered intake of cows on different photoperiods is that of feeding time. That is, does light exposure influence the amount of time that cows spend feeding? Studies in heifers and some of our own work in dry cows suggest that shifts in total feeding time do not account for differences in intake (Zinn et al., 1986; Karvetski et al., 2006). However, there may be altered distribution of feeding bouts throughout the day on different photoperiods. For example, dry cows on LDPP spend more time feeding directly after feed is offered relative to those on SDPP that distributed feeding bouts more evenly throughout the day (Karvetski et al., 2006). That observation may be useful in barn design and feeding area management, because the peak utilization of the feedbunk would differ between groups.

Implementing Photoperiod Management

Light exposure is easily manipulated during lactation as it requires extending the amount of light beyond the typical natural exposure. Light intensity of 150 to 200 lux is necessary to produce the response, and placement of the lamps should ensure that all areas of the barn achieve that illumination level, not just the feedbunk. A consistent duration of 16 to 18 hrs of light is needed, and it is critical that a continuous 6 to 8 hour period of darkness occur to sustain the response. That is, continuous light exposure should be avoided in lactating cows.

For dry cows, limiting light exposure to 8 hours/day can be achieved using well-ventilated, enclosed barns. Cows can be outside and exposed to natural daylight for up to 8 hours, but should be in darkness for the remaining 16 hours/day. Low intensity red lighting, such as that from 7 to 15 W incandescent bulbs, can be used for observation during dark periods in both lactating and dry cows, as dim illumination in the red range is not perceived as light by cows. More information on lighting design and approaches is available at: http://www.traill.uiuc.edu/photoperiod/.

Conclusions

In summary, photoperiod manipulation during lactation and the dry period offers an effective, non-invasive approach to stimulate milk yield and performance. Light exposure alters DMI directly and indirectly depending on the stage of the lactation cycle. Photoperiod management is easily integrated into most types of confinement dairy production systems and can be combined with other stimulators of yield.

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Field Responses to the Feeding of Rumensin®

Elvin E. Thomas[1]
Elanco Animal Health

What is Rumensin and How Does it work?

Monensin is classified as an ionophore, which by definition is a compound that transports metal ions and protons **(H^+)** across cellular membranes. Monensin is the active compound in Rumensin.[2]

Monensin's mode of action begins with an initial attachment to the cell membrane of gram positive ruminal microorganisms. An immediate loss of cellular potassium and an influx of H^+ occurs (Russell, 1997). Monensin then catalyzes the influx of sodium and an efflux of protons. In spite of this action, protons generally accumulate inside the cell, resulting in decreased intracellular pH. An attempt by the cell is made to pump the excess protons out of the cell, resulting in depletion of the cellular adenosine triphosphate **(ATP)**. Lack of ATP prevents the cells from growing and contributes to decreased numbers of gram positive bacteria. Due to the nature of their cell membranes, gram positive ruminal bacteria are more sensitive to monensin than are gram negative bacteria. The gram negative organisms are largely responsible for production of propionic acid in the rumen. Thus, the net effect of including Rumensin in the feed is that the concentration of propionate increases in contrast to acetate and butyrate that decrease. Propionate is either used directly as an energy source or may be used for gluconeogenesis by the animal.

What has Happened Since Rumensin was Approved for Dairy Cows?

Rumensin was approved by the Food and Drug Administration **(FDA)** on October 28, 2005 for feeding to dairy cows (both lactating and dry) fed total mixed rations. The indication was improvement of milk production efficiency[3] with monensin levels ranging from 11 to 22 g/ton of total mixed ration dry matter. Since that time, many nutritionists have learned how to adjust diets to allow dairy farmers to successfully reap the benefits from Rumensin and increase profitability.

Label Changes Since the Initial Approval

Two changes have been made following the initial approval and include:

- Allow feeding Rumensin to component-fed herds (including top dress). This change in the Rumensin 80 label allows for feeding 185 to 660 mg/head/day to lactating dairy cows or 115 to 410 mg/head/day to dry cows in a component of the total diet or as a top-dress. This provides added flexibility to the feeding program by allowing the producer to start feeding 185 mg/head/day, then increase the amount in the diet to the 275 to 350 mg/head/day range or desired level. The component feed concentration of monensin must be within the 11 to 400 g/ton range and be fed in a minimum of one pound of feed per cow per day.

[1]Contact at: 2001 W. Main, Greenfield, IN 46140, (317) 277-4950, FAX: 317-651-6484, Email: e.thomas@lilly.com
[2]Rumensin® is a trademark for Elanco's brand of monensin sodium.
[3]Production of marketable solids-corrected milk per unit of feed intake.

- Type B Bluebird label was changed to allow monensin concentrations ranging from 23 to 80,000 g/ton in Type B feeds.

These rapid changes to the label speak highly for the willingness of the FDA to quickly evaluate the label and help make it more user-friendly for the dairy industry yet maintain the rigid requirements for feeding correct levels of monensin.

What Have we Seen Regarding Rumensin Response Versus Diet Composition?

During the past year, some dairy farmers have incorporated Rumensin into their feeding programs and experienced only minimal changes in milk component composition. Some dairy farmers, however, have seen a marked reduction in milk fat percentage. Also, some producers observed milk production to increase from 1 to 8 lb/cow/day after incorporating Rumensin into the diet, while other producers maintained current production. (Note: Rumensin is approved for increased milk production efficiency which is more salable solids corrected milk per unit of feed intake.)

Nutritionists have commented that Rumensin will help evaluate whether a diet fed is pushing the limit of too much starch and/or lack of effective fiber. Based on the data package submitted to the FDA for approval and field observations, the effect on milk components is a manageable effect when feeding Rumensin. When milk components are negatively impacted in the presence of Rumensin, the level of starch and unsaturated oils may need to be reduced while effective fiber is increased.

Diets Containing Finely Ground, High Moisture Corn

Diets fed to lactating cows from the upper midwest and eastern regions of the U.S. typically are based on high moisture corn (finely ground and sometimes containing 30% moisture or higher), corn silage, and haylage. Evaluation of feeding programs in which milk components (fat and protein percentages) were minimally impacted and milk production either remained the same or increased showed that typical levels of NDF were 30 to 32% and starch was 21 to 23% with an absolute maximum of 25% (dry matter basis). Unsaturated oil sources, such as corn distillers, were sometimes included in these diets at rates of approximately 2 to 3 lb/cow/day of dry matter.

In contrast, feeding programs in which milk fat percentages were reduced markedly showed that NDF was lower (typically 27 to 29%) and contained much higher starch levels (26 to 32%). Those diets often contained high levels of unsaturated oil sources, such as corn distillers or soybeans, in addition to high levels of finely ground high moisture corn.

Two nutritionists reported that milk fat was depressed markedly when Rumensin was fed in conjunction with small grain silage, such as rye, or ryegrass. Those forages often contribute a sizable quantity of unsaturated oils, which in the presence of sufficient amounts of starch could theoretically have a negative impact on biohydrogenation in the rumen. Others nutritionists have reported no problems with milk fat depression when feeding small grain silages. In those cases, starch levels were maintained at a maximum of 23 to 25% of dry matter, which would theoretically have limited impact on the biohydrogenation process.

Diets Containing Dry Corn

Field observations in which Rumensin is included in lactating diets containing dry corn (in contrast to finely ground, high moisture corn) has shown that starch levels can be higher (compared to diets containing high moisture corn) with minimal impact on milk components. It has also been reported that more unsaturated oils may be contained in the diet without negatively impacting

milk fat levels compared to those diets containing finely ground high moisture corn.

Maintaining the Balance Between Starch, Fiber, and Unsaturated Oils in the Diet Appears to be Critical

Research has shown that biohydrogenation of unsaturated fatty acids is reduced significantly in the presence of high levels of starch. From field reports, higher levels of unsaturated fatty acids may be included in the diet without milk fat depression if starch levels are held around 21 to 23% of dry matter in diets that contain finely ground high moisture corn. These reports are supported by Griinari et al. (1998) in which addition of corn oil to a low fiber, high starch diet reduced milk fat ($P < 0.05$) but had no effect when added to a high fiber diet. Kalscheur et al. (1997) compared ruminal trans 18:1 production and duodenal flow in diets containing either high forage or high concentrate with or without dietary buffers. In the high concentrate diet, trans 18:1 production was reduced and milk fat increased with buffer addition (increased rumen pH). These papers illustrate that both low rumen pH and a source of unsaturated fatty acids are required for synthesis of fatty acid intermediates involved in milk fat depression.

What has been the Response from Including Unsaturated Fat Sources in the Diet?

Field reports from nutritionists suggest that milk components may be affected as a result of the interaction between the amount of unsaturated oils, the availability of the oil sources, and both the amount and source of starch contained in the diet. In a Rumensin-fed herd (11 g/ton of dry matter), cows were producing 85 lb/day of milk, and milk fat percentage was reduced from 3.55 to 3.2% in response to fine grinding of roasted soybeans. When the grind was changed back from finely ground to coarse rolled (breaking the beans into 5 to 8 pieces), milk fat returned to 3.5% within 5 days. The roasted beans were fed at a rate of 6 to 8 lb/cow/day in a diet containing a low level of starch (22 to 23% of dry matter). This suggests that highly available sources of unsaturated fats (finely ground vs. coarse cracked) may impact milk fat percentage to a greater extent than less available sources in some situations.

Some nutritionists are using the guideline of including a maximum of 5% total unsaturated fat sources (dry matter basis) when high moisture corn is contained in the diet. A typical diet based on corn, corn silage, and haylage contains approximately 3% unsaturated fat, thereby allowing 2% additional unsaturated fat from sources such as oil seeds. If dry matter intake is assumed to be 50 lb/cow/day, this equates to adding 1.0 lb of unsaturated fat from either 5 lb of soybeans or whole cottonseed. Some are including 2 to 3.5 lb of dry matter from distillers grains in diets containing approximately 32% NDF without reducing milk fat percentage. The fat in distillers is readily available in contrast to that from whole cottonseed or coarsely cracked roasted soybeans. Surveys have shown that the amount of fat in distillers grains varies widely (ranges reported from 10 to 34% fat) and must be monitored closely.

In diets containing dry corn, some nutritionists are including up to 6% total unsaturated fat (dry matter basis) with little effect on milk components.

What Starch Levels are Working?

Reports from nutritionists show a wide range in starch content (21 to 30%) of diets fed to lactating dairy cows. Closer examination shows that higher starch contents ranging from 26 to 30% may be fed with Rumensin when dry corn is sourced in the diet along with good effective fiber. However, when finely ground high moisture corn is fed, starch levels need to be reduced to a maximum of 25%, with many nutritionists targeting starch at 21 to 23% and observing no reduction in milk fat percentage

while maintaining milk production. The difference in recommended starch levels surely stems from the fact that high moisture corn ferments more rapidly and to a greater extent than dry corn with a resultant lower rumen pH. The process of biohydrogenation of unsaturated fatty acids is reduced when rumen pH is low and contributes to lower milk fat production.

What About Feeding By-product Feedstuffs?

Substituting 3 to 4 lb of beet pulp or soybean hulls in place of high moisture corn has corrected some cases of milk fat depression. This correction is probably due to providing a more desirable level of starch in the rumen because of the composition difference between those by-products compared to the corn it replaced. These by-products contain low levels of starch (5% or less vs. 72% for corn) and unsaturated oils (2.4% or less vs. 4.3% for corn) yet are highly digestible energy sources (1.79 Mcal NE_L/lb vs. 1.84 Mcal NE_L/lb for corn). Including citrus pulp has also shown to be beneficial. Both beet pulp and citrus pulp contain sugars that may also contribute to improvement in milk components and yield.

What Levels of Monensin are Being Fed Today?

Many herds feeding a TMR are targeting 11 g/ton of dry matter. Assuming dry matter intake of 50 to 59 lb/day, monensin consumption will range from 275 to 325 mg/head/day. Some have gradually increased to 16 g/ton which would equate to an intake of around 400 mg/day of monensin with an intake of 50 lb of dry matter. Increasing from 11 g/ton has been done in a slow step-wise manner, while closely monitoring milk components.

During the dry period, many nutritionists are increasing Rumensin levels to provide approximately 275 to 325 mg/head/day of monensin during the close-up period. Their reasoning is to get the rumen adjusted to a similar amount of Rumensin that will be consumed during early lactation.

During the far-off dry period, Rumensin is typically being fed to increase efficiency. During the Elanco Rumensin trials, dry cows fed 22 g/ton of Rumensin ate less feed yet maintained the same body weight and body condition. Thus, the efficiency of feed utilization was improved.

What is the Energy Equivalent from Feeding Rumensin?

Using data from the Rumensin clinical trials, the following formula was used to calculate the energy content of the control and Rumensin-containing rations:

$$\text{Energy density} = \frac{\text{SCM energy + NEm +/- energy for BW change,}}{\text{DMI}}$$

where SCM is solids corrected milk, NEm is the net energy for maintenance requirement of cows, BW is body weight change, and DMI is dry matter intake (kg/head/day).

Based on this formula, the energy content of the diets increased with increasing levels of Rumensin in the diet. From that, it was calculated that the increased energy content of the diets was equivalent to what would have been achieved by feeding 1 to 2 lb of corn grain. Some nutritionists are attributing an energy value to Rumensin in their ration balancing program and finding that Rumensin is usually brought into rations for lactating and dry cows due to its relatively low cost.

What's Working for Getting Rumensin Introduced into the Herd for the First Time?

Some nutritionists are establishing the NDF level at 34% and the starch level at a maximum of 25% (when high moisture corn is fed) before

introducing Rumensin. The fiber may then be gradually reduced with 3 weeks between changes while monitoring milk component composition. This adjustment of the diet before inclusion of Rumensin is highly recommended.

Some nutritionists are using a step-up program in which Rumensin is introduced into the diet at the lowest cleared level (185 mg/head/day for component-fed herds or 11 g/ton dry matter basis for TMR herds) then stepped up in 3 stages, each lasting 5 to 10 days until the desired level is reached. This allows slow adjustment of the ruminal microbial population to Rumensin and is recommended.

How Do I Measure the Response from Rumensin?

The indication for Rumensin in dairy cows is for "improvement of milk production efficiency". To calculate milk production efficiency in the clinical trials, the following formula was employed:

$$\text{Milk Production Efficiency} = \frac{\text{Marketable solids-corrected milk}}{\text{Total } NE_L \text{ intake (adjusted for body-weight change)}}$$

In the 9 clinical trials, milk production efficiency was improved approximately 2 to 4% compared to controls by including Rumensin at 11 to 22 g/ton of dry matter. As indicated in the formula, cow weight changes were considered in the energetics of production and required that the cows be weighed monthly throughout the trial. Feed offered and refused daily was also measured to allow calculation of dry matter and NE_L intakes.

The method employed in the 9 clinical trials employed very precise procedures of measuring feed offered, feed refusal, and cow body weight changes that may be beyond the scope of some dairy producers. In an effort to evaluate the effect of Rumensin on efficiency, some dairy farmers are measuring the amount of feed offered and assuming a constant refusal rate (not weighing refused feed). In addition, they calculate production of energy corrected milk to allow for variation in milk composition that may occur.

This procedure is being used to measure the effect of Rumensin on milk production efficiency on a large (4000 cows) dairy farm in Idaho. Dry matter offered was recorded daily with a constant amount of feed refusal assumed. Milk yield was adjusted to 3.5% fat and 3.2% protein using the following equation (Bernard, 1997):

ECM = (0.3246 * lb milk) + (12.86 * lb milk fat) + (7.04 * lb milk protein)

The goal for that dairy farm is to produce energy corrected milk **(ECM)** per pound of dry matter consumed with an efficiency of 1.60. Efficiency was improved from 1.52 to 1.64 after inclusion of Rumensin into the lactating cow feeding program.

Guidelines for Feeding Rumensin Based on Field Reports and Observations

The following guidelines have been reported by nutritionists successfully using Rumensin in dairy cow diets (dry period and lactation).

- NDF inclusion levels of 35% in close up cows and greater than 28% (typically 29 to 32%) during lactation. Some nutritionists balance the diet using NDF as the primary parameter.
- Starch inclusion levels of a maximum of 25% (typically 21 to 23%) with high moisture corn and 26 to 28% (range of 26 to 30%) with dry corn. Some nutritionists balance the diet using starch content as the primary parameter.
- Reduce the amount of high moisture corn or replace 3 to 4 lb of it with soy hulls or beet pulp if milk fat is markedly reduced.

- Starch is often reduced by increasing the proportion of corn silage and reducing high moisture corn while being sure there is adequate effective fiber.

- Limit total fats to 6% (typical range of 5 to 6%) of dietary dry matter. Limit addition of unsaturated oils to a maximum of 2 to 3% of dietary dry matter.

- When introducing Rumensin into a feeding program, start with NDF at 33 or 34% and gradually decrease it while monitoring milk components. Wait 3 weeks between changes in fiber content.

- Use a step-up program for introduction of Rumensin into component-fed herds. Start with 185 mg/head/day, then increase in three stages to the final desired level, each stage lasting 5 to 10 days.

The Future: What is Needed for Diet Formulation?

With our knowledge of the importance of achieving a balance of dietary ingredients to optimize milk production and composition, it is apparent that the amount, particle size, and the extent of gelatinazation and fermentability of starch sources must be considered. Rapid, reliable, and repeatable techniques for measuring rumen fermentable and total starch are also needed. With increasing use of technology to enhance ruminal digestibility of corn, there is a greater need for information to evaluate the extent of expected increase of starch digestibility to help determine the proper amounts of starch and fiber in dairy rations (Firkins, 1997). It is also apparent that a good practical way of evaluating the amount of effective fiber in a ration is needed. The amount of unsaturated fat must also be known so that a measure, such as the iodine value, may be employed during ration balancing. The industry is quickly moving to the point of formulating diets based on the balance of fermentable starch, effective fiber, and unsaturated oils to support milk production and optimization of milk components. Knowledge of the requirements for and the ability to accurately balance these ration parameters will reduce the negative associative effects on fermentation in the rumen and result in greater milk production efficiency.

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Appropriate Methods of Diagnosing Mineral Deficiencies in Cattle

Jeffery O. Hall[1]
Utah Veterinary Diagnostic Laboratory
Department of Animal, Dairy, and Veterinary Sciences
Utah State University

Introduction

Many minerals have been proven in research studies to be essential for optimal growth, physiologic function, and productivity in ruminants. Historically, testing for these minerals has been performed on diets and/or dietary components to ensure "adequate" concentrations of specific minerals in the diet. However, general mineral analysis does not identify the chemical forms of these minerals, which can dramatically alter their bioavailability and utilization.

Although not possible for some of the minerals, the most specific means of diagnosing a mineral deficiency is by testing animals for unique functional deficits or deficiencies of specific mineral containing proteins or enzymes. This type of testing is often impractical from a field perspective due to individual test costs or rigorous sample handling requirements. But, when possible, this type of testing eliminates the need to know the specific molecular characteristics of a dietary mineral and the potential for competitive interactions of antagonistic minerals for absorption/utilization. For minerals that do not have identified physiologic indices for which testing can be performed, direct quantification from animal tissues or serum may provide a reliable indication of the overall mineral status of the animal or herd.

Mineral deficiencies can be suggestively diagnosed by the development of clinical disease or by post-mortem identification of tissue lesions. But, proof of mineral deficiencies often requires analytical verification since most do not have very unique clinical signs or lesions. In some instances, circumstantial proof of a deficiency can be provided by positive response to supplementation of a suspected deficient mineral. But, positive response may have nothing to do with the supplementation and may be just a time responsive correction of some other clinical condition.

An individual mineral may have multiple means of measurement for identification of deficiencies, but most have one that is more specific than the others. For example, dietary concentrations may or may not be reflective of the amount of bioavailable minerals. Or, an individual tissue concentration may or may not reflect functionally available mineral concentrations at the target or functional site.

The age of the animal being tested also is important for proper interpretation of mineral status. For example, feti accumulate some minerals at different rates during gestation, necessitating adequate aging of the fetus for interpretation. In addition, some minerals, for which little is provided in milk, accumulate at higher concentrations during gestation in order to provide neonates with adequate body reserves for survival until they begin foraging. This is especially prevalent with copper, iron, selenium, and zinc. Thus, the "normal range" for these minerals in body storage tissues would be higher in early neonates than in an adult animal.

[1]Contact at: 950 East 1400 North, Logan, UT 84322-5700, (435) 797-0238, FAX: (435) 797-2805, Email: jhall@cc.usu.edu

When individual animals are tested, the prior health status must be considered in interpreting mineral concentration of tissues. Disease states can shift mineral from tissues to serum or serum to tissues. For example, diarrhea can result in significant loss of sodium, potassium, and calcium from the body. Or, acidosis will cause electrolyte shifts between tissues and circulating blood. It is known that infectious disease, stress, fever, endocrine dysfunction, and trauma can alter both tissue and circulating serum/blood concentrations of certain minerals and electrolytes. Thus, evaluation of multiple animals is much more reflective of mineral status within a group than testing individual animals that are ill or have died from other disease states.

This paper is directed at the animal testing side of diagnosing mineral deficiencies and provides a summarization of the most commonly utilized tissues and fluids that are used for diagnosing specific mineral deficiencies in animals.

Live Animal Sampling

A variety of samples are available from live animals that can be analyzed for mineral content. The most common samples from live animals are serum and whole blood. These samples are adequate for measurement of several minerals, but it must be recognized that some disease states, as well as feeding times, can result in altered or fluctuating serum concentrations. Other samples from live animals that are occasionally used for analyses include liver biopsies, urine, and milk. But, since milk mineral content can vary through lactation, vary across lactations, and be affected by disease, it is not typically used to evaluate whole animal mineral status. Furthermore, hydration status significantly affects urinary mineral concentrations, rendering it a poor sample for evaluation of mineral status.

Serum should be separated from the red/white blood cell clot within 1 to 2 hours of collection. If the serum sets on the clot for long periods of time, minerals that have higher intracellular content than serum can leach into the serum and falsely increase the serum content. Minerals for which this commonly occurs include potassium and zinc. In addition, hemolysis from both natural disease and due to collection technique can result in increased serum concentrations of iron, manganese, potassium, selenium, and zinc.

The best type of collection tube for serum or whole blood is the royal blue-top vaccutainer tubes, as they are trace-metal free. Typical red-top clot tubes will give abnormally increased concentrations of zinc as a zinc containing lubricant is commonly used on the rubber stoppers. For minerals other than zinc, serum samples from the typical red-top clot tubes are adequate. Similarly, serum separator tubes are typically adequate for mineral analyses, except for zinc. But, I also have found tin contamination in serum samples collected into some brands of serum separator tubes.

Samples should be appropriately stored for preservation. Liver biopsies, urine, and serum can be stored frozen long term or refrigerated if analyses are to be completed within a few days. Whole blood and milk should be refrigerated but not frozen, as cell lysis or coagulation of solids, respectively, will result.

Post-Mortem Animal Sampling

A variety of post-mortem animal samples are available that can be analyzed for mineral content. The most common tissue analyzed for mineral content is liver, as it is the primary storage organ for many of the essential minerals. In addition, bone is used as the primary storage organ for calcium, phosphorous, and magnesium. Other post-mortem samples that can be beneficial in diagnosing mineral deficiencies include urine and ocular fluid.

Post mortem samples should be stored frozen until analyzed to prevent tissue degradation. If samples are to be analyzed within 1 to 2 days, they can be stored under refrigerated conditions.

Calcium

Analysis for calcium deficiency falls into two distinct classes. The first of which is metabolic calcium deficiency, often referred to as "milk fever". The second is due to a true nutritional deficiency which is associated with long term dietary calcium deficits.

Analysis for metabolic calcium deficiency is aimed at detection of low systemic or circulating calcium concentration. In live animals, testing is performed on serum to determine circulating calcium concentration. However, in dead animals, testing is more difficult as serum collected post-mortem will not accurately reflect true serum calcium concentration prior to death. But, circulating serum calcium concentration can be approximated from analysis of ocular fluid, with a vitreous to serum ratio of approximately 0.54 (McLaughlin and McLaughlin, 1987). The Utah Veterinary Diagnostic Laboratory has been able to confirm and disprove clinical hypocalcemia in numerous post-mortem cases via vitreous fluid analysis.

True, nutritional calcium deficiency is associated with weak, poor doing animals that have swollen joints, lameness, weak bones, and a propensity for broken bones (Puls, 1994). Analytical verification of calcium deficiency requires analysis of bone, since approximately 98 to 99% of the body calcium content is in bone and serum concentrations are maintained by both diet and turnover of bone matrix. The bone analysis should be performed as fat-free, dry weight to remove the age variability of moisture and fat concentrations.

Cobalt

Cobalt deficiency is associated with deficiency of vitamin B_{12} (cobalamin) in ruminants. Deficiency is associated with decreased feed intake, lowered feed conversion, reduced growth, weight loss, hepatic lipidosis, anemia, immunosuppression, and impaired reproductive function (Graham, 1991; Puls, 1994). Cobalt deficiency can also lead to decreased copper retention in the liver.

Tissue and serum concentrations of cobalt are generally quite small, as the B_{12} is produced in the rumen by the microflora. Since cobalt concentrations may not truly reflect the B_{12} concentrations, the most appropriate analysis for cobalt deficiency is the direct quantification of serum or liver vitamin B_{12}. But, there are numerous forms of cobalamins that ruminants produce with differing bioactivity, making interpretation of analytical results difficult (Mills, 1987). Cobalamin is absorbed into circulation and small amounts are stored in the liver. Of the tissues available, the liver cobalt concentration best reflects the animal's overall status, but it may not be truly reflective of vitamin B_{12} content.

Copper

Copper deficiency is a commonly encountered nutritional problem in ruminants, but copper excess is also commonly encountered, especially in sheep. Clinical signs of deficiency can present as a large array of adverse effects (Graham, 1991; Puls, 1994). Reduced growth rates, decreased feed conversion, abomasal ulcers, lameness, poor immune function, sudden death, achromotrichia, and impaired reproductive function are commonly encountered with copper deficiency.

The best method for diagnosing copper status is via analysis of liver tissue, although much testing is performed on serum. Deficiency within a herd will result in some animals that have low serum

copper concentrations, but serum concentration does not fall until liver copper is significantly depleted. In herds that have tested liver and found a high incidence of deficiency, it is not uncommon for a high percentage of the animals to have "normal" serum concentrations. At the Utah Veterinary Diagnostic Laboratory, it is commonly recommended that 10% of a herd or a minimum of 10 to15 animals be tested in order to have a higher probability of diagnosing a copper deficiency via serum quantification. Even with herd deficiency, low serum copper concentrations may only be seen in 20% or more of the individuals. Herds that may be classified as marginally deficient based on liver testing may have predominantly "normal" serum copper concentrations. Thus, serum copper analysis should be viewed as a screening method only. Another factor that can influence diagnosis of copper deficiency in serum is the presence of high serum molybdenum. As the copper-sulfur-molybdenum complex that forms is not physiologically available for tissue use, "normal" serum copper content in the presence of high serum molybdenum should always be considered suspect. In addition, the form of selenium supplementation can alter the normal range for interpretation of serum copper status, with selenite supplemented cows having a lowered normal range for serum copper.

Copper deficiency can be diagnosed via analysis of copper containing enzymes. The two most common enzymes that are utilized are ceruloplasmin and superoxide dismutase (Suttle, 1986; Mills, 1987). Low concentrations of these enzymes in serum and whole blood, respectively, are diagnostic for copper deficiency. But, ceruloplasmin concentrations can increase with inflammatory disease states. Higher costs for analysis of these enzymes, than that of liver copper analysis, often limits their utilization.

Excessive supplementation of copper in dairy cattle is a relatively common finding at the Utah Veterinary Diagnostic Laboratory. Liver copper concentrations greater than 200 ppm are routinely identified. In comparison, the recommended adequate liver copper concentration range in cattle is 25 to 100 ppm.

Iron

As an essential component of proteins involved in the electron transport chain and oxygen transport, iron is essential for normal cellular function of all cell types. Iron deficiency is associated with reduced growth, poor immune function, weakness, and anemia (Graham, 1991; Puls, 1994). Although offspring are typically born with liver reserves of iron, providing the mother had adequate iron reserves, milk has low iron concentration which results in iron deficiency over time in animals fed a diet of only milk, as is the case in veal animals.

Both liver and serum concentrations are commonly utilized to diagnose iron deficiency. When using serum to measure iron concentration, samples that have evidence of hemolysis should not be used, as they will have artificially increased iron concentration from the ruptured red blood cells. In addition, disease states can alter serum and liver iron concentrations as the body both tries to limit availability of iron to growing organisms and increases the availability of iron to the body's immune cells. Thus, interpretation of iron status should be made with consideration of the overall health of the animal.

Other factors that can be used to assist with diagnosis of iron status include serum iron binding capacity, serum iron binding saturation, red blood cell count, packed cell volume, serum hemoglobin concentration, and ferritin concentration (Smith, 1989). But a variety of clinical conditions can cause these values to vary, including bacterial infections, viral infections, other types of inflammation, hemorrhage, bleeding disorders, and immune mediated disorders.

Magnesium

Similar to calcium, analysis for magnesium deficiency falls into two distinct classes. The first is of which is metabolic magnesium deficiency often referred to as "grass tetany". The second is due to a true nutritional deficiency, which is associated with long term dietary magnesium deficits.

Analysis for metabolic magnesium deficiency is aimed at detection of low systemic or circulating concentration. In live animals, testing is performed on serum to determine circulating magnesium concentration. But, it must be noted that ruminants that are displaying recumbency or tetany may have normal serum magnesium, as tissue damage that occurs releases magnesium into the serum from the soft tissues. And, testing in dead animals is even more difficult, as serum collected post-mortem will not accurately reflect true serum magnesium concentration prior to death. Circulating serum magnesium concentration can be approximated from analysis of ocular fluid, with a vitreous to serum ratio of 1.05 (McLaughlin and McLaughlin, 1987). The Utah Veterinary Diagnostic Laboratory has been able to confirm clinical cases of hypomagnesemia in numerous post-mortem cases via vitreous fluid analysis. Urine is another post-mortem sample that can be analyzed, since at times of low serum magnesium, the kidneys minimize magnesium loss in the urine.

True nutritional magnesium deficiency is not recognized in ruminants, except under experimental conditions. This syndrome is associated with weak, poor doing animals that have weak bones, low bone ash, and calcification of soft tissues. Analytical verification of true magnesium deficiency would require analysis of bone for verification, since approximately 70% of the body magnesium content is in bone. The bone analysis should be performed as fat-free, dry weight to remove the age variability of moisture and fat content.

Manganese

Manganese deficiency in ruminants is associated with impaired reproductive function, skeletal abnormalities in calves, and less than optimal productivity (Graham, 1991; Puls, 1994). Cystic ovaries, silent heat, reduced conception rates, and abortions are reported reproductive effects. Calves that are manganese deficient can be weak, small, and develop enlarged joints or limb deformities.

Manganese deficiency, although not reported often, is identified routinely in dairy cattle when tested. Of interest is the fact that most testing of beef cattle finds normal manganese concentrations in liver, blood, and serum, but in these same matrices, greater than 50%, 75%, and 95%, respectively, of dairy cattle tested are below recommended normal concentrations (unpublished data). This may, in part, be due to high calcium and phosphorous concentrations in dairy rations, which can be antagonistic to the bioavailability of manganese.

Of the samples available, liver is the most indicative of whole body status, followed by whole blood, and then serum. As red blood cells have higher manganese concentration than serum, hemolysis can result in increased serum concentration. Since the normal serum concentration of manganese is quite low, many laboratories do not offer this analysis because of inadequate sensitivity. Overall, response to supplementation has frequently been used as a means of verifying manganese deficiency, but it is critical that a bioavailable form be utilized.

Phosphorous

Phosphorous status is somewhat difficult to measure in animal tissues. Serum and urine phosphorous concentrations can aid in diagnosing deficiency, but with mobilization of bone phosphorous to maintain serum concentration,

significant drops in serum and urine may take weeks to develop. Serum phosphorous measurement should be as inorganic phosphorous for adequate interpretation. Longer term phosphorous deficiency can be diagnosed post-mortem by measuring bone or bone ash phosphorous concentrations. Dietary phosphorous and/or response to supplementation are better indicators of deficiency than tissue concentrations unless severe long term deficiency has occurred.

The predominant effects of low dietary phosphorous are associated with diminished appetite and its resultant effects. Depressed feed intake, poor growth, and weight loss are common with phosphorous deficient diets. Longer term phosphorous deficiency results in impaired reproductive performance, diminished immune function, bone abnormalities, and pica.

Potassium

Tissue concentrations of potassium poorly correlate with dietary status. Of the animal samples available, serum potassium is the best indicator of deficiency, but disease states can cause electrolyte shifts that result in lowered serum potassium when dietary deficiency has not occurred. In addition, serum that is hemolyzed or left on the clot too long may have falsely increased potassium concentration due to loss from the red blood cells. In addition, renal disease can result in increased serum potassium. Thus, dietary potassium concentrations are a better guide to potassium status.

Dietary potassium deficiency affects intake, productivity, heart function, and muscle function. Common clinical signs of severe potassium deficiency include diminished feed intake, reduced water intake, pica, poor productivity, weakness, and recumbency.

Selenium

As an essential mineral, selenium is commonly identified as deficient in ruminants, but infrequently in dairy cattle. Selenium deficiency in ruminants is associated with adverse effects on growth, reproduction, immune system function, offspring, and muscle tissues (Graham, 1991; Puls, 1994). "White muscle disease", a necrosis and scaring of cardiac and/or skeletal muscle, is linked to severe selenium deficiency; although, it can be caused by vitamin E deficiency as well. Reduced growth rates, poor immune function, and impaired reproductive performance can be observed with less severe selenium deficiency.

Diagnosis of a deficiency can be made by analysis of liver, whole blood, or serum for selenium concentration or by analysis of whole blood for glutathione peroxidase, a selenium dependent enzyme, activity (Ullrey, 1987). The most specific analysis is that of whole blood glutathione peroxidase, as it verifies true functional selenium status. Liver is the optimal tissue to analyze for selenium concentration as it is a primary storage tissue. With serum and whole blood, the former better reflects recent intake, while the latter better reflects long term status. Since seleno-proteins are incorporated into the red blood cells when they are made and the cells have a long half-life, selenium concentration is a reflection of intake over the previous months.

In order to adequately diagnose selenium deficiency, the dietary form of the selenium consumed by the animals is important. Natural selenium, predominantly in the form of selenomethionine, is metabolized and incorporated into selenium dependent proteins but can also be incorporated into non-specific proteins in place of methionine. Inorganic selenium is metabolized and only incorporated into selenium dependent proteins. Thus, "normal" concentrations in serum and whole blood differ depending on whether the dietary

selenium is a natural organic form or an inorganic supplement.

Sodium

Tissue concentrations of sodium poorly correlate with dietary deficiency. Of the animal samples available, serum and urine are the best for measuring sodium deficiency, but disease states can cause electrolyte shifts that result in lowered serum or urinary sodium even when dietary concentrations are adequate. Thus, dietary sodium concentrations are a better guide to diagnosing a deficiency.

Dietary sodium deficiency affects feed intake and productivity. Common clinical signs of severe sodium deficiency include diminished feed intake, reduced water intake, poor productivity, and pica.

Zinc

Zinc is an essential mineral that is required by all cells in animals. Zinc plays a role in numerous enzymatic reactions (Graham, 1991; Puls, 1994). Deficiencies of zinc are associated with reduced growth, poor immune function, diminished reproductive performance, and poor offspring viability, as well as skin lesions in severe cases.

Tissue zinc concentrations do not reflect body status well (Mills, 1987). Of the common samples tested, liver and serum are the best indicators of zinc status. But, serum and liver zinc can be altered by age, infectious diseases, trauma, fever, and stress. It has been suggested that pancreas zinc concentration is the best means of truly identifying zinc deficiency. Response to zinc supplementation has shown that some animals having low-end normal liver or serum zinc can still show improvement in some clinical conditions. Thus, liver and serum only verify deficiency when these samples have very low zinc concentration.

Summary

A variety of samples can be tested for mineral content but may not provide any indication of the overall mineral status of the animal. Appropriate diagnosis of mineral status involves thorough evaluation of groups of animals. The evaluation should include a thorough health history, feeding history, supplementation history, and analysis of several animals for their mineral status.

Dietary mineral evaluation should augment the mineral evaluation of animal groups. If minerals are deemed to be adequate in the diet, but the animals are found to be deficient, antagonistic interactive effects of other minerals need to be investigated. As an example, high sulfur or iron in the diet can cause deficiencies in copper and selenium, even when there are adequate concentrations in the diet.

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Water Soluble Vitamins for Dairy Cattle

William P. Weiss[1] and Gonzalo Ferreira
Department of Animal Sciences
The Ohio State University

Abstract

Research on effects of water soluble vitamins when fed to dairy cows and field supplementation of some water soluble vitamins has increased markedly in the past few years. In research studies, biotin supplementation (20 mg/day) has consistently improved hoof health of cows and often increased milk production. Supplementation of niacin, although common, has little effect on milk production unless the supplementation rate is 12 g/day and then the response is often not profitable. Adding rumen-protected choline (50 g/day) often increases milk production in early lactation and the average response is profitable. Research on folic acid, vitamin B-12, and vitamin C for dairy cows is continuing, but at the present time, inadequate data are available to recommend routine supplementation.

Introduction

Vitamins are organic compounds needed in minute amounts that are essential for life. A vitamin must be in the diet (dietary essential) or be synthesized by microorganisms in the digestive system and then absorbed by the host animal. Currently, there are 14 recognized vitamins of which 4 are fat-soluble and 10 are water-soluble, but not all animals require all 14 vitamins (Table 1). When an animal absorbs an inadequate quantity of a particular vitamin, various responses are observed depending on the vitamin and the degree and duration of deficiency. The most severe situation (seldom observed in U.S. dairy cows) is a clinical deficiency. For example, rickets result from a clinical deficiency of vitamin D. Marginal deficiencies of vitamins usually have more subtle and less defined signs. Unthriftiness, reduced growth rate, milk production, or fertility, and increased prevalence of infectious diseases can be observed when animals absorb inadequate amounts of vitamins.

It is not known definitively whether cows have an absolute dietary requirement for any of the water soluble vitamins. The liver and kidney of the cow can synthesize vitamin C, and ruminal and intestinal bacteria synthesize most, if not all, of the B-vitamins. The concentrations of many B-vitamins are relatively high in many common feeds; therefore, in the vast majority of situations, cows do not need to consume any supplemental water soluble vitamins to prevent *clinical deficiency*. In a survey (Kellogg et al., 2001) of the highest producing dairy herds in the US (data collected in 2000), niacin was the only water soluble vitamin fed to a substantial number of herds (43% of the herds reported that at least one group of cows was fed niacin). Choline and biotin was also fed, but they were used by less than 4% of the surveyed herds. Even though clinical deficiencies of water soluble vitamins are extremely rare in dairy cows, research and field interest in water soluble vitamins has increased markedly in the past few years.

[1]Contact at: 1680 Madison Ave., Wooster OH 44691, (330) 263-3622, FAX: (330) 263-3949, Email: weiss.6@osu.edu

Why the Increased Interest in Water-Soluble Vitamins?

The predominant function of the B-vitamins is to act as co-factors for enzymes that are involved in amino acid, energy, fatty acid, and nucleic acid metabolism (Table 1). Many of these enzymes are involved directly in the production of milk and milk components. Therefore, as milk production increases, the need for these enzymes (and the associated co-factors) increase. In the past 15 years, average milk yield per cow has increased from about 14,500 lb per year to almost 19,500 lb, and herds (not individual cows) that average 28,000 lb or more per cow are not uncommon. Assuming average milk composition (for a Holstein cow) and assuming composition has not changed over time, the average cow in 2005 must synthesize approximately 0.4 lb more milk fatty acids (assuming 50% of milk fatty acids come from the diet), 0.6 lb more milk protein, and 0.9 lb more lactose each day than the average cow in 1990. During that same 15 year period, average dry matter intake has increased from about 44 lb to about 50 lb/day. In other words, the yield of milk and milk components has increased about 33%, but dry matter intake has increased only about 15%. Because most B-vitamins are not supplemented, supply to the cow would mostly be a function of intake of typical feed ingredients whereas their need would be a function of milk production. The potential imbalance between supply and need in today's high producing cow increases the likelihood that responses will be observed when B-vitamins are supplemented. The interest in B-vitamins has increased because cows have changed.

B-Vitamin Supply

As with all nutrients, a response to supplemental B-vitamins will only be observed if: 1) supplementation actually increases the supply of vitamin to the tissues that require it, and 2) the nutrient is first limiting. Vitamin supply is defined as the amount (micrograms or milligrams) of a vitamin that is absorbed from the digestive system each day. Supply is a function of the amount of the vitamin consumed (vitamin concentration times dry matter intake), ruminal synthesis and degradation of the vitamin, and its bioavailability (i.e., its ability to be absorbed, mainly by the small intestine).

Dietary concentrations

Because of the difficulty in measuring many of the B-vitamins, we have very limited data on their concentrations in common feedstuffs. Inadequate information is available to discuss differences in concentrations of B-vitamins among feedstuffs, but data for some feeds are available (Schwab et al., 2006). Ranges in reported concentrations of various B-vitamins in diets fed to lactating cows are in Table 2. Most of the data represent 7 relatively diverse diets from 3 experiments (Santschi et al., 2005a; Santschi et al., 2005b; Schwab et al., 2006), but it is important to note that all the analyses were conducted in a single laboratory. Considering the analytical and sampling error usually observed when trace nutrients are measured, concentrations of most of the B-vitamins were relatively consistent across the diverse diets with the clear exception of niacin. The concentration of dietary niacin was mostly a function of the amount of soyhulls included in the diet. In the 4 diets that contained little or no soyhulls, niacin concentrations were <30 mg/kg, and in the 3 diets that contained appreciable concentrations of soyhulls, niacin concentrations were >60 mg/kg. Additional data are needed to confirm whether soyhulls typically contains such high concentrations of niacin. The biotin concentration of different diets within an analytical method did not vary greatly, but method of analysis had a substantial effect (Table 2). Biotin concentrations in 3 studies that used one analytical procedure averaged about 7 mg/kg, and in 3 other studies using a different procedure, it was almost 20 times lower (about 0.4 mg/kg). At the current time, we do not know which method is accurate.

Ruminal metabolism

The flow of B-vitamins measured at the duodenum can be substantially different from intake (Zinn et al., 1987; Santschi et al., 2005a; Schwab et al., 2006). The difference between intake and duodenal flow is called net synthesis because it reflects both ruminal degradation and synthesis. For most B-vitamins, flow out of the rumen exceeds intake indicating net synthesis of the vitamin (Table 3). With the exception of biotin and vitamin B-6, ruminal synthesis appears to provide the majority of the B-vitamins that reach the small intestine (Table 3). Both studies that used dairy cows (Santschi et al., 2005a; Schwab et al., 2006) reported no net synthesis of biotin or that ruminal degradation was slightly higher than synthesis (i.e., flow to the duodenum was statistically lower than biotin intake). Those 2 studies also were among those that reported very high concentrations of biotin in the diet (Table 2). If the concentration of biotin in the diet was overestimated, net synthesis would be underestimated. Net ruminal synthesis of biotin in beef cattle (Miller et al., 1986; Zinn et al., 1987) and in in vitro ruminal systems (Abel et al., 2001) was positive. In addition, the development of clinical signs of biotin deficiency was prevented when chicks were fed ruminal contents but not when fed the same diet fed to the cow from which the ruminal contents were obtained (McElroy and Jukes, 1940). Additional research is needed to clarify the question regarding biotin synthesis in the rumen.

Only one study that used modern analytical techniques has evaluated how dietary factors influenced duodenal flow of B-vitamins (Schwab et al., 2006). In that study, diets had either 40 or 60% forage (50:50 mix of corn silage and hay) with low (approximately 6.5%) or moderate (approximately 20%) starch concentrations. Starch concentration was varied by replacing dry ground corn with soyhulls. Cows fed the low forage diets consumed about 5 lb/day more ($P < 0.01$) dry matter than cows fed the high forage diet and duodenal flow of thiamin, niacin, B-6, folic acid, and B-12 were also higher. Much of the increased flow of those vitamins was a direct result of increased intake, but apparent ruminal synthesis of folic acid and B-12 were also increased when low forage diets were fed. Cows fed the moderate starch diets consumed more dry matter than cows fed the low starch diets, and duodenal flows of B-6, biotin, and folic acid were also higher. Apparent ruminal synthesis of niacin was more than doubled when the moderate starch diets were fed, but because the low starch diets contained much higher concentrations of niacin, duodenal flow was not affected. Apparent ruminal synthesis of folic acid and B-6 was increased with moderate starch diets, but synthesis of B-12 was reduced. Overall, it appears that ruminal synthesis of most B-vitamins is related to microbial fermentation in the rumen. Diets that have a higher concentration of rumen fermentable matter promote increased synthesis of many of the B-vitamins but may reduce synthesis of B-12.

Apparent ruminal synthesis of B-vitamins equals: [synthesis of the vitamins by ruminal microorganisms - (degradation of the vitamins by ruminal microorganisms + ruminal and omasal absorption of the vitamin)]. To measure ruminal disappearance of B-vitamins, diets with and without supplemental vitamins are fed and duodenal flows of the vitamins are compared. If supplementation resulted in no increase in duodenal flow of that vitamin, apparent disappearance equals 100%. Degradation of B-vitamins contained in feedstuffs may or may not be the same as disappearance of supplemental B-vitamins. Santschi et al. (2005a) measured apparent ruminal disappearance of supplemental B-vitamins in dairy cows. Approximately 100% of supplemental riboflavin, niacin, and folic acid disappeared in the rumen. Approximately two-thirds of the supplemental thiamin and B-12, and 40 to 45% of the supplemental B-6 and biotin (the variation in biotin disappearance was extremely high) disappeared.

Because apparent ruminal disappearance is caused by both microbial degradation and absorption, high disappearance values do not necessarily mean that responses to supplementation are unlikely. The rumen and omasum do not appear to be major absorptive sites for most B-vitamins, but high supplementation rates still might increase systemic concentrations of some vitamins. In addition, some rumen microorganisms require B-vitamins; therefore, ruminal effects can occur even if a substantial amount of the supplemental vitamin disappears in the rumen.

Intestinal absorption

Apparent intestinal absorption is calculated by subtracting the flow of a vitamin at the ileum from flow measured at the duodenum. Bacteria can inhabit the terminal portion of the small intestine (ileum); therefore, apparent intestinal absorption measured in this way would underestimate true absorption if B-vitamins are synthesized by those bacteria. In addition, some vitamins that are absorbed are secreted in bile which would result in lower apparent intestinal absorption. Only one study is available that used dairy cows and modern analytical techniques (Santschi et al., 2005a). They measured apparent intestinal absorption of several B-vitamins from supplemented and unsupplemented diets. Overall, few differences were observed between supplemented and unsupplemented treatments, suggesting the absorption of basal and supplemental vitamins was similar. Apparent intestinal absorption of thiamin, niacin, and B-6 averaged 70 to 85%, for riboflavin and biotin it averaged about 35%, and about 13% for B-12. Apparent intestinal absorption of folic acid was negligible, probably because of bilary secretion.

Clinical and Production Responses to B-Vitamins

Niacin

No requirement for dairy cows has been established for niacin, but niacin is involved in most energy-yielding pathways and for amino acid and fatty acid synthesis and therefore is important for milk production. Niacin has been evaluated for possible prophylactic and therapeutic effects on ketosis and fatty liver syndrome. Although a few studies reported that niacin supplementation during the periparturient period (usually 6 to 12 g/day) reduced blood ketones and plasma nonesterified fatty acids (NEFA), the vast majority of studies (see page 171 of NRC, 2001 for listing of the studies) showed no effect (a few actually found increased ketones and NEFA with niacin supplementation). In a recent study published only in abstract form (French, 2004), Jersey cows were fed 48 g/day of nicotinic acid from 30 days prepartum until calving. The day before calving, cows fed supplemental niacin had greater dry matter intakes (22.0 vs. 14.7 lb) and lower plasma NEFA (491 vs. 1244 umol/L).

Several summaries of production studies evaluating niacin supplementation have been published (Drackley, 1992; Erdman, 1992; Girard, 1998; NRC, 2001; Schwab et al., 2005). The recent summary by Schwab et al (2005) was conducted using a new statistical method and is probably the best current summary. They concluded that supplementing 6 g/day of niacin (commonly used supplementation rate) had no effect on milk production or milk composition. At 12 g/day of supplemental niacin, 3.5% fat-corrected milk increased about 1 lb/day, fat yield was increased 26 g/day, and milk protein yield was increased 17 g/day. Based on the current cost of niacin, this response would often not be profitable. The likelihood of a profitable response can be increased by targeting specific animals. Positive responses

appear more likely in early lactation, high producing cows, and responses are almost never observed in mid and late lactation cows (Girard, 1998). Supplemental niacin often had negative effects when fed with diets that contained supplemental fat (Drackley, 1992). Possible reasons for the limited response to supplemental niacin include: 1) basal diets provide adequate niacin to the intestine (Tables 2 and 3), or 2) supplementation at 6 to 12 g/day does not increase flow of niacin because of extensive ruminal metabolism. Increasing flow of niacin to the duodenum could be accomplished by feeding rumen-protected niacin or perhaps by greatly increasing supplementation rate. A rumen-protected form of niacin is available, but published data evaluating the product with dairy cows are not available. Additional research is needed to study production and other responses to higher supplementation rates.

Biotin

A dietary biotin requirement has not been established for dairy cows. Six clinical trials have been published that examined the effect of supplemental biotin on hoof horn lesions and lameness in dairy cows (reviewed by Weiss, 2005). Although the response variable varied among experiments, all studies reported reduced prevalence of specific lesions or clinical lameness when biotin was supplemented. The supplementation rate was 20 mg/day in most studies, but one study with beef cows fed only 10 mg/day and reported a positive response, and all studies involved long term (months) biotin supplementation. Biotin supplementation usually reduces hoof lesions in 2 to 3 months, but 6 months of supplementation may be required to reduce clinical lameness. The mechanisms by which biotin affects foot health are not well understood. Increased keratin synthesis by keratinocytes from the hoof might be a possible mechanism by which biotin improves foot health. Keratinocytes are cells responsible for the synthesis of proteins known as keratins, and keratin synthesis is a main determinant of hoof integrity. Keratin synthesis by human skin keratinocytes was increased when cultured with supra-physiological concentrations of biotin (Fritsche et al., 1991). Increased fatty acid synthesis via increased activity of acetyl-CoA carboxylase might be another mechanism by which biotin improves foot health. The keratinocytes are embedded in a lipid-rich extracellular matrix composed of cholesterol, fatty acids, and ceramides. Higuchi et al. (2004) reported that biotin supplementation decreased the concentration of water and increased the concentration of lipids in the sole of dairy cows.

Milk yield responses to supplemental biotin are less consistent than hoof responses, but the majority of studies reported increased production (Table 4). Low producing cows and/or cows in late lactation are unlikely to increase milk yield when biotin is supplemented. A recent 14-day study from our laboratory (Ferreira, 2006) found that biotin increased milk yield when supplemented to high-producing dairy cows (control cows average production = 89 lb/day and 136±56 days in milk) but not when supplemented to low-producing cows (average production for control cows = 52 lb/day and 267±53 days in milk). The lack of a production response by low-producing cows in that study agrees with data from Australia (Fitzgerald et al., 2000). The reason cows in the Rosendo et al. (2004) experiment did not respond is not known (milk production of control cows averaged 79 lb/day). Across all studies, the median increase in milk yield was 2 to 3 lb/day. Whereas months of supplementation are required to observe improved hoof health, the milk yield response appears very rapidly (Figure 1). The mechanism by which biotin supplementation increases milk yield is still not known, but supplemental biotin can increase the activity of one gluconeogenic enzyme in the liver of dairy cows (Ferreira, 2006).

Folic acid and vitamin B-12

A substantial amount of research has been conducted in Canada on folic acid and B-12 nutrition of dairy cows (Girard and Matte, 1998; Girard and Matte, 1999; Girard et al., 2005; Girard and Matte, 2005). Vitamin B-12 is essential for folic acid to work properly, and therefore, these 2 vitamins must be considered together. Both vitamins are involved in methionine metabolism. Vitamin B-12 can be synthesized by rumen bacteria if adequate cobalt is in the diet (NRC requirement is 0.11 mg/kg of dietary DM, but newer research suggests that 0.2 to 0.3 mg/kg may be better). The effect of folic acid supplementation (typical rates are between 2 and 3 g/day) on milk production has been variable. In one study, milk production of multiparous cows increased by 4 to 6 lb/day when folic acid was supplemented, but no effect was observed with first lactation cows. In other experiments, folic acid has not affected milk production. One reason for the variable responses may be that vitamin B-12 status was limiting. If cows are limited in B-12, they are unlikely to respond to folic acid supplementation. Interactions between methionine supply, folic acid, and B-12 are likely. Both vitamin B-12 and folic acid are expensive, and we still do not understand all the factors that influence responses to supplementation. Additional research is needed before routine supplementation of these vitamins is recommended.

Other B-vitamins

Research is extremely limited on the effects of supplementing B-vitamins (other than biotin and niacin) to dairy cows. In a study (Majee et al., 2003) in which a mixture of B-vitamins (biotin, folic acid, niacin, pantothenic acid, B-6, riboflavin, thiamin, and B-12) was fed, milk production was increased compared with the control but was not different from a treatment in which only biotin was supplemented. When the amount of supplemental B-vitamins was doubled, intake and milk production was similar to control cows (i.e., lower than the 1-X supplementation treatment). Shaver and Bal (2000) examined the effects of supplemental thiamin on milk production. In one experiment, yield of milk, milk fat, and milk protein increased when cows were fed 150 mg/day of thiamin. In 2 other experiments, cows fed thiamin at 300 mg/day had similar milk yields as control cows. Overall, the available data do not support routine supplementation of 'other' B-vitamins. However, as productivity of cows continues to increase and as new experiments are conducted, this conclusion may change.

Choline

Choline does not fit the definition of a vitamin. It is required in gram quantities (not milligram or microgram quantities), and it is synthesized by the cow. Very little, if any, dietary choline (with the exception of rumen-protected supplements) is absorbed from the gut because it is degraded in the rumen. At the 2002 Tri-State Conference, Donkin (2002) summarized previous data on milk yield responses (10 comparisons) to supplemental choline. Six comparisons (60%) reported a statistical increase in milk production. Two additional studies have since been published and one paper (Janovick Guretzky et al., 2006) reported no response while the other (Piepenbrink and Overton, 2003) reported increased milk production. Across all 12 comparisons, all but one reported a numerical increase in milk production and the median increase to choline supplementation was about 5 lb/day. Supplemental choline during the transition period may reduce liver fat, but results have not been consistent. Because choline can be synthesized from methionine, diets that provide marginal amounts of metabolizable methionine may be more likely to respond to choline supplementation. Choline must be rumen-protected to be effective.

Vitamin C

Vitamin C also does not fit the definition of a vitamin for dairy cows because its tissues can synthesize ascorbic acid. Vitamin C is probably the most important water soluble antioxidant in mammals. Most forms of vitamin C are extensively degraded in the rumen (Macleod et al., 1999); therefore, the cow must rely on tissue synthesis of vitamin C. The concentration of ascorbic acid is high in neutrophils and increases as much as 30-fold when the neutrophil is stimulated by the presence of bacteria (Wang et al., 1997). Santos et al. (2001) reported that plasma ascorbic acid concentrations in dairy cows were not correlated with somatic cell count **(SCC)**. However, the range in SCC was limited (67,000 to 158,000/ml) and cows were only sampled once. Another experiment evaluated the therapeutic use of ascorbic acid following intramammary challenge with endotoxin (Chaiyotwittayakun et al., 2002). One quarter from each cow was infused with endotoxin and the ascorbic acid was injected IV at 3 and 5 hours post challenge (25 g/dose). Vitamin C therapy had only limited effects on clinical signs. Because of the way vitamin C works, an endotoxin challenge may not be a very good model to evaluate effects of vitamin C on mastitis. Studies in which mammary glands were either experimentally or naturally infected with bacteria clearly show a relationship between plasma vitamin C concentrations and infection. Cows with a mammary gland infection had lower concentrations of vitamin C in plasma than did healthy cows (Weiss et al., 2004; Kleczkowski et al., 2005; Ranjan et al., 2005). In addition, we (Weiss et al., 2004) observed significant correlations between vitamin C concentrations in plasma and milk and clinical signs of mastitis caused by *E. coli*. Greater decreases in vitamin C concentrations were related to longer duration of clinical mastitis and greater decreases in milk production. Data from these experiments do not mean that increasing vitamin C status of cows will reduce the prevalence or severity of mastitis. We do not know whether lower vitamin C status allowed cows to become infected or whether the infection depleted body vitamin C.

Conclusions and Recommendations

1. Supplemental biotin provided at about 20 mg/day has consistently improved hoof health, and has increased milk production in several, but not all, studies. For improvements in hoof health, biotin must be fed for several months (including the dry period), but increased production will happen within a very short period. Feeding 20 mg/day of biotin to lactating and dry cows is recommended because of its effects on foot health.

2. Rumen-protected choline fed at 50 g/day (actual product, not choline) has resulted in increased milk production in most studies and reduced liver fat in some studies. The cost of supplementation is substantial, but the median response to supplementation was about 5 lb/day of milk. A response in milk production is most likely in early lactation (up to about 60 days in milk) and to maximize the likelihood of a profitable return on investment, supplementation should be limited to early lactation cows.

3. A milk production response to niacin supplementation at 6 g/day is unlikely, but supplementation at 12 g/day can increase milk production by about 1 lb/day (likely not a profitable return on investment). A positive return on investment is more likely when supplementation is limited to early lactation cows. The use of supplemental niacin in herds that feed a single diet to all cows is unlikely to have a positive return on investment.

4. At this time, insufficient data are available to recommend supplementation of other B-vitamins and vitamin C to dairy cows.

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Table 1. Compounds currently recognized as vitamins.

	General function
Fat-soluble vitamins	
Vitamin A	Gene regulation, immunity, vision
Vitamin D	Ca and P metabolism, gene regulation
Vitamin E	Antioxidant
Vitamin K	Blood clotting
Water-soluble vitamins	
Biotin	Carbohydrate, fat, and protein metabolism
Choline	Fat metabolism and transport
Folic acid[1]	Nucleic and amino acid metabolism
Niacin	Energy metabolism
Pantothenic acid	Carbohydrate and fat metabolism
Pyridoxine (vitamin B-6)	Amino acid metabolism
Riboflavin	Energy metabolism
Thiamin	Carbohydrate and protein metabolism
Vitamin B-12	Nucleic and amino acid metabolism
Vitamin C	Antioxidant, amino acid metabolism

[1]In this paper, the term folic acid is used to describe total folates.

Table 2. Concentrations of B-vitamins in cattle diets and typical vitamin intakes by dairy cattle. Data are from 7 different diets fed in 3 experiments (Santschi et al., 2005a; Santschi et al., 2005b; Schwab et al., 2006). All analyses (except where noted) were conducted in a single laboratory.

Vitamin	Average, mg/kg DM	Range, mg/kg DM	Mean Intake, mg/day[1]
Thiamin	2.0	1.5 to 2.6	45
Riboflavin	5.4	4.3 to 6.7	123
Total niacin	46.0	22.6 to 94.8	1045
Vitamin B-6	5.2	3.2 to 8.5	118
Total folates	0.5	0.4 to 0.7	11
Biotin	6.9	6.3 to 7.8	157
Biotin[2]	0.37	0.33 to 0.41	8

[1]Based on an average dry matter intake of 50 lb/day.
[2]Biotin data in this row are from three different diets (Zinn et al., 1987; Frigg et al., 1993; Midla et al., 1998) and the analytical methods used were different from those used in the other experiments.

Table 3. Net ruminal synthesis of B-vitamins by dairy cattle [data derived from Santschi et al., (2005a); and Schwab et al.(2006)]. Synthesis values are the means of 5 different dietary treatments.

Vitamin	Net ruminal synthesis		Total flow[1,2], mg/day	Ruminal synthesis, % of total flow
	mg/kg of DM intake	mg/day[1]		
Thiamin	2.3	51	96	53.1
Riboflavin	12.1	274	397	69.0
Total niacin	62.8	1425	2470	57.7
Vitamin B-6	0.9	21	139	15.1
Total folates	0.9	19	30	63.3
Biotin	0	0	157 (8)[3]	0
Vitamin B-12	3.9	88	88	100

[1]Based on an assumed DM intake of 50 lb/day.
[2]Flow measured at the duodenum and equals the sum of vitamin intake (Table 2) and net synthesis.
[3]The number in parenthesis is intake based on a different analytical technique (see Table 2).

Table 4. Summary of reports on effects of biotin supplementation on milk yield[1].

Treatment	Results	Ref[2]
0 or 20 mg/day until 300 DIM	Treatment increased 305-day ME by 680 lb ($P < 0.05$), Control ME = 25,900 lb	1
0 or 20 mg/day for 13 months	No effect on milk yield. Yield was 42 lb/day for control	2
0 or 20 mg/day for first 120 DIM	Treatment increased ($P < 0.05$) yield from 82 to 86 lb/day	3
0 or 20 mg/day for 14 months	Treatment increased 305-day milk by 1060 lb ($P < 0.05$), Control milk = 22,200 lb	4
0, 10, or 20 mg/day until 100 DIM	Linear ($P < 0.05$) effect. Yields were 81, 83, and 87 lb/day	5
0 or 20 mg/day for 28 day periods	Treatment increased ($P < 0.05$) yield from 82 to 84 lb/day	6
20 or 40 mg/day for 28 day periods	No effect, average yield = 90 lb/day	6
0 or 30 mg/day until 70 DIM	No effect on 4% FCM yield, average = 76 lb/day	7
0 or 20 mg/day for 14 days starting at 136 DIM	Treatment increased ($P < 0.05$) yield from 92 to 98 lb/day	8
0 or 20 mg/day for 14 days starting at 267 DIM	No effect, average yield = 53 lb/day	8

[1]DIM = days in milk, FCM = fat-corrected milk, and ME = mature equivalent in milk.
[2]References were: 1) Midla et al., 1998; 2) Fitzgerald et al., 2000; 3) Margerison et al., 2002; 4) Bergsten et al., 2003; 5) Zimmerly and Weiss, 2001; 6) Majee et al., 2003; 7) Rosendo et al., 2004; and 8) Ferreira, 2006.

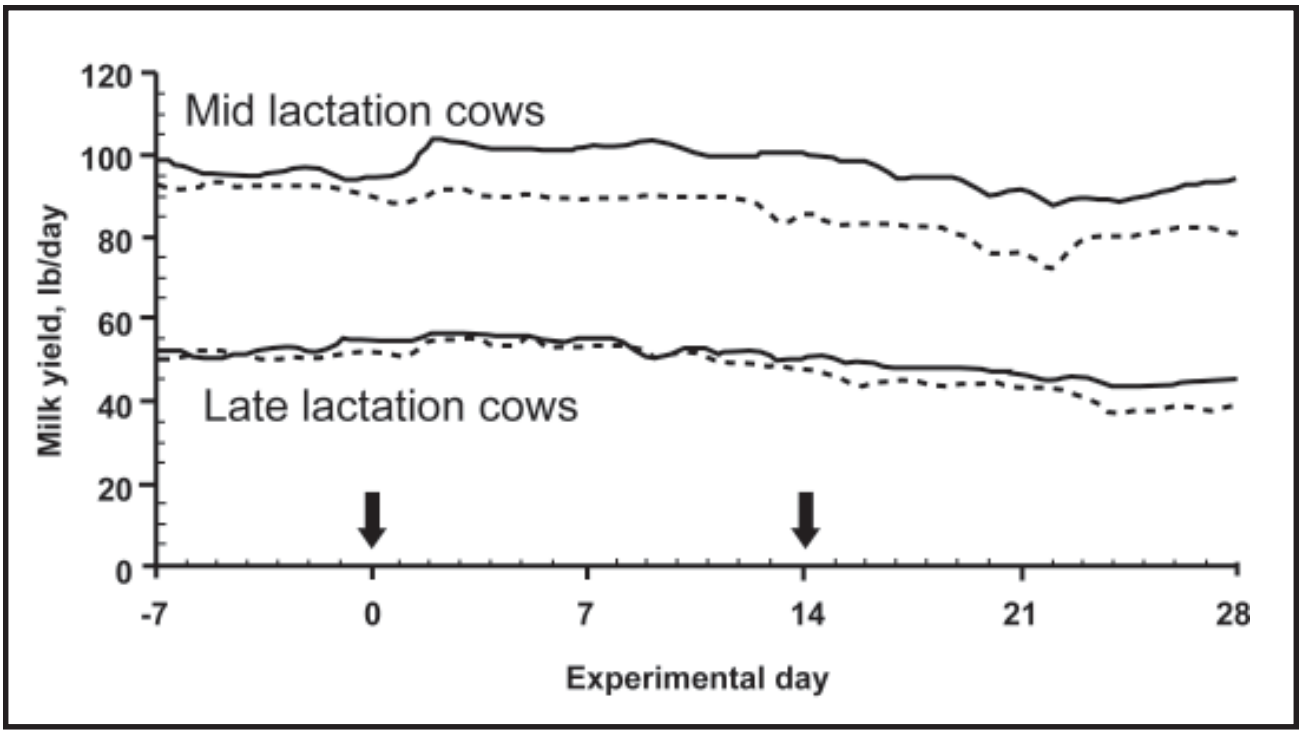

Figure 1. Milk yield response when cows in mid (136 days in milk) or late (267 days in milk) were supplemented with 20 mg/day of biotin. Dashed lines represent control cows and solid lines represent supplemented cows. Arrows designate when supplementation started and ended.

Why Should I Know About Animal Welfare Audits?

Susan D. Eicher[1]
Livestock Behavior Research Unit
USDA-ARS

Abstract

Market driven demands for verification that animals used to produce food receive humane care have resulted in welfare audits becoming a reality for beef and chickens raised for products sold to fast food chains. The dairy industry is targeted for similar audits and the Dairy Quality Assurance Center (2002) was approved by the retail organizations Food Marketing Institute and the National Council of Chain Restaurants in 2002 for that purpose. Assessments are performed by a 3rd party with producer interaction. The audit is an on farm evaluation by a 3rd party checking compliance or noncompliance with written policies with no producer interaction during the audit. Several auditing organizations are available for dairy audits and certification, and one program can provide USDA recognized "Certified Humanely Raised & Handled" labeling. Bunk space; appropriate diet composition; feeding and feed storage; and water cleanliness and availability are covered in the audits. Although the programs cover similar topics, their outcome may be distinctly different based on the design, purpose, certification criteria, and standards on specific topics. Knowing what auditing tools are available and which programs fit your production needs will become imperative as audits become necessary for dairy product marketing.

Introduction

Concern for animal well-being (welfare) is not new to animal and veterinary science. However, documentation programs of animal well-being are relatively new. The term "animal welfare" should not strike fear in our hearts, just because activists groups have used it negatively against animal agriculture (Dairy Herd Management, 2006). Animal agriculture has been addressing animal welfare for decades, seeking to find the best housing and feeds among many other areas. We continue to address these issues with scientific research of practices that best fit our current livestock. Animal well-being has a variety of definitions, depending on the perception of the observer. "Animals can suffer" is an animal oriented definition, "animals are special" is a species orientation, and people's ideal image is human oriented. Animals have specific needs, as we are all aware. Among those needs are nutrients specific to species and age, social contacts, exploration, thermoregulation, rest, safety, and psychological (security and novel environments).

Well-being is an ongoing process dependent on balancing stress. Positively, stress satisfies a need for excitement. Negatively, it interferes with homeostasis and life functions. The familiar thermal neutral zone diagram can be used to examine many well-being needs of animals (Figure 1). For instance, if this is applied to the number of pen mates, we know that cattle are social animals and

[1]Contact at: 125 S. Russell St., 219 Poultry Bldg., West Lafayette, IN 47907, (765) 496-3665, FAX: (765) 496-1993, Email: spruiett@purdue.edu

isolation is extremely stressful for them. Alternatively, an overcrowded pen is known to result in increased fighting and reduced and variable feed intake. Finding the parameters of a condition that we plan to evaluate (such as social contact) is essential before it can be evaluated in the field. When confronted by one of these extremes past the critical point, animals can no longer adapt or tolerate the extreme and their welfare becomes compromised.

We must keep in mind that animal well-being is not just a physiological or psychological reality, but includes public perception. That public perception will likely involve anthropomorphism (attributing human needs/characteristics to animals) as the population making the perception is further removed from the farm.

The Evolution of Welfare Audits

Previously, not much progress was made by activist groups by addressing producers and packers, but a vulnerable link in the marketing process was found. Audits were initiated in response to demands of the People for the Ethical Treatment of Animals made of retailers. In 1999, the "McCruelty" campaign began and then ended when McDonald's developed animal welfare standards. The year 2001 brought "Murder King" and later in 2001 "Wicked Wendys". Both of these campaigns against the restaurants were ended as welfare standards were developed.

From these points of conflict, 3rd party audits were developed. Third party audits measure a producer's or packer's level of compliance against a prescribed set of animal care criteria. The driving force of the 3rd party audits has been the Food Marketing Institute **(FMI)** and the National Council of Chain Restaurants **(NCCR)**. Eighty five percent of food is sold in U.S. groceries through these organizations (FMI-NCCR Animal Welfare Program, 2003).

Presently the major programs available for dairy (Table 1) include: the Dairy Quality Assurance Center **(DQAC)** 2002, Humane Farm Animal Care **(HFAC)** 2003, Validus (formerly Environmental Management Solutions, LLC, 2004), and Farm Animal Care Training and Auditing **(FACTA)** has audited humanely raised veal farms (Reynolds, 2005). California has developed its own program, California Dairy Quality Assurance Program (based on DQAC). The DQAC was developed in 1990. It features internal audits and 3rd party certification by DQA auditors. It was expanded to include animal care in 1995. In 2002, DQAC agreed to revise "Caring for Dairy Animals reference guide" to incorporate FMI/NCCR recommendations. The 2002 revision included: 1) adding a space allocation guideline for a cow to free stall ratio of 1.2, 2) recommended switch trimming to be used rather than tail-docking, and 3) specific guidelines regarding ages and methods for castration and dehorning.

The HFAC is the primary niche market auditing program. It is an independent non-profit organization developed through funding from the Humane Society of the United States, the American Society for the Prevention of Cruelty to Animals, and regional and local animal welfare organizations. The standards were based on the Royal Society for the Prevention of Cruelty to Animals' Freedom Foods program in the United Kingdom and the Federation of Animal Science Societies' Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999) and were customized to U.S. farms by a scientific advisory committee. It requires additional measures that are related to organic farming, such as no use of hormones and traceability of feeds and products used. Guidelines for calves prohibit tethering, muzzles, or physical alterations to prevent inappropriate suckling. A "Certified Humane Raised and Handled" label can be issued which will add market value and the USDA-recognized label can be used for the products in stores.

Nutritional Aspect of the Audit Programs

Access to water and quality of water are evaluated in the audits. Accessibility and non-slip flooring in the watering area are scored. The animals' approach to and use of waterers are observed. Feed quality and quantity are determined by observations and by records. All animals on the farm will be assessed for life stage appropriate feed. The percentage of cows that can eat at once and the percentage of the day spent at the feed bunk may be scored. Adequate feeders and bunk space are observed, and when possible, it is determined whether or not old feed is removed on a daily basis (checking for moldy or dampened feeds). Proper feed storage, including protection from the elements, proper labeling, and vermin control, as well as separate storage of medicated feeds, are scored. Toxic compounds must be kept outside of the feeding and resting areas. Each audit will have specific paperwork that is requested from the producer (and therefore nutritionist).

What Will it Cost to Become Part of an Audit Program?

Costs to participate can vary from $200 to 1,500 annually, depending on which program is used and other variables (Table 1). Auditor's fees, travel costs, administration fees, and frequency of audits can all contribute to the cost. Presently, the cost is on the packers and producers with no incentive, yet. Because of the marketing system, agriculture can not fix prices to recuperate the costs.

What are the Advantages and Pitfalls of Audit Programs?

Problems with the audits include standardizing the system; presently they are not standardized by species, auditing firms differ, and auditor qualifications vary. A study highlighting difference of the DQAC, HFAC, and University of California-Davis **(UCD)** program indicated that selection of the available assessment programs for welfare of animals on commercial dairy farms is important to determine outcomes (Stull et al., 2005). Although the three programs that were assessed covered similar topics, the outcomes reflected the design, purpose for assessment, certification criteria, and differences in specific standards of each assessment tool (Table 2). Because of this type of problem, a national oversight program has been developed, Professional Animal Auditors Certification Organization (**PAACO;** http://www.animal auditor.org). This group is comprised of animal scientists and veterinarians with the goal "to promote the humane treatment of animals through education and certification of animal auditors and to promote the profession of animal auditors".

Summary

Animal husbandry should be equivalent to animal welfare, providing clean, dry and comfortable housing, nutrition balanced for stage of life, trained employees, pain control, euthanasia programs, and verification that these needs are being met. But back to the original question, why should I know about animal welfare audits? Firstly, to make an informed decision regarding participation in the programs that are offered. Dairy Quality Assurance 5-Star program is an assessment and verification/audit program. Validus is an assessment and audit program. Humane Farm Animal Care provides an opportunity to become certified for a niche market prior to participation in that market. Presently, you will need to ask yourself, which program will benefit your operation? Do you want or need to sell to a market requiring audits? You will probably eventually need to participate to remain competitive. Cost is presently being covered by producers. However, in due course, the question will become "Can I afford not to participate?".

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Table 1. Audit programs, internet addresses, phone number, and e-mail addresses.

Program	Internet address	Phone	E-mail
DQAC[1]	www.dqacenter.org/dairy%care.htm	1-800-55-DAIRY	info@dqacenter.org
HFAC[2]	www.certifiedhumane.org	703-435-3883	
AWAP[3]("SES", Inc.)	www.awaudit.org	1-800-897-1163	
AWARE[4](Validus)	www.emsllc.org	1-515-278-8002	voldl@validusservices.com

[1]Dairy Quality Assurance Center
[2]Humane Farm Animal Care
[3]Animal Welfare Auditing Program
[4]Animal Welfare Assurance Review and Evaluation

Table 2. Ranking of 10 dairy farms in California using 3 audit programs: Dairy Quality Assurance Center (DQAC), Humane Farm Animal Care (HFAC) and the University of California Davis (UCD) (Stull et al., 2005). Bolded numbers are ranked equally across audits.

Dairy[1]	DQAC, rank	HFAC, rank	UCD, rank
A	7	4	3
B	5	2	8
C	3	1	4/5
D	6	3	4/5
E	8	7	2
F	2	8	7
G	1	6	1
H	**10**	**10**	**10**
I	4	5	6
J	**9**	**9**	**9**

[1] Dairy farms were designated with a letter to preserve confidentiality.

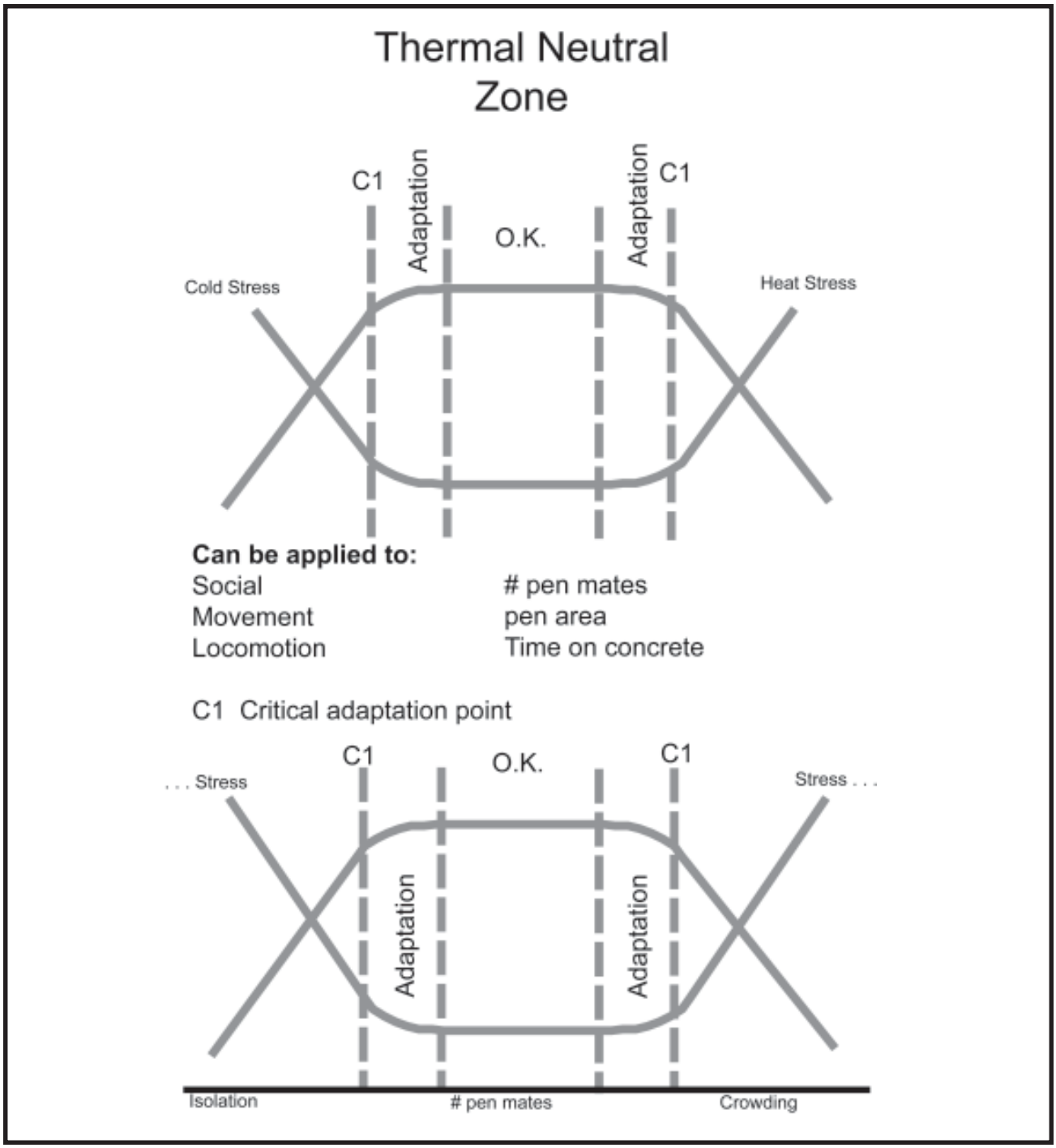

Figure 1. Thermal neutral zone diagram in the top panel shows critical (C1) temperatures, thermal neutral zone (O.K.), and areas where the animal has to adapt. The lower diagram shows how this concept can be applied to social stress. Isolation and crowding are the extremes, with C1 areas defining the critical number of pen mates to which the animal can adapt.

Can We Feed More Distillers Grains?

David J. Schingoethe[1]
Dairy Science Department
South Dakota State University

Abstract

Distillers grains is a very good protein source (>30% crude protein) which is high in ruminally undegradable protein and is very good energy source (NE_L ~1.02 Mcal/lb of dry matter; **[DM]**) for lactating cows and growing cattle. The modest fat concentration (10 to 12% of DM) and the readily digestible fiber (39% neutral detergent fiber; **NDF**) contribute to the high energy in distillers grains. The current large supply and competitive prices for distillers grains make it economically attractive to feed as much as possible. One can easily formulate nutritionally balanced diets for lactating cows that contain approximately 20% of the ration DM as distillers grains, an amount that is more than the conservative amounts that some people recommend, but less than the 30 to 40% that has been fed in some dairy studies or the 40 to 50% that has been included in diets of finishing cattle. This presentation summarizes the results of feeding distillers grains, especially larger amounts, to dairy cattle, points out where the maximums may occur, and points to possible differences to consider when feeding wet versus dried distillers grains.

Introduction

Distillers grains have been fed for more than 100 years; however, it is just during recent times that large quantities are becoming available and at competitive prices. Also, the products available today usually contain more protein and energy (Birkelo et al., 2004) than older "book values", even more than listed in the recent dairy NRC (2001), and can be of uniformly good quality. This reflects the improved fermentation efficiency of the new generation ethanol plants (Spiehs et al., 2002).

For several years, I as well as others have recommended that one can feed 20% of the ration DM as distillers grains. This may be considered as a sizable amount; approximately 10 to 13 lb/head/day of dried or 30 to 40 lb/day of wet distillers grains, but an amount that can be easily fed in nutritionally balanced diets and with very good animal performance. This recommendation is based on research by others and us, some of which will be reviewed in this presentation. Research will also be reviewed in which greater amounts of distillers grains were fed, pointing out some limitations but also indicating that the "20% of DM figure" may actually be conservative.

Virtually all of the distillers grains available today is distillers grains with solubles **(DGS)** because, while the solubles can be fed separately, they are usually blended back with the distillers grains. In fact, many research studies don't designate whether the product used was with or without solubles. The composition of corn distillers grains is essentially the same with or without solubles added, except for a lower phosphorus content (~0.4%) without solubles because the solubles are quite high (~1.35%) in phosphorus. The protein content may be slightly lower and the fat content slightly higher

[1]Contact at: 303B Dairy-Microbiology Bldg., Box 2104, Brookings, SD 57007-0647, (605) 688-5483, FAX: (605) 688-6276, Email: david.schingoethe@sdstate.edu

with solubles, reflecting the slightly lower protein and higher fat content of the solubles. If a DGS product contains substantially more fat (e.g. > 15%) and/or phosphorus (e.g. >1.0%), it is very likely that more than normal amounts of distillers solubles were blended with the distillers grains, or that the processor had problems with separation of materials during the handling of solubles. Such variations also point out the importance of obtaining analytical data on the specific product being received from a supplier and the importance of suppliers providing uniform, standardized products.

This presentation will discuss primarily the research with feeding DGS, both wet and dried, and especially look at the feeding of large amounts of DGS. Other distillers coproducts, such as condensed corn distillers solubles **(CCDS),** will be mentioned only briefly. In the future, there will likely be a host of new and improved products. For instance, improvements in fermentation technology already provide DDGS today that contain more protein and energy than DGS of previous years. It is also becoming feasible to "fractionate" in some manner DGS into products that are higher in protein, other products that are higher in fat or in fiber, and products that are higher or lower in phosphorus (Rausch and Belyea, 2006). And some products from ethanol production may find their way into non-food uses.

Production Response to Distillers Grains

More than two-dozen research trials have been conducted since 1982 in which distillers grains, either wet or dried, were fed to lactating cows. Amounts fed ranged from 4.2% of total dietary DM (Broderick et al., 1990) to 41.6% of DM (Van Horn et al., 1985). Kalscheur (2005) conducted a meta analysis of 24 studies reported from 1982 to 2005, involving 98 treatment comparisons. An abbreviated summary of this extensive survey of virtually all of the modern research data available about feeding DGS to lactating cows is listed in Table 1.

Distillers grains are palatable and readily consumed whether wet or dried; however, some decreases in DM intake can occur when cows are fed high amounts of DGS, especially wet DGS. Dry matter intakes were as high as or higher than intakes of control diets even with more than 20% DGS in the diet (Table 1). While DM intakes were not affected by inclusion of even high amounts of dried DGS (Kalscheur et al., 2004b; Kalscheur, 2005), DM intakes with wet DGS diets (46.1, 52.2, 50.5, 47.0, and 41.0 lb/day, respectively, for 0, <10, 10 to 20, 20 to 30, and >30% wet DGS) tended to decrease with more than 20% of the DM as wet DGS and significantly decreased ($P < 0.05$) with more than 30% of DM as wet DGS. Gut fill may limit DM intake when diets contain less than 50% DM, which is likely to occur when diets contain more than 20% of DM as wet DGS in diets that already contain other moist feeds, such as corn silage or haylage. Indeed, Hippen et al. (2003) observed decreased DM intake with a corresponding decrease in milk production when wet DGS supplied more than 20% of the dietary DM in diets that contained only 40 to 46% DM. Schingoethe et al. (1999) also observed decreased DM intake when diets contained 31% of DM as wet DGS in a 47% DM diet, but milk production was similar to the control diet.

Milk production was usually similar to production with control diets, and in many cases, higher when fed any amount of DGS (Table 1). With dried DGS, production tended to be highest for diets containing up to 30% DGS, while with wet DGS, production was highest when fed up to 20% DGS (Kalscheur, 2005). To illustrate this point, Kleinschmit et al. (2006b) used a standard, good quality dried DGS to evaluate the response to 2 specially processed dried DGS products intended to have even better quality. Milk production was higher for all 3 dried DGS products than for soybean meal-based control diet, with only small additional differences in response due to the improved dried DGS quality. Florida research (Powers et al., 1995)

indicated higher production when dried DGS were fed from either whiskey or fuel ethanol plants than when soybean meal was fed. However, to point out the importance of protein quality when a dried DGS product was darker and possibly heat damaged, milk production was lower than when fed the lighter, golden colored dried DGS but still similar to production achieved when soybean meal was fed (Powers et al., 1995).

Most distillers grains in the U.S. today is made from corn and the quality of protein in corn DGS is fairly good. As with most corn products, lysine is the first limiting amino acid in corn DGS for lactating cows, but corn DGS is a very good source of methionine. Therefore, sometimes (Nichols et al., 1998), but not always (Liu et al., 2000), milk production increased when fed supplemental ruminally protected lysine and methionine with dried DGS, or when the dried DGS was blended with other protein supplements that contained more lysine. Kleinschmit et al. (2006b) showed that, while there may be differences in protein quality of various sources of dried DGS present today (Kleinschmit et al., 2006a), differences in yields of milk and milk protein may be slight, unless a product is greatly heat-damaged. In all 3 of the above referenced lactation studies, dried DGS supplied 20% of the dietary DM.

Feed efficiency, as measured by fat-corrected or energy-corrected milk yield per pound of DM intake, when cows were fed DGS is the same as or higher than when cows were fed a control diet. Research with beef cattle (Larson et al., 1993; Ham et al., 1994) often showed increased feed efficiency when fed distillers grains products in place of corn. They concluded that this may in part be due to fewer off-feed problems and reduced subacute acidosis. Similar results were observed when feeding wet corn gluten feed (Krehbiel et al., 1995), another byproduct feed that also contains high amounts of digestible fiber. That is because, even though the DGS contains similar amounts or more energy than corn, the energy in DGS is primarily in the form of digestible fiber and fat; in corn, most of the energy is in the form of starch. Ruminal starch fermentation is more likely to result in acidosis, laminitis, and fatty liver. Most studies with dairy cattle have been short-term studies, which may not allow for detection of such responses. A continuous trial with lactating cows is currently in progress at SDSU (Hippen et al., unpublished results) in which lactating cows are being fed 15% of dietary DM as wet DGS for the entire lactation, during the dry period, and the first 70 days of the next lactation. An intent is to also evaluate any possible health issues that may occur with long-term feeding of DGS. Results from the first year of this study (Mpapho et al., 2006) indicate similar milk production, milk composition, feed intake, and reproductive efficiency with wet DGS as with the control diet.

Wet Versus Dried DGS

Very few trials compared wet versus dried DGS; most trials simply compared DGS to a control diet. The meta analysis (Kalscheur, 2005) indicated similar DM intake, milk yield, and milk composition when cows were fed wet or dried DGS; however, most of those experiments cited had no direct comparison between wet and dried DGS. When Al-Suwaiegh et al. (2002) directly compared 15% of DM as wet versus dried corn or sorghum DGS for lactating cows, they observed similar production for both wet and dried DGS but 6% more milk ($P < 0.13$) with corn versus sorghum DGS. There was no control, non-DGS diet fed in that experiment. Research by Anderson et al. (2006) observed greater production when cows were fed either wet or dried DGS, each fed at 10 and 20% of dietary DM, than when cows were fed the control diet. They observed a tendency ($P = 0.13$) for greater production when cows were fed wet DGS instead of dried DGS, and a tendency ($P = 0.12$) for greater production when cows were fed 20% of the ration DM as DGS versus 10%, either wet or dried.

Digestibilities of wet and dried DGS are usually considered to be similar; however, few studies have actually compared the digestibilities of wet and dried DGS. Lodge et al. (1997) determined that corn wet DGS was more digestible than was sorghum wet DGS, and wet DGS products were more digestible than dried DGS. Firkins et al. (1984) observed similar ruminal digestibility with wet and dried DGS but higher ruminally degradable protein in the wet product.

The main considerations regarding the use of wet versus dried DGS are handling and costs. Dried products can be stored for extended periods of time, can be shipped greater distances more economically and conveniently than wet DGS, and can be easily blended with other dietary ingredients. Feeding wet DGS avoids the costs of drying the product, but there are other factors to consider when feeding wet DGS that are not concerns when feeding dried DGS. Wet DGS will not remain fresh and palatable for extended periods of time; 5 to 7 days is the norm. This storage time span will vary somewhat with environmental temperature as products will spoil and become unpalatable more rapidly in hot weather, but may be kept in an acceptable form as long as 3 weeks under cool conditions. Surface molds occasionally occur, thus there is usually some feed lost; a problem that wouldn't be a consideration with dried DGS. The addition of preservatives such as propionic acid or other organic acids may extend the shelf life of wet DGS (Spangler et al., 2005), but refereed journal publications that document such results are limited. We at SDSU (Kalscheur et al., 2002; 2003; 2004ab) successfully stored wet DGS for more than 6 months in silo bags. The wet DGS was stored alone or blended with soyhulls (Kalscheur et al., 2002), with corn silage (Kalscheur et al., 2003), and with beet pulp (Kalscheur et al., 2004a). Some field reports indicate successful preservation of wet DGS for more than a year in silo bags.

Milk Composition

The composition of milk is usually not affected by feeding DGS unless routinely recommended ration formulation guidelines, such as feeding sufficient amounts of forage fiber, are not followed. Some field reports indicated milk fat depression when diets contained more than 10% of ration DM as wet DGS (Hutjens, 2004); however, those observations are not supported by research results. The data summarized in Table 1 from the meta analysis of 24 studies (Kalscheur, 2005) showed that there were no decreases in milk fat percentage when diets contained wet or dried DGS at any level, even as high as 40% of DM intake. The only time when milk fat percentage may have been lower with DGS was when diets contained less than 50% forage (3.21% fat versus 3.50 and 3.45% with 50% and >50% forage, respectively (Kalscheur, 2005). This result hints at why field observations of milk fat depression may have occurred. Because DGS contains an abundance of NDF, one is often tempted to decrease the amounts of forage fed when formulations indicate more than sufficient amounts of NDF are present in the diet. However, the small particle size of DGS means that its "effective fiber" is not as great as that of the forage fiber it replaced.

A recent study at SDSU supports the observations from the meta analysis. Cyriac et al. (2005) observed a linear decrease in milk fat concentration when cows were fed 0, 7, 14, and 21% of DM as dried DGS in place of corn silage, although milk production remained unchanged and milk protein percentage increased. The control diet contained 40% corn silage, 15% alfalfa hay, and 45% concentrate mix. When cows were fed low forage diets (45% of DM) that already caused a modest milk fat depression (3.38% fat), the feeding of increasing amounts of dried DGS resulted in a modest additional drop in milk fat percentage to 3.24% fat with 15% DGS (Leonardi et al., 2005). This slight response was less drastic than the

response observed by Cyriac et al. (2005). Thus, nonforage fiber sources of NDF, such as in DGS, can partially replace forages at times when forage supplies may be limited; however, one must realize that some milk fat depression may occur under these conditions.

Some have surmised that there is a lot of "free oil" in DGS that may be more likely than "bound fat" to interfere with ruminal fermentation and cause milk fat depression. However, any free oil in DGS is most likely in the solubles, which may account for 15 to 30% of the fat in DGS. If that is the case, feeding CCDS should cause a milk fat depression. When we fed 5 or 10% CCDS, we observed only a slight decrease in milk fat tests, going from 3.54 to 3.38% fat (DaCruz et al., 2005). In a recently completed experiment (Sasikala-Appukuttan et al., 2006), feeding 10 or 20% CCDS (2 and 4% added dietary fat) caused no milk fat depression, although milk fat tests tended to be low with all diets, including the control, in that experiment.

The fatty acid content of milk fat when cows are fed DGS is not expected to be affected greatly but has been evaluated in a couple of studies. Because the fat in DGS is quite unsaturated with typically more than 60% linoleic acid, it is logical to expect a modest increase in the concentration of unsaturated fatty acids in the milk produced as observed by Schingoethe et al. (1999). Leonardi et al. (2005) and Anderson et al. (2006) also reported modest increases in the healthful fatty acid cis-9,trans-11 conjugated linoleic acid **(CLA)** and its precursor vaccenic acid (trans-11 C18:1) in milk. They observed little or no change in the fatty acids that may be related to milk fat depression, trans-10 C18:1 and trans-10. cis-12 CLA.

Milk protein content is seldom affected by feeding DGS unless protein is limiting in the diet. Then the lysine limitation in DGS may cause a slight decrease in milk protein percentage (Kleinschmit et al., 2006b). This effect may be more noticeable in diets that contain more than 30% DGS (Kalscheur, 2005) because DGS is high in ruminally undegradable protein and limiting in lysine (Kleinschmit et al., 2006a). Milk protein percentage is typically decreased about 0.1% when cows are fed added fat from any source, so that can be a minor consideration when feeding DGS; however, most studies with DGS showed no effect on milk protein percentage.

Summary and Recommendations

One can easily formulate nutritionally balanced diets for lactating cows that contain approximately 20% of the ration DM as distillers grains. Optimal feed intake and productivity often occurs with 20% or more DGS in the diet. In diets that contain higher proportions of corn silage, even greater amounts of sGS may be usable without feeding excessive amounts of protein. However, the need for some other protein supplement, protein quality (e.g. lysine limitation), and phosphorus concentration may become factors to consider. In diets that contain higher proportions of alfalfa, less than 20% DGS may be needed to supply the protein required in the diet, thus the diet may not be able to utilize as much DGS without feeding excess protein. When feeding more than 20% distillers grains, one is likely to feed excess protein, unless forages are all or mostly corn silage and/or grass hay, and feeding excess phosphorus may become a consideration. Wet DGS can be well utilized up to 20% of dietary DM; however, if the diet also contains other moist feeds, such as corn silage or haylage, gut fill may limit total DM intake and production with diets that contain more than 20% of DM as wet DGS. Decreased DM intake is likely with more than 30% of DM as wet DGS. Milk fat depression is not a problem with the feeding of any amount of DGS unless the diet does not contain adequate amounts of forage fiber.

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Table 1. Dry matter intake (DMI), milk yield, and milk fat and protein percentages from cows fed diets containing wet or dried distillers grains with solubles.[1]

Inclusion level	DMI	Milk	Fat	Protein
(% of DM)	(lb/day)		(%)	
0	48.9[b]	72.8[ab]	3.39	2.95[a]
4 to 10	52.2[a]	73.6[a]	3.43	2.96[a]
10 to 20	51.6[ab]	73.2[ab]	3.41	2.94[a]
20 to 30	50.3[ab]	73.9[a]	3.33	2.97[a]
> 30	46.1[c]	71.0[b]	3.47	2.82[b]
SEM	1.8	3.0	0.08	0.06

[a,b,c]Values within a column followed by a different superscript differ ($P < 0.05$).

[1]Adapted from Kalscheur (2005).

Update on Development of the Spartan Dairy Ration Evaluator/Balancer Version 3

Michael J. VandeHaar[1] and Robert D. Kriegel
Animal Sciences Department
Michigan State University

Abstract

Version 2 of the Spartan Dairy Ration program was widely used because it was user-friendly and gave reasonable diets relatively quickly. In Version 3 of Spartan Dairy, we have tried to retain those aspects of Version 2 which made it successful, while also incorporating the best science for on-farm nutrition. The nutrition model is largely based on the 2001 version of the Nutrient Requirements of Dairy Cattle by the National Research Council **(NRC)**. The 2001 NRC made fundamental changes in the submodels for energy and protein. The NRC was designed as an evaluation model, and some of these changes created challenges for a user-friendly program that was designed for routine use in formulating diets on dairy farms. Version 3 of Spartan Dairy incorporates the 2001 NRC system as written, as well as modifications that enhance its use in ration formulation and state-of-the-art features for a Windows application.

Nutrition Model

The team working on Spartan Dairy 3 includes Mike VandeHaar, Robert Kriegel, Dave Beede, Herb Bucholtz, and Mike Allen. Robert Kriegel is the programmer. Spartan Dairy 3 is largely based on the 2001 Dairy NRC. A critique of the nutrition model of the 2001 Dairy NRC and challenges in using this model in ration evalution were presented at the TriState Dairy Nutrition Conference in 2002 (VandeHaar, 2002).

Energy

The energy system of the 2001 Dairy NRC is considerably more complicated than that of the 1989 NRC. The 2001 NRC was developed to be a retrospective evaluation program. Whereas a retrospective program examines a diet that has already been consumed by a cow and thus is at least reasonable, the prospective ration formulation program must be able to develop a new diet without prior knowledge of how the cow will eat it. Estimation of feed energy values using the composition of ingredients is likely an improvement over the previous system of book NE_L values. However, protein is overvalued in the model, with an energy value of 5.6 kcal/g of digested protein but with the same constant conversion of digestible energy **(DE)** to metabolizable energy **(ME)** as in 1989. More importantly, the digestibility discount is now adjusted for level of intake, and, although feed factors are not used in predicting feed intake, they are used in predicting digestibility. Nonfat feeds with the highest total digestible nutrients **(TDN)** values at 1X maintenance are discounted the most with increasing intake. As an evaluation program, this may work well. However, as a formulation program for high producing cows, the new system predicts nearly the same energy-allowable milk with a high grain diet as with a high forage diet. Because the feed intake equation does not use feed factors, the implicit assumption is that a cow can eat as much of a high forage diet as a high grain diet. Thus, least-cost formulation programs would be unjustly

[1]Contact at: 2265 Anthony Hall, Michigan State University, East Lansing, MI 48824-1225, (517) 355-8489, FAX: (517) 432-0147, Email: mikevh@msu.edu

biased toward high forage diets for high-producing cows. As a diet formulator, the 2001 NRC model favors diets for high producing cows that are higher in fat, protein, and fiber than are optimal for high production.

Whereas Spartan Dairy 3 includes the energy system as designed in 2001 NRC, it also includes a revised energy system. Key features of the revised system include a lower energy value for digested protein, using protein fractions to calculate the amount of digestible protein instead of acid detergent insoluble crude protein **(ADICP)** and separate tables for specific feeds, and the removal of feed factors from the equation for predicting the digestibility discount. Requirements for energy in the new Spartan program are largely as written in the 2001 NRC, but include revisions to the requirements for work and also include adjustments for environmental temperature and Rumensin (Elanco, Greenfield, IN).

Protein

The protein system of the 2001 Dairy NRC also is considerably more complicated than that of the 1989 NRC. On the requirement side for NRC, the metabolic fecal protein requirement was decreased, a requirement for secreted gut proteins was added, the protein requirement for pregnancy increases with day of gestation, and the protein required for growth or body condition gain is affected by body weight **(BW)** as a percentage of mature BW and the actual body condition score of the animal. On the supply side, the fraction of protein that is rumen undegradable protein **(RUP)** is a function of its protein fractions (A, B, and C) and the competition of digestion and passage for fraction B. All of the C fraction is assumed to be RUP, and all of the A fraction is assumed to be rumen-degraded protein **(RDP)**. The RUP value of the B fraction depends on its digestion rate (k_d, which is a fixed value for each feedstuff) and the passage rate (k_p) for the feed. In addition, the percent of RUP that is digested is no longer assumed to be 80% for all feeds but is a fixed value for each feedstuff. The supply of metabolizable protein from microbial protein is a function of the fat-corrected, discounted TDN intake of the animal. As in 1989, microbial crude protein is considered to be 80% true protein and 80% digestible. The equation for microbial protein yield has no intercept, so it works much better for young heifers. Finally, the new NRC also considers amino acid requirements and supply.

As with energy, Spartan Dairy 3 allows the use of the NRC system but also provides an alternative. In the Spartan Dairy protein system, requirements are largely as in NRC, but slight changes were made in requirements for pregnancy, work, and thermoregulation. The equations for microbial yield were altered, and microbial yield is slightly greater with the Spartan system than with NRC. Finally, the equations for the supply of lysine and methionine were altered slightly; and methionine supply is generally greater with the Spartan equations.

Feed intake

In the 2001 NRC, expected feed intake for lactating cows is predicted from metabolic body weight, fat-corrected milk yield, and days-in-milk. Expected feed intake is predicted from body weight, lactation number, and days-til-calving for dry cows and from metabolic weight and dietary energy density for heifers. Predicted feed intake is not altered by activity, growth rate, temperature, or ionophore. Thus, increasing the work level of a cow can greatly increase the required energy density in her diet if the predicted intake is used for formulating a diet. In Spartan 3, the equation for predicting feed intake is consistent across all animals and is based on metabolic body weight, energy-corrected milk yield, and energy requirements for daily gain, pregnancy, and work, with adjustments for days-in-milk, days-til-calving, temperature stress, and ionophore feeding. In most cases, the

dry matter intake **(DMI)** predicted by Spartan 3 is slightly higher than that predicted by NRC or Spartan 2.

Other nutritional features

In addition to the new equations of the Spartan 3 system, the Spartan 3 program also provides the user with values for energy and protein supply from the 2001 NRC and the Spartan 2 (1989 NRC). Dietary fiber fractions included neutral detergent fiber **(NDF)**, effective NDF, forage NDF, and soluble fiber. The user may do an accounting of carbohydrate fractions. Minerals are balanced on a total or absorbed basis.

The User Interface

Spartan Dairy 3 was designed from the start as a Windows application. It is a stand-alone program that will run best on a Windows XP operating system (or later). The program uses a spreadsheet interface similar to that of Spartan 2. All data are stored in MS Access database files. Several rations and feed library windows may be open simultaneously. Feeds can easily be copied and pasted from one file to another, and from or to MS Excel files. The program includes an optional transcript window that lists a complete audit trail of equations and calculations for the advanced user who wants more information.

Progress

Currently a working version of the program is undergoing testing; this program includes the feed library, animal description, animal requirements, and ration worksheet. Rations can be balanced manually, but further enhancements are being made to improve the user-interface and performance. The program lacks dialogs for reports and printing, user set-up, help, and the linear program. We are currently on target to release a product for sale by July, 2007. Updates will occasionally be posted on our website (www.msu.edu/ssl).

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Silage Management: Common Problems and Their Solution

Keith K. Bolsen[1]
Department of Animal Sciences and Industry
Kansas State University

Introduction

Regardless of the size of an operation, dairy producers know problems occur in every silage program. This paper describes possible causes and solutions for 10 common problems, which include:

- Safety issues for bunker silos and drive-over piles
- Effluent
- Large variation in the dry matter **(DM)** content and/or nutritional quality of the ensiled forage
- Missing the optimum harvest window for whole-plant corn
- Clostridial, butyric acid-containing hay-crop silage
- High levels of acetic acid, particularly in wet corn silage
- Heat-damaged silage
- Aerobically unstable corn silage during feedout
- Excessive surface-spoiled silage in sealed bunker silos and drive-over piles
- High 'forage in' versus 'silage out' losses in bunker silos, drive-over piles, and bags

Beef and dairy producers (and their nutritionist) should discuss these problems and solutions with everyone on their silage team as a reminder to implement the best possible silage management practices (Bolsen, 1995).

Safety Issues for Bunker Silos and Drive-Over Piles

Consistently protecting workers, livestock, equipment, and property at harvest, filling, and feeding does not occur without thought, preparation, and training. You have nothing to lose by practicing safety; you have everything to lose by not practicing it (Murphy and Harshman, 2006).

Major hazards and preventive measures

- Tractor roll over
  - √ Roll over protective structures (**ROPS**) create a zone of protection around the tractor operator. When used with a seat belt, ROPS prevent the operator from being thrown from the protective zone and crushed by the tractor or equipment mounted on or drawn by the tractor.
  - √ A straight drop off a concrete retaining wall is a significant risk so never fill higher than the top of a wall.
  - √ Install sighting rails on above ground walls. These rails indicate the location of the wall to the pack tractor operator but are not to hold an over-turning tractor.
  - √ Consider adding lights to the rail if filling will occur at night.
  - √ Form a progressive wedge of forage when filling bunkers or piles. The wedge provides a slope for packing, and a maximum 3 to 1 slope minimizes the risk of a tractor roll-over.

[1]Contact at: 6106 Tasajillo Trail, Austin, TX 78739, (512) 301-2281, FAX: (866) 230-2970, Email: keithbolsen@hotmail.com

- √ Backing up the slope can prevent roll backs on steep slopes.
- √ Use low-clearance, wide front end tractors and add weights to the front and back of the tractors to improve stability.
- √ When using front-end loaders to carry feed into the silo, do not carry bucket any higher than necessary to help keep the center of gravity low.
- √ Front-wheel and front wheel-assist drive tractors provide extra traction and stability.
- √ When two or more pack tractors are used, establish a driving procedure to prevent collisions.
- √ Dump trucks, which are used to transport chopped forage in large-scale operations, can roll over on steep forage slopes, particularly if the forage in not loaded and packed uniformly.
- √ Raise the dump body only while the truck is on a rigid floor of the storage area to prevent turn overs.

• Entangled in machinery
- √ Keep machine guards and shields in place to protect the operator from an assortment of rotating shafts, chain and v-belt drives, gears and pulley wheels, and rotating knives on tractors, pull-type and self-propelled harvesters, unloading wagons, and feeding equipment.
- √ "The accident happened on Saturday June 14, 1974 while making wheat silage at Kansas State University's Beef Cattle Research Unit. The blower pipe plugged for about the 10th time that afternoon. I started to dig the forage out from the 'throat' of the blower, and the PTO shaft was making one more revolution … zap! The blower blade cut off the ends off three fingers on my right hand" (Bolsen, 2006).

• Run-over by machinery
- √ Never allow people on foot (especially children) in or near a bunker or pile during the filling operation.
- √ Properly adjust rear view mirrors on all tractors and trucks.

• Fall from height
- √ It is easy to slip on plastic when covering a bunker, especially in wet weather, so install guardrails on all above ground level walls.
- √ Use caution when removing plastic and tires, especially near the edge of the feeding face.
- √ Never stand on top of a silage overhang in bunkers and piles, as a person's weight can cause it to collapse.

• Crushed by an avalanche/collapsing silage
- √ The number one factor contributing to injuries or deaths from silage avalanches is overfilled bunkers and drive-over piles!
- √ Do not fill higher than the unloading equipment can reach safely, and typically, an unloader can reach a height of 12 to 15 feet.
- √ Use proper unloading technique that includes shaving silage down the feeding face and never 'dig' the bucket into the bottom of the silage. Undercutting, a situation that is quite common when the unloader bucket cannot reach the top of an over-filled bunker or pile, creates an overhang of silage that can loosen and tumble to the floor.
- √ Never allow people to stand near the feeding face, and a rule-of-thumb is never be closer to the feeding face than three times its height.
- √ Fence the perimeter of bunkers and piles and post a sign, "Danger: Do Not Enter. Authorized Personnel Only".

• Complacency
- √ Mac Rickels, a dairy nutritionist in Comanche, TX, almost lost his life the day he took silage samples from a bunker silo with a 32-foot high feedout face

(Schoonmaker, 2000). Rickels said, "Even though I was standing 20 feet from the feedout face, 12 tons of silage collapsed on me. I didn't see or hear anything. I had been in silage pits hundreds of times, and you just become kind of complacent because nothing ever happens. It just took that one time."

√ Think safety first! Even the best employee can become frustrated with malfunctioning equipment and poor weather conditions and take a hazardous shortcut, or misjudge a situation and take a risky action (Murphy, 1994).

√ It is always best to take steps to eliminate or control hazards ahead of time rather than to rely upon yourself or others to make the correct decision or execute the perfect action when a hazard is encountered.

Effluent

Effluent has a very high biochemical oxygen demand. It should always be contained near the silo of origin and never allowed to enter groundwater and/or a nearby pond or watercourse. When seepage occurs, the plant materials that threaten water quality are also nutrients that are lost from the silage.

Causes

- Forage ensiled at too low DM content for the type and size of silo.
- Forage was not pre-conditioned when cut.
- Forage was in a windrow that was too bulky for the time allowed for field-wilting.
- Weather did not allow the forage to be field-wilted properly before chopping.
- Person(s) responsible for determining the DM content of the forage made a mistake.
- Whole-plant corn, sorghum, or cereal was harvested at an immature stage of growth.
  - √ Silage contractor does not arrive at the scheduled time.
  - √ Chopping began too early because of the number of acres to harvest.

Solutions

- Use weather forecasts to make forage management decisions.
- Take advantage of new mowing, cutting, and conditioning equipment technologies.
- Coordinate the merging of windows with the time of chopping.
- Monitor the dry-down rate and whole-plant moisture content of each field of corn or sorghum so the harvest can begin at the proper time.
- Select a range of corn or sorghum hybrids with differing maturities to widen the effective harvest window.

Large Variation in the DM Content and/or Nutritional Quality of the Ensiled Forage

Causes

- Interseeded crops of different maturity.
- Multiple cuttings or multiple forages ensiled in the same silo.
- Delays in harvest activities because of a breakdown or shortage of machinery and equipment.
- Seasonal or daily weather affects crop maturing and field-wilting rates.
- Differences among corn hybrids. Hybrids with the stay-green trait tend to be wetter at a given kernel maturity than non stay-green hybrids.

Solutions

- Use multiple silos and smaller silos that improve forage inventory control.
- Ensile only one cutting and/or variety of 'hay-crop', field-wilted forage per silo.
- Minimize the number of corn and/or sorghum hybrids per silo.
- Shorten the filling-time but do not compromise packing density.

Missing the Optimum Harvest Window for Whole-Plant Corn

Causes

- Harvest equipment capacity is inadequate.
- The crop matures in a small harvest window.
- Warm, dry weather can speed the maturing process and dry-down rate of the grain and forage parts of the plant.
- Wet weather can keep harvesting equipment out of the field.
- Sometimes it is difficult to schedule the silage contractor.

Solutions

- Plant multiple corn or sorghum hybrids with different season lengths.
- Improve the communication between the beef or dairy producer, crop grower, and silage contractor.
- Change harvest strategy, which might include kernel processing, shorter theoretical length of cut (**TLC**), or adding a pack tractor.

Clostridial, Butyric Acid-Containing Hay-Crop Silage

Causes

- The forage is ensiled too wet and undergoes a fermentation dominated by clostridia.
- Alfalfa and other legumes, which experience a rain event in the field after mowing, are at a higher risk because rain leaches soluble sugars from the forage.
- The forage is harvested too wet for the type and size of storage.

Solutions

- Chop and ensile all forages at the correct DM content for the type and size of silo.
- Proper packing to achieve a minimum density of 15 lb of DM per ft^3 excludes oxygen and limits the loss of plant sugars during the aerobic phase (Visser, 2005; Holmes, 2006).
- Apply a homolactic bacterial inoculant (**HLAB**) to all forages to ensure an efficient conversion of plant sugars to lactic acid.
- Do not contaminate the forage with soil or manure at harvest.
- If it is not possible to control the DM content by wilting, the addition of soluble sugars can reduce the chance of clostridial fermentation and the problems associated with butyric acid silages.

High Levels of Acetic Acid, Particularly in Wet Corn Silage

Causes and symptoms

- When the whole-plant has a low DM content at harvest, it is predisposed to undergo a prolonged, heterolactic fermentation.
- This silage has a strong 'vinegar' smell, and there will be a 2 to 3 feet layer of bright yellow, sour smelling silage near the floor of a bunker silo or drive-over pile.

Solutions

- Ensile all forages at the correct DM content and especially not too wet.
- Use a HLAB inoculant to ensure an efficient conversion of plant sugar to lactic acid.

Heat-Damaged Silage

Causes and symptoms

- This silage has a dark brown color and a burnt caramel/tobacco smell.
- Heat-damaged silage typically has reduced digestibility of the protein and energy components.

- In well-managed silage, the temperature of the ensiled forage should not increase more than 8 to 10° F above the ambient temperature at harvest, and when the temperature of the ensiled forage exceeds 115 to 120° F during the first 1 to 2 weeks, heat-damage can occur.
- Most of the heat is from plant and microbial respiration, which continues as long as oxygen is present in the ensiled mass.
- Chemical reactions, called Maillard or 'browning', bind plant sugars and hemicellulose with proteins and amino acids.

Solutions

- Before filling a bunker silo, seal cracks in the walls and/or line walls with polyethylene.
- Harvest at the correct stage of maturity and especially not too mature.
- Ensile all forages at the correct DM content and especially not too dry.
- Do not chop forages too long, which would typically be longer than 1-inch TLC for field-wilted forages and ½-inch to ¾-inch TLC for whole-plant corn or sorghum.
- Achieve anaerobic conditions as quickly as possible in the ensiled forage mass.
- Fill silos in a timely manner and distribute the forage evenly in the silo.
- Achieve a minimum packing density of 15 lb of DM per ft^3.
- Cover/seal the surface as quickly as possible following filling (within 24 hours).

Aerobically Unstable Corn Silage During Feedout

Research into the processes of aerobic deterioration has not explained why corn silages differ in their susceptibility to aerobic deterioration. Microbes, primarily lactate utilizing yeast, as well as forage and silage management practices contribute to aerobic stability of an individual corn silage (Uriarte-Archundia et al., 2002).

Solutions

- Harvest at the correct stage of kernel maturity and especially not too mature.
- Ensile at the correct DM content and especially not too dry.
- In normal conditions, do not chop longer than ¾-inch TLC if the crop is processed or ½-inch if not processed.
- Achieve a minimum packing density of 15 lb of DM per ft^3.
- Maintain a uniform and rapid progression through the silage during the entire feedout period**.** Remove a minimum of 6 to 12 inches per day in cold weather months and 12 to 18 inches per day in warm weather months.
- Minimize the amount of time corn silage stays in the commodity area before adding it to the ration. It might be necessary to remove silage from a bunker or drive-over pile and move it the commodity area twice daily.
- Do not leave corn silage rations in the feed bunk too long, especially in warm, humid weather.
- Add about 2 to 4 lb of a buffered propionic acid product per ton of total mixed ration if heating does occur.
- Consider re-sizing a silo and subsequent feedout face for the time of year a silage will be feedout.
- Feed from 'larger feedout faces areas' in cold weather months.
- Feed from 'smaller feedout faces areas' in warm weather months.

Excessive Surface-Spoiled Silage in Sealed Bunker Silos and Drive-Over Piles

Solutions

- Achieve an optimum packing density (minimum of 15 lb of DM per ft^3) within the top 3 feet of the silage surface.
- Shape all surfaces so water drains off the bunker or pile, and the back, front, and side slopes should not exceed a 3 to 1 slope.

- Seal the forage surface immediately after filling is finished.
- Two sheets of polyethylene or a single sheet of oxygen barrier (**OB**) film is preferred to a single sheet of plastic (Bolsen, 2004; Berger and Bolsen, 2006).
- Overlap the sheets that cover the forage surface by a minimum of 3 to 4 feet.
- Arrange plastic sheets so runoff water does not contact the silage.
- Sheets should reach 4 to 6 feet off the forage surface around the perimeter of a drive-over pile.
- Put uniform weight on the sheets over the entire surface of a bunker or pile, and double the weight placed on the overlapping sheets.
  - √ Bias-ply truck sidewall disks, with or without a lacework of holes, are the most common alternative to full-casing tires.
  - √ Sandbags, filled with pea gravel, are an effective way to anchor the overlapping sheets, and sandbags provide a heavy, uniform weight at the interface of the sheets and bunker wall.
  - √ Sidewall disks and sandbags can be stacked, and if placed on pallets, they can be moved easily and lifted to the top of a bunker wall when the silo is being sealed and lifted to the top of the feedout face when the cover is removed.
  - √ A 6- to 12-inch layer of sand or soil or sandbags is an effective way to anchor sheets around the perimeter of drive-over piles.

- Prevent damage to the sheet or film during the entire storage period.
  - √ Mow the area surrounding a bunker or pile and put up temporary fencing as safe guards against domesticated and wild animals.
  - √ Develop a rodent control program for the farm.
  - √ Use a mesh or resistant secondary cover to exclude birds.
  - √ Store waste polyethylene and cover weighting materials so it does not harbor vermin.
  - √ Regular inspection and repair is recommended because extensive spoilage can develop quickly if air and water penetrate the silage mass.

- Discard all surface-spoiled silage because it has a significant negative effect on DM intake and nutrient digestibility (Whitlock et al., 2000; Bolsen, 2002).
- Full-casing discarded tires were the standard for many years to anchor polyethylene sheets on bunker silos. These waste tires are cumbersome to handle, messy, and standing water in full-casing tires can help spread the West Nile virus, which is another reason to avoid using full-casing tires on beef and dairy operations (Jones et al., 2004).

High 'Forage In' vs. 'Silage Out' Losses in Bunker Silos, Drive-Over Piles, and Bags

Solutions

- Select the right forage hybrid or variety.
- Harvest at the optimum whole-plant DM content.
- Use the correct size of bunker or pile, and do not over-fill bunkers or piles.
- Employ well-trained, experienced people, especially those who operate the forage harvester, pack tractor, or bagging machine. Provide training as needed.
- Apply a HLAB inoculant.
- Achieve an optimum and uniform packing density in bunkers and piles (a minimum of 15 lb of DM per ft^3).
- Provide an effective seal to the surface of bunkers and piles and consider using double polyethylene sheets or OB film.
- Follow proper face management practices during the entire feedout period.

- Start a silage quality control program and schedule regular meetings with your team.

Profitability of HLAB-Treated Corn Silage for Growing Cattle and Lactating Dairy Cows

Many dairy producers, nutritionists, and custom silage operators are concerned about whether it is economical to use a HLAB when making corn silage. Presented in Tables 1 and 2 are examples from spreadsheets, which show the profitability of inoculating whole-plant corn silage with HLAB.

Growing cattle

The cattle in this example had an average weight of 650 lb, a DM intake of 2.62% of body weight, a ration DM intake to gain ratio of 7.1, and an average daily gain of 2.39 lb. The cattle performance responses to HLAB-treated corn silage were a 0.05 lb increase in DM intake (17.0 vs. 17.05 lb/day) and an improved ration DM to gain ratio of 0.15 (6.95 vs. 7.1). The DM recovery response was 1.3 percentage units for HLAB - treated silage compared to the untreated silage (83.8 vs. 82.5). The gain per ton of 'as-fed' whole-plant corn ensiled was 91.78 lb for the HLAB-treated vs. 88.45 lb for untreated corn silage, which was an increase of 3.33 lb. With a cattle price of $1.20 per lb and a HLAB cost of $0.75 per ton of crop ensiled, the net benefit per ton of crop ensiled was $3.25.

Lactating dairy cows

The dairy herd in this example had an average milk production 75 lb/day per cow and a DM intake of 52 lb/day. The increase in net income with HLAB-treated corn silage, calculated on a 'per cow per day' and 'per cow per year' basis, came from increases in both forage preservation and silage utilization improvements. The additional 'cow days' per ton of crop ensiled because of the increased silage recovery (1.5 percentage units) and in-creased milk per cow per day (0.25 lb) gave an increased net income of 16.2¢ per cow per day and $49.50 per cow per year. The increased net return per ton of whole-plant corn ensiled was $6.99.

Profitability of Sealing Bunker Silos and Drive-Over Piles

A spreadsheet to calculate the profitability of sealing corn and alfalfa silages in bunker silos and drive-over piles was developed from research conducted at Kansas State University from 1990 to 1995 and equations published by Huck et al. (1997). Huck et al. (1997) noted that about 75% of the total tons of corn and sorghum silage made in Kansas from 1994 to 1996 were not sealed, and the value of silage lost to surface spoilage was $7 to 9 million annually. Presented in Table 3 are examples from the spreadsheet. The profitability of properly sealing bunkers and piles with 6-mil standard plastic or an improved OB film makes it clear that producers should pay close attention to the details of this 'highly troublesome' task.

Dagano (1999) introduced the OB film as an alternative to standard plastic at the XII International Silage Conference in 1999. Wilkinson and Rimini (2002) reported virtually no visible surface mold and a markedly lower percentage of inedible silage for OB film-sealed pilot silos compared to the single and double standard film-sealed silos.

Bolsen (2004) compared the OB film to 6-mil standard black plastic in two field trials conducted from September 2003 to May 2004. The first trial was with whole-plant corn at a commercial feedyard near Dimmit, TX; the second trial, with high moisture (**HM**) corn was at a feedyard near Garden City, KS. In Trial 1, the OB film and standard plastic were applied to side-by-side, 40 ft wide x 60 ft

long areas of the bunker surface; in Trial 2, the OB film and standard plastic were applied to side-by-side, 130 ft wide x 60 ft long areas. The standard plastic and OB film was weighted with either full-casing, discarded car tires (Trial 1) or truck sidewall disks (Trial 2). A thin tarpaulin was put on the film ahead of the tires or sidewalls because the OB film did not have protection from ultraviolet light. The sealing materials were removed about 240 day post-filling and samples taken at 0 to 6, 6 to 12, and 12 to 18 inches from the surface at four locations across the width of each test area.

There was virtually no visible discoloration or surface spoilage in the OB film-sealed bunkers; however, there was visible mold and aerobic spoilage in the standard plastic-sealed bunkers, particularly in the top 12 inches of corn silage. The corn silage and HM corn in the top 0 to 18 inches under the OB film had better fermentation profiles and lower estimated additional spoilage losses of OM compared to the corn silage and HM corn under the standard plastic (Table 4).

When compared to standard plastic in a 1,152-ton capacity bunker silo, OB film would result in the net saving of $490 of corn silage in the original top three feet (Table 3). In a 180 x 280 drive-over pile of corn silage, OB film would produce a net savings of $6,140 of silage in the original top three feet compared to standard plastic (Table 3). In a 100 x 150 drive-over pile of alfalfa haylage, OB film would produce a net savings of $18,600 of haylage in the original top three feet. Additional information about the OB film is located at www.silostop.com.

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Table 1. Profitability of HLAB-treated corn silage for growing cattle.[1]

Ration ingredients	DM basis (%)	Untreated ration (DM, %)	HLAB ration (DM, %)	Untreated ration (lb/day)	HLAB Response[2]	HLAB ration (lb/day)
Corn silage	**87.5**	**33.3**	**33.3**	14.88		14.92
Other silage or hay	**0**	**90.0**	**90.0**	0		0
Grain or supplement	**12.5**	**90.0**	**90.0**	2.12		2.13
Total	100			17.0		17.05
Avg. cattle wt, lb	**650**					
Cattle price, $ per lb	**1.20**					
Avg daily gain, lb				2.39		2.45
DM intake, lb per day				**17.0**	+ 0.05	17.05
Ration DM per lb of gain, lb				**7.1**	- 0.15	6.95
Silage per lb of gain, lb of DM				6.21		6.08
Silage per lb of gain, lb as-fed				18.7		18.3
DM recovery, % of the ensiled crop				**82.5**	+ 1.3	83.8
Gain per ton of as-fed crop ensiled, lb				88.45		91.78
Value of the extra gain per ton of crop ensiled, $				---		4.00
Cost of HLAB per ton of crop ensiled, $				---		**0.75**
Net benefit per ton of HLAB-treated crop ensiled, $				---		3.25

[1]Numbers in **bold** are user inputs and changeable; HLAB = homoladic bacterial inoculant and DM = dry matter.

[2]Response is a 19-trial average across all HLAB products (Bolsen et al., 1992).

Table 2. Profitability of HLAB-treated corn silage for lactating dairy cows.[1]

Ration ingredient	DM intake, lb/day	DM, %	As-fed, lb/day	$ per lb	Feed cost, $ per day
Corn silage	**15.0**	**33.3**	45.0	**0.0175**	0.79
Other silage/haylage	**9.0**	**45.0**	20.0	**0.030**	0.60
Other forage/hay	**4.0**	**88.0**	4.6	**0.060**	0.27
Grain/supplement	**24.0**	**88.0**	27.3	**0.075**	2.05
Total	52.0		96.9		3.71
Corn silage required per cow per year, tons					7.94
HLAB cost per ton of crop, $					**0.75**

Component	Untreated corn silage		HLAB corn silage
Preservation efficiency:			
Silage recovery, % of crop ensiled[2]	**85.0**	**(1.5)**	86.5
Silage recovered per ton of crop ensiled, lb	1,700		1,730
Amount of corn silage fed per cow per day, lb	45.0		45.0
Cow days per ton of crop ensiled	37.74		38.41
Extra cow days per ton of crop ensiled			0.67
Milk production per cow per day, lb			**75.0**
Milk gained per ton of crop ensiled, lb			49.9
Milk price, $ per lb			**0.15**
Increased milk value per ton of crop ensiled, $			7.49
Utilization efficiency:			
Increased milk per cow per day, lb			**0.25**
Increased milk value per ton of crop ensiled, $			1.44
Preservation + utilization efficiency:			
Extra milk value per ton of crop ensiled, $			8.93
Increased feed cost per extra cow day, $			2.92
Increased feed cost per ton of crop ensiled, $			1.94
Increase net return per ton of crop ensiled, $			6.99
Added cost of HLAB: per cow per day, $			0.020
per cow per year, $			5.96
Added income as milk: per cow per day, $			0.182
per cow per year, $			55.50
Net benefit with HLAB: per cow per day, $			0.162
per cow per year, $			49.50

[1]Numbers in **bold** are inputs by the producer and changeable; HLAB = homolactic bacterial inoculant and DM = dry matter.

[2]Shown in parenthesis is the response to HLAB expressed in percentage units.

Table 3. Profitability of sealing corn silage and alfalfa haylage in bunker silos and drive-over piles with standard plastic and oxygen barrier **(OB)** film.[1]

Inputs and calculations	Bunker 1 corn std plastic	Bunker 2 corn OB film	Pile 1 corn std plastic	Pile 2 corn OB film	Pile 3 alfalfa OB film
Silage value, $ per ton	**32.50**	**32.50**	**32.50**	**32.50**	**60**
Silage density, lb per ft^3 as-fed basis	**48**	**48**	**48**	**48**	**40**
Silo width, ft	**40**	**40**	**180**	**180**	**100**
Silo length, ft	**100**	**100**	**280**	**280**	**150**
Silage lost in the original top 3 feet:					
unsealed, % of the crop ensiled	**50**	**50**	**50**	**50**	**50**
sealed, % of the crop ensiled	**20**[a]	**12**[a]	**20**[a]	**12**[a]	**10**
Cost of covering sheet, ¢ per sq. ft	**3.5**	**10.0**	**3.5**	**10.0**	**10.0**
Silage in the original top 3 ft, tons	288	288	3,630	3,630	900
Value of silage in original top 3 ft, $	9,360	9,360	117,975	117,975	54,000
Silage lost if unsealed, $ per silo	4,680	4,680	58,970	58,970	27,000
Silage lost if sealed, $ per silo	1,870	1,120	23,590	14,150	5,400
Sealing cost, $ per silo	560	800	6,800	10,100	3,000
Silage saved by sealing, $ per silo	2,270	2,760	28,580	34,720	18,600

[1]Numbers in **bold** are inputs by the producer and changeable.

[a]Unpublished field trial data comparing standard plastic and OB film on bunker silos of corn silage and high moisture corn (Bolsen, 2004).

Table 4. Effects of standard plastic and oxygen barrier **(OB)** film on pH, fermentation profile, estimated additional spoilage loss of organic matter **(OM)**, and ash content in corn silage and high moisture **(HM)** corn at 0 to 18 inches from the surface at 240 days post-filling.

Item	Corn silage		HM corn	
	std plastic	OB film	std plastic	OB film
DM content, %	29.2	31.6	72.3	73.2
pH	4.28	3.78	4.70	4.09
Estimated OM loss[1,2]	27.3	8.4	12.6	7.2
	% of the silage DM			
Lactic acid	2.7	6.8	0.86	1.08
Acetic acid	2.6	2.2	0.25	0.31
Ash	11.2	9.1	2.10	1.98

[1]Values are estimated additional spoilage loss of OM, calculated from ash content using the equations described by Dickerson et al. (1992).

[2]Ash content of the face samples was 8.4% for the corn silage and 1.85% for HM corn.

In Vivo Digestibility of Forages

Gabriella A. Varga[1]
Department of Dairy and Animal Science
Pennsylvania State University

Introduction

There has been a trend for dairy producers to feed higher forage rations over the last 5 to 10 years. A primary reason is that producers are doing a better job of harvesting and storing larger quantities of high quality forages. The use of the neutral detergent fiber **(NDF)** digestibility concept has also provided additional information to assist feed professionals in formulating dairy rations with higher levels of forage. There have also been improvements in the corn hybrids and forage varieties available in terms of NDF digestibility. A key reason for including more forage in the ration in many herds is an attempt to minimize herd health disorders related to feeding high nonfiber carbohydrates and starch levels in dairy rations. In addition, incorporating a greater proportion of higher quality forages in the diet reduces feed costs. In some instances, it may also have the added benefit of increased nitrogen use by the cow and thereby strategically improve nutrient management on the farm.

However, many factors affect the quality and quantity of forages that can be incorporated into lactating cow rations. Variation in forage quality can impact dry matter **(DM)** intake, diet energy density, dietary grain and protein supplementation amounts, feed costs, lactation performance, and cow health. Forage quality is highly variable among and within forage types (NRC, 2001). Forage species, variety or hybrid, stage of maturity at harvest, cutting, environmental factors, production and harvest practices, storage method, and ensiling practices all are factors that contribute to this variation (Shaver et al, 2002). These are many of the forage variables. There are then many factors that affect the fiber requirements of lactating dairy cows and the amount of forage DM that can be incorporated into the ration. These include level of intake, quality and type of the forage source, amount and type of nonstructural and structural carbohydrates in the ration DM, particle size and processing method of forages and grains, rate and extent of fermentability of the fiber source, ruminal fermentation characteristics, and management of feed allocation. The challenge for the nutritionists is to provide guidance in ration formulation that allows for a high incorporation of forages in the ration without compromising milk yield or components.

All factors affecting in vivo forage digestibility certainly cannot be addressed in one paper; therefore, efforts will be placed on understanding the factors that contribute to the use of quality forages in lactating cow rations. The topics that will be covered include: factors affecting forage fiber digestion, such as DM intake, the interaction of concentrates and other fiber sources in the ration with forage sources, and processing and particle size of forages, and influences on performance and milk components.

[1]Contact at: 324 Henning Building, University Park, PA 16802, (814) 863-4195, FAX: (814) 865-7442, Email: GVarga@psu.edu

Why is Knowing Forage Fiber Digestibility Important?

Oba and Allen (1999) evaluated the relationship between fiber digestibility and animal performance using 45 sets of treatment means from 27 articles published in the Journal of Dairy Science. These 27 articles had reported significant differences in NDF digestibility in vivo, in situ, or in vitro. Experiments with cows averaging less than 100 days in milk **(DIM)** at the midpoint were classified as early lactation and classified mid-lactation otherwise. There was a 5.2% increase in fiber digestibility of the diets evaluated for the early cows and a 9% unit increase in digestibility for the mid-lactation cow data sets. Cows in early lactation fed high-fiber digestibility forages consumed 2.6 lb/day more DM ($n = 16$; $P < 0.004$) and produced 2.7 lb/day more fat-corrected milk **(FCM)** than cows fed the lower digestible forage diets. Dry matter intake **(DMI)** was not affected by forage digestibility for mid-lactation cows. Differences in fiber digestibility effects on DMI may be related to stage of lactation. When cows were in negative energy balance, intake was found to be controlled by physical fill when high forage diets were fed (Dado and Allen, 1996). Level of NDF concentration in a diet is negatively correlated to DMI since fiber ferments slowly and stays in the rumen longer than other feed components. However, fiber that is more digestible might stimulate intake as it disappears from the rumen, creating space for another meal sooner. The DMI of mid and late lactation cows, however, is less likely to be limited by physical fill but more by the ability of the metabolic processes of the cow to utilize absorbed nutrients for productive purposes. Therefore, depending on production level, mid and late lactation cows would be expected to respond less to an increase in DMI due to an increase in fiber digestibility (Robinson and McQueen, 1997). Allen and Oba (1996) demonstrated from these studies that a one unit increase in NDF digestion resulted in a 0.51 lb/day increase in milk yield.

More recently Grant (2004) fed cows diets containing forage with 58% NDF digestibility and cows produced 76 lb/day of milk, while cows fed a higher digestible NDF forage (67%) produced only 78 lb/day. When the high producing cows (i.e., >80 lb/day) were separated out, these cows actually produced an additional 6 lb/day of milk when provided the higher digestible NDF forage versus cows producing less than 60 lb/day of milk. Therefore, knowing information regarding forage digestibility is critical as it allows producers the opportunity to allocate higher digestible forages to higher producing cows and accordingly plan harvesting and storage structures.

Forage source will also impact forage allocation to other groups of animals on the farm. Sutherland (1988) demonstrated that as much as half of the particles in the rumen are smaller than the largest particles in the feces. Particles that have low concentrations of fermentable fiber that ferment quickly, such as from alfalfa, might pass more quickly than particles that have more fermentable fiber, which ferment slowly, such as from grasses (Jung and Allen, 1995; Allen and Oba, 1996). If it is assumed that the ruminal retention time is affected by stage of lactation, an early lactation cow may have a ruminal retention for NDF of 30 hours, while that for a late lactation cow approximately 45 hours. The potentially digestible NDF fraction of alfalfa may be nearly digested in the rumen of an early lactation cow while that of grass may only be 65% complete. At lower ruminal retention times, legumes may have greater DM digestibility because of their lower NDF contents and lower NDF digestibility than grasses (Varga et al, 1990). Faster rate of digestion of the potentially digestible fiber for alfalfa may promote greater intake via faster passage rate. However, the grass may have greater NDF digestibility when fed to cows with longer retention times, such as late lactation or dry cows. Grasses, therefore, may have similar or greater digestibility than legumes when offered to cows with longer ruminal retention times. Forage inventories can be varied to accommodate

animals in different physiological states, such as late lactation and dry cows or heifers.

Broderick et al. (2002) demonstrated that DM and nitrogen efficiency, and total tract NDF digestibility, were greater for diets containing ryegrass silage compared to alfalfa silage. However, apparent digestibility of acid detergent fiber **(ADF)** was greater for alfalfa than ryegrass, which led to greater DMI and milk yield for the legume silage. Apparent digestibility of the ADF averaged 63% for ryegrass vs. 43% for alfalfa; however, apparent digestibility of the digestible fraction of ADF was actually greater for alfalfa than ryegrass. This indicated that microbial attack of digestible alfalfa fiber proceeded more rapidly in the rumen, despite higher intakes and presumably greater rate of passage.

In Vivo Versus *In Vitro* or *In Situ* Forage Fiber Digestibility

Due to many confounding factors, it is likely that digestibility of forage fiber measured in vitro or in situ is a better indicator of the potential of forages to enhance DMI than NDF digestibility measured in vivo. The NDF digestibility is a function of the potentially digestible fraction and its rate of digestion and rate of passage. Digestibility of NDF measured in vivo is confounded by different retention times in the rumen, which can be affected by differences in DMI (Oba and Allen, 1999). In addition, exposure to acidic conditions in the small intestine and fermentation in the large intestine in vivo might reduce differences observed for fermentation by rumen microbes in vitro or in situ. For this reason, NDF digestibility measured in vitro or in situ is an important measure of forage quality and should be distinguished from NDF digestibility in vivo. In addition, there is great variability in the estimate of in vivo digestibility, as there are many methods that have been employed throughout the years in many research trials. These include total fecal collection, use of chromic oxide as a marker, indigestible ADF or NDF as a marker, and rare earths that have been sprayed on or adsorbed onto fiber or indigestible fiber (Church, 1993). Rarely is recovery of these markers measured.

There has been a great deal of attention paid to measurement of in vitro NDF digestibility of forages and various corn hybrids, especially in the last 5 to 8 years. In many cases, especially when evaluating NDF digestibility of corn hybrids other than brown midrib **(BMR)** varieties, NDF digestibility differences may vary only by 2 to 3 percentage units among hybrids. When considering the associative effects of feedstuffs and the discussion above regarding the difference in in vitro versus in vivo fiber digestibility, it is not surprising that a production response may not be observed on a farm when in vitro analyses may indicate a 2 to 3% differences in NDF digestibility. In addition, it is clear that grouping of animals can dilute out or enhance the performance or milk component response on the farm.

The use of *in vitro* or *in situ* estimates of forage fiber digestibility is useful and should be continued; however, they have their own limitations (Oba and Allen, 2005). It is important that in vivo estimates of forage fiber digestibility are not related back to in vitro measures. For example, in the data set used by Oba and Allen (1999), the in situ or in vitro forage fiber digestibility of the high NDF digestible forage was 62.9% and for the low NDF digestible forage 54.5%. In that same data set, in vivo estimates of total tract NDF digestibility were also provided and were 54.8 vs. 51.5% for the high versus low NDF digestible diets.

Fiber Requirements

Allen (1997) summarized several studies on the effect of NDF on ruminal pH and found that overall dietary NDF concentration was not correlated with ruminal pH. The concentration of NDF provided by forage as a percentage of DM

had a strong positive relationship with ruminal pH. However, Allen (1997) also demonstrated that fermentability of the fiber portion of the ration was more critical to the amount of acid produced in the rumen than either changing forage NDF as a percentage of DM or total NDF of the ration. Differences in sources of NDF, particle size of the forage, source and amount of nonstructural carbohydrates **(NSC)**, and the interaction among those factors have a large influence on ruminal pH. Due to these and other factors, it is difficult to provide a single value for the minimum concentration of NDF in the ration required to maintain ruminal health. Studies to evaluate minimum fiber requirements of lactating dairy cows were conducted by Clark and Armentano (1993), Colenbrander et al. (1991), and Depies and Armentano (1995). Combined, these studies suggest that when alfalfa is the primary forage source and provides approximately 65 to 75% of the total dietary NDF and corn grain is the predominant starch source, diets with 25% NDF are acceptable and appropriate when the forage is not finely chopped. Few studies have evaluated the minimum amount of NDF needed with corn silage based diets. Similar results were obtained for milk yield when corn silage based diets varied in NDF content from 24 to 29% (Bal et al, 1997). The NDF from corn silage elicits similar or greater chewing times than alfalfa silage (Mertens, 1997). Therefore minimum amount of NDF needed to maintain rumen function when diets are based on corn silage is probably similar or slightly higher than for diets with alfalfa silage assuming particle size is adequate. Forage source along with other factors play an important role in determining fiber requirements of the lactating cow, and this is especially important in early lactation.

The formulation of diets based on NDF of the ration DM has been recommended because of the positive relationship between NDF and rumen fill and the negative relationship between NDF and energy density (Mertens, 1994). A large portion of the fiber in the diet of lactating dairy cows needs to come from forage to maintain rumen function, milk fat percentage, and overall animal health. Previous NRC (1989) recommendations to ensure adequate fiber intake were a minimum of 25 to 28% dietary NDF with 75% of it supplied from forage. Therefore, a minimum recommendation for forage NDF on a DM basis is 18.75% (25% NDF X 75% = 18.75% forage NDF). However, the percentage of dietary NDF from forage might not adequately reflect the presence of effective fiber when by-products feeds that are high in fiber are incorporated into the ration. Even when NDF from forage is used as an index of adequate fiber, particle size (Woodford and Murphy, 1988), and species of forage must be evaluated. When forages are harvested at different stages of maturity, this is especially evident. Hoffman et al (1993) demonstrated that the digestibility of NDF for legumes decreased approximately 20% from late vegetative to midbloom, rate of digestion decreased almost 35%, and the indigestible portion of the NDF increased 30%. The effect of forage maturity on DMI is presented in Figure 1.

Factors Affecting Fiber Digestibility

Fiber digestibility is usually defined as the proportion of ingested fiber that is not excreted in the feces. Fiber contains an indigestible fraction and one or more potentially digestible fractions, each of which is degraded at its own rate. The process of fiber digestion consists of hydrolysis of polysaccharides and the conversion of monosaccharides to volatile fatty acids **(VFA)**, fermentation gasses, and heat (Tamminga, 1993). The rate of hydrolysis is generally the limiting factor in fiber digestion in the rumen (Varga and Kolver, 1997). The rate of hydrolysis is limited by penetration of the enzymes that degrade the cell wall deep into lignin-polysaccharide complexes. The extent of fiber digestion depends on the size of the indigestible fraction and the competition between the rate of degradation and the rate of passage out

of the rumen. Excellent reviews on factors affecting fiber rate and extent of fiber digestibility are available (Mertens, 1994 and 1997; Firkins, 1997; Allen, 1997).

The indigestible fraction of NDF is a major factor affecting the utilization of carbohydrate sources as it varies greatly and may exceed more than one half of the total NDF in the rumen. Glenn and Canale (1990) demonstrated that particulate matter leaving the rumen has a high ratio of ruminally undigestible fiber to digestible fiber. They proposed that the rate grass and legume cell walls reach this ratio might serve as a regulator of particulate turnover from the rumen. Although information on the size of the indigestible fiber fraction of some forages are available, information is still needed on other nonforage fiber sources **(NFFS)**, as well as on the portion of the potentially digestible fraction that is actually digested. The rate at which the potentially fermentable NDF is fermented is another major factor affecting fiber utilization. Though most forages are higher in fiber content than NFFS, some forages can be digested at higher rates than some NFFS (Firkins, 1997). Therefore, replacement of forage sources, such as very high quality alfalfa haylage, for NFFS to reduce fermentation rate in the rumen has advantages.

Varga et al. (1984) fed diets to early lactation cows that were formulated to be low or high in fiber fill value and that had been formulated to differ in rate and extent of NDF digestion. Although cows produced significantly more milk and milk protein on the low fill diet and had almost two-fold fewer kilograms of DM in the rumen, they did not consume more feed than the cows fed the high fill diet. Robinson and McQueen (1997) observed when mid lactation cows were fed forages of varying fermentability and level of concentrate, cows responded by increasing DMI and milk production. The variation in the outcome of these studies can be related to a combination of factors. Milk production potential of the cows was different as was the forage fiber and NFFS used in the rations. In addition, the physiological state of the cows differed. Finally, maybe the most important factor still unknown is the contribution of the indigestible fiber pool on intake, as well as the digestibility of the potentially digestible pool. The main reason for lack of an effect on DMI is probably that small particle potentially digestible NDF may not promote rumination activity and therefore retains much of its bulk characteristics and contributes to rumen fill. Additional research is needed to measure the contribution of forage fiber and various NFFS to total chewing activity and bulk in the rumen and their impact on forage fiber digestibility.

A great deal of attention is paid to in vitro and in situ NDF digestibility information of forages that may have NDF concentration between 35 to 40% on a DM basis. Perhaps as much, if not more, attention should be placed on the other components that clearly contribute energy and protein to the ration. As an example, Varga et al. (1990) and Aldrich et al. (1996) determined the in situ disappearance of all feed ingredients for fiber, starch, and protein of the TMR fed to cows. Using this information on individual feed ingredients allowed for closer prediction of whole animal diet digestibility.

Interaction of Concentrates and Nonforage Fiber Sources on Forage Digestibility

Ruminal fiber digestibility is also affected by the rate of passage of particulate matter out of the rumen. Rate of passage is affected primarily by intake. However, feed particle size, concentrations of dietary fiber and NSC level, and rate of digestion of the potentially digestible fiber fraction may also affect passage rate. Interference of NSC with fiber digestion has been observed frequently, and the main effect is a drop in ruminal pH with a negative effect on fiber digestion (Tamminga, 1993). The effect of starch on fiber digestion does vary with starch source. Replacing corn with barley has been shown

to have a negative effect on fiber digestibility (McCarthy et al., 1989; Herrara-Saldana et al, 1990). When the starch sources, cassava, barley and corn, were studied, cassava and barley starch sources had more of a pronounced effect on the amount of fiber in the rumen over time after feeding (Tamminga, 1993). Apparent digestibilities of fiber were 55.1 and 56.3% for barley and cassava-containing diets, respectively, and 63.6% for the corn-containing diet. Concentration and type of NSC will affect the rate of passage of potentially digestible fiber from the rumen. Many experiments have also shown that NFFS forage sources, such as beet pulp, almond hulls, citrus pulp, and cottonseed, have a positive effect on fiber digestion as fiber concentration in the ration is increased using these fiber sources.

Adding sugar as dried molasses (2.4 to 7.2% total sugar) to diets formulated to contain 60% forage on a DM basis (65% corn silage and 35% alfalfa haylage) resulted in a 4% unit increase in total tract NDF digestibility in Holstein dairy cows (Broderick and Radloff, 2004). In the same paper when adding liquid molasses to provide 2.6 to 10% total sugars, these authors observed an 8% unit increase in NDF total tract fiber digestibility. Sugar source and amount can affect fiber digestibility. Sugar addition to the diet has been shown to enhance fiber digestibility, especially for poorer quality forages (Varga, 2003).

Grinding and pelleting usually results in decreased rate and extent of ruminal fiber digestion (Shaver et al., 1988; Uden, 1988). Although grinding increases the surface available for microbial attack, retention time of the particles is reduced, and the net result is often reduced total tract digestibility. Grinding and pelleting results in an increase in the size of the calculated undegradable fiber fraction and an increased length of the lag phase (Tamminga, 1993). Reduced ruminal pH caused by a decrease in rumination reduces production and flow of saliva which impacts ruminal fiber digestibility by the cellulolytic organisms (Shaver et al., 1988).

Effects of Forage Particle Size on DMI, Digestibility, and Milk Yield

Few authors have observed particle size effects of alfalfa silage on DMI when well balanced rations were fed to mid-lactation cows. Positive effects with reduced particle size on DMI have been reported in some studies feeding corn silage of different particle sizes (Stockdale and Beavis, 1994) but have not been observed in others. Positive effects with reduced particle size have been observed when poor quality forages containing high cell walls were fed (Kusmartono et al., 1996). Although several authors have reported increased DMI with reduced forage particle size while feeding high quality forages (Beauchemin et al., 1997), most authors report no effect on DMI when good quality forage is fed. Taken together, most reports support the hypothesis that DMI is influenced by particle size reduction only when a poorly digestible feed with a high cell wall content is fed, and no effects occur when good quality forages are fed.

For alfalfa based diets, forage particle size has been shown to significantly affect both yield and composition; however, most differences are reported when forage is in the dehydrated form (Shaver et al., 1988; Woodford and Murphy, 1988). When forage is in silage form and different lengths of cut are fed, differences have been observed in milk fat (Shaver et al., 1988; Grant et al., 1990; Fisher et al., 1994) and protein percentage (Beauchemin et al., 1994). No effect of particle size on milk components was observed by Colebrander et al. (1991) when altering forage particle size.

Fiber Sources

Various NFFS, such as soybean hulls, beet pulp, corn gluten feed, whole linted cottonseed, dried distillers grains, and wheat middlings, have been used in the diets of lactating cows to supplement conventional forage fiber. Many of these contain more NDF than some forage sources (Firkins,

1997). One of the major differences between nonforage and forage sources of NDF is particle size. Although differences exist among feedstuffs, nonforage fiber is less effective at maintaining good chewing activity and ruminal health compared with forage fiber of adequate particle size. Allen (1997) demonstrated that NDF from forage was 2.8 times more effective at increasing pH than was NDF from nonforage sources. Firkins (1997) concluded that forage fiber was about 1.6 times more effective at maintaining total tract fiber digestibility than was nonforage fiber. Based on chewing activity, Mertens (1997) presented information that forage NDF was approximately two fold more effective at buffering the rumen environment than nonforage NDF. Therefore, when forage NDF is replaced by nonforage NDF, it is on the average 50% as effective in stimulating chewing activity and/or milk fat percentage as that of forage NDF. For a diet based on forage with adequate particle size and dry corn, the minimum NDF should be 25% total NDF with 75% of the NDF from forage (approximately 19% of dietary DM). A method to calculate minimum concentrations of total NDF and forage NDF and maximums for nonfiber carbohydrates **(NFC)** is presented in the dairy NRC (2001).

What Do We Know About In Vivo Forage Fiber Digestibility for Dairy Cows?

First of all, forage NDF is needed in diets to maximize milk yield, efficiency of feed utilization, and animal health. Forages provide longer particles than other feed ingredients, which are needed to form a rumen mat that entraps smaller particles, thus increasing their digestibility (Allen, 2005). Forage NDF is retained in the rumen longer and is therefore more filling than other feed components. High yielding cows are challenged to meet their energy requirements, and DMI of these cows is limited by the filling effects of diets to a greater extent than for low yielding cows consuming the same diet. Therefore, a greater advantage might be expected for forages with high NDF digestibility when included in high forage NDF rations. Gut fill is more of a limitation to DMI as diet forage NDF concentration increases. Enhanced NDF digestibility of BMR corn silage compared to its isogenic control led to an increase in DMI and milk yield to a greater extent when fed in higher forage (39% NDF) compared to lower forage (29% NDF) diets (Oba and Allen, 2000).

There is some concern, however, when digestibility of forages is improved, as many dairy rations are already formulated for minimum dietary NDF and forage NDF content (25 and 19%, respectively; NRC, 2001) and contain 50% or greater highly fermentable carbohydrates. Therefore, increasing the digestibility of the forage fraction and/or increasing the amount of highly digestible forage into the ration could increase the problem observed when feeding higher concentrate diets. Therefore, a high NDF corn silage might be beneficial if the increased NDF did not limit intake through decreased NDF digestibility and rumen fill. Higher NDF corn silage would allow for a greater incorporation of forage into the ration. Ivan et al. (2005) nicely demonstrated the benefits of replacing a corn hybrid with high NDF and high NDF digestibility for a hybrid with lower NDF and lower NDF digestibility on DMI, milk yield and digestibility in lactating dairy cows. These researchers demonstrated an increase in DMI, milk yield, and total tract NDF digestibility for high NDF corn silage with a high NDF digestibility compared to the low NDF corn silage with a low NDF digestibility. It has been thought that increasing the NDF content of the diet, as was the case for the high NDF corn silage hybrid, would decrease passage rate of the diet, but apparently the higher rate of NDF digestion in the rumen was able to overcome the presumed decrease in passage rate due to higher NDF concentration (Shaver et al., 1988). The wet digesta weight, ruminal volume, and digesta DM and NDF were all lower for the higher NDF diet, indicating that higher NDF digestibility of this diet was decreasing ruminal fill. This all agrees nicely with

the data of Broderick et al. (2002) when they compared alfalfa versus ryegrass silages fed to lactating dairy cows.

Fiber digestibility can be affected by forage source, as well as fermentability of other carbohydrate sources in the ration (i.e., corn). A study recently completed by Brown et al. (2006; Table 1) formulated diets to contain 50% of the ration DM as forage, of which 50% was made up of either alfalfa or grass silage and the remainder as corn silage. Within each forage source, either fine ground corn or coarse corn was evaluated for effects on milk yield and components and nutrient digestibility. Dry matter intake and FCM were significantly higher for the alfalfa silage based diets. However, apparent NDF digestibility was not different between forage sources but was enhanced when corn was finely ground. In situ NDF digestibility was 40% lower for the grass silage, while in situ digestibility of the total mixed diets were not different among treatment and reflected the data observed for apparent NDF digestibility. Though not measured, it is possible, based on previous discussions, that rate of fermentation, rate of passage, rumen fill, and ultimately DMI affected milk yield.

Conclusions

In addition to careful selection of corn silage hybrids for lactating dairy cows, source of forage and level of incorporation into the dietary DM are important areas needed for future research. The inclusion of greater quantities of high quality forages into lactating cow rations is justified and can be accomplished using forage analyses and digestibility information. However, the interaction of level of forage inclusion, forage source, DMI, concentrate sources, and how they are processed clearly impact forage fiber digestibility. Due to many confounding factors, it is likely that digestibility of forage fiber measured in vitro or in situ is a better indicator of the potential of forages to enhance DMI than NDF digestibility measured in vivo. Digestibility of NDF measured in vivo is confounded by different retention times in the rumen, which can be affected by differences in DMI. In addition, exposure to acidic conditions in the small intestine and fermentation in the large intestine in vivo might reduce differences observed for fermentation by rumen microbes in vitro or in situ. For this reason, NDF digestibility measured in vitro or in situ is an important measure of forage quality and should be distinguished from NDF digestibility in vivo.

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Table 1. Effect of alfalfa versus grass silages with coarse or fine ground corn in diets for lactating dairy cows on DMI, FCM, milk components, and nutrient digestibility.[1,2]

Item	Alfalfa Fine Corn	Alfalfa Coarse Corn	Grass Fine Corn	Grass Coarse Corn	Significant effect
DMI, lb/day	61.4	61.4	48.6	48.4	Forage effect
FCM, lb/day	80.7	77.2	64.5	67.5	Forage effect
Milk fat, %	3.66	3.85	3.75	3.71	NS
Milk protein, %	3.11	3.09	3.07	3.03	Forage effect
Apparent DM digestibility, %	57.6	57.3	60.0	52.9	Forage X Corn
Apparent NDF digestibility, %	32.6	29.0	36.9	29.4	Corn effect

[1]Taken from Brown et al. (2006).
[2]DM = dry matter, DMI = dry matter intake, FCM = fat corrected milk, NDF = neutral detergent fiber, and NS = not significant.

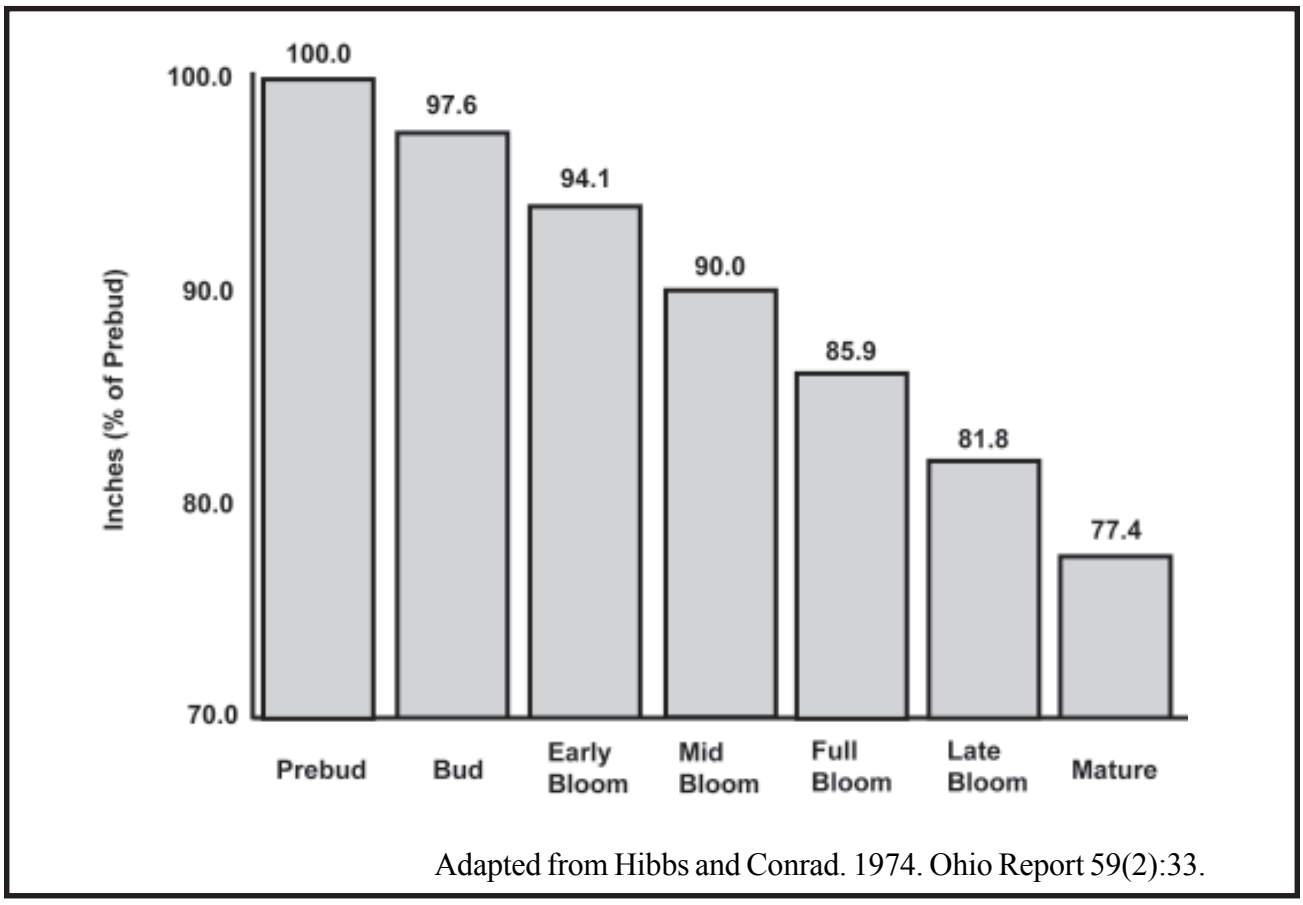

Figure 1. Effect of alfalfa-brome greenchop stage of maturity on dry matter intake.

Starch Digestibility of Corn - Silage and Grain

Jeffrey L. Firkins[1]
Department of Animal Sciences
The Ohio State University

Introduction

Nonstructural carbohydrates **(NSC)** have been a discussed in numerous proceedings from this Conference. Major sources include starch, sugars, and neutral detergent solubles, depending on the type of laboratory measurement made, which also has been widely discussed. Clearly, the amount of NSC fed depends on the amount of effective NDF in the diet and that which was actually consumed and not sorted. Effective NDF can be broken down into the ability of NDF to stimulate chewing to stimulate salivary buffer secretion and that to dilute NSC to prevent excessive rate of volatile fatty acid **(VFA)** production. Moreover, the amount of NSC that should be fed depends on the amount of rumen-degraded protein and other factors affecting the efficiency of usage of energy available from fermentation. Although we know much more in general about these topics, there remains a major limitation in improving our feeding strategies on dairy farms: how do we reliably predict the amount of rumen-degraded starch **(RDS)** reliably and use that information to make rations work more consistently with diverse forages and bunk management capabilities? My major emphases will be to discuss recent research: 1) relating starch digestibility in corn silages differing in processing methods and nutritional qualities and 2) describing how processing of corn grain affects site of digestion. My goal is that this information will help nutrition advisors diagnose differences among farms to help improve the amount or efficiency of milk production under their particular circumstances.

Starch Digestibility and Kernel Processing of Corn Silage

In the past 10 years, there has been considerable research on kernel processing **(KP)**. Building from the foundational work of comparing KP versus unprocessed silages (Johnson et al., 1999), more current research has been documenting interactions in the efficacy of KP among chop length, maturity, and corn cultivars. Weiss and Wyatt (2000) found that KP increased apparent total tract starch digestibility from kernels while still allowing greater length of chop to increase the effectiveness of the NDF portion. The benefit of KP was more pronounced with a conventional hybrid than with a high-oil hybrid, which already maintained higher starch digestibility. If high-oil hybrids increased in frequency of planting, further work would be needed because increasing amylose/amylopectin ratio of corn could decrease starch digestibility in part because of increased chemical interactions with lipid (Svihus et al., 2005).

Based on a series of experiments by Wisconsin and Washington State researchers, it is well documented that KP is more beneficial with advancing maturity of the corn. In fact, the dry matter **(DM)** percentage remains a key diagnostic indicator, even with KP silages (Johnson et al., 2002a). Moreover, increasing DM was highly correlated with vitreousness of the corn kernel in the silage. Vitreousness is a term describing the type of endosperm in the corn kernel. Researchers

[1]Contact at: 223 Animal Science Bldg., 2029 Fyffe Rd., Columbus, OH 43210, (614) 688-3089, FAX: (614) 292-1515, Email: firkins.1@osu.edu

manually fractionate the components of the starch kernel and measure the proportion of starch in the endosperm that is floury versus vitreous. The latter is associated with decreased susceptibility to amylase either originating from microbes or from the animal, in part because of increasing interaction with corn protein. However, when these two hybrids were selected to be different in vitreousness, there wasn't a major difference in ruminal starch digestibility, perhaps because they stated that vitreousness changed from before to after ensiling. In a companion study (Johnson et al., 2002b), the hybrid that was supposed to have kernels that were more vitreous (based on planting strategy) actually had greater apparent ruminal starch digestibilities. When the two different hybrids were harvested at 2/3 milkline, both had very comparable vitreousness values (48% of starch). The KP increased their measure of RDS in both hybrids but more in one than another. Still, differences were relatively minor in total tract starch digestibility. Moreover, data in their paper documented moderate relationships when total tract starch digestibility was regressed against the percentage of post-ensiling vitreousness values, but the low scale for digestibility (only a couple percentage units range) compared against the scale for vitreousness (about 70 percentage units range) and the dispersion (variability) about the regression document little applicability of vitreousness as a predictor of starch digestibility for silages. Further work (Johnson et al., 2003) demonstrated that total tract starch digestibility was primarily a function of the amount of intact kernels remaining in silage, which obviously depends on whether or not the silage was processed.

Ferreira and Mertens (2005) selected 32 samples of widely diverse chemical and physical corn silages being used in the field. The percentage of starch in the particle fraction from a sieve of 4.75-mm (0.187 of an inch) pore size ranged from 8.7 to 100%. They developed an index based on the starch remaining in this sieve (roughly 1/4 or larger of a kernel) to represent slowly degraded starch, which correlated with in vitro disappearance of starch and non-fiber carbohydrate (the by-difference calculation). Although they stated that further in vivo testing is needed, this index should be considered for application. Because the Penn State Shaker tends to separate higher (0.31 of an inch for the middle screen) and lower (0.05 of an inch) than 0.187 of an inch, it is unclear if this index is adaptable to that current Penn State system. In addition, Stone (2004) elaborated on the necessity of calibration of the Penn State shaker system; to be consistent, calibration probably also would be needed for the 4.75-mm index or any other index as it is adapted from the originating lab to any commercial lab (see later comments).

Other sources of variation might not be explained by individual experiments. Cooke and Bernard (2005) documented reasons for varying particle size (theoretical length of cut, TLC) and magnitude of KP (different roller clearance) in the field as farmers try to speed up the time for harvest (Table 1). Decreasing roller clearance from 8 to 2 mm increased total tract starch digestibility, regardless of TLC. However, the combination of the greater clearance and TLC seemed to negate the benefit on NDF digestibility. These latter values are lower than expected and might be a result of the digestibility marker used (indigestible ADF) or from climate (Georgia). However, the interaction of TLC and roller clearance seemed to be largely explained by lower energy-corrected milk and milk efficiency in that last treatment. Clearly, either 1" of TLC is too coarse, unless the extra power and time are used to fully process the kernels. Moreover, it would be expected that results would be further exaggerated when cows are in free stalls and sorting behavior would be worsened with increased chop length. This trial certainly documents why there could be differences among trials due to actual efficacy of chopping and rolling.

Starch Digestibility and Corn Silage Hybrid

Differences in total tract starch digestibility might result from differing types of corn silage hybrids. When a higher fiber hybrid was compared to a conventional hybrid, starch digestibility increased, although part of the response could have been due to varying corn grain concentration in the diet (Weiss and Wyatt, 2002). In a study comparing unprocessed or kernel-processed brown midrib **(BMR)** silage to KP conventional silage (Ebling and Kung, 2004), milk production was only increased by BMR compared with conventional KP silage if the BMR was also KP (Table 2). Total tract digestibility of starch was decreased when BMR silage was not processed, and this result seemed to be related to the number of intact kernels excreted in the feces. In fact, this trend also was seen in the Washington State and Wisconsin studies. Interestingly, if an in situ assay for starch digestibility of manually processed samples was extended too long, this difference was not significant even though the data more closely approximated the in vivo data. Moreover, fecal pH decreased with decreasing total tract starch digestibility, indicating continuing hindgut fermentation. Personally, I don't have much faith in fecal pH as a diagnostic for rumen acidosis because shifting digestion from the rumen to the intestines should decrease pH. The in situ data highlight the need for standardization among protocols like this for improved predictability over a wide range of silages and conditions.

Kinetics of digestion and passage of BMR hybrids (and probably other corn hybrids) need to be taken into consideration. Oba and Allen (2000a,b) factorialized conventional versus BMR silage with dietary NDF level. The BMR treatments decreased the apparent rumen digestibility of starch, perhaps because the BMR hybrid was drier or had less corn grain in the diet. Interestingly, increasing the amount of either silage (i.e., increased NDF) decreased both the digestion and passage rates of starch from the rumen. Clearly, the interaction of silage with rumen turnover is a factor that needs further research (and will be developed more in the next section).

Starch Digestibility of Processed Corn Grain

Considerable work has been done to characterize grain structure, including chemical and physical aspects of starch composition and interrelationship with other components of the kernel (Svihus et al., 2005). Firkins et al. (2001) have quantified differences in corn processing methods for ruminal and total tract digestibility, microbial N flow to the duodenum, and milk production characteristics (Tables 3 and 4). In general, more aggressive processing of corn grain will increase ruminal digestibility of starch, but this tends to be at the expense of ruminal NDF digestibility. There was considerable compensation of digestion in the intestines such that the overall benefit on total tract organic matter digestibility was relatively minor: about 3 to 5 % units might be expected. Previously, I determined that this improvement might provide enough energy to support about 5 lb/day of milk (Firkins, 1997a). However, these studies were nearly all with fixed levels of corn in the diets within studies, and most studies provided low or no corn silage. Therefore, negative rumen effects from highly processed corn might have been overestimated, and any benefits of using less aggressively processed corn grain in heavy corn silage diets was not ascertained. Although we showed that increasing DM intake decreased the percentage of corn grain starch that was degraded in the rumen, increasing intake still increased the amount of starch being broken down and fermented in the rumen on a lb/day basis. Consequently, it is becoming more and more important to balance diets to account for RDS and perhaps diluting highly available starch with slower degrading byproducts, especially when cows are likely to sort against forage and slug feed in free stalls.

Site of Starch Digestion

We must remember that simply shifting starch digestion out of the rumen has several ramifications that might negate its effect. As explained previously, theoretically it is more efficient to digest starch and absorb glucose from the small intestine than to produce VFA, of which only propionate and a minor amount of branched chain VFA can be net precursors for glucose synthesis. However, with the fast passage rates of high producing dairy cattle, most processing methods that decrease RDS also decrease total tract digestibility of starch by 5 to 10% units (Firkins et al., 2001). Also, it is becoming clearer that increasing protein supply to the small intestine increases intestinal digestibility of starch (Abramson et al., 2005). Therefore, shifting too much starch to the small intestine without coupling RDS to energy available for microbial protein synthesis could theoretically further decrease the benefit of shifting starch digestibility to the small intestine. Further, calculations estimating efficiency of energy availability from digestibility of starch in the rumen versus the small intestine might be exacerbated by assuming a constant methane output (Harmon and McLeod, 2001). For example, even if molar proportion of propionate were increased from 20 to 25% of total VFA, this might seem only moderately significant. However, increasing RDS should increase total VFA production. Also, if the corn grain were 1/3 of the diet, this 5% unit increase was actually diluted to the extent that non-grain components of the diet would be fermented to propionate (and not changed by corn processing). With propionate fermentation, there is no stoichiometric possibility for methane being produced. Consequently, I think that increasing RDS probably has a much less negative effect on energy or nutrients available to support milk production than currently projected.

Optimizing Rumen Degraded Starch

The amount of RDS consumed must be maintained to an adequate level to prevent ruminal acidosis and to prevent a major decrease in the efficiency of microbial protein synthesis. The former situation is very obvious to all readers of this paper and won't be addressed (although it is highly important). In contrast with other corn processing methods (grinding, flaking, and rolling) in Tables 3 and 4, there have been several more recent studies reinforcing our summary that feeding high-moisture shelled corn **(HMC)** versus dry ground corn has either decreased the amount of microbial protein flowing to the duodenum or decreased its efficiency (see papers discussed later). Given that protein from soybean meal or other more expensive rumen undegradable protein **(RUP)** sources is more costly than energy from grain and also that microbial protein has an excellent profile of amino acids (NRC, 2001), depressed efficiency of microbial protein synthesis really translates to depressed efficiency of conversion of dietary protein into milk protein (Firkins and Reynolds, 2005). Conversely, increased substitution of beet pulp for HMC linearly decreased the amount of microbial protein flowing to the duodenum (Voelker and Allen, 2003c). Insufficient RDS clearly would therefore limit metabolizable protein for the cow, even though total organic matter digestibility was increased (Voelker and Allen, 2003b). Had those researchers prepared diets lower in crude protein (18.0%), it is tempting to speculate that milk protein yield might have been decreased. Taken in total, both excessive and insufficient RDS should decrease the amount of microbial protein flowing to the duodenum.

Considerations for High-Moisture Corn in Silage and Grain

Balancing diets for an optimum RDS depends on predictions for processed grains (Tables 3 and 4) but also on predicting the RDS from corn silage, reducing forage particle sorting, adequate feeding frequency, etc. Unfortunately, there are not very firm estimates for RDS in corn silage. Consequently, many of you are using or wondering about using in vitro estimates for forages or grains

on your clients' farms. Although Weiss and Wyatt (2002) reported no net benefit for in vitro NDF digestibility compared with summative prediction equations, Oba and Allen (1999) developed a prediction equation for the benefits of increasing in vitro NDF digestibility on DM intake and milk production. I am aware of only one study with published equations predicting improvement of starch digestibility and corresponding feed intake and milk production based on in vitro analyses. Ferreira and Mertens (2005) have several predictions that might be of use for some laboratories. However, I caution that these have not been scrutinized with diverse in vivo data; and even so, when adapting these published equations to commercial labs, considerable care must be taken to standardize grinding procedure, amount of feed incubated, incubation time, and other variables. Taylor and Allen (2005a) suggested that in vitro starch digestibility should be used only as a general ranking of corn grains. With any in vitro procedure, there are considerable differences among run (even within labs and this would be more severe among labs) (Firkins, 1997b). In particular, users should be aware that a standard forage should be run with each in vitro batch to make sure that the results are standardized to an average or common calibration value. Moreover, some Michigan State publications have explained that rate of starch digestibility might not be constant with varying concentrations of starch (i.e., second order kinetics). Therefore, I also conclude that these types of procedures should be used mainly to rank corn silages and corn grains.

Oba and Allen (2003b) reported an interaction in the rate of starch digestibility in the rumen when two levels of corn grain (either dry ground or high moisture) were fed; this would mean that a rate being inputted into a model such as Cornell Penn Minor **(CPM)** might need to be manually changed depending on the feeding level. Also, users evaluating kinetics of grain degradation in situ should be aware that fine-grinding also increases the instantaneously soluble fraction (Rémond et al., 2004). Clearly, the ramifications of trying to transfer information like this into practice would be a real challenge with today's resources. Therefore, despite the increasing sophistication of ration evaluation software, there are still some limits regarding their inputs and the continuing need to retain the services of a good, discerning nutrition consultant.

Rumen Digestion Characteristics of High-Moisture Corn

As stated previously, there have been much more data collected for HMC since data in Tables 3 and 4 were generated. I caution that the data for HMC might exceed your general expectations because HMC sources on farms are probably of lower quality than those from these research trials. When HMC or dry ground corn was fed at 21 or 32% of the diet (Oba and Allen, 2003a,c), the HMC decreased meal size. This corn source was only 63% DM, indicating a very highly available source. Interestingly, they noted an interaction in the average amount of starch consumed per meal. At the lower inclusion level, cows consumed nearly 1 lb of starch per meal. However, at the 32% level, those fed dry ground corn consumed 1.6 lb/meal and for HMC 1.3 lb/meal (a statistical interaction). These data indicate that the amount of RDS impacts meal size. However, the mean ruminal pH still was greater than 6.1, which might seem less indicative of rumen issues. If we stop there, we could conclude that the cow regulates her own RDS (Allen's group has a series of experiments documenting why short-term intake is regulated by the amount of propionate being produced in the rumen to be metabolized in the liver). For my perspective, though, even an average pH of 6.12 was related to over 9 hours in which the pH was below 6.0, and his group has shown that increasing volatility of pH is correlated with reduced efficiency of microbial protein synthesis. This kind of roller coaster up and down swings of pH and energy availability for ruminal microbes caused the efficiency of microbial protein synthesis to be about

20% higher for dry ground corn. In addition, they noted that microbial efficiency was positively related to ruminal starch passage rate, which was faster for dry ground corn than HMC.

Voelker and Allen (2003a,b,c) replaced HMC with beet pulp, which decreased the digestion rate and increased the passage rate of starch from the rumen. This effect might have been a result from decreasing the percentage of starch from HMC grain compared with that from corn silage. This treatment structure shifted starch digestibility from the rumen to the intestines, resulting in no net decrease in total tract digestibility. Efficiency of microbial protein synthesis was not affected, but again, it was negatively correlated to digestion rate of starch and positively correlated with starch passage rate. Increasing passage rate should increase passage of adherent bacteria and increase efficiency of microbial protein synthesis by decreasing the fraction of energy spent on cell maintenance functions. For such a dramatic response in starch digestibility, though, the digestibility of starch from their corn silage must have been very low in the rumen yet high in the total tract. This underscores the need for more definitive information on starch digestion kinetics for corn silage.

When HMC and dry cracked corn were processed to the same particle size, the retention time in the rumen was greater for cracked corn (Krause et al., 2002). They described the effects of particle size and hydration on digestion and passage kinetics. Even though the retention time in the rumen and the total tract was longer for the dry cracked corn, total tract starch digestibility was still lower. This trial is important because it is one of the few to standardize against the effects of particle size. Yet, passage rate did not decrease significantly when semiflint corn grain was processed to have increasing particle size (Rémond et al., 2004). In our review (Firkins et al., 2001), HMC seemed to stimulate chewing time, indicating that the larger corn kernels are residing in the rumen longer and being remasticated. Therefore, the greater potential from low pH resulting from increased ruminal availability of coarser rolled HMC typically fed would be compensated at least in part by increased salivary buffering. Consequently, the risk of acidosis might not be as great as thought, particularly if feed intake or meal pattern are affected. In contrast, nutrition advisors should strongly consider that the amount of microbial protein contributing to metabolizable protein will likely be lower in diets with high amounts of HMC, and protein supplementation might need to be modified for high producers.

Vitreousness of Corn Grain

In addition to increasing the surface area of starch granules, grinding also helps to break up protein complexes that inhibit starch degradation. In particular, corn kernels that have a greater contribution of vitreous endosperm have more zein protein, which is more resistant to proteolytic attack than other proteins that are greater in floury endosperm. Taylor and Allen (2005a) selected two corn grains widely different in percentage of vitreousness (Table 5). The site of digestion was shifted from the rumen to the intestines for the vitreous corn. Because the postruminal digestibility was about 8% lower when expressed relative to that entering the intestine, the total tract digestibility was about 5% lower. In a companion study (Taylor and Allen, 2005b), the vitreous corn tended ($P < 0.12$) to increase efficiency of microbial protein synthesis. Their correlation analysis showed that this efficiency was inversely correlated with starch digestion rate and positively related to ruminal pH and starch passage rate, which would be expected based on other reports from Allen's group. Even though these grains were selected to be diversely different, the differences were within the ranges of values seen for unselected corn grains comprising much of the literature (Firkins et al., 2001). As I interpret these data and compile the MSU results, I conclude that increased vitreousness is not a dramatic problem, particularly in diets with moderate to high

levels of corn silage and with RDS approaching the maximum that can be used efficiently in the rumen. Slowing of ruminal availability of starch should reduce energy spilling and perhaps even provide a vehicle for adherent bacteria to pass from the rumen. A main factor to consider is total tract digestibility of starch and total organic matter, which were 4.6 and 3.3% units different, respectively. In this study, the mean particle sizes of the two corn grains were 1.4 and 1.6 mm.

In another experiment (Rémond et al., 2004), a semiflint corn grain was ground to differing mean particle sizes (0.7, 1.8, and 3.7 mm). Increasing mean particle size of this corn grain decreased the apparent digestibility of starch in the rumen from 58.6 to 49.8 to 35.5%, but there was no compensation in the intestines because total tract digestibility still decreased from 91.4 to 86.0 to 69.5%. In contrast, when dent corn was ground (3-mm screen) or coarsely rolled (0.6 or 3.5 mm), there was a lower difference in rumen (69.8 and 53.5%) and total tract starch digestibility (97.3 and 89.2%), but results from such a large range in particle size were clearly much less than in the previous experiment with semiflint corn. Combined with the previously discussed effects of vitreousness, I conclude that dry corn can be poorly digested in the total tract if it contains a lot of vitreous starch, unless it is ground to be less than about 1.5 mm mean particle size. And then, based on their data, grinding to less than about 1 mm seems to further improve starch digestibility.

There are laboratory measurements that can help improve the characterization of corn grain sources. Vitreousness can be visually appraised, and the assay often reported is from dissecting out the endosperm and determining the starch contribution as a percentage of the total. I recommend that if this procedure is done, then its main purpose would be to help nutrition advisors know when to fine-grind corn. Gelatinization is being evaluated, but I have not seen conclusive published data for a wide range of conditions. However, gelatinization is clearly related to ruminal availability (Svihus et al., 2005), and extrusion of corn seems to increase gelatinization compared to dry grinding. The question remaining to be answered, though, is how well is gelatinization related to total tract digestibility? I would suspect that unless gelatinization potential is moderate to high, the only major result is a shift in site of digestion from the intestines to the rumen with minor impact on nutrition and milk production; if gelatinization potential is low, then it might be a valuable diagnostic, but its use as a predictor might best be used to convince users to grind corn finely. When assessing gelatinization, it is important to note that the sample needs to be taken from the grain actually consumed because starches can undergo retrogradation as processed grain dries and cools (Svihus et al., 2005), and such a process might leave it as bad or worse than before.

Some Considerations for Sugars and Rumen-Degraded Starch

Feeding smaller amounts of molasses and other products with sugars might compensate for slower degradation rate in some corn grains. In the studies by Broderick and Radloff (2004), an optimum amount of about 6 and 5% total sugar was proposed for dried and liquid molasses experiments. However, when I looked at their data (Table 6), I noted that increasing total sugar increased organic matter digestibility much more in the first than the second trial. Assuming 100% digestibility of the molasses product, I used a statistical procedure to estimate the total tract digestibility of organic matter of the HMC that was replaced by molasses products. This substitution procedure predicted the total tract OM digestibility of the HMC to be only about 65 and 89% in the trial with dried and wet molasses, respectively. Even if these values are not absolutely accurate, this exercise indicates that substitution of sugars for grain that is less digestible should provide more benefit than when the grain it replaces is already highly available in the total tract (and by correlation, in the rumen).

Conclusions

Taken together with other studies described, I conclude that there should be an optimum NSC availability in the rumen that is consistent with efficient rumen microbial metabolism. Clearly, the amount of RDS depends on the maturity, DM percentage, and processing of corn silage. Some silages might be lower in RDS than others, so we still need to develop or improve methodology to predict starch availability in silages in a systematic laboratory analysis that will help nutrition advisors to better account for varying RDS. Until more work is done, the Wisconsin index (Ferreira and Mertens, 2005) for particle size should be considered to predict total tract starch digestibility. Vitreousness of corn grain in silage seems to be of relatively little value. In contrast, vitreousness or perhaps gelatinization of dry corn grain should be considered, particularly to help users know when to grind corn more finely. Using these considerations for coarse adjustment of rations, the amount of RDS can be fine-tuned with more slowly available byproducts or increased moderately with small amounts of sugars according to individual herd or group needs. As ration balancing and feeding systems continue to improve in reliability and repeatability, nutrition advisors will still have to use their knowledge of nutrition to continue to keep pace with other feeding management practices.

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Table 1. Interaction of chop length and kernel processing on digestibility and lactation performance.[1]

Item	TLC (inch): / Roller clearance (mm):	¾ None	¾ 2	¾ 8	1 2	1 8	Contrast
Starch digestibility, %		79.4	83.1	75.8	87.7	75.3	R
NDF digestibility, %		20.1	29.7	30.6	35.4	23.2	P, I
DMI, lb/day		48.2	48.0	48.8	48.6	48.2	
ECM, lb/day		81.0	81.0	82.9	83.8	75.7	I
ECM/DMI		1.70	1.72	1.71	1.74	1.56	I

[1]From Cooke and Bernard (2005). TLC = theoretical length of cut.
[2]R = main effect of roller clearance, P = processing effect (first treatment versus average of last four), and I = interaction of TLC and R for the last four treatments. DMI = dry matter intake, ECM = energy-corrected milk, and NDF = neutral detergent fiber.

Table 2. Starch digestibility and milk production by dairy cattle fed processed conventional or brown midrib (BMR) corn silage.[1]

	Control	BMR	
	Processed	Processed	Unprocessed
In situ starch disappearance, %			
3 h	73.9[ab]	77.9[a]	69.9[ab]
12 h	85.7[a]	89.4[a]	74.2[b]
30 h	97.4	97.2	90.7
Total tract starch digestibility, %	99.0[a]	98.7[a]	88.5[b]
Number corn kernels per lb feces	7.3[b]	5.5[b]	36.4[a]
Fecal pH	6.92[a]	6.97[a]	6.81[b]
Dry matter intake, lb/day	51.5[b]	57.0	53.9[ab]
Milk, lb/day	91.1[b]	97.5[a]	93.5[ab]

[ab]Means in the same row with dissimilar superscripts differ statistically ($P < 0.05$).
[1]From Ebling and Kung (2004).

Table 3. Ruminal and total tract digestibilities of nutrients and duodenal flow of microbial N by lactating dairy cows fed different corn sources.[1]

	Rumen, %				Total tract, %		
Corn Source	Starch, Apparent	NDF	OM, True	Microbial N, g/day	Starch, Apparent	NDF	OM, Apparent
Dry, cracked or rolled	44.6	48.1	52.3	276	85.0	52.0	66.6
Dry, ground	52.3	44.9	48.6	257	90.7	49.0	67.8
Dry, ground finely					91.4	51.2	69.8
Steam-rolled					88.8	49.8	67.2
Steam-flaked	56.9	41.9	52.8	296	94.2	48.2	68.6
High-moisture, rolled	86.8	47.1	60.1	236	94.2	50.0	71.9
High-moisture, ground					98.8	50.4	73.9

[1]Adjusted for effects of experiment and other significant variables (Firkins et al., 2001). All data are on an apparent basis (not accounting for endogenous or microbial contributions) except organic matter (OM) digestibility in the rumen. Note that 56.9% for steam-flaked corn is probably too low (should be around 65%), as explained in the paper.

Table 4. Lactation performance for Holstein cows fed different corn sources.[1]

Corn Source	DMI, lb/day	Milk, lb/day	Protein, %	Fat, %
Dry, cracked or rolled	49.5	68.0	3.09	3.59
Dry, ground	50.8	69.3	3.18	3.53
Dry, ground finely	48.2	71.3	3.02	3.49
Steam-rolled	48.6	70.2	3.10	3.49
Steam-flaked	50.2	71.5	3.10	3.36
High-moisture, rolled	49.9	71.5	3.17	3.54
High-moisture, ground	50.8	74.6	3.17	3.37

[1]Data are adjusted for effects of experiment and other significant variables (Firkins et al., 2001). To interpret these data for milk, for example, the actual data were scaled to an average dry matter intake (DMI).

Table 5. Differences in digestibility between floury and vitreous corn grains in dairy cattle.[1]

	Floury	Vitreous
Vitreousness, %	3.0	67.2
Starch disappearance in vitro, %/hour	7.7	1.8
True starch digestibility in the rumen, % of intake	62.1	46.3
Apparent postruminal digestibility		
% of intake	39.3	56.8
% of duodenal flow	90.8	83.6
Apparent total tract digestibility, % of intake	96.3	91.7

[1]All means were $P < 0.05$ except disappearance rate (statistics not done). Taylor and Allen (2005a).

Table 6. Digestibility and lactation performance when high-moisture corn grains was replaced by increasing levels of dried or liquid molasses in two trials.[1]

	Dried, % of DM					Liquid, % of DM				
	0	4	8	12	Contrast	0	3	6	9	Contrast
Total sugar, % of DM	2.4	4.2	5.6	7.2		2.6	4.9	7.4	10.0	
pH	5.63	5.80	5.66	5.82	NS	6.07	5.90	6.02	6.06	NS
OMD, %	58.8	60.1	61.1	63.1	L	64.7	63.1	66.4	65.1	C
NDFD, %	37.5	37.8	38.6	41.1	L	36.6	36.3	44.6	37.2	L
DMI, lb/day	55.7	56.5	57.9	57.2	L	55.9	61.8	57.4	59.0	Q,C
Milk, lb/day	83.6	82.5	85.6	80.7	C	95.9	100.1	96.8	93.3	Q
3.5% FCM, lb/day	90.6	92.8	93.9	88.7	Q	101.2	102.7	96.8	93.3	L
Protein, lb/day	2.62	2.53	2.64	2.44	C	2.90	3.15	3.01	2.84	Q

[1]From Broderick and Radloff (2004). NS = not significant, L = linear, C = cubic, and Q = quadratic responses ($P<0.05$). OMD = organic matter digestibility, NDFD = NDF digestibility, DMI = dry matter intake, and FCM = fat-corrected milk.

Dairy Nutrition and Air Quality

David K. Beede[1]
Department of Animal Science
Michigan State University

Introduction

The time is fast approaching when dairy farmers and their nutritionists will be more obligated than in the past to manage and even reduce gaseous air pollutants and odors in their farms. Some emissions are considered harmful to the environment or to human health. Air quality standards as a result of the Air Quality Consent Agreement will come from the U.S. Environmental Protection Agency **(EPA)** in accordance with the federal Clean Air Act **(CAA)** enacted in 1990. Additional new rules may be set by state or local governments.

In the long run, as environmental certification becomes a more and more important (if not mandatory) global trading factor, compliance with worldwide air quality standards will be crucial for the U.S. dairy industry to be competitive. Investing today in the search for solutions to reduce air emissions will have environmental and potential economic benefits tomorrow. Doubtless, proper nutrition will play a significant role in minimizing emissions to the furthest extent biologically possible, now and into the future.

The objectives of this paper are: to provide a short background on the current pursuit to establish air emission standards for animal agriculture; to briefly summarize information about air emissions currently considered important in regulation, and thus, to the U.S. dairy industry; and to briefly review known nutrition and feeding strategies that have been suggested or employed to reduce specific air emissions from livestock or dairy operations.

Background

Why air quality and animal agriculture?

Although, the federal CAA was enacted in 1990, it typically has not been enforced within animal agriculture by federal or state agencies. However, the Act also did not specifically exempt agriculture or concentrated animal feeding operations **(CAFO)** from regulation or compliance. Through a complicated set of evolving circumstances over the last 20 to 30 years involving both changes in rural demographics (e.g., many more people with little or not previous experience or acceptance of the odors associated with animal agriculture) and relatively recent increased concentrations of animals and use of new manure collection and storage systems in some farms, odors have become an unavoidable social and political contention. Whereas odors generally are addressed at the local level (e.g., township, county, and state) through nuisance laws (often with some protection of animal agriculture from nuisance lawsuits, depending on the state), other gaseous emissions are known to have broader specific negative impacts on the environment and human health. The latter category of emissions are typically thought to have consequences in greater spatial dimensions, and thus, have received more focused attention and actions of federal and in some cases state legislation

[1]Contact at: 2265K Anthony Hall, East Lansing, MI 48824, (517) 432-5400, FAX: (517) 432-0147, Email: beede@msu.edu

and regulatory agencies based on the specific gaseous species emitted, their relative perceived or estimated impacts, and the amounts emitted (Tables 1 and 2).

Air Emissions of Interest

Gaseous emissions generally can be classified in two categories based on spatial scale (Table 1; NRC, 2003): those with which concerns are local and those which have potential regional, national and global impacts.

Gaseous emissions of local concern are those which raise issues, often with neighbors, most frequently regarding a number of compounds emitting human-detectable odors affecting quality of life (whether perceived or real), hydrogen sulfide **(H_2S)**, and particulate matter **(PM)** which include particles with properties that cause haze and affect human health. Volatile organic compounds **(VOC)**, although perhaps not presently considered a significant or primary health or environmental concern, are important because they may be odorous and because the EPA must monitor their concentrations according to the CAA.

Gaseous emissions with potential regional, national, or global impact and major concern include atmospheric deposition of ammonia and the haze it causes, atmospheric deposition and haze produced by nitrous oxide (N_20), and the effects on global climate of NO_x (nitric oxide + nitrogen dioxide) and methane.

On the national and global scales, agriculture and particularly animal agriculture is considered a major emitter of ammonia (50% of total) and N_2O (25% of total), and a significant (18% of the total) emitter of methane in the U.S. (Table 2). Additionally, about 25% of the nitrogen (N) in dairy manure is lost as ammonia emission with current manure management practices (Pinder et al., 2003 as cited by Broderick, 2005). Based on model estimates from 100 years of data, only fossil fuel combustion and production, industrial processes, and landfills rival animal agriculture in air emissions (Table 2; van Aardenne et al., 2001). Additionally, reliable (quantified) estimates of animal agriculture's contributions to VOC (and odor) have not been established, yet work is under way currently to determine VOC contributions from dairy operations.

To better understand and explore the potential for reduction of air emissions from animal agriculture systems, a summary of the chemicals and their transfer and conversion characteristics is useful (NRC, 2003).

Ammonia ***(NH_3^+)***

Agricultural animals are the single largest source of ammonia (Table 2) and ruminants are the single biggest contributor among farm livestock to overall ammonia-N emissions (Bouwmann, et al., 1996). Ammonia results from hydrolysis of urinary urea (the main N-containing excretory product of ruminants) via microbial urease which is ubiquitous in manure and in the environment. When the ammonia is emitted into the air, it can be converted (hydrolyzed) into ammonium (NH_4) and removed from the atmosphere by dry or wet deposition. Once removed from the atmosphere, both chemical species contribute to ecosystem fertilization, acidification, and eutrophication, and can impact visibility, soil acidity, stream acidity, and natural and cultivated aquatic and terrestrial ecosystems' biodiversity and productivity (Galloway and Cowling, 2002). Ammonia also contributes indirectly to the formation of $PM_{2.5}$ (particles with diameters up to 2.5 microns) via airborne ammonium salt crystals.

Nitrous oxide ***(N_2O)***

Nitrous oxide is formed through microbial nitrification and denitrification and contributes to depletion of stratospheric ozone and increased global warming. Animal agriculture contributes significantly (Table 2).

Nitric oxide ***(NO)***

Direct emission of nitric oxide from animal systems is estimated to be small (compared with fossil fuel combustion and refinement/production; Table 2). However, N-fertilizer applied to cropland soils in dairy systems can result in the emission of NO. Nitric oxide and nitrogen dioxide (signified together as NO_x in the literature) are readily inter-convertible and removed from the atmosphere by dry and wet deposition. The NO_x is an important precursor of ozone production and aerosol nitrate is a contributor to $PM_{2.5}$ and N deposition as HNO_3.

Methane ***(CH_4)***

Methane (CH_4) is produced through anaerobic fermentation in the rumen and anaerobic digestion of manure. When emitted into the air, which currently happens with the vast majority of the world's methane production, methane is an important greenhouse gas contributing to global warming. In the U.S. and globally, animals are believed to contribute about 18 and 29% of total methane emissions, respectively; fossil fuel burning and landfills both contribute more or similar proportions (Table 2).

Volatile organic compounds ***(VOC)***

Though not well-quantified from animal operations at this time, VOC from livestock operations include organic sulfides, disulfides, aldehydes of 3- through 7-carbon lengths, trimethylamine, C_4 amines, quinoline, demethylpyrazine, short-chained organic acids, and aromatic compounds. The quantitative significance of VOC emissions in dairy production systems is not yet known but suspected to be significant. The CAA requires that EPA monitor VOC from industrial operations, which will include some or all livestock (dairy) farms once the standards are set (discussed below).

Hydrogen sulfide ***(H_2S)***

Hydrogen sulfide is formed during the anaerobic reduction of sulfate in aqueous solutions and suspensions, and decomposition of S-containing compounds in manure. Once released into the atmosphere, H_2S is oxidized to sulfur dioxide and removed by wet or dry deposition from the air. On a global basis, it does not appear that hydrogen sulfide has much environmental impact. However, effects in more highly concentrated animal feeding systems are of special interest because hydrogen sulfide also has been used as an odor indicator in some instances (e.g., in Minnesota).

Particulate matter ***(PM)***

Particulate matter indirectly or directly occurs from livestock operations through animal activities, housing fans, incorporation of air into materials from scruf, soil, and manure, and conversion of aerosols of ammonia, nitric oxide, and hydrogen sulfide to crystalline forms. Respiratory health from deposition in the airways of animals and humans and visibility can be affected deleteriously by both $PM_{2.5}$ and PM_{10}.

Odors

Odors result from a variety of compounds emitted from animal operations. They have been difficult to quantify but include, among many other compounds, VOC and perhaps hydrogen sulfide. Nonetheless, they are of significant local societal concern in some areas and likely will continue to be

the focus of environmental research and regulation if humans continue to spread into agricultural areas and animal production units continue to increase animal densities and chemical (nutrients and waste products) concentrations.

U.S. Animal (Dairy) Industries' Current Engagement

Various legal actions have challenged animal agriculture to comply with standards set as part of the 1990 federal CAA. Currently, CAFO are asked to comply with CERCLA (the Comprehensive Environmental Response, Compensation, and Liability Act) that covers ammonia and hydrogen sulfide emissions and EPCRA (the Emergency Planning and Community Right-to-Know Act) that addresses monitoring of ammonia and hydrogen sulfide emissions. The CAA also specifies the monitoring of VOC, particulate matter (PM of up to 10 microns in diameter [PM_{10}] and $PM_{2.5}$ up to 2.5-micron diameter particles, and total suspended particulate [TSP]), and NO_x (various N-oxygen species).

Based largely on the paucity of reliable information from which to develop workable standards for air emission (NRC, 2003), **e**arly in 2005 the EPA announced the Air Quality Consent Agreement between EPA and some major segments of the U.S. livestock industry with the following objectives: to monitor emissions; to develop protocols for emission monitoring from various livestock production operations (varying in size, animal species, and housing and management systems); to determine what sizes of operations (within livestock species) are likely to exceed regulatory thresholds; and to determine what enforcement will be required (EPA, 2005). The national swine, layer (poultry), and dairy industries have engaged to participate in the Consent Agreement to gather air emissions data. Very sizable investments have been budgeted by each participating industry to collect needed emission data, with hopes of gaining some additional information about mitigation of emissions later in the study period. To date, other major segments of the U.S. livestock industry (e.g., beef, broiler, turkey, and sheep) have not engaged.

Dairy industry investment

In 2005, a task force of dairy producers from across the U.S. facilitated through the National Milk Producers Federation (NMPF) met several times to determine the best ways for the dairy industry to engage in the EPA Consent Agreement efforts. In January 2006, the U.S. Congress approved a one-time amendment to the National Dairy Promotion Act to allow the National Dairy Board (NDB) to authorize (only for fiscal year 2006) expenditure of funds from the national dairy check-off to address environmental and public health considerations. Following, the NDB voted to authorize $6 million to fund air quality research to help establish baseline standards of air emissions from some types of U.S. dairy farms and to explore strategies to help mitigate a portion of emissions as part of the EPA Consent Agreement. The identity of the six dairy sites around the U.S. has not been publicized, and the actual on-farm measurements have not begun as of this writing, late March 2006.

Doubtless, the stage is set. Likely, some significant portion of the U.S. dairy industry will have to respond to regulations of CAA — even with the multitude of variable conditions such as different housing, management, and manure storage and application systems — once the baseline standards are set from work through the Consent Agreement.

Potential Strategies to Reduce Emissions

In general, 5 on-farm operational categories are considered to reduce air emissions from dairy operations: housing system; manure handling, treatment, and storage; manure disposal, distribution and land application; conversion of the components

of manure into value-added products; and emission mitigation through nutrition and feeding management. Considering the previously listed compounds released into the air from dairy operations, what can be done to reduce the rate and amounts of various emissions? To address this question, the following must be considered: the potential relative amount of emission (relative to that from other sources of the compound, e.g., in Table 2); the potential environmental impact of the emitted compound; the potential relative emphasis in both small and larger spatial scale regulatory actions; and the potential for success in mitigation as well as risk if mitigation proves difficult. Starting to address emission mitigation at the beginning of the nutrient and emission compound flow— with the nutrition and feeding of the animals— would seem logical.

Nutritional Strategies to Reduce Emissions from Dairy Farms

The no brainer

To varying extents, livestock producers have often over-fed some nutrients and energy relative to animals' nutritional requirements to be more certain that requirements were actually met and because of real or perceived issues with variability in operational practices (e.g., ability to repeatedly mix and deliver rations accurately and precisely through time) to meet requirements. Feeding in excess of true nutrient requirements will not minimize air emissions. For example, crude protein **(CP)** supplied in the diet in excess of cows' requirements can have profound effects to increase N losses and ammonia release from manure (Swensson, 2003).

Broderick (2005) showed in one experiment using diets with CP concentrations varying from (13.5 to 19.4%, dry basis) and typical midwestern feeds that, in general, there were no improvements in actual milk yield (ranged between 80 to 85 lb/cow/day), fat-corrected yield (ranged between 75 to 81 lb/day), or milk protein yield with more than 16.5% CP. Similar responses were observed in other work when CP concentration of the ration exceeded that needed to meet requirements. It is not uncommon for the CP concentration in practical rations to exceed that needed (NRC, 2003). Dairy producers, nutrition consultants, and extension professionals have as an immediate tool the ability to reduce excess dietary CP as a way to reduce N emissions from dairy operations. The related topic of efficiency of dietary protein utilization will be mentioned subsequently.

Precision feeding

Perhaps, the second most obvious strategy to reduce excretion and air emissions of N is to group animals by productivity level or other distinguishable characteristic (e.g., gender or body weight) to improve dietary N utilization. Grouping dairy cows into separate production/management groups decreased N excretion by 6% compared with feeding all lactating cows the same ration (St-Pierre and Thraen, 1999).

Increase efficiency of nutrient and energy utilization

Feed efficiency (3.5% fat-corrected milk yield/unit of feed dry matter [DM] consumed) has gotten special recent attention as a nutritional management monitoring tool to help optimize nutrient and energy utilization and profitability (Hutjens, 2005; Shirley, 2006). Nutritional efficiency certainly is not a new concept to dairy producers, nutritionists, or scientists. However, improving the efficiency of nutrient utilization for environmental management and reduction of excretion of pollutants (e.g., unutilized nutrients or their components or byproducts of energy metabolism) into air and water will receive even greater management attention in the future. Efficiency equals nutrient or energy in usable product divided by nutrient or energy intake. Thus, a reduction of the denominator or an increase in the

numerator will enhance efficiency - for example, increasing the nutrients in milk per unit of intake (e.g., milk protein/protein consumed).

Dairy nutritionists and researchers have worked for a long, long time to improve the efficiency of dietary N utilization. It is well known that the amount of metabolizable protein and the profile of potentially absorbed essential amino acids are very important. In addition to ruminally synthesized metabolizable protein, dietary protein as rumen undegraded protein **(RUP)** and rumen-protected methionine or lysine, when in short supply, limit lactational performance and the overall efficiency of dietary N utilization (NRC, 2001; Noftsger and St-Pierre, 2003; Broderick, 2005; among many others). The resulting inefficiency of N utilization increases emission of N-containing compounds, especially ammonia from urea excretion.

Whereas some progress has been made, brilliant (e.g., greater than 30 to 35%) improvements in the efficiency of ration protein conversion to milk protein seem unlikely and certainly will not happen until we achieve a much greater basic understanding of the influences of various feed and animal variables (e.g., intake rates, types and interactions of feed carbohydrates and proteins, etc.) on ruminal fermentation kinetics (e.g., pool sizes, flow and turnover rates, and microbial protein synthesis rates) and animal performance to affect N excretion.

Increasing productivity to reduce relative emissions (e.g., reduce emissions per unit of edible food produced).

Van Horn et al. (1994) used a modeling approach to illustrate conversion of dietary N to milk N and the excretion of N as affected by level of herd productivity. The overall conversion of intake N to milk N ranged from about 25% to nearly 30% as milk yield per cow per year increased from 18,000 to 26,000 lb, respectively. Concomitantly, the absolute amount of N excreted per unit of milk produced decreased from about 6.5 g/lb to about 5.5 g/lb; about a 15% reduction in excretion. This analysis illustrated the potential power of high productivity, at presumably similar (fixed) biological and operational maintenance inputs, to lessen emissions per unit of edible product.

Thus, some effective management practices that increase herd productivity also might be expected to reduce N excretion per unit of milk produced. Jonker et al. (2002) examined a set of dairy management practices with modeling in combination with survey data of 454 dairy farms in the Chesapeake Bay Basin. On average, this set of dairy farms fed nearly 7% more N than recommended by the National Research Council (NRC, 2001), resulting in a 16% increase in urinary N and nearly a 3% increase in fecal N. The overall efficiency of conversion of dietary N to milk N was 28.4% (standard deviation = 3.9). The following herd management tools (some expected to increase lactation performance per cow) reduced N losses in manure per unit of milk N produced: use of bovine somatotropin; routine use of milk urea N testing; use of a complete feed; management of the photoperiod with artificial lighting; and being a member of the Dairy Herd Improvement Association. Factors in their analysis that did not affect conversion of dietary N to milk N included use of a total mixed ration, milking three times per day, seasonal calving, use of cover crops, and having a nutrient management plan for N.

In another study, Dunlap et al. (2000) demonstrated that increasing milk yield of dairy cows by bovine somatotropin **(bST)**, 3-time versus 2-time/day milking, and increasing photoperiod with artificial lighting reduced manure N excretion by 16% for a given amount of milk produced.

Increased productivity, often gained through more intensively managed herds, actually does not increase air emissions, but in fact, can result in a considerable decrease in waste nutrient excretion

per unit of milk produced - even considered in a herd size-neutral context. Emissions per unit of edible product may be a logical index to consider as a standard for regulatory compliance. However, until actual air emission data from actual farms with known (definable) environmental and herd characteristics are known, it is unlikely to receive much consideration.

Methane

To this point, the discussion has focused mainly on N. However, of the approximately 19.8 lb of carbon consumed daily by a lactating cow (66 lb/day of milk), about 40% is expired as carbon dioxide and about 3% is converted to methane (Pfeffer and Hristov, 2005). However, the increase in methane emissions associated with U.S. and global animal agriculture is of potential concern because this molecule is chemically relatively stable in the atmosphere and contributes to the increase and acceleration of global warming.

To date, only modest advancement has been made to reduce methane emissions from dairy cattle. In Canada, Sauer et al. (1997) studied the effects of feeding monensin (ELANCO, Greenfield, IN) (24 ppm of dietary dry matter (DM) after a gradual step-up introduction of monensin over 1 week) by lactating dairy cows (n = 88 to 109) fed a TMR (corn silage, alfalfa haylage, hay, and concentrate in DM proportions of 30: 26: 9: 35, respectively) continuously. Methane and CO_2 emissions were sampled and measured continuously with infrared gas analyzers in the tie stall barn environment under a practical management routine. In the first trial, monensin reduced methane production by 21% per cow during the 3 week period it was fed compared with the previous period of over 3 months before monensin-feeding. Also, improved feed conversion efficiency, increased milk production, reduced ruminal fluid ratio of acetate to propionate (A:P), and reduced milk fat percentage were observed. Monensin was then removed from the diet for a period of about 160 days. When monensin was reintroduced in a second trial (67 of the cows that had received monensin in the first trial plus 21 cows that had calved recently and had never received monensin), there was neither a significant reduction in methane production, ruminal A:P, milk fat percentage, nor an increase in milk yield and feed conversion efficiency due to monensin. The authors suggested that there might be some adaptive mechanism that is not understood and that rotating use of different ionophores (if and when approved) or other feed additives might be helpful.

Other nutritional factors known to reduce methane production include type of dietary carbohydrate and higher concentrations of concentrate feeds (e.g., grains with more nonstructural carbohydrates reduce methane production compared with forages). Also, methane production from ruminants can be reduced by feeding more digestible (higher quality) forages by harvesting at earlier stages of maturity or processing methods that increase digestibility (Johnson and Johnson, 1995). Supplemental fat is known to reduce the amount of methane produced. Similarly, the quantity of feed consumed also affects methane production. Each of these strategies also reduces the maintenance subsidy per unit of edible product relative to the quantity of methane produced.

Odors

To date, most concerns over air emissions related to animal agriculture have been evidenced through state-level discussions about odor regulations (Powers, 2003). There are numerous compounds that are known to impart human-detectable odors if in sufficient concentrations in air. For example, over 330 odor-causing compounds have been identified in swine manure (Schiffman et al., 2001). Comparable data have not been found for dairy facilities (NRC, 2003); however, far fewer odor-causing compounds likely be expected. Thus far, nutrition or feeding strategies backed by sound,

unbiased research to appreciably reduce odors have not been advanced.

Other emissions

The amounts of hydrogen sulfide and VOC emitted from dairy operations (from the animals themselves plus the manure handling and storage systems) have not been quantified; research is under way.

Summary

- Dairy producers and nutritionists will have an important role and increasingly important function in air quality management in commercial dairy farms in the Tri-State area.
- Concerns about gaseous emissions exist in two categories. Concerns with odor, VOC, H_2S, particulate matter, and haze are raised locally; whereas, ammonia, nitric oxide, and nitrogen dioxide emissions are strong greenhouse gases of concern for our regional, national, and global climates.
- In 2005, the EPA announced the Air Quality Consent Agreement with U.S. animal agriculture aimed at studying emissions from farms. Several animal industries have agreed to participate to gather scientific data for use in setting standards, including the national dairy industry (via the National Dairy Board), which pledged $6 million to fund air quality studies.
- On-farm operational categories considered to reduce air emissions from dairy operations are: housing systems; manure handling, treatment, and storage; manure disposal, distribution, and land application; conversion of the components of manure into value-added products; and, emission mitigation through nutrition and feeding management.
- Some nutrition strategies for reducing emissions include not overfeeding livestock, precision (targeted group) feeding, increasing efficiency of nutrient and energy utilization, and increasing productivity.

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Table 1. Proposed relative importance (in rank order within spatial scale) of air emissions from animal feeding operations based on the NRC (2003) special committee's scientific evaluation.[1]

	Spatial Scale		
Emission	Local – property line, or nearest dwelling	Global, national and regional	Primary Concern
Ammonia (NH_3)	Minor	Major	Atmospheric deposition, haze
Nitrous oxide, N_20	Insignificant	Significant	Global climate change
NO_x[2]	Minor	Significant	Atmospheric deposition, smog
Methane (CH_4)	Insignificant	Significant	Global climate change
VOC[3]	Minor	Insignificant	Quality of human life
Hydrogen sulfide (H_2S)	Significant	Insignificant	Quality of human life
PM_{10} (μm)[4]	Significant	Insignificant	Haze
$PM_{2.5}$ (μm)[5]	Significant	Insignificant	Health, haze
Odor	Major	Insignificant	Quality of human life

[1]Rank order (high to low) of concern = major, significant, and insignificant.
[2]NO_x = nitric oxide + nitrogen dioxide ($N0_2$).
[3]VOC = volatile organic compounds.
[4]PM_{10} = particulate matter includes particles with aerodynamic equivalent diameters up to 10 micrometers.
[5]$PM_{2.5}$ = particulate matter includes particles with aerodynamic equivalent diameters up to 2.5 micrometers.

Table 2. Annual estimated percentages and total amounts of some air emissions from most known sources in the United States and globally in 1990.[1]

Source	NH_3-N		N_2O-N		NO-N[2]		CH_4-C		VOC-mass[3]	
	U.S.	Global	U.S.	Global	U.S.	Global	U.S.	Global	U.S.	Global
	% of total emissions									
Agriculture										
Agriculture and natural land	36	29	25	33	5	14	1	18	NA	NA
Animals	50	49	25	33	1	3	18	29	NA	NA
Biomass burning										
Savannah burning	0	4	0	3	0	8	0	2	0	3
Deforestation	0	3	0	0	0	3	0	2	0	4
Energy										
Fossil fuel combustion + production	0	0.2	25	7	88	58	53	29	42	37
Biofuel combustion	7	5	0	3	1	4	1	5	4	17
Industrial processes	0	0.5	25	17	1	4	0	0	49	31
Waste										
Agriculture waste burning	4	3	0	3	3	6	2	4	5	8
Landfills	4	6	0	0	0	0	24	11	0	0
Total amount of source, Tg[4]	2.8	43.4	0.4	3	7.6	36.6	30.9	239.7	24.3	181.1

[1]Adapted from NRC (2003) as adapted from van Aardenne et al. (2001). Percentages may not sum exactly to 100% because of rounding. NH_3-N = nitrogen in ammonia; N_2O-N = nitrogen in nitrous oxide; NO-N = nitrogen in nitric oxide; CH_4-C = carbon in methane; VOC = volatile organic compounds; and NA = not available. The H_2S emissions are not available for the level of disaggregation shown for other emission species, but they are small relative to other sulfur sources (e.g., SO_2 from fossil fuel combustion) on a national and global basis. They might be important on a regional basis in some areas.

[2]Estimates of NO emissions from manure applied to fields vary substantially. Reported values for the fraction of manure nitrogen lost as NO have been as high as 5.4%, but 2% was selected as a mid-range value in the calculations (uncertainity is about a factor of two).

[3]VOC = volatile organic compounds; quantities of these emissions are not available for agricultural sources except agriculture burning.[4]Tg = 1 teragram = 1 million metric tonnes.

Current Status of Amino Acid Requirement Models for Lactating Dairy Cows

M.D. Hanigan[1] and J. Cyriac
Department of Dairy Science
Virginia PolyTechnic Institute

Abstract

The lactating dairy cow is fairly inefficient at converting dietary nitrogen to milk protein. When cows are fed to National Research Council (NRC, 2001) requirements, they convert approximately 25% of dietary nitrogen to milk protein. There may be some inherent limitations to the efficiency that can be achieved by a ruminant; however, examination of the NRC model with respect to available data suggest several areas where requirement or supply predictions could be improved. Ruminally degradable protein **(RDP)** requirements appear to be too high. Reducing these requirements would allow reductions in dietary protein and improved animal efficiency. Amino acid **(AA)** flow to the small intestine appears to be predicted with good accuracy provided diets are within current NRC requirements for RDP. Absorption of AA from the gut lumen is not constant across AA within an ingredient or across ingredients as assumed by NRC. Thus, absorbed AA may be predicted with bias. The gut tissues as represented in the portal-drained viscera and the liver remove approximately 2/3 of the available AA on a daily basis, and this removal is dependent on supply. Variable use by these maintenance tissues violates the NRC assumption of fixed maintenance use and introduces a significant bias in our current predictions. Mammary AA removal is highly regulated and counters supply, i.e. as supply declines, transport activity increases and vice versa. Variable transport activity with respect to AA supply violates the NRC assumption of fixed conversion efficiencies for absorbed AA and introduces bias into predictions. Thus, our ability to successfully balance diets for adequate AA while minimizing dietary nitrogen inputs is currently hampered by a lack of data with respect to AA digestibility in the small intestine and poor representations of postabsorptive use of AA. Amino acid digestibility coefficients for individual ingredients are needed to address the first challenge and an alternate representation of postabsorptive use and recycling of AA is required to address the second challenge.

Introduction

Production per cow continues to increase annually (APHIS, 2002). Much of this improvement is associated with genetic gain. However, management and nutrition must keep pace with genetic gain to allow expression of full genetic merit. Certainly, there is constant economic pressure to identify nutritional and management factors that may be limiting cow performance and correct those deficiencies.

In many areas of the country, including the mid-Atlantic region, environmental pressure has also increased. The Chesapeake Bay Watershed is an environmentally sensitive area with large concentrations of livestock (Gollehon et al., 2001). Considerable emphasis is being placed on reducing nitrogen **(N)** and phosphorus influx into the watershed. A significant component of these efforts

[1]Contact at: Blacksburg, VA 24061, (540) 231-0967, FAX: (540) 231-5014, Email: mhanigan@vt.edu

is related to nutritional management of production species, including the dairy cow.

Nitrogen required by the ruminant to synthesis products and maintain the animal is derived from the protein synthesized by microbes in the rumen and from undegraded feed sources. As compared to other species, the dairy cow appears to be fairly inefficient at converting dietary N to milk N (Figure 1). However, this may simply reflect our lack of knowledge in the area. Animals in the U.S. are generally fed to requirements as set by NRC (2001). As these requirements reflect our knowledge base, the fact that current N efficiencies are low is not necessarily indicative of an inherent limitation in the species relative to N efficiency. It is possible our requirements are set too high relative to the real needs of the cow, causing us to overfeed N.

Over the past 100 years, investigators have derived dose-response relationships between animal performance and dietary N inputs. Such work has demonstrated that requirements must be considered relative to both ruminal and postabsorptive needs. Ruminal requirements reflect the needs of the microbes inhabiting the rumen and the postabsorptive requirements reflect the animal needs. Thus, the overall N needs of the animal are comprised of the N available in the rumen for microbial use, and the N or protein that escapes the rumen in the form of undigested feed protein and as microbial protein and becomes available for animal use.

These requirements historically have not reflected the need for specific AA by ruminal microbes or by the cow. The most recent release of the NRC (2001) provides predictions of absorbed essential AA and suggestions regarding the quantity of absorbed lysine and methionine required to meet animal needs, and thus reflects some progress in the area.

This review will summarize some key components of that progress and highlight some deficiencies in our current knowledge base and in our requirement system. As identification of deficiencies in our prediction systems is critical to making further progress, more time will be spent discussing these. This should not be construed as a condemnation of our current systems. They have served the industry well and continue to do so today. But to improve these systems for the future, it is critical to understand the deficiencies. Additionally, such knowledge helps users of the current system recognize the potential limitations of the system so they can avoid making mistakes when using the system.

Ruminal Nitrogen Metabolism and Requirements

Mammalian species do not possess the ability to digest and absorb structural fiber, e.g. fiber insoluble in acid detergent. However, ruminants possess a large population of microbes that reside in the rumen that are able to ferment fiber as well as other nutrients. As the end-products of fermentation represent approximately half of the energy supply of the animal, this is a critical component of their digestive system. Another product of this microbial growth is microbial protein which represents approximately half of the N absorbed from the digestive tract of the ruminant. Thus, microbial growth and metabolism are an essential component of ruminant metabolism, and the nutritional needs of these microbes must be considered when designing diets if cows are to achieve their genetic potential for production. In general, this equates to maximizing or attempting to maximize microbial growth as that will result in the greatest nutrient supply (energy and protein) to the animal when significant forage is included in the diet. Greater energy densities can be achieved on low or zero forage diets, but such diets are inconsistent with long-term cow health in a dairy production setting.

Microbial N Requirements

To maximize microbial growth, mixed ruminal microbes appear to require peptides, free AA (Argyle and Baldwin, 1989), and ammonia (Roffler and Satter, 1975). All 3 of these entities can be generated via protein degradation, which occurs in an ordered process (protein - peptides - AA - ammonia) via microbial activity (Figure 2). As peptides, AA, and ammonia are all generated from ruminally degradable N, one can aggregate the requirements for the 3 entities into a single RDP or ruminally degradable N requirement. In taking this approach, one must recognize that the apparent RDP requirement will reflect the most limiting nutrient of the 3 N classes (peptides, AA, or ammonia). Thus, there is potential for improved ruminal N efficiency if the individual requirements are better reflected in requirement systems so that feeding programs can be devised to meet each requirement independently.

The RDP supply is assessed using duodenally cannulated animals and can be estimated from the solubility and degradation extent of dietary proteins. The standard for the latter, as set by NRC (2001), is the in situ or in sacco technique. The challenge with this method is that it requires the use of a ruminally cannulated animal and has significant cost. Samples cannot be assessed in real-time, and thus, it is not always possible to determine RDP content prior to ingredient use. Expected mean values have been derived from existing literature and tabulated (NRC, 2001). These values are commonly used in formulation, although they do not necessarily reflect the true value of the ingredient being fed.

As most ruminal microbes can synthesize all 20 primary AA, the requirement for AA appears to reflect a need for amino acid N rather than a given AA, per se (Atasoglu et al., 2003). Work in the 1970's suggested that branch-chain AA (leucine, isoleucine, and valine) or their keto-acid precursors could limit microbial growth (Bryant, 1973). Addition of branched chain keto-acids that can be converted to branched-chain amino acids were observed to increase fiber digestion, microbial protein production, and microbial growth efficiencies (Russell and Sniffen, 1984). However, subsequent work was equivocal and a comprehensive study at the Univ. of Illinois showed no significant effects on a broad range of biological processes (Klusmeyer et al., 1987).

Recent work with methionine analogs has hinted at a potential methionine or methionine metabolite requirement (Noftsger et al., 2005). But, statistically significant responses have not been observed in all studies (Noftsger et al., 2003). Responses to other AA have not been consistently observed. Therefore, excepting a potential role for methionine analogs in supporting microbial growth, it appears that given an adequate supply of total AA, mixed microbial populations can synthesize the mix of AA needed to support growth and metabolism.

In addition to total AA supply, some populations of microbes appear to require AA in peptide form. Addition of peptides to ruminal fluid has been observed to increase fiber digestion, microbial protein production, and microbial growth efficiencies (Russell and Sniffen, 1984). In vitro work has demonstrated responses to individual peptides (Argyle and Baldwin, 1989); however, in vivo concentrations of peptides appear to be much greater than those required to maximize growth (Chen et al., 1987). Given the huge number of potential peptides that could exist, it is a daunting task to test all of them for growth stimulation effects, and thus, one cannot rule out the potential for discovery of one or more peptides that would stimulate microbial growth rates in the rumen. However, based on current knowledge, the known AA and peptide requirements of mixed ruminal microbes appear to be much lower than prevailing ruminal concentrations of these substrates when typical dairy diets are fed.

Work by several investigators has clearly identified a requirement for ammonia by mixed ruminal populations and further work associated this requirement with the fiber digesting bacterial population (Bryant, 1973). Thus, failure to meet minimal ammonia requirements could compromise microbial yield and fiber digestibility, leading to a loss in animal productivity. The work of Satter and colleagues (Satter and Slyter, 1974; Roffler and Satter, 1975; Satter and Roffler, 1975) demonstrated that this requirement was met at a ruminal ammonia concentration of approximately 5 mg/dl, which occurred at a dietary crude protein **(CP)** level of 14%. This requirement could be met by provision of ruminal degradable protein or of non-protein N **(NPN)** sources such as urea. However, provision of the latter in amounts that resulted in ammonia concentrations greater than 5 mg/dl would not result in incorporation of NPN into microbial protein, i.e. it would be absorbed as ammonia and excreted in urine as urea.

Ruminal ammonia can also be derived from urea that has been transferred from blood to the rumen. The balance of absorption of ammonia from the rumen to blood and blood urea to the rumen determines whether the ruminal NPN balance is positive (ammonia N absorption exceeds urea N influx) or negative (urea N influx exceeds ammonia N absorption). The balance of N across the rumen wall has been observed to be 0 when ruminal ammonia concentrations were approximately 9.5 mg/dl (Remond et al., 2002), and thus, a net influx of N occurs from blood at concentration below that range. This helps buffer ruminal ammonia concentrations, helping prevent a deficiency when low protein diets are fed.

Although ruminal ammonia requirements of 5 mg/dl are well supported by the work of the Satter group, Klusmeyer et al. (1990) observed no loss of fiber digestibility or animal performance when diets with 11% CP were fed to lactating cows, even though ruminal ammonia concentrations were as low as 2 mg/dl. Thus, there are apparently certain dietary conditions that will support maximal microbial growth and fiber digestibility at ruminal ammonia concentrations much less than the commonly accepted requirement. Undoubtedly, movement of blood urea into the digestive tract is a key component of this ability. Such a mechanism is not represented in the NRC model (NRC, 2001), and thus, it cannot be used to design diets, such as those of Klusmeyer et al. (1990) for use in a production setting.

As noted above, requirements for peptides, AA, and ammonia can be expressed in aggregate as a RDP or N requirement. The RDP requirements have been derived by NRC (2001) and found to generally range from 9.5 to 10.5% of dietary DM. It would appear that such a requirement range is clearly adequate given the accuracy in predicting microbial yield (Figure 3). However, they may also be excessive as there are few observations in the literature where diets less than 9.5% RDP were fed. For example, if the true requirement were 7% RDP, then any diets with greater than 7% RDP would result in equal microbial growth as RDP is not a limiting nutrient. The low protein diets of Klusmeyer et al. (1990) had predicted RDP contents of 6.6 and 5.7% of DM as calculated from NRC (2001), and these diets did not appear to compromise microbial flow to the small intestine or fiber digestibility as compared to a diet with 8.7% RDP. We have recently tested RDP contents ranging down to 7.6% RDP (571 g/day deficit) and found no significant effects of RDP on milk production at 8.8% RDP (280 g/day deficit) and only a trend for a reduction at 7.6% RDP. Thus, it would appear that microbial needs for RDP are clearly met with diets containing 8.8% predicted RDP, likely met with 7.6% RDP, and may be met under some cases at 5.5% predicted RDP. As reduction in RDP feeding could be achieved by reductions in CP feeding, there is significant room for improving the N efficiency of the dairy cow if the current requirement system more accurately reflected ruminal N metabolism.

Ruminally Undegraded Nitrogen

Accurate predictions of ruminally undegraded protein **(RUP)** and microbial protein flows to the small intestine are required to accurately predict duodenal flows of AA. Predictions of these flows have apparently improved over time (Figure 3) reflecting the steady increase in knowledge. Current predictions of RUP and microbial N flows have a prediction error of approximately 20 and 17%, respectively. These prediction errors appear to be partially offsetting as the prediction error for total duodenal flow is approximately 10%. Relative to absorbed protein supply, the latter error reflects the current status of our knowledge, and this is well within the expected biological variation for that measurement.

Unexplained variation in RUP flow presumably arises from the 2 key components of the system: 1) estimates of the intrinsic protein degradation rate for individual ingredients and 2) estimates of the rate of passage. Error in either of these estimates will result in biased estimates of RUP and RDP.

Passage rate equations were developed by NRC (2001) from existing data; however, the accuracy of the equations was not reported. Driving variables were reported as dry matter intake **(DMI)**, the percentage of concentrate in the diet, the neutral detergent fiber **(NDF)** content, and the class of feed where the latter were wet forage, dry forage, or concentrate. The NRC committee (2001) recognized the potential affects of particle size and density, functional specific gravity, processing, and the rumen environment as potential factors influencing passage rate and cited the lack of data density relative to these effects as the limitation in considering them in prediction equations. As little data exists relative to these effects on passage rate, it is unlikely that significant improvement in passage rate prediction accuracy will occur in the near future.

Another contributing factor to RUP errors is our limited ability to evaluate the intrinsic rate of protein degradation in the rumen. In sacco techniques are laborious and difficult to standardize across evaluation runs. Some of the variation may be associated with differences in DMI. Bateman et al. (2005) developed a linear adjustment method that significantly improved predictions of non-microbial N flow at the duodenum. This adjustment method if used when reporting RUP values may improve the accuracy of the system.

Current RUP assessment methods also do not account for interactions among ingredients. It is not clear to what extent such interactions influence rates of degradation, but the amount of CP and RDP offered appears to influence RUP content. Klusmeyer et al. (1990) observed a reduction in RUP flow from 28 to 21% of intake N as dietary protein was reduced from 14.5 to 11.0% via replacement of soybean meal with ground corn. In both cases, observed RDP content (72 and 79%, respectively) was significantly greater than the constant 60% predicted for both diets by NRC (2001). Further gains in prediction accuracy for RUP flow will require more accurate methods of assessing both the intrinsic rate of degradation and the rate of passage.

Amino Acid Flow from the Rumen

As microbial protein and RUP represent greater than 80% of the total protein flow to the small intestine, accurate predictions of the flows of these proteins and the AA composition of them is required to predict AA flow from the rumen. Thus, predictions of these AA flows would be subject to the same errors noted above for the parent proteins. However, for diets that meet NRC (2001) requirements, the accuracy of predicting flow of AA to the small intestine appears to be quite good (NRC, 2001). This assessment is supported by the work of Pacheco and Lapierre (2004) that demonstrated reasonably good correlations among

essential AA predicted to be absorbed from the digestive tract by the NRC (2001) and the observed appearance of the same AA in the portal vein. Thus, it would appear that our ability to predict the supply of AA for diets with RDP contents greater than 9% is fairly good. However, for diets with RDP contents less than NRC requirements, predictions will likely be in error given the apparent over-prediction of RDP needs of microbes.

Animal Requirements

Protein that passes from the rumen is digested to AA and peptides and mostly absorbed from the digestive tract, although evidence of absorption of peptides from the omasum exists (Matthews and Webb, 1995). After absorption, the AA are released into portal blood and eventually into general circulation where they are available for all tissues to utilize for maintenance and productive purposes.

Although the animal requires AA, they are not convenient to measure. An estimate of the aggregated AA content of the diet or a feed can be derived by measuring the N content with corrections for a NPN sources in the feed. This will yield no information relative to the AA composition of the feedstuff, but given the knowledge that feed AA generally have a mean N content of 16% allows one to estimate the overall AA content. In a similar manner, microbial protein that can be used by the animal is estimated from the total N content with corrections for NPN components. Absorbed protein that is utilizable by the animal is labeled metabolizable protein.

Current metabolizable protein requirements are calculated as additive functions of that required for maintenance, growth, reproduction, and lactation with minimal consideration of the AA composition required for each of those functions. Maintenance is assumed to remain fixed, regardless of the level of production. Growth, reproduction, and lactation are derived by multiplying the protein deposited in product by a conversion factor to account for inefficiencies. The partial efficiency assumed by NRC (2001) for milk protein synthesis is 65%, which is significantly greater than the partial efficiency observed for lactating cows (Figure 4). So there are some intrinsic limitations in our current representations of post-absorption N metabolism. Exploration of these limitations provides a framework for future progress. As most of the limitations of our current systems revolve around the aggregation of individual AA requirements into a metabolizable protein requirement, the remainder of the discussion will focus mostly on AA metabolism.

Although some AA can be synthesized by post-absorptive tissues, 10 essential AA (EAA) cannot be synthesized in quantities adequate to supply needs. These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. And some of the nonessential AA can only be synthesized from EAA. Thus the EAA must be obtained from gut absorption in quantities adequate to support inherent losses associated with metabolism and net deposition in tissue and milk protein.

In conducting dose:response experiments to determine metabolizable protein requirements, a variety of different ingredients were used, and thus a range of dietary AA inputs occurred. This approach ensured that individual AA requirements are generally met as any deficiencies would have been observed as a deficiency in metabolizable protein. Of course, there may be dietary conditions where one or more EAA are not provided in adequate quantities, but this should be a rare occurrence.

Although there are probably limited opportunities for increasing production through manipulation of AA content on N sufficient diets, there is likely significant progress that can be

achieved in N efficiency by reducing the input of AA that are provided in excess of needs. This could be achieved by reducing the amount of protein in the diet and supplementing with the AA that are most limiting to production. Such an approach has been adopted by the swine and poultry industries. Identification of the limiting AA can be achieved by trial and error, but given that all 10 EAA must be considered and the large number of ingredients fed to ruminants, testing for limitations of each AA and combinations of them under all dietary conditions would be an overwhelming task. Thus, it is critical to develop an understanding of the metabolism of at least the EAA in order to derive a model that will allow predictions of limitations.

While AA absorption capacity in the intestine is not thought to be limiting, individual AA are digested with varying efficiencies, and the efficiency for any given AA varies by ingredient (Figure 5). Such variation is not reflected in the current NRC (2001), and data appear to be inadequate to derive robust prediction equations. Thus, the constant digestion coefficient assumed by NRC (2001) likely does not reflect all the variation for absorbed AA. Such variation may explain a portion of the variation in absorbed AA relative to predictions observed by Pacheco and Lapierre (2004).

Recent work has suggested that the portal-drained viscera **(PDV)** catabolizes the equivalent of 1/3 of the AA that are absorbed on a net basis from the gut lumen (Hanigan et al., 2004b). This catabolism occurs primarily from blood and is responsive to blood concentrations and, to a lesser extent, blood flow. Hepatic tissue has been found to catabolize another 1/3 of the absorptive supply, and as for PDV, this catabolism is responsive to blood concentrations and blood flow (Hanigan et al., 2004a). But, removal by the splanchnic tissues is not constant for all AA, and thus, the composition of the post-splanchnic AA is altered relative to absorbed AA (Figure 6). Thus, the activity of the splanchnic tissues (PDV plus liver) results in the clearance of approximately 2/3 of the AA available to the post-absorptive tissues, and most of this clearance and use is derived from blood supplies rather than directly from the absorption stream of AA.

More importantly, these tissues represent the maintenance component of NRC (2001), and their AA clearance rates are not fixed as assumed in that model. As the absorbed supply of protein from the gut lumen increases, clearance of AA from blood also increases. This leads to significant bias in predicting AA availability for productive use in the current NRC (2001) as the maintenance component is not fixed as assumed in that system. Rather, it appears to be a variable component that is a function of the balance of AA supply and use for productive purposes.

Other work has demonstrated that the mammary AA transport activity for several EAA is adjusted to help buffer deficiencies or excesses in AA availability (Bequette et al., 2000). For example, when animals were made histidine deficient, they increased their histidine transport activity more than 40-fold. This buffered the drop in mammary histidine uptake that would have occurred due to declining arterial concentrations. At the same time, the transport activity for other EAA declined, thereby reducing their removal so that the composition of the EAA taken up by the udder remained very similar to the histidine sufficient state.

Variable AA extraction efficiencies by mammary tissue result in variable rates of return of AA to general circulation where they are subject to clearance by the splanchnic tissues. This explains the variable efficiency of N use for milk production as summarized by Hanigan et al. (1998) and is consistent with the apparent linkage of post-splanchnic AA supply and mammary AA use (Bequette et al., 2003). But, it also violates the assumption of a constant efficiency of conversion of AA to product used in construction of the NRC (2001) model.

Work examining the relationships between AA supplied to the mammary and milk protein production has demonstrated that the oft-used first-limiting AA model of milk protein synthesis explains very little variation in milk protein synthesis. Clark et al. (1978) demonstrated that multiple AA could be limiting at the same time. This observation was consistent with the work of Hanigan et al. (2000), indicating that representations of protein synthesis as a function of the first-limiting AA explained very little of the observed variation in a large data set including AA infusions. The data set contained a number of observations where a single AA was infused which introduced independent variation. The first-limiting model appears to work when used with data derived from protein infusion or dietary protein manipulation experiments. The lack of independent variation in AA supply imposed by infusion of complete proteins does not allow identification of the problem. Thus, this approach may appear to work for dietary manipulations, but bias is being introduced into those predictions which reduces model reliability. A more accurate representation of the process is needed for use in ration balancing software.

In summary, predictions of AA flow at the duodenum have improved in accuracy tremendously over the past 20 years and are likely adequate for field use. Additional improvements in supply predictions could be achieved by accounting for interactions among nutrients and ingredients. Absorption of AA from the intestinal tract varies by AA and ingredient, and this variation is not currently represented in our prediction systems. The splanchnic tissues catabolize significant quantities of AA and thus represent a major source of AA loss. Mammary tissue has the ability to change its expression of AA transport capacity to match its needs for AA. Our requirement models need to be updated to account for variable absorption rates of AA, variable use of AA by splanchnic tissues, and variable efficiency of use by mammary tissue. In the absence of such updates, our ability to design diets to maximize milk production and N efficiency will be hampered.

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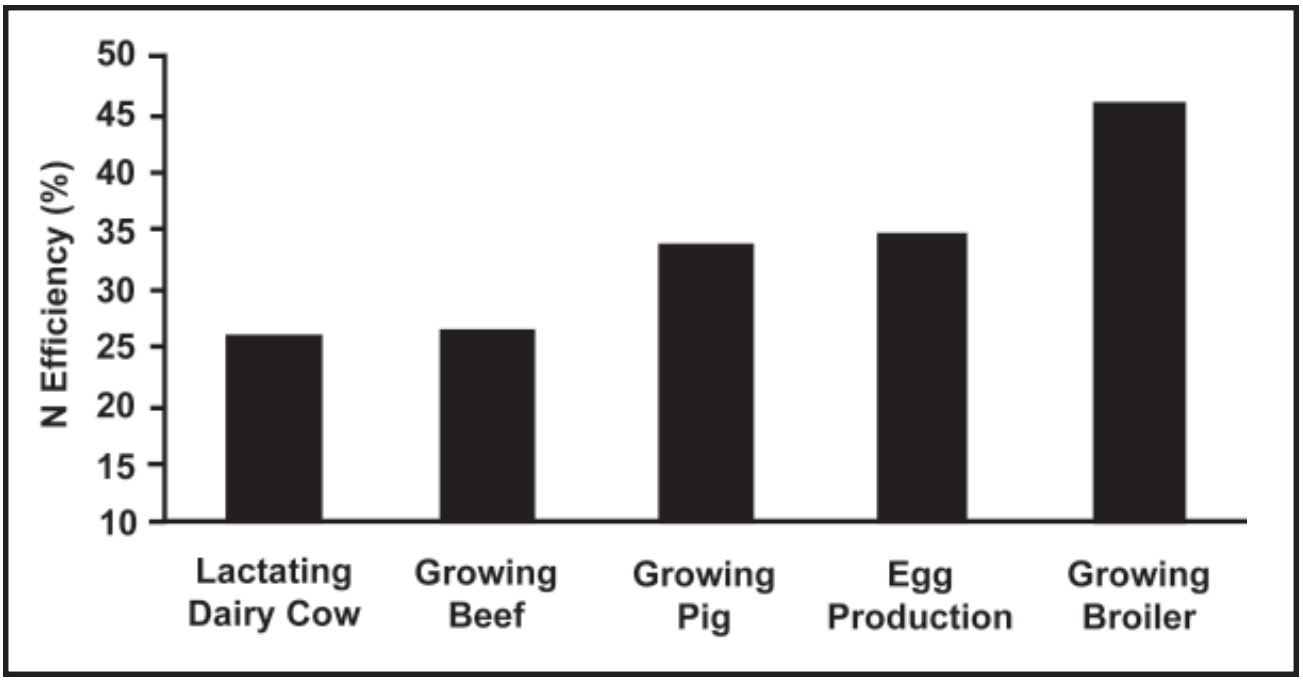

Figure 1. Efficiencies of conversion of dietary nitrogen (N) to product N (meat, milk, and eggs) in various species. Adapted from Bequette et al. (2003).

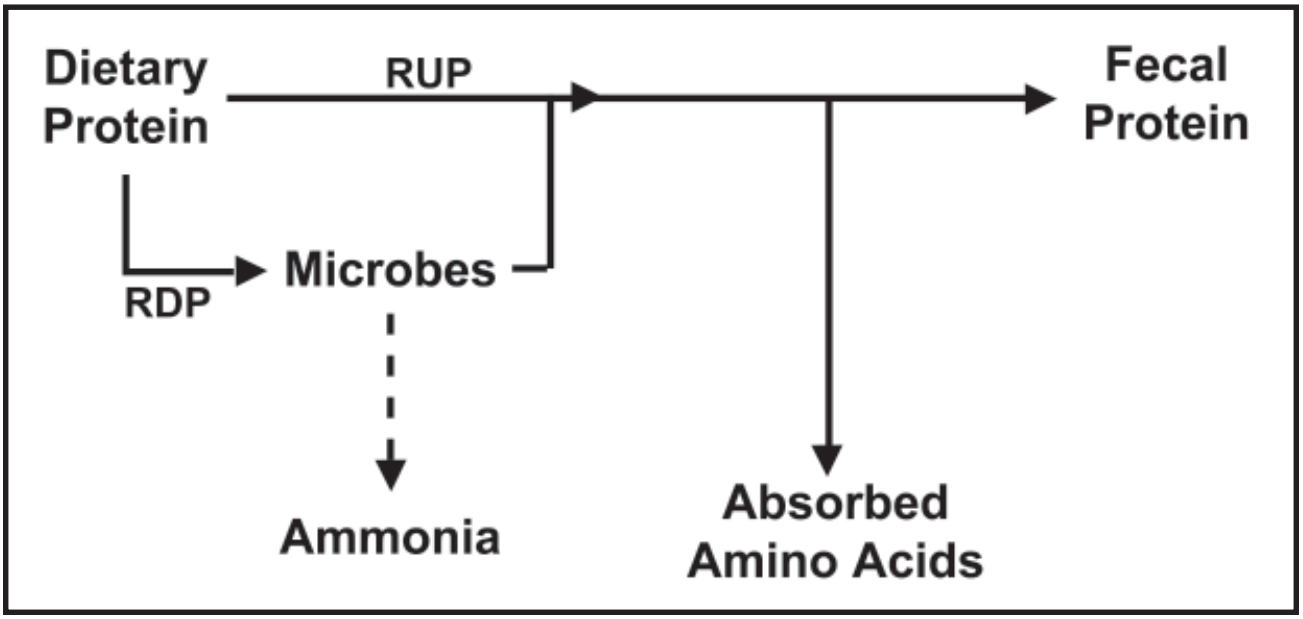

Figure 2. Schematic of protein and nitrogen flows in the digestive tract of cattle. RUP and RDP represent ruminally undegradable and degradable protein, respectively.

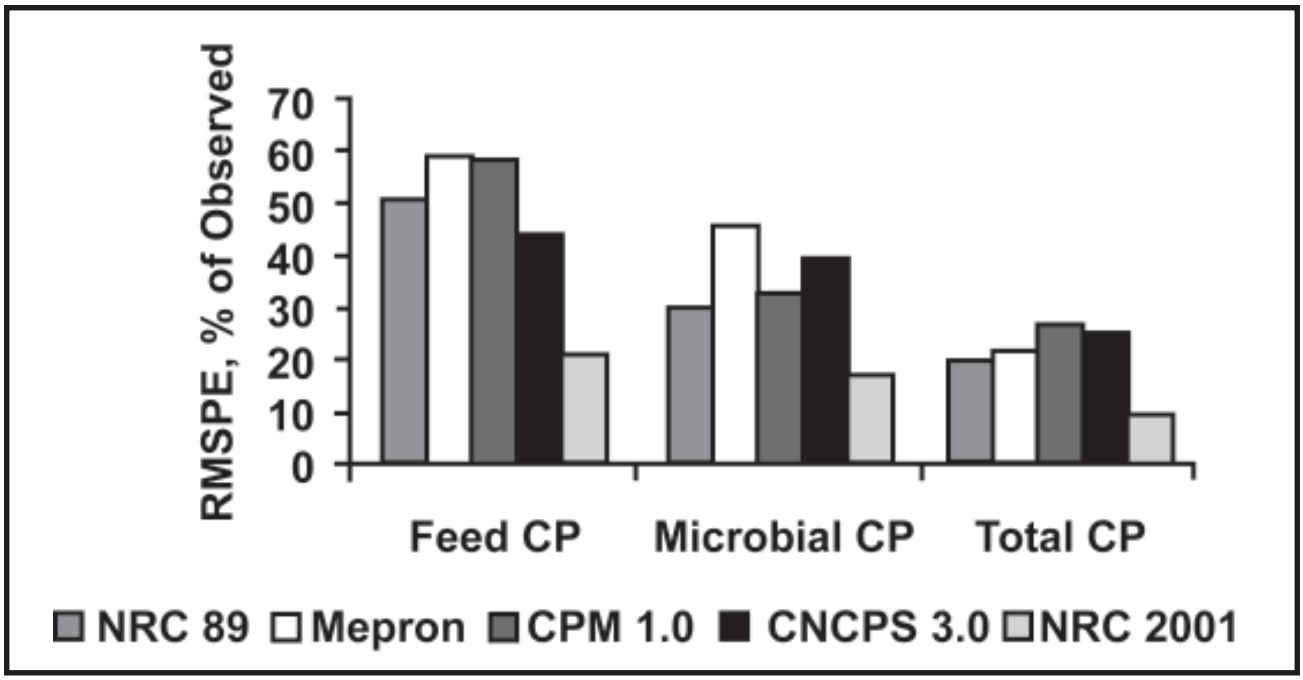

Figure 3. Prediction errors for several dairy models used in the industry. Entities predicted were total, microbial, and feed crude protein (CP) flows to the duodenum of lactating dairy cows. Errors are expressed as root mean square prediction errors (RMSPE). Adapted from Bateman et al. (2001) and NRC (2001). NRC = National Research Council, CPM = Cornell-Penn-Miner program, CNCPS = Cornell Net Carbohydrate and Protein System, and Mepron refers to a commercial model developed by Degussa, Inc (Parsippany, NJ).

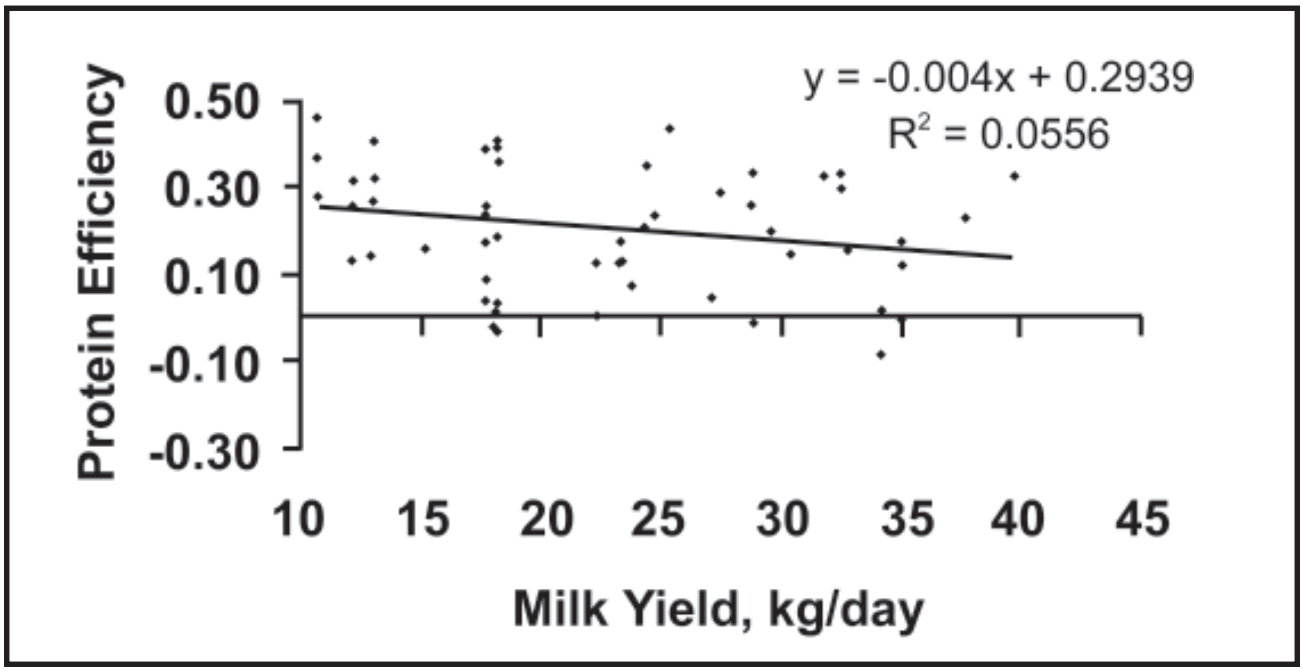

Figure 4. The efficiency of use of infused casein for milk protein synthesis. From Hanigan et al. (1998).

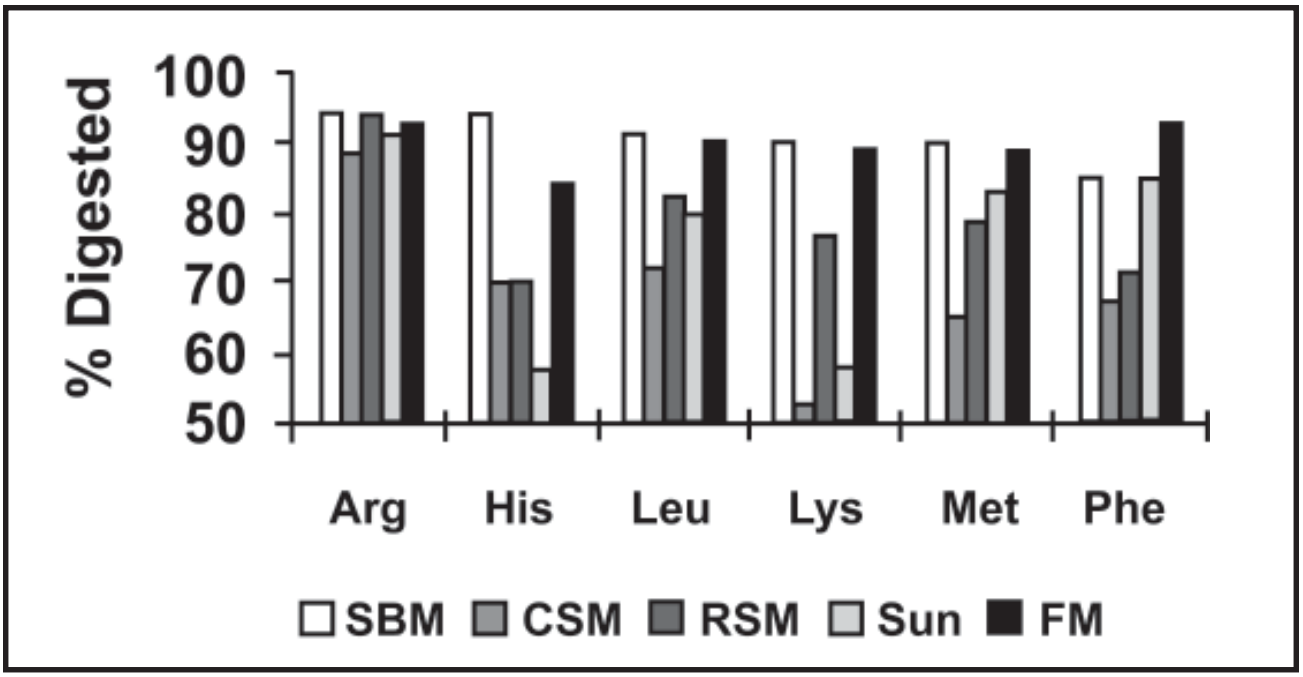

Figure 5. Amino acid digestion of various ingredients in the small intestine. Adapted from Hvelplund and Hesselholt (1987). Arg = arginine, His = histidine, Leu = leucine, Lys = lysine, met = methionine, and Phe + phenylalanine. SBM = soybean meal, CSM = cottonseed meal, RSM = rapeseed meal, Sun = sunflower meal, and FM = fishmeal.

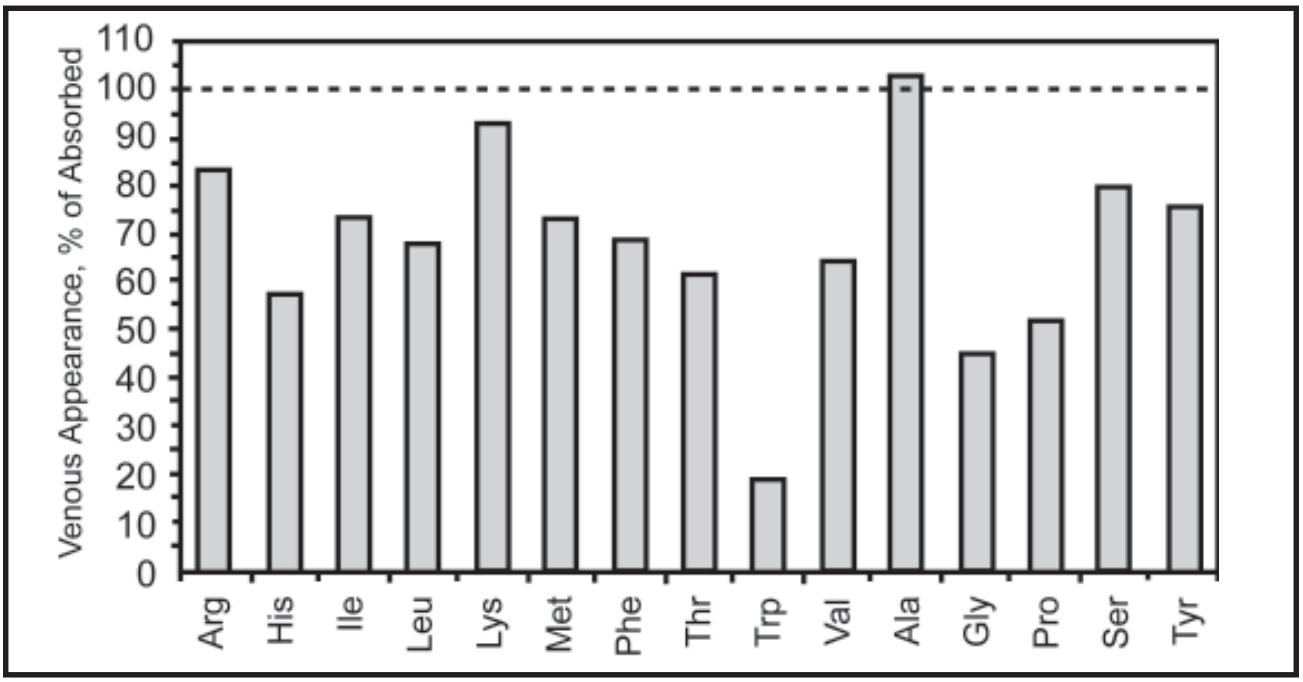

Figure 6. Hepatic vein amino acid appearance as a percentage of that absorbed from the digestive tract. From Hanigan (2005). Arg = arginine, His = histidine, ILE = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = Threonine, Trp = tryptophan, Val = valine, Ala = alanine, Gly = glycine, Pro = proline, Ser = serine, and Tyr = tyrosine.