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Cornell University

Ithaca, New York

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2010 Cornell Nutrition Conference for Feed Manufacturers

Conference Program

Tuesday, October 19

Pre-Conference Symposium hosted by H.J. Baker Bro., Inc.

Theme: Proteins and Amino Acids

1:00 PM	Welcome and Introduction
1:15 PM	Balancing for Amino Acids beyond Lysine and Methionine *Charlie Sniffen, Fencrest, LLC and William Chalupa, University of Pennsylvania*
2:10 PM	Marine-Animal Dairy Protein Concentrate: Achieving Optimum Amino Acid Balance *Paul Chandler, Chandler & Associates*
3:00 PM	Break
3:20 PM	From Crude Protein Intake to Amino Acids...to Milk Protein *Hélène Lapierre, Agriculture and Agri-Food Canada*
4:15 PM	Value of Milk Protein to the Producer, Processor and Future Opportunities *Dean Ellinwood, Dairy Farmers of America*
5:00 PM	Questions & Wrap-Up
6:00 PM	Evening Reception

Wednesday, October 20

6:30 AM	**Breakfast, sponsored by Kemin and The Old Mill-Troy**
	Chromium in Dairy Cattle Nutrition *Jerry Spears, North Carolina State University*

Morning Session – Larry Chase presiding

8:30 AM	National Air Emissions Monitoring Study - Dairy Component Findings *Curt Gooch, Cornell University*
9:00 AM	Livestock's Long Shadow (FAO Report): The Whole Story *Frank Mitloehner, University of California at Davis*
9:40 AM	Presentation of Maynard Award *Dr. Dale Bauman, Cornell University*
9:50 AM	Break
10:10 AM	Refinement of the Estimation of NDF Pool Size and Implications for Intake *Emiliano Raffrenato, Cornell University*
10:40 AM	Food Chain Mycotoxins 2010: Threats and Solutions *Dan Brown, Cornell University*
11:10 AM	Measuring the Effect of Stress During the Transition Period on Subsequent Health and Performance of Dairy Cattle *Julie Huzzey, Cornell University*
11:40 AM	Raw Milk Bacteriology Tests: Do They Predict Consumer Dairy Product Performance? *Kathryn Boor, Cornell University, Dean, College of Agriculture and Life Sciences*
12:20 PM	Lunch

Afternoon Session – Mike Van Amburgh presiding

1:30 PM How Well Do We Really Understand Silage Fermentation?
Limin Kung, University of Delaware

2:00 PM Developing Biotechnology to Convert Poultry Feathers into High-Quality Feed Protein Supplement
Xingen Lei, Cornell University

2:30 PM Break

The J. B. Russell Symposium

3:00 PM The CNCPS Legacy
Charlie Sniffen, Fencrest LLC

3:45 PM The Microbiologist Colleague
Paul Weimer, USDA-ARS, Dairy Forage Research Center, Madison, Wisconsin

4:30 PM Contributions to Ruminal Microbiology
Rod Mackie, University of Illinois

5:15 PM A Career as a Student and a Scholar
Todd Callaway, USDA-ARS, Southern Plains Agricultural Research Station, College Station, Texas

6:00 PM Evening Reception & Dinner

Thursday, October 21

6:30 AM **Breakfast, sponsored by Biozyme, Inc.**

6:45 AM The Impact of NDFd on Dairy Performance and Opportunities for Improving Forage Digestibility
James Nocek, Spruce Haven Farm and Research Center

Morning Session – Tom Overton presiding

8:15 AM Is there opportunity to boost milk protein production?
Laurie Winkelman, Cornell University

8:45 AM Current and Future Applications of Nutrigenomics in the Horse
Samantha Brooks, Cornell University

9:15 AM Filling Feed Holes: Advances and Current Issues in Forages and Grazing Management
Woody Lane, Lane Livestock Services

9:45 AM Changes and Implications of Updates to the CNCPS Biology
Mike Van Amburgh, Cornell University

10:20 AM Break

10:45 AM NDF and DMI - Has Anything Changed?
David Mertens, Mertens Innovation and Research, LLC

11:25 AM The Changing Roles of Insulin During the Transition Period
Katie Schoenberg, Cornell University

11:50 AM How Much Gas Do Cows Produce?
Larry Chase, Cornell University

12:50 PM Adjourn

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

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BALANCING FOR AMINO ACIDS BEYOND LYSINE AND METHIONINE

W. Chalupa[1] and C. Sniffen[2]
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INTRODUCTION

Several nutrition models (NRC, 2001; Fox et al. 2004; Tedeschi et al. 2008; Tylutki, 2010) allow for formulation of rations for lactating cows on the basis of AA, primarily Met and Lys. This report examines AA beyond Met and Lys.

AMINO ACID REQUIREMENTS

Requirements for absorbed EAA can be defined using the classical factorial method (Chalupa and Sniffen, 2006; O'Connor et al. 1993) and by an ideal protein method (Chalupa and Sniffen, 2006; Rulquin and Verite, 1993; NRC, 2001).

The factorial method requires knowledge of the amino acid content of products and the efficiency of amino acid use. AA content of milk and tissues can be estimated reliably but an estimate of the efficiency of AA use is difficult.

The ideal protein approach proposed by Rulquin and Verite (1993) is based on responses of milk protein to Met and Lys expressed as percentages of PDI (equivalent to MP). The ideal protein method gives a rectilinear response to Met and Lys that is expected in biology. Schwab and Foster (2009) recently presented new response curves for the NRC (2001), CPM-Dairy (Tedeschi et al. 2008) and AMTS (Tylutki, 2020) models.

FURTHER RESEARCH ON THE IDEAL PROTEIN METHOD

Since the report by Rulquin and Verite (1993), the ideal protein concept has been expanded to other AA. Sniffen et al. (2001) applied non linear Neural Net regression procedures using the JMP discovery software (SAS Institute) to cow experiments (calving through 4-8 weeks postpartum) designed to study the efficacy of rumen protected Met and Lys. Rulquin et al. (2001) used an AA profiling prediction system of intestinal contents to include experiments where AA were not infused post rumen or fed in protected form.

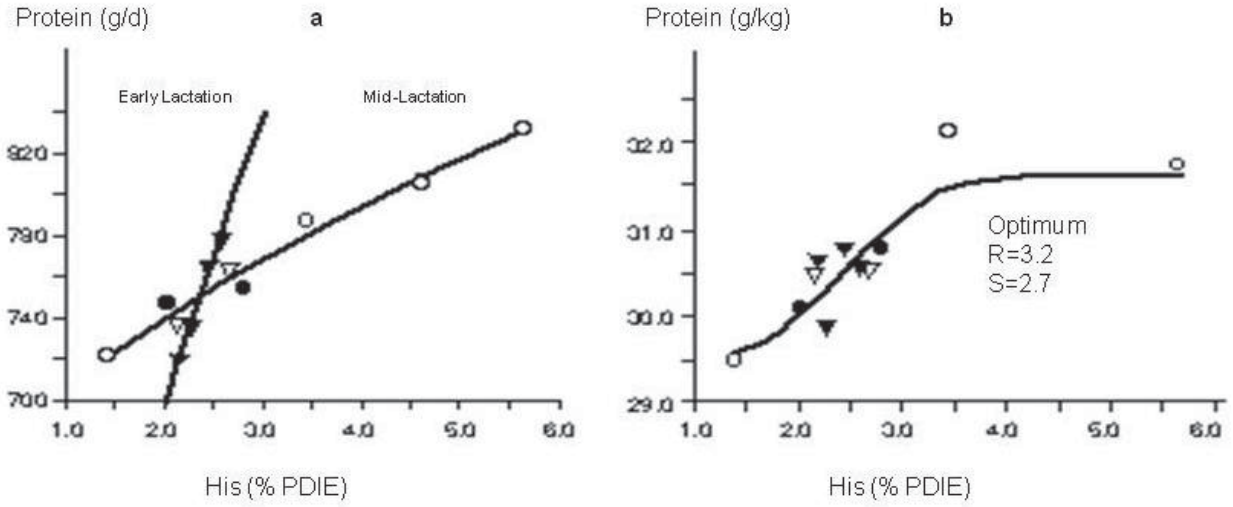
Abbreviations: MP = metabolizable protein; AA = amino acids; EAA = essential amino acids; NEAA = nonessential amino acids; Met = methionine; Lys = Lysine; Arg = arginine; His = histidine; Thr = threonine; Val = valine; Leu = leucine; Ile = isoleucine; Phe = phenylalanine; Trp = tryptophane; Ala = alanine; SBM = soybean meal; DDGS =

distillers grains; SB = processed soy beans (Soy Best); CM = canola meal; BM = blood meal; AMP = animal marine protein (Prolak).

Histidine

Increasing His in PDIE increased total protein output (g/d). However, this was due mainly to an increased milk yield so that protein content in milk (g/kg) plateaued at 3.2% His in PDIE (Figure 1). Sniffen et al. (2001) suggested 2.7% His in MP.

Figure 1. Production (a: g/day) and ratio (b:g/kg) of milk protein as a function of His in protein digestible in the intestines in early lactation and mid lactation cows (Rulquin et al. 2001).



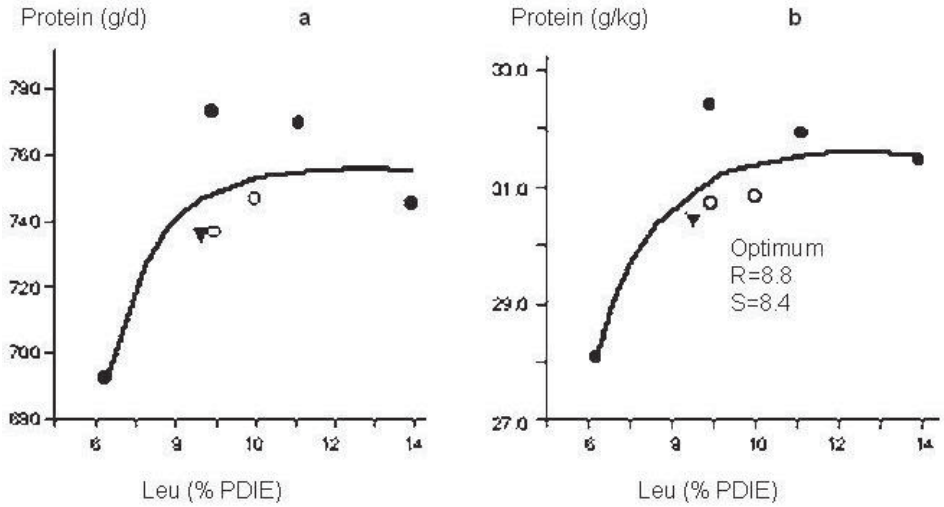
R = Rulquin et al. 2001; S = Sniffen et al. 2001

After finding no responses to abomasal infusion of Met and Lys in cows fed grass silage plus a barley-oats supplement (Varvikko et al. 1999), His was identified as the first limiting AA even when feather meal was the source of RUP (Kim et al. 1999, 2000, 2001a, 2001b; Huhtanen et al. 2002; Korhonen et al. 2000; Vanhatalo et al. 1999). In cows fed a corn and alfalfa silage based total mixed ration, milk yield increased by 1.7 L/d when His was added to drinking water (35 g/d post ruminal His). Lactose yield increased by 90 g/d, and there were tendencies for protein yield to increase, fat percentage to decrease, and protein to fat ratio to increase (Dolman et al. 2008).

Leucine

The curves for Leu in PDIE versus total protein output (g/d) and protein content in milk (g/kg) are similar (Figure 2). Thus, Leu does not appear to affect milk volume but has an impact on concentration of protein in milk. Leu may limit milk protein concentration when less than 8.8% of PDIE. Sniffen et al. (2001) found that 8.4% Leu in MP was needed. Leu may be below suggested values with grass and barley based rations (Rulquin et al. 2001). Leu was not a second limiting AA to His in cows fed grass silage rations (Korhonen et al. 2002).

Figure 2. Production (a: g/d) and ratio (b: g/kg) of milk protein as a function of Leu in protein digestible in the intestines. (Rulquin et al. 2001).



R = Rulquin et al. 2001; S = Sniffen et al. 2001

Isoleucine

Optimum Leu in PDIE (MP) reported by Rulquin et al. (2001) and Sniffen et al. (2001) was 5.0% and 4.7%. Ile was not a second limiting AA to His in cows fed grass silage rations (Korhonen et al. 2002). Abomasal infusion of Ile to cows fed a ration formulated to be deficient in absorbable Ile, Met and Lys increased milk yield but when the Ile infusion was combined with feeding rumen protected Met and Lys, there was no response in milk yield or milk protein content (Robinson et al. 1999).

Arginine

Intravenously (IV) infused Arg during late pregnancy increased prolactin, growth hormone and insulin and increased milk yield during the subsequent 22 weeks of lactation (Chew et al. (1984). Arg IV, but not into the abomasum increased somatotropin and insulin in plasma but Arg abomasally or IV did not stimulate synthesis of milk or milk components in cows already lactating (Vicini et al. 1988). Feeding rumen protected Arg plus Lys did not did not increase plasma growth hormone, but decrease in milk yield of cows fed the rumen protected AA was 0.9 kg/d less than control cows (Kirchgessner et al. 1993). Arg in PDIE of 4.3% seemed sufficient (Rulquin et al. (2001). However, Sniffen et al. (2001) reported that about 6% Arg in MP was optimum.

Valine

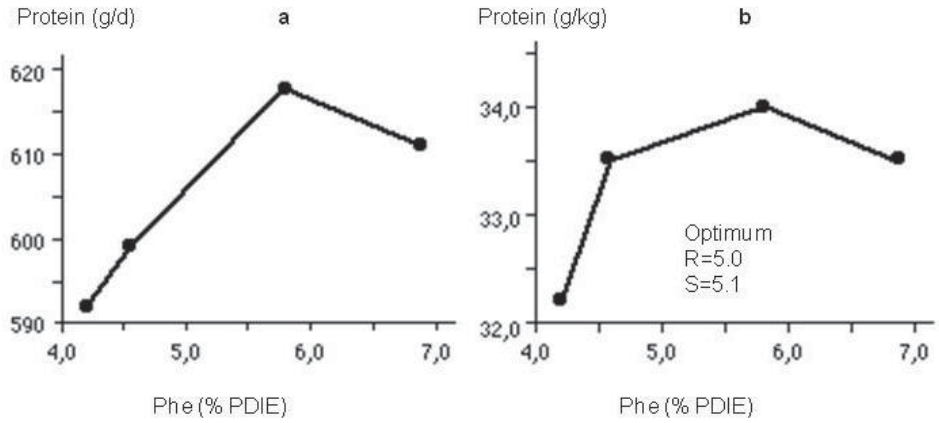
Rulquin et al. (2001) reported that Val was not limiting so long as the concentration in PDIE was greater than 5.3%. Sniffen et al. (2001) reported that concentration of

protein in milk was optimized with 5.75% Val in MP. Val was not a second limiting AA to His in cows fed grass silage rations (Korhonen et al. 2002).

Phenylalanine

The curves for in Phe in PDIE versus total protein output (g/d) and protein content in milk (g/kg) are similar (Figure 3). Thus, Phe does not have much of an affect on milk volume but has an impact on concentration of protein in milk. Phe in PDIE of about 5% seems to be sufficient, (Rulquin et al. 2001). The optimum Phe reported by Sniffen et al (2001) was 5.1% in MP.

Figure 3. Production (a: g/d) and ratio (b: g/kg) of milk protein as a function of Phe in protein digestible in the intestines. (Rulquin et al. 2001).



R = Rulquin et al. 2001; S = Sniffen et al. 2001

Rulquin et al. (2001) suggested that Tyr might lower the requirement for Phe. Tyr is insoluble in water so it may not require protection to reach the small intestine.

Tryptophan

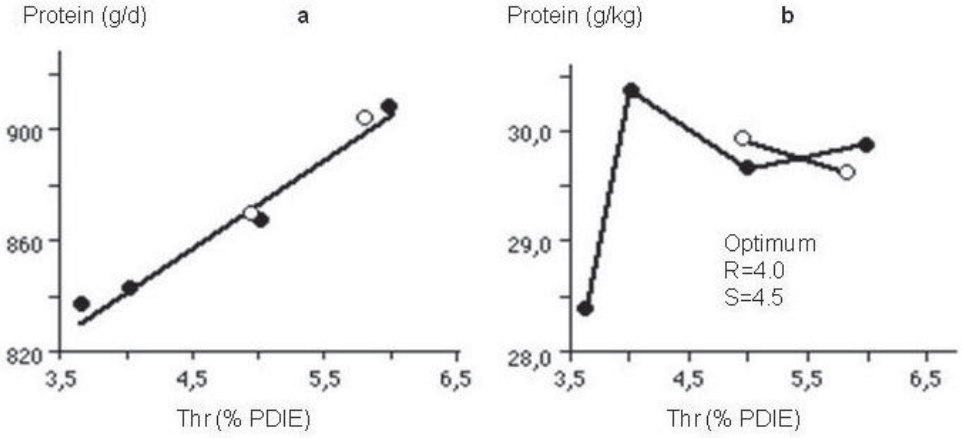
According to Rulquin et al. (2001), Trp does not appear to be a limiting amino acid with hay and corn-based rations. Sniffen et al. (2001) reported that 1.37% Trp in MP was needed.

Threonine

Thr in PDIE increased total protein output. However, this was due mainly to an increased milk yield (Figure 4). Thr in PDIE of about 4% seems to be sufficient, (Rulquin et al. 2001). This is similar to the 4.5% suggested by Sniffen et al. (2001).

Comparisons of amino acid requirements

Figure 4. Production (a: g/d) and ratio (b: g/kg) of milk protein as a function of Thr in protein digestible in the intestine. (Rulquin et al. 2001).



R = Rulquin et al. 2001; S = Sniffen et al. 2001

Comparisons of AA in PDIE (MP) to maximize the concentration of protein in milk are presented in Table 1. Optimum Met and Lys concentrations show variation but this would be expected because there are differences in how models calculate bacterial protein and rumen escape protein flows to the small intestine. For the other AA, ideal values reported by Sniffen et al. (2001) and Rulquin et al. (2001) are similar.

Table 1. Comparisons of percentages of AA in PDIE (MP) to maximize the concentration of protein in milk.

AA	Sniffen	Rulquin	NRC[1]	CPM[1]	AMTS[1]
Met	2.02	2.5	2.29	2.57	2.40
Lys	7.05	7.3	6.80	7.46	6.68
Thr	4.54	>4.3			
Val	5.75	>5.3			
Leu	8.37	<8.8			
Ile	4.73	>5.0			
Phe	5.10	4.9-5.0			
Trp	1.37	Not limiting			
His	2.72	3.2			
Arg	6.22	>4.3			

1.Schwab and Foster (2009)

Nonessential amino acids.

When compared to AA in milk protein, NEAA uptake by the mammary gland does not account for the amount in milk. Uptake of EAA by the mammary gland is sufficient for His, Met, Phe and Trp, and in excess for Ile, Leu, Lys and Val. (Clark, 1975; Mephan, 1982; Doepel et al. 2007). EAA extracted in excess provides N and C for mammary synthesis of NEAA and perhaps a source of energy (Wholt et al. 1997; Lapierre et al. 2003).

Infusion of NEAA plus EAA did not increase milk protein output above that obtained with infusion of EAA only in cows fed rations adequate (Metcalf et al. 1996) or deficient (Doepel and Lapierre, 2010) in MP. In general, if MP requirements are met, requirements for total NEAA will be met before the requirements for the most limiting EAA (Schwab and Foster 2009).

An exception might occur in early lactation. The increased demand for glucose after calving requires metabolic adaptations that may be enhanced by NEAA (Overton, 1998). Propionate is the main substrate for gluconeogenesis but after calving conversion of alanine (an indicator of gluconeogenesis from AA) to glucose increases more than the conversion of propionate to glucose (Overton et al. 1998). Since glucose uptake by the mammary gland is a major determinant of milk volume, limiting the supply of NEAA by reducing MP could compromise acceleration of milk yield.

APPLICATION

Sources of amino acids

To be considered a good source of AA, the supply should be greater than the requirement. Other than cost, Met and Lys are not problems as there are several rumen protected products available. Crude protein, rumen escape of the crude protein and intestinal digestibility of the rumen escape protein are important considerations as they determine the MP value. MP values and the AA profile of the MP for rumen bacteria and several protein ingredients are in Table 2.

Rumen bacteria are a good source of MP (37% DM) and except for small deficits of Leu and His, have a good profile of EAA. With good quality forages, rumen bacteria provide 50 to 55% of a lactating cow's required MP. However, with poor quality forages, this can drop to 40 to 45% of the MP requirement resulting in a greater reliance upon rumen escape protein from ration ingredients.

SBM, CM and DDGS have low MP values (19, 16 and 13% DM). Beyond Met and Lys, SBM is deficient in all EAA except Arg, DDGS is deficient in all EAA except Leu and CM is marginally deficient only in Leu. SB has a higher MP value (35% DM) with a better amino acid profile than SBM. SBM and CM provide rumen available N (peptides and ammonia) needed for growth of bacteria. Animal and marine proteins are often included in rations. BM has a high MP value (67% DM) but is extremely deficient in Ile. AMP has a moderate MP value (44% DM) but is also deficient in Ile.

Ration formulation

To examine when AA other than Met and Lys might be limiting, we used CPM-Dairy (Tedeschi et al. 2008) to formulate rations for a cow producing 45.4 kg milk with 3.75 fat and 2.95% true protein. The efficiency of MP into milk protein was increased from 65 to 67%. Rations were formulated with the optimizer using SBM, CM, DDGS, SB, BM and AMP as forced protein sources at generally accepted amounts. Rations can seldom be formulated with a single protein source, so we allowed the selection of

Table 2. Metabolizable protein and amino acid profiles of rumen bacteria and selected protein ingredients.

Protein		Bact	SBM	SB	CM	DDGS	BM	AMP
CP (%DM)		62	55	49	41	30	93	79
RUP (%CP)		60[1]	36	76	44	61	81	74
DP (%RUP)		100[1]	94	95	87	72	89	75
MP (%DM)		37	19	35	16	13	67	44
Amino Acid	Req[2]			Supply (% Requirement)				
Met/ RUP[3]	2.57	107	33	63	56	48	43	67
Lys/ RUP	7.46	112	83	86	91	28	128	84
Arg/ RUP	6.22	132	151	135	129	79	95	107
Thr/ RUP	4.54	123	67	87	107	69	104	91
Leu/ RUP	8.80	85	70	92	91	103	152	100
Ile/ RUP	5.00	118	85	97	99	56	18	57
Val/ RUP	5.75	107	66	90	112	91	158	112
His/ RUP	2.96	91	77	90	136	61	218	109
Phe/ RUP	5.00	103	78	103	94	84	157	99

1. Bact TP (%BCP), Digestible BTP (%BTP).

2. Schwab and Foster (2009); Rulquin et al. (2001) and Sniffen et al. (2001).

3. AA/RUP.

SBM and DDGS into all rations. To have at least 30% of the CP as soluble protein, urea was allowed. Rations were formulated using good and poor quality forages. So that meeting amino acid requirements were not compromised by Met and Lys in the ingredients, we allowed the inclusion of rumen protected products. To be conservative, because of the limited data base, AA were considered deficient if they were less than 85% of requirements.

As expected, MP from bacteria decreased when poor quality alfalfa silage and corn silage were used. More MP had to come from the feed protein sources, causing increases in ration CP (Tables 3 and 4).

With SBM, CM and DDGS, Leu and His were often below 85% of requirements. This was exacerbated with low quality forages (Table 3). With SB, BM and AMP, there were instances where Leu and His were slightly below 85% of requirements (Table 4). As mentioned before, His has been identified as the first limiting AA when grass silage plus barley oat diets were fed (Kim et al., 1999, 2000, 2001a, 2001b; Varvikko, et al. 1999; Vanhatalo et al.1999; Korhonen et al. 2000). Also, cows fed a corn and alfalfa silage-based ration produced more milk when drinking water contained His.

Table 3. Formulations using SBM, CM and DDGS with different quality forages.

Forced Protein		SBM	SBM	CM	CM	DDGS	DDGS
Forage Quality[1]		Good	Poor	Good	Poor	Good	Poor
SBM (kg DM)		**3.83**	**5.55**	2.75	4.62	3.14	4.95
DDGS (kg DM)		1.00	1.00	0.00	0.00	**2.30**	**2.50**
CM Meal (kg DM)		0.00	0.00	**2.30**	**2.30**	0.00	0.00
CP (%DM)		19.1	21.8	18.9	21.6	19.0	20.9
MP Bacteria (g)		1482	1233	1448	1195	1449	1185
MP RUP (g)		1345	1609	1322	1658	1362	1668
Amino Acid	Req[2]	Supply (% Requirement)					
Arg/ MP	6.22	104	105	105	106	100	101
Thr/ MP	4.54	96	89	101	93	95	88
Leu/ MP	8.80	82	80	84	81	84	81
Ile/ MP	5.00	97	92	100	95	95	90
Val/ MP	5.75	89	84	94	88	90	84
His/ MP	2.96	81	78	89	86	80	77
Phe/ MP	5.00	91	87	93	89	91	86

1. **Alfalfa silage. Good**: 20% CP, 40% NDF, 1.43 Mcal/kg NEL; **Poor**: 17% CP, 46% NDF, 1.16 Mcal/kg NEL. **Corn silage. Good**: 30% DM, 9.2% CP, 40% NDF, 1.56 Mcal/kg NEL. **Poor**: 40% DM, 9.2% CP, 45% NDF, 1.15 Mcal/kg NEL.

2. Rulquin et al. (2001) and Sniffen et al. (2001).

Table 4. Formulations using SB, BM and AMB with different quality forages.

Forced Protein		SB	SB	BM	BM	AMP	AMP
Forage Quality[1]		Good	Poor	Good	Poor	Good	Poor
SB (kg DM)		**1.80**	**1.80**	0.00	0.00	0.00	0.00
BM (kg DM)		0.00	0.00	**0.45**	**0.45**	0.00	0.00
AMP (kg DM)		0.00	0.00	0.00	0.00	**1.00**	**1.00**
SBM (kg DM)		1.00	2.63	2.60	3.67	1.65	3.03
DDGS (kg DM)		1.00	0.00	0.26	0.70	0.23	0.00
CP (%DM)		18.1	18.6	18.6	19.7	17.7	19.5
MP Bacteria (g)		1365	1262	1410	1289	1471	1234
MP RUP (g)		1494	1612	1442	1568	1421	1631
Amino Acid	Req[2]	Supply (% Requirement)					
Arg/ MP	6.22	102	104	99	100	99	99
Thr/ MP	4.54	99	94	98	94	99	93
Leu/ MP	8.80	88	84	91	88	87	85
Ile/ MP	5.00	99	97	89	86	92	88
Val/ MP	5.75	94	89	98	94	97	91
His/ MP	2.96	84	82	96	93	87	86
Phe/ MP	5.00	97	93	99	95	94	90

1. **Alfalfa silage. Good**: 20% CP, 40% NDF, 1.43 Mcal/kg NEL; **Poor**: 17% CP, 46% NDF, 1.16 Mcal/kg NEL. **Corn silage. Good**: 30% DM, 9.2% CP, 40% NDF, 1.56 Mcal/kg NEL. **Poor**: 40% DM, 9.2% CP, 45% NDF, 1.15 Mcal/kg NEL.

2. Rulquin et al. (2001) and Sniffen et al. (2001).

SUMMARY

We have all been frustrated with occasional low production on rations that seem to be well balanced, including Met and Lys. His and Leu supplies may not be adequate in some rations. We can use these ratios in the field to fine tune rations for optimum response in milk yield and protein yield. Good practices of ration formulation like maximizing bacterial growth by feeding good quality forages, using high RUP ingredients with good EAA profiles and high intestinal digestibility, and providing sufficient rumen available nitrogen may solve unexplained low production.

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MARINE-ANIMAL DAIRY PROTEIN CONCENTRATE: ACHIEVING OPTIMUM AMINO ACID BALANCE

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INTRODUCTION

In the early 1990's a project was initiated to formulate a protein supplement from marine-animal protein ingredients that would augment and enhance the metabolizable protein pool for lactating dairy cows. At that time the Nutrient Requirements of Dairy Cattle as presented in the 6th Revised Edition (NRC 1989) clearly demonstrated the importance of amino acid quality for the rumen escape or by-pass component of metabolizable protein for high producing dairy cows.

Early on in this exercise it was recognized that, if the special formulated protein supplement was to have a chance of altering and improving the metabolic protein pool for milk production, then the concentration of supplied protein nutrients or amino acids must be high in both amounts and bioavailability. Typically the 650 kg cow producing 45 kg milk requires in excess of 4 kg of protein of which >70% is associated with production requirement (The typical 100 pound milk producing mature cow). A product with a rumen escape of 70% and formulated to be highly available with a 70% protein guarantee would provide only 490g/kg and that would be only 12+% of the total requirement. It was immediately recognized that the formulated product must be high protein and high rumen escape, but still maintain amino acid balance and protein quality.

The goal was the achievement of a formulated supplement to the desired protein content and rumen escape values that placed emphasis on the 10 amino acids that are classified as dietary essential. Experiences with feed product formulations had demonstrated that frequently, compromises or modifications must be made. The "no feasible solution" message was encountered routinely as real activity components failed to supply requested right hand side values in correct amounts and ratios. It was obvious that adjustments in right hand side values had to be made, but it was important that the system used for adjustment was based on scientific knowledge that was available.

Thus, the primary objective of this paper is to describe the necessary steps to follow in formulating a protein supplement that places emphasis on total amino acid balance for achieving improvement in the biological value or amino acid quality of the metabolizable amino acid pool, used to drive milk protein synthesis. Where appropriate the base data used for the initial formulation will be presented. This will be followed by introducing information that has emerged following the initial effort, which will bring the approach in line with the current status of scientific knowledge existing in the area.

FORMULATION PROCESS

The process begins by obtaining an adequate understanding of the composition of the product that is to be produced, specifically milk or the protein components of milk. Since our subject relates to amino acids, the understanding must focus on the amino acid content of milk proteins.

Composition of Milk

Milk is approximately 87% water and 13% solids of which proteins constitute around one-quarter of the solid component. The specific proteins within the milk protein component are the caseins, albumin and globulins. Since a unique genetic code is involved for each of these protein components it is very likely that the amino acid profiles for the proteins differ. But for nutritional programming involved in product formulation, it seems appropriate to rely on one profile representing the amino acid content of bovine milk protein.

Consideration of milk protein composition is critical as the amino acid content determines the need for specific amounts and ratios of amino acids in the metabolizable amino acid pool. It is not easy to come upon a standard accepted amino acid profile for bovine milk protein. Part of the reason for this is that the compositions presented were derived for activities that were not devoted entirely to dairy cattle nutrition. Table 1 presents an amino acid profile of milk protein derived from five published sources.

The most dominant amino acid within milk protein is glutamic acid, constituting at least one fifth of the total protein. Amino acids of a gluconeogenic nature, glutamic acid, aspartic acid, alanine, isoleucine, leucine and valine comprise more than 50 percent of the amino acid content of milk protein. This points to the interaction between energy metabolism and protein nutrition and illustrates the importance of adequate protein and amino acid nutrition especially in the early lactation phases of fresh cows, prior to the establishment of strong fermentation activity and feed intakes.

Lysine and methionine, the classically considered first and second limiting amino acid for lactating cows constitutes around 10 percent of the amino acid content of milk. Adequate and optimum amino acid nutrition for lactation must consider all of the 20 amino acids that are involved in the building of the proteins contained within milk.

Milk Protein Synthesis

We are aided by the established fact that the sole precursor(s) for milk protein synthesis are the free amino acids extracted from the mammary gland blood supply and this extraction process is consistent around the daily cycles of the cows (Larson 1969). But the amino acid extraction from the arterial blood supply to the mammary gland does not follow a 1 to 1 ratio with respect to the amounts extracted and the amounts present in synthesized milk protein (Cant et al. 1993, Clark et al. 1977, Derrig et al. 1974, Guinard and Rulquin 1994 a and b, and Spires et al. 1975).

Table 1. Amino acid composition of milk protein (as % of protein).

	(Source)					
AA	**[1]**	**[2]**	**[3]**	**[4]**	**[5]**	**Mean**
Arg	3.28	3.34	3.35	3.37	3.61	3.35
His	2.53	2.41	2.56	2.84	2.82	2.60
Ile	5.53	5.83	5.30	5.13	5.22	5.33
Leu	9.10	8.96	9.06	9.84	10.08	9.29
Lys	7.60	7.10	7.44	7.71	8.86	7.64
Met	2.44	2.23	2.56	2.49	2.43	2.40
Phe	4.60	4.42	4.65	4.88	5.22	4.69
Thr	4.32	4.21	4.14	4.41	4.04	4.21
Trp	1.31	1.29	1.39	1.37	1.61	1.38
Val	6.19	6.27	6.04	6.32	6.82	6.25
EAA	**46.9**	**46.1**	**46.5**	**48.4**	**50.7**	**47.2**
Ala	3.19	3.15				3.13
Asp	7.41	6.66				6.95
Cys	0.75	0.82		0.79	1.57	0.78
Glu	20.54	21.33				20.68
Gly	1.88	1.80				1.82
Pro	9.29	10.15				9.60
Ser	5.25	5.38				5.25
Tyr	4.78	4.65				4.66
NEAA	**53.1**	**53.9**	**53.5**	**51.6**	**49.3**	**52.8**

Source Note:

[1] Metabolism Handbook, pages 7-8, Altman and Dittmer (1968).

[2] Metabolism Handbook, pages 53-57, Altman and Dittmer (1968).

[3] NRC (2001) Table 5-10.

[4] NRC (2001) Table 15-2.

[5] Feedstuffs Reference Issue (Batal and Dale, 2010).

Based on bovine mammary cell culture, the so called dietary essential amino acids (Crampton and Harris 1969) are also essential for milk protein synthesis (Table 2). Among the 10 dietary essential amino acids, some are extracted from arterial blood flow in direct portion to the amount present in synthesized protein, but for others, extraction rate far exceeds the amount in the synthesized protein. The obvious question for consideration is, “why does the mammary gland extract amino acids beyond the amounts needed to balance the amino acids appearing in the synthesized product”? It is documented and understood that certain amino acids are transformed into other amino acids. And the term “dietary essential amino acids” results because this intra metabolic transformation is limited, forcing the supply of some or all of the needs of some amino acids to originate from the diet.

Table 2. Amino acides essential for milk protein synthesis in the in vitro secretory cell (Schingoethe et al. 1967)

	Not required by:	
Usual essential amino acids	Bovine	Rat
Arginine, cystine, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, valine	Glutamine Tyrosine	Cystine Glutamine

Table 3, provides a summary of the amount of amino acid in synthesized milk protein compared to the amount extracted from arterial blood flowing to the mammary gland. The remarkable point illustrated from this table is the consistency of the results from study to study. These studies were published over a 20 year period, involving different investigators, feeds, cows and techniques. And arterial venous studies of this type are not easy, as blood flows as well as metabolite concentrations must be determined to achieve the projected transfers.

Table 3. Amino acid in milk protein expressed as a percentage of the amount extracted from the arterial blood supply.

AA	Study 1[1]	Study 2[2]	Study 3[3]	Study 4[4]	Study 5[5]	Study 6[6]	Mean
Arg	**30**	32	**39**	36	33	35	34
His	**68**	100	76	82	97	**107**	88
Ile	58	**55**	65	68	**70**	68	64
Leu	65	63	69	**60**	**81**	80	70
Lys	70	82	**86**	66	64	**57**	71
Met	**78**	91	97	**115**	100	86	94
Phe	100	100	100	100	100	100	100
Thr	**67**	77	74	67	**91**	90	78
Trp	--	--	--	253	--	--	--
Val	46	50	63	**43**	**76**	**76**	59

1 Derrig et al., 1974; 2 Spires et al., 1975; 3 Clark et al., 1977; 4 Cant et al., 1993; 5 Guinard & Rulquin, 1994a; & Guinard & Rulquin, 1994b.

Common across the six studies is the establishment of phenylalanine as the reference amino acid and adjusting the other amino acids relative to the phenylalanine value. Justification for this correction is the exclusive use of phenylalanine as a supplier of protein synthetic needs for phenylalanine and tyrosine (Derrig et al. 1974).

Among the dietary essential amino acids, arginine is by far the least efficient with respect to the amount extracted and the quantity used to synthesize milk proteins. Histidine, methionine and phenylalanine are extracted and exported as components of milk protein in almost a 1 to 1 ratio. Lysine averages a 71% appearance in milk proteins, but among the six studies there is a range of from 57 to 86% of the extracted lysine appearance in milk proteins. The branched chain amino acids, consisting of leucine, isoleucine and valine vary among studies from a low of 43 to a high of 80% appearance in milk proteins.

Tryptophan was reported in only one of the six studies and the value given is likely not relevant as much error can be involved with the chemical detection of this amino acid and blood flow values. The true appearance rate of this amino acid relative to the extracted amount is generally accepted as being in the 85% range (Clark et al. 1977).

Metabolizable amino acids for milk protein synthesis

The free amino acids within arterial blood originate from the amino acids absorbed from the digestive tract as well as the amino acids that are recycled from other metabolic processes. For the most part digestive absorption is the main contributor and within ruminants the small intestine is the major site of absorption. But the type of protein material that is digested to provide amino acids for absorption is quite variable. The first and most dominant protein source is the microbial fraction, resulting from fermentation activity and flow of microbial cells from the rumen. In properly fed cows this component can easily provide 60% of the total metabolizable protein required and in numerous situations encountered within the dairy industry, ruminal contribution can be much higher than the 60%.

Amino acid composition of ruminal bacteria is not constant as documented by Clark et al. (1992) from average composition of 441 bacterial samples for animals fed 61 dietary treatments in 35 experiments. Coefficients of variation exceeded 20% for histidine and methionine and 10% for arginine, leucine, lysine, valine, proline and tyrosine. If the objective becomes that of influencing the amino acid content of metabolizable protein, then the space for working action is beyond the rumen microbial content and likely no more that 40% of the metabolizable protein amount. And because of the variation in certain amino acids it is likely that results will vary.

There are feed proteins escaping rumen fermentation and contributing amino acids to the metabolic protein pool. Large variations exist among feed sources and for some sources there are large variations within. If a feed contribution stands a chance of eliciting a significant improvement in the amino acid composition of the metabolizable protein pool, then it must be present in sufficient amounts or be a supplier of only a few amino acids. Our approach for a protein blend has been to set aside at least 30% of the rumen escape metabolizable protein pool to have an opportunity of inducing a positive influence on total amino acid balance or biological value.

Variable vs. fixed models

Correct feeding of a herd of dairy cows requires the use or consideration of a variable model. Things vary, cows are at different stages of lactation, some cows are still completing their growth process, and mature body size is a large variable. Feed types, amounts, and compositions will vary between and within dairy operations. Considerations of all of these important factors are possible only through a variable approach.

But things differ for the feed industry where demands are placed on producing products that maintain quality and consistency throughout the year on a load by load basis for large areas of the country. The term of "what is on the tag must be in the bag" is of extreme importance for a quality producer from the feed industry. If the product is promoted as supplying a certain number of nutrient units then those units must be present at all times. Production of such products requires the use of fixed models where nutrient amounts described as "right hand side values" are set in place.

Right hand side values

The objective of this exercise is the development of a protein blend that will enhance the amino acid nutrition for the dairy cow. Information of three types are required for this exercise, (a) amino acid profile of milk (Table 1), (b) efficiency of mammary use of blood amino acids to produce milk protein (Table 3), and (c) amino acid profile of rumen microbial protein. Rumen microbial protein was estimated from the review publication of Clark et al. (1992) and TABLE 5-10 of NRC (2001). Cystine estimate for rumen microbes was made from the reported methionine and the data presented on TABLE 5-9 of NRC (2001). Table 4 presented on the following page, provides estimates for right hand target values for the escape protein at varying rumen microbial outputs.

Adjusting the milk profile by the production/transfer efficiency coefficients increases the EAA value from 47 to 70%. This value is related to an efficiency factor of 67%. Target ratio values for milk are obtained by maintaining the same EAA ratios as are present after adjustments for efficiency, but placing the ratios within a 47 EAA space. Rumen escape or target values for amino acids generally decline as more dependence is placed on microbial production, but there are exceptions. Arginine, glutamic acid and proline increase with greater demand placed on microbial protein and valine is variable. These values result from setting needs at zero and higher and not allowing negative values to be expressed in amino acid profile calculations.

Matrix solution

Linear programming for cost minimization, subject to nutrient constraints was used for solution. Constraints were established for protein, soluble and rumen escape protein and the ten essential amino acids. Real and artificial activities were used to solve the nutrient restraint equations.

Real activities of marine and animal protein sources, consisting of fish meals, meat and bone meals, blood meal, feather meal, poultry by-product meal, and synthetic methionine served as suppliers of nutrients. For achievement of solutions under all constraint sets, artificial activities were introduced for each of the amino acids. These artificial activities provided 100 percent of the nutrient at very unreasonable prices like $10,000 per kilogram. The appearance of any sufficient amount of these artificial activities signaled a nutrient constraint that was not in the practical solution range and dictated the adjustment down for that specific nutrient factor. Repetitive solutions with a

10% reduction in problem constraints were conducted until the cumulative use of artificial activities was below 0.5 percent of the formulation.

This approach in formulation closely follows the concepts promoted by Oser, as described in Chapter 4 of Crampton and Harris (1969). The essential amino acid index (EAA index) method was introduced because of the view that all essential amino acids should be considered rather than the one that is the most deficient. Formulations produced by the described approach consistently produced profiles with EAA index values that equaled or exceeded the values achieved, when rumen microbial protein was used against the reference profile.

FORMULATION TESTING

Maintaining an extensive quality assurance program is absolutely essential, when working with products from the marine and animal protein product industry. Emphasis must be placed equally across incoming ingredient supplies as well as the finished product blends. The situation with the dairy protein supplement was somewhat unique in that not only the product required testing, but also the concept of by-pass protein feeding within the dairy industry was not adequately understood.

Large scale field test

More than 33,000 milk records and 7,000 cows in 35 herds were evaluated with respect to a significant response to the inclusion of the test formulation at a 2 to 4 percent addition (Ferguson et al. 2000). Of the 35 herds, 19 were classed as having increased milk yield, 12 herds as having no change, and 4 herds as having decreased milk yield. The overall population response was 1.24 kg/d more milk. The results from this study confirmed that herd responses would be variable and substantiated some of the variability concerns that had been raised earlier (Clark et al. 1992).

University studies

The blend has been tested under varied conditions and some milk and feed intake results are summarized in Table 5.

In some studies significant differences have been established for actual milk, fat or energy corrected milk, feed intakes, or ratio’s involving the mentioned production factors. Overall it seems appropriate to concentrate on milk production and feed efficiency as summarized in Table 5. Currently an 8% improvement in feed efficiency can be noted and 3.5% FCM increased by more than 6%. There are unresolved questions concerning energy contributions from changes in body reserves. In the early lactation study at Illinois where a 14+% improvement was noted, it is likely that, if non-significant changes in early lactation body weights are considered, the true feed efficiency improvement is in the range of 5% rather than the calculated 14.13%.

Table 4. Right hand side target values at varying rumen microbial dependence (% of protein).

EAA	Milk Profile (g/100g)	AA Milk/ AA Blood (Transfer Coefficient)	(g/100g)	Target Ratio (% of Protein)	Rumen Microbes AA (g/100g)	Rumen Escape or By-Pass Target values at: 50/50	**60/40**	70/30	80/20
Arg	3.35	34%	9.84	6.63	5.02	7.98%	**8.37%**	8.55%	8.71%
His	2.60	88%	2.95	1.99	1.97	1.95%	**1.87%**	1.68%	1.38%
Ile	5.33	64%	8.33	5.61	5.61	5.44%	**5.20%**	4.63%	3.75%
Leu	9.29	70%	13.27	8.94	7.97	9.59%	**9.62%**	9.22%	8.54%
Lys	7.64	71%	10.77	7.25	7.77	6.52%	**5.99%**	4.97%	3.43%
Met	2.40	94%	2.55	1.72	1.92	1.47%	**1.31%**	1.03%	0.61%
Phe	4.69	100%	4.69	3.16	5.02	1.26%	**0.34%**	0.00%	0.00%
Thr	4.21	78%	5.40	3.64	5.71	1.52%	**0.49%**	0.00%	0.00%
Trp	1.38	85%	1.62	1.09	1.29	0.86%	**0.74%**	0.52%	0.20%
Val	6.25	59%	10.59	7.13	6.10	7.91%	**8.04%**	7.86%	7.51%
Ala	3.13			3.13	7.38	0.00%	**0.00%**	0.00%	0.00%
Asp	6.95			6.95	12.01	1.83%	**0.00%**	0.00%	0.00%
Cys	0.78			0.78	0.64	0.88%	**0.91%**	0.90%	0.88%
Glu	20.68			20.68	12.89	27.58%	**29.98%**	32.02%	34.57%
Gly	1.82			1.82	5.71	0.00%	**0.00%**	0.00%	0.00%
Pro	9.60			9.60	3.64	15.07%	**17.17%**	19.37%	22.30%
Ser	5.25			5.25	4.53	5.79%	**5.87%**	5.72%	5.44%
Tyr	4.66			4.66	4.82	4.36%	**4.09%**	3.53%	2.67%
EAA	47.15		70.03	47.15	48.25	44.62%	**42.16%**	38.70%	34.47%
NEAA	52.85			52.85	51.75	55.38%	**57.84%**	61.30%	65.53%
	100			100	100	100	**100**	100	100
		Efficiency 67.3%							

Table 5. Marine - animal protein blend improves milk yield and feed efficiency[1].

Study	FCM[2], kg	DMI, kg	Blend Amount, kg	Feed Efficiency	% Increase
Arizona - SFC - Control	34.83	26.30	0.00	1.32	
Arizona - SFC - By-Pass Blend	37.04	26.80	1.34	1.38	4.35%
[J. Dairy Sci., 82:728-737, 1999.]					
Canada - 2 Meals - Control	27.48	19.58	0.00	1.40	
Canada - 2 Meals - By-Pass Blend	31.04	19.38	1.71	1.60	14.11%
Canada - 7 Meals - Control	28.20	19.48	0.00	1.45	
Canada - 7 Meals - By-Pass Blend	31.30	19.18	1.71	1.63	12.74%
[J. Dairy Sci., 83: Supp. 1:289, 2000.]					
Illinois - Control	34.32	19.88	0.00	1.73	
Illinois - By-Pass Blend	37.19	18.88	0.82	1.97	14.13%
[J. Dairy Sci., 84: Supp. 1:364, 2001.]					
Illinois - Low Control	35.97	24.50	0.00	1.47	
Illinois - Low By-Pass Blend	35.59	23.90	0.41	1.49	1.43%
Illinois - Medium Control	35.26	24.70	0.00	1.43	
Illinois - Medium By-Pass Blend	37.98	25.10	0.83	1.51	5.98%
Illinois - High Control	39.09	26.00	0.00	1.50	
Illinois - High By-Pass Blend	36.61	23.50	1.15	1.56	3.63%
[J. Dairy Sci., 87: Supp. 1:339, 2004.]					
					8.05%

1/Dairy Efficiency Calculated as FCM/DMI

2/ 3.50% FCM Calculated as (16.216*kg Fat)+(0.4324*kg Milk)

Evaluation with herd feeding models

An evaluation of the marine-animal protein blend with modern feeding models that are extensively used by professional nutritionist seems appropriate. Two models were chosen, CPM Dairy, version 3.0.10 and CNCPS, version 6.1. Diets and actual performance from a selected dairy herd was chosen, where feeds, and nutrition was available. The diets and model evaluations are presented on the following pages.

Overall there was a high degree of consistency in the manner in which the two models projected the nutrition of the diets (Tables 6 and 7). The largest difference noted was the estimate of rumen escape or by-pass protein. The **CPM** model was at least 5 percentage points lower in this estimate compared to the **CNCPS** model.

Amino acid nutrition values are almost identical estimates between the two models (Table 7). Requirement satisfaction at 100% of ratio requirement was noted for methionine, lysine and arginine. Leucine, isoleucine and valine were satisfied in the 83 to 98 percentage range. The large over supply estimates for phenylalanine and tryptophan may be related to the use of phenylalanine as the reference amino acid in estimating blood transfers and the overall errors associated with tryptophan.

The marine-animal protein blend formulated by the described procedures has been demonstrated to be effective in dairy herd feeding. An improvement of 6% in milk yield with feed efficiency increases of 5 to 8% has been noted. These responses place this dairy supplement formulation concept in the environmental friendly class.

Amino acid nutrition certainly extends beyond the first or second limiting concept. Oser's EAA Index method takes the stand that all essential amino acids should be considered, rather than the single one that is most deficient relative to a standard (Crampton and Harris 1969). The results from mammary cells in culture clearly document a rather complex system (Park et. al, 1976 and Clark et. al, 1978). Lysine, methionine, valine, arginine, threonine and cystine could all be justified as having significant roles in milk protein synthesis.

And on a final note, the performance of the marine-animal protein blend in the nutrition of an aggressive milking dairy herd along with the evaluation through two popular dairy feeding models of this time (Tables 6 and 7) supports the delivery of balanced amino acid nutrition. Protein blends, when properly developed offers the opportunity to overcome the natural variability in quality that is inherent within the use of single feed ingredient sources.

SUMMARY AND CONCLUSIONS

The procedures that were followed for the production of a marine-animal dairy protein concentrate with emphasis on optimum amino acid balance are presented. This formulation process is conducted with the recognition of the contribution of rumen metabolizable amino acid output to the requirements for lactation. The formulation process must consider amino acid profiles of milk protein, transfer efficiencies of amino acids from the blood system into the mammary gland, and the working space available within the metabolizable amino acid pool, after accounting for the contributions from the rumen system.

A matrix solution process must be followed that allows real feed activities the opportunity to satisfy all nutrient constraints as close as practically possible, considering cost, quantity, consistency and quality assurance. Such a process requires down toning of right hand side requirement values, when the use of artificial activities with very high prices are selected to solve nutrient equations.

Table 6. Actual diets and nutrition (dry matter basis) for 27,000+ dairy herd.

Item	Sep-96		Oct-96		Dec-96		Jan-97	
DHIA 202								
Avg Milk	85.6		78.0		87.3		86.3	
Peak milk	106		98		109		109	
Fat %	3.3		3.4		3.1		3.4	
Protein %	3.1		3.1		3.2		3.0	
Diets, lb/d								
Corn Silage	12.50		12.50		14.25		10.50	
Haylage	10.50		10.50		8.75		8.50	
DBG	-----		-----		-----		3.75	
HMSC	6.37		6.37		8.34		8.84	
CORN	7.48		7.73		5.04		4.14	
WCS	5.60		5.60		5.60		5.60	
SBM-48	2.85		2.60		3.40		4.93	
Pro-Lak	2.69		2.69		2.49		1.59	
MEGALAC	1.00		1.00		1.00		1.00	
MINERAL	1.20		1.20		1.14		1.16	
DMI	50		50		50		50	
Nutrition, %	CPM	CNCPS	CPM	CNCPS	CPM	CNCPS	CPM	CNCPS
CP	18.5	18.3	18.4	18.3	18.5	18.3	19.7	19.5
RUP	41.2	46.2	41.3	46.1	40.1	45.6	40.4	46.3
EE	7.3	7.3	7.3	7.2	7.2	7.2	7.5	7.5
NDF	30.0	30.0	29.9	30.0	29.7	29.6	30.0	30.1
eNDF	23.7	23.3	23.6	23.3	23.4	23.0	22.3	22.0
starch	29.0	29.9	30.1	29.9	30.0	30.2	28.0	28.0
sugar	2.6	2.5	2.6	2.5	2.6	2.5	3.1	3.0
NFC	40.1	38.7	40.3	38.7	40.5	39.2	39.0	37.2

Table 7. Model estimates of amino acid nutrition for the herd.

	Sep-96				Oct-96			
	CPM		CNCPS		CPM		CNCPS	
AA	RR*	%Rqd	RR*	%Rqd	RR*	%Rqd	RR*	%Rqd
Met	1.92	100	1.79	103	1.93	100	1.79	103
Lys	6.34	101	6.04	105	6.34	101	6.04	105
Arg	6.13	101	6.13	96	6.13	102	6.13	96
Thr	4.60	132	4.49	141	4.60	133	4.49	141
Leu	7.91	93	7.82	98	7.91	93	7.83	98
Ile	4.39	83	4.30	85	4.40	83	4.30	85
Val	5.63	94	5.54	97	5.63	94	5.54	97
His	2.66	123	2.68	136	2.66	123	2.68	136
Phe	4.90	142	4.96	155	4.90	143	4.96	156
Trp	1.50	143	1.54	136	1.50	144	1.54	136
	Dec-96				**Jan-97**			
Met	1.92	99	1.80	105	1.92	99	1.79	106
Lys	6.39	101	6.12	107	6.23	99	5.93	106
Arg	6.20	102	6.20	98	6.12	101	6.10	98
Thr	4.63	133	4.53	144	4.62	133	4.50	145
Leu	7.90	92	7.83	99	7.96	93	7.92	102
Ile	4.42	83	4.35	87	4.51	85	4.39	89
Val	5.61	93	5.51	98	5.41	90	5.30	96
His	2.66	122	2.68	138	2.57	118	2.57	134
Phe	4.92	142	4.98	158	4.99	145	5.03	162
Trp	1.48	141	1.51	134	1.45	139	1.48	133

Note: RR* = Rulquin Ratio (Amino acids as a percentage of metabolizable protein).

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FROM CRUDE PROTEIN INTAKE TO ABSORBED AMINO ACIDS… TO MILK PROTEIN

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Over the last decade, the awareness of those involved in the dairy sector on the possibilities to reduce the economical and environmental costs of feeding excess nitrogen (N) has been steadily increasing. The most effective strategy to increase the efficiency of N utilization is to decrease the amount fed, but obviously, on a dairy farm, this should be done without detrimental effects on milk production and cow health. A better knowledge of the fate of ingested N has definitively allowed moving toward this direction. Improvements have been proposed to refine our assessment of protein supply (and requirement) to dairy cows: first, we moved from crude protein (CP) to degradable and undegradable protein. But none of these is a direct assessment of the real supply to the cow: the former represents the N available to the rumen micro-organisms whereas the latter only represents a fraction of what is available to the animal. Finally, the most recent versions of predictive models propose an estimation of the amount of protein truly available to the animal, i.e. digested in the small intestine, called metabolizable protein (MP; e.g. Amino Cow; CNCPS (Fox et al., 2004 and Tylutki et al., 2008); NRC, 2001). Although theoretically MP would represent a closer estimation of the true supply of protein to the animal, there is still much reluctance to use it to balance dairy rations as it is an estimation and not a direct measurement as is CP. And even if most of the predictive models have focussed their effort in improving the prediction of the supply of protein and concurrently amino acids (AA), this is only the first step to balance a ration. The second step is to determine how this supply is used to fulfill the requirements. For this important transfer, most of the models still use a simple, fixed coefficient of transfer, either for MP or for single AA.

We will examine the previous statements using studies conducted in dairy cows where net portal absorption (NPA) of AA and subsequent metabolism across the liver and the mammary gland (MG) and final delivery into milk protein have been measured. 1) Is the estimation of total AA supply improved when using MP compared to CP? 2) Once the AA are digested, is their fate across the portal-drained viscera (PDV), the liver, and the MG really a straight proportion of their supply (fixed efficiency)? As models predict only the digestive flows of essential AA (EAA) and due to the more limited data on non-essential AA (NEAA) splanchnic fluxes in dairy cows, when considered individually, this presentation will focus on the fate of individual EAA.

FROM CRUDE PROTEIN TO ABSORBED AMINO ACIDS

Crude Protein vs. Metabolizable Protein

To assess the supply of protein to a cow, nothing can be simpler than CP intake, which is just the sum of intake of each ingredient times its N concentration multiplied by 6.25. But the true biological value of a well defined chemical analysis needs to be questioned. Indeed, the term “crude protein” is highly misleading, as this so-called ‘protein’ comprises all the N sources found in plants and other feed ingredients. In addition to true protein, these N sources also include nucleic acids, nitrates, AA and peptides plus a variety of compounds formed during the forage ensiling process, such as ammonia and amines (NRC, 2001). For example, in silages, the contribution of non protein-N can be more than 50% of the total CP estimation (NRC, 2001). In addition, extensive rumen fermentation greatly alters rumen protein flow, with microbial protein representing more than 50% of duodenal protein supply (Clark et al., 1992). Therefore, MP has been developed within most recent predictive models to better assess the true protein supply to the cow. As the focus of this presentation is the supply and utilization of AA beyond the rumen, we refer readers to existing rumen sub-models (e.g. AminoCow, CNCPS, NRC) that detail the complexity of the various rumen transformations, estimate these processes and predict MP supply. Supply of MP refers to the flow of true protein digested across the small intestine: it sums the flows of the RUP fraction, microbial protein and endogenous secretions, each associated with a digestibility coefficient. Metabolizable protein, however, is an estimation and as such, there is still much reluctance to use it. Two outputs can be used to compare CP vs. MP: ideally, a comparison with total flow of absorbed AA should give an unequivocal answer, but the number of studies reporting NPA of AA in dairy cows is quite limited, so the comparison between CP and MP will be expanded using milk protein yield as the end result.

Using all the control treatments (n = 34) from studies used by Doepel et al. (2004), milk protein yield was estimated as a function of CP intake of MP supply. In this dataset, milk protein yield was more closely related (higher R^2, lower Syx) to MP supply than CP intake (Table 1). This can be explained by the fact that, although the relationship between CP intake and MP supply is generally not too bad (r = 0.96 in this database), in specific situations, MP much better represents the availability of AA than CP intake. This is best exemplified by the study of Blouin et al. (2002) in which an increase in the estimated MP from 1654 to 1930 g/d in two diets each containing 16% CP increased NPA of AA from 1194 to 1381 g/d. Indeed, in a recent meta-analysis, energy content of the diet, either assessed by total digestible nutrients (TDN, %DM) or inversely by NDF (%DM), significantly interfered on the relationship between N intake and NPA of AA, both in sheep and cattle; furthermore, the CP concentration of the diet also had a negative impact on that relationship (Martineau et al., 2009 & submitted). To continue the investigation, all studies reporting NPA of AA (either individually or as α-N) conducted in dairy cows for which MP supply could be estimated were used to assess the relationship between NPA of AA and CP intake or MP supply (Reynolds et al., 1988; McGuire et al., 1989; Bach et al., 2000a & b; Blouin et al., 2002; Tagari et al., 2004 &

2008; Hammon et al., 2008; Doepel et al., 2009; Larsen and Kristensen, 2009). Similar to milk protein yield, MP supply was better related than CP intake with NPA of AA (Table 1), but the improvement was not as significant as observed for milk protein yield. Overall, a similar CP intake is not a warrant of similar AA absorption and CP intake might be a poor indicator of AA supply and, therefore, should not be used to assess AA availability. This is as far as we can go to link protein supply and output, as after digestion, what is absorbed and used by the tissues to support protein synthesis are the individual AA. So, the next question is: "How do the predicted digestive flows of AA compare with the amount of AA absorbed in the portal vein?"

Table 1. Relationship between milk protein yield (MPY) or net portal absorption of AA (NPA-AA) and crude protein intake (CPi) or metabolizable protein supply (MPs)

Y	X	b_0 (SE)	b_1 (SE)	b_2 (SE)	R^2adj	Syx
MPY[a]	CPi	-179 (206)	0.49 (0.14)**	-0.00005 (0.00002)*	57.2	117.4
	MPs	-350 (163)*	0.99 (0.19)**	-0.0002 (0.00005)**	71.4	95.8
NPA-AA[b]	CPi	133 (127)	0.31 (0.05)**	--	95.8	95.1
	MPs	81 (120)	0.51 (0.07)**	--	96.5	87.0

[a]$Y = b_0 + b_1X + b_2X^2$ + error; * and **: b is significant at $P < 0.05$ and 0.01, respectively; 32 control treatments were used; all values are expressed in g/d.
[b]$Y = b_0 + b_1X$ + study + error; * and **: b is significant at $P < 0.05$ and 0.01, respectively; 12 studies and 27 treatments were used; all values are expressed in g/d.

From Digestion to Absorption

Theoretically, due to catabolism of AA across the PDV (Lobley and Lapierre, 2003), NPA of AA should be lower than the net amount digested. Indeed, this is what has been observed when direct measurements of digested AA in the small intestine (between the duodenum and the ileum) were compared with NPA of AA, although the apparent losses were larger than what would be expected from AA metabolism across the PDV. An initial comparison in sheep reported ratios of NPA of AA on AA digested ranging from 30% for Lys to 109% for His (Tagari and Bergman, 1978): such large variations in the losses were probably more technical than related to real biological difference. Nevertheless, more recent studies conducted in sheep and in dairy cows reported lower losses but still with ratios lower than unity, ranging from 43% for Thr to 95% for His (MacRae et al., 1997; Berthiaume et al., 2001).

Two reasons can mainly explain the differences between small intestinal disappearance and NPA. First, not only the NEAA, but some of the EAA are oxidized across the PDV. In dairy cows, oxidation of Leu across the PDV has been measured (Lapierre et al., 2002), whereas in sheep, Leu and Met were oxidized, but not Phe and Lys, at least from systemic source (Lobley et al., 2003). Independently if the AA oxidized across the PDV is from arterial or digestive source, the oxidation diminishes the NPA (Lapierre et al., 2002; Lobley and Lapierre, 2003). But oxidation cannot explain all the loss of AA across the PDV. A second reason also contributes to the discrepancy between small intestinal disappearance and NPA. As previously mentioned,

Table 2. Ratios between net mesenteric appearance (NMA) or net portal appearance (NPA) and small intestinal digestibility (SID) in ruminants

	Dairy cows[a]		sheep fed 800 g/d[b]		sheep fed 1200 g/d[b]	
AA	NMA/SID	NPA/SID	NMA/SID	NPA/SID	NMA/SID	NPA/SID
Arg	1.03	0.63				
His	1.27	0.95	1.14	0.69	1.11	0.63
Ile	1.02	0.62	1.05	0.72	1.02	0.63
Leu	0.92	0.62	1.18	0.68	1.03	0.71
Lys	0.76	0.55				
Met	1.01	0.67				
Phe	1.00	0.76	1.25	0.85	1.12	0.91
Thr	1.15	0.43	1.09	0.80	0.85	0.63
Val	1.11	0.51	1.16	0.62	0.76	0.46

[a]From Berthiaume et al., 2001
[b]From MacRae et al., 1997

endogenous secretions contribute to duodenal protein flow and in dairy cows, up to 20% of the duodenal flow originates from endogenous proteins (Ouellet et al., 2002 & 2007). This endogenous fraction constitutes a recycling of AA previously absorbed from the small intestine, returned to the gut tissue via arterial circulation and used to build proteins that are returned into the lumen of the gut prior to the duodenum. As such, their digestion/absorption does not represent a net input into the portal circulation. Therefore, although they are present in the duodenal digesta, their digestion/absorption just returns into blood circulation constituents that have been withdrawn from arterial supply for a null net result (Lapierre et al., 2006). These reasons also explain why net mesenteric appearance (draining only most of the small intestine) is close to small intestinal digestibility (Table 2). First, the endogenous proteins secreted pre-duodenum do not impose a “penalty” on the mesenteric absorption and second, with a contribution of 25% to the total mass of PDV (Reynolds et al., 2004), the mesenteric-drained viscera probably contribute only to this proportion to PDV oxidation. General conclusions are, however, uneasy to draw, due to the scarcity of studies that have done these direct comparisons.

Another way to estimate AA metabolism across the PDV is to calculate the recovery of a known supply of AA into the portal circulation. The recovery does not indicate basal losses, but could be used to quantify losses associated with increased supply. In sheep, mesenteric absorption of EAA was linearly related to the rate of casein-AA infusion into the duodenum with a slope not different from unity (except for Trp). Portal absorption of EAA, however, presented a slope lower than unity for the branched-chain AA (BCAA: Ile, Leu and Val) but yet not different from unity for the other EAA, albeit all numerically lower than 1 (El-Kadi et al., 2006): in other words, a slope equal to 1 means that all the dose of AA infused was recovered in portal absorption, ie. there was no loss across PDV metabolism whereas a slope lower than unity means that there was catabolism of these AA across the PDV. In a recent study (Freetly et al., 2010), infusions of increasing amounts of an isolated soy protein increased NPA of Ile, Leu, Met, Phe but did not alter NPA of His, Lys, Thr and Val. No clear pattern could be proposed form the latter study, but results from the former suggested an increasing loss of the BCAA with increased

supply, as previously measured for Leu in dairy cows (Lapierre et al., 2002). Increased BCAA supply increases concentrations (under the same conditions) and Hanigan et al. (2004) suggested that catabolism across the PDV results from a mass action, i.e. is related to amount presented to the tissue: for a same blood flow, the higher are the concentrations, the larger is the removal.

Using a similar approach, we have attempted to determine the NPA of AA with the ingestion of a known source of RUP, feeding dairy cows with Pro-Lak®, a high RUP protein source. Results of two studies are presented in Table 3. For the first study, 4 cows, averaging 650 ± 92 (SD) kg and 131 ± 12 DIM, were fed a fixed amount of TMR balanced to provide sufficient crude, degradable and non-degradable protein, energy and MP every 2 h in equal meals plus 1 kg/d of hay (NRC, 2001) for the first period. For the second period, cows were fed, in addition to the same TMR allocation, 1.8 kg/d (as is) of Pro-Lak®. The basal TMR supplied 1819 g MP/d (16.5% CP) whereas supplementation with Pro-Lak® increased MP supply to 2641 g MP/d (21.8% CP). The amount fed purposely exceeded the requirements in order to generate a large increment of circulating concentrations of AA. NPA of EAA all increased ($P < 0.10$) in response to RUP supplementation whereas milk protein yield increased only numerically from 977 to 1035 g/d. The second study is part of an already published study (Raggio et al., 2004): only the Medium (2264 g MP/d; 14.7% CP) and High (2517 g MP/d; 16.6% CP) MP treatments are compared. Comparison of the Low MP (1922 g MP/d; 12.7 %CP) to the Medium MP treatment is not adequate, as microbial protein synthesis was predicted to increase between the 2 treatments (due to the increased RDP supply; NRC, 2001) and therefore, the increment in NPA of AA would not only be the result of increased RUP availability. Intake of Pro-Lak® increased by 610 g (DM basis) between these 2 treatments. Expected NPA of AA from Pro-Lak® intake was estimated using a factor of 70% of RUP and 80% of digestibility and the following AA concentrations (mg AA/g CP): His: 33.9; Ile: 29.8; Leu: 89.2; Lys: 60.9; Met: 15.7; Phe: 50.0; Thr: 40.7 and Val: 66.4.

Although this approach uses more unknown (e.g. true RUP, true digestibility) than the straight infusion of a single protein source, on average, all EAA showed a good recovery of the increased RUP supply in the portal circulation, ranging from 66 to 91% of the expected increment. Unexpectedly, this recovery was higher than unity for Phe and Thr in the second study, and was the lowest for Lys and Met, which should present limited catabolism across the PDV. Real recovery of the BCAA could be lower than these estimations due to underestimation of the true concentration of these AA in the RUP fraction of the protein with a single 24h hydrolysis (see discussion below). Overall, however, these data support that, as a result of an increased supply of AA, catabolism of EAA across the PDV also increases, probably, as outlined by Hanigan et al. (2004) due to increased concentrations of AA (data not shown). Unfortunately, with the technical limitations of NPA measurements and estimations of digestibility, it has not been possible yet to clearly delineate the differences between individual AA. This is where estimations of MP become even more useful, as from this digestive flow of protein can be estimated the digestive flows of individual AA, and increase the data available for comparison between the digestive flow and NPA of AA.

Models use different approaches to estimate the flow of individual AA available to the dairy cow, either assessing an AA composition to each duodenal fraction (RUP, microbial protein: e.g. AminoCow, CNCPS) or using regression equations linking the percentage of an EAA in duodenal protein to the percentage of this AA in RUP and the percentage of RUP into duodenal protein (NRC, 2001). A first attempt was made to compare measured NPA of individual AA with the estimations of digestive flows, either obtained with NRC (2001) or CNCPS (version 5.0.34) models (Pacheco et al., 2006). The slopes are expected to be lower than unity, as for the comparison between small intestinal disappearance and NPA, although the role of the endogenous secretions is different with this type of estimation. Only endogenous secretions that are not reabsorbed and which flow at the ileum level, either fermented by hindgut bacteria or excreted in the feces represent a loss for the animal. Overall, the NRC model presented lower root mean square prediction errors (as % of the mean: the lowest the better is the predictive model) than CNCPS, but most of the slopes of observed vs. predicted AA fluxes were higher than 1, albeit known losses of AA across the PDV (Lobley and Lapierre, 2003; Pacheco et al., 2006). Suggestions have been made to increase the estimated digestive flow of AA, including using the factorial approach and increasing the digestibility of some RUP fractions and microbial true protein: increasing the digestive flows of AA resulted in slopes of observed NPA vs. predicted digestive flow of AA closer to expectations (Pacheco et al., 2006). Nevertheless, the ranking amongst AA in terms of potential losses (indicated by the magnitude of the slope), except for Met and Phe, followed the expected metabolism of EAA across the PDV: loss of all EAA through the non-reabsorption of intestinal endogenous secretions, minimal oxidation of His and limited Lys, considerable oxidation of the BCAA, and a loss of Thr larger than those of other EAA due to the relatively high contribution of Thr to endogenous proteins. Except for His for which a major use across PDV was reported, this ranking also agrees with the model developed by Hanigan et al. (2004). A technical pitfall may be worth mentioning to explain the apparent deficiency of the digestive flow of AA, especially the BCAA and maybe Thr. All measurements or estimations of digestive flows of AA use, at some time point, AA concentrations of feed ingredients or digesta obtained after hydrolysis of the proteins. Time of hydrolysis has been standardized to 24h, albeit it is known that for some AA, this time is not sufficient to break all the peptide links: the BCAA would be the most underestimated in digesta (Darragh et al., 1996). When comparing treatments or feed ingredients, it might be considered that this does not induce a bias, but when trying to integrate estimations of flows from hydrolysed proteins, with measurements of free AA absorbed, that might create a discrepancy. In the same line, AA concentrations of milk used to estimate AA requirement are estimated based on the AA composition of the proteins (Swaisgood, 1995) and are not those obtained with a 24h hydrolysis, as these systematically underestimate the concentrations of Ile, Val and Thr (Rutherfurd et al., 2008). This might also create a bias in the calculation of AA balances, between a supply measured from a 24h hydrolysis, a NPA obtained from free AA analyses and a requirement for milk output obtained with the real AA concentration.

Table 3. Net fluxes of essential AA (g/d) across the portal-drained viscera (PDV), the liver, the splanchnic tissues (TSP) and the mammary gland (MG) in dairy cows fed Pro-Lak®

		Study 1[a]				Study 2[b]					Avg.
AA	Tissue	Ctrl	Pro-Lak	Exp. diff.[c]	obs./ exp.	Low MP	Medium MP	High MP	Exp. diff.	obs./ exp.	obs./ exp.
His	PDV	28	45	25	0.68	29	45	53	9	0.89	0.79
	Liver	-11	-19			-11	-16	-27			
	TSP	17	26			18	28	26			
Ileu	PDV	73	91	22	0.83	66	84	91	8	0.90	0.86
	Liver	8	9			8	14	5			
	TSP	81	100			74	97	96			
Leu	PDV	123	188	65	1.00	112	136	156	24	0.85	0.93
	Liver	16	-19			3	19	7			
	TSP	139	169			115	155	164			
Lys	PDV	97	134	44	0.83	102	133	141	16	0.49	0.66
	Liver	9	3			0	3	-17			
	TSP	106	136			102	135	124			
	MG					-87	-93	-105			
	Milk	81	86			70	75	80			
Met	PDV	35	42	11	0.66	36	48	51	4	0.67	0.66
	Liver	-9	-9			-11	-15	-24			
	TSP	26	33			25	33	28			
	MG					-24	-25	-28			
	Milk	27	29			24	25	27			
Phe	PDV	79	112	36	0.89	97	118	140	13	1.67	1.28
	Liver	-33	-52			-47	-60	-82			
	TSP	46	59			50	58	58			
Thr	PDV	57	81	30	0.78	58	83	99	11	1.48	1.13
	Liver	-11	-21			-19	-33	-42			
	TSP	47	59			39	50	57			
Val	PDV	73	114	48	0.84	80	97	114	18	0.98	0.91
	Liver	10	-21			4	20	17			
	TSP	83	92			84	117	132			

[a]Difference in Pro-Lak intake of 1680 g/d.
[b]From Raggio et al., 2004: Low, Medium and High MP refer to a supply 1922 g MP/d (12.7% CP), 2264 g MP/d (14.7% CP) and 2517 g MP/d (16.6% CP); only the Medium and High MP treatments are compared (see text), with a difference in Pro-Lak intake of 610 g/d; PDV Thr (Medium MP), adjusted.
[c]Expected difference from the intake of Pro-Lak®: see text for calculations

FLOWING ACROSS THE LIVER

If all AA are considered together, then the liver removes, on average, between 45 and 50% of portal absorption (see reviews: Lapierre et al., 2005; Reynolds, 2006). This statement, however, hides two major points. First, there is a considerable variation in how each individual AA is handled and, an average is far from representative of the fate of individual AA. Second, although the ratio of liver removal on portal absorption is convenient to give a quick figure on the active metabolism of the liver, it is somewhat

misleading, as the primary factor driving hepatic removal is not the amount absorbed but the total inflow, which makes a balance between the amount absorbed and the amount used by other tissues. It should be kept in mind that although one function of the liver is to prevent hyperaminoacidaemia, involving deamination of excess AA and synthesis of urea, followed by catabolism of the AA carbon skeleton, some of the removed AA are directed towards vital hepatic anabolic purposes (e.g. gluconeogenesis mainly from NEAA, synthesis of plasma export proteins).

The first point can easily be assessed with numbers presented in Table 3. Clearly hepatic metabolism divides the EAA into two categories: those that are extensively removed and those that are, on a net basis, barely removed. The first category, referred to as Group 1, includes His, Met, Phe and Thr whereas the Group 2 includes the BCAA and Lys. Hepatic removal of AA of the first group usually varies between 30 and 40% of absorption but can be as high as 50% of absorption for some AA under large supply. On the other hand, net removal of EAA of Group 2 is almost nil, even positive in some cases, especially under limited protein supply. Reynolds (2006) has suggested that some of the BCAA could be synthesized within the liver by transamination of their respective keto-acids, or catabolism of plasma peptides; another possibility would be synthesis from absorption of branched-chain volatile fatty acids.

The second point needs specific conditions to be demonstrated. Indeed, under normal circumstances, circulating concentrations of AA reflect portal absorption and therefore, liver removal of EAA is related to both portal absorption and total inflow (mainly driven from concentration). However, under peculiar circumstances, circulating concentrations do not follow anymore portal absorption and then offer a nice model to test the concept. Induction of lactation is one of these situations where concentrations of AA decrease in spite of increased intake. In dairy cows, despite an increment of NPA of EAA from 63 to 104 g N/d, liver removal tended to decrease from 14 to 9 g N/d: therefore, the liver removal of Group 1 AA represented 60% of absorption before calving and decreased to 31% of absorption after calving (Doepel et al., 2009). The hepatic extraction, calculated on total inflow to the liver, numerically decreased from 6.3 to 4.6%. Similar observations have been reported by Reynolds (2006), where hepatic removal of EAA decreased from 63 to 12% of net portal absorption in dry and early lactating cows. This indicates that although the liver is the first organ that the absorbed nutrients “meet”, it does not have a first “cut” on absorbed AA. The liver “sees” the total inflow, a combination of arterial concentration plus portal absorption, removes a certain proportion of that inflow, usually not exceeding 10% for Group 1 AA in dairy cows. Then, these AA are circulated through the whole body and other tissues have access to these AA. The “unused” AA are brought back to the liver with a proportion removed again, and that way, demands of all tissues for AA (and other nutrients) are integrated to meet metabolic priority.

MAMMARY GLAND AND MILK

Data for mammary metabolism of Met and Lys have been added to Table 3, being representative of observations made elsewhere on Group 1 and Group 2 AA,

respectively (see: Lapierre et al., 2005). Generally, the MG and the liver have complementary actions on EAA. The Group 1 AA (e.g. Met), removed significantly by the liver, are quantitatively removed by the MG, on a net basis, only to fulfill the requirement for milk protein secretion. On the other hand, Group 2 AA (e.g. Lys), which are not taken up by the liver, are extracted by the MG to a greater extent than that needed to support milk protein secretion. The excess uptake relative to milk output of these AA increases with supply (Rulquin et al., 2007) and the difference between net uptake and milk protein output was oxidized when measured for Leu (Raggio et al., 2006). It is important to note that these only represent net movements. Total uptake of AA is greater than net uptake. For Group 1 AA, part of the total uptake is used for the synthesis of constitutive proteins with simultaneous release of an equivalent amount in the mammary vein of AA originating from the breakdown of constitutive proteins. For Group 2 AA, as previously mentioned for Leu, in addition to the synthesis of protein, the excess uptake relative to milk protein secretion is oxidized. But what are the excess AA taken by the MG used for?

Partial oxidation of these AA can supply energy to the MG. Also, excess N and C skeletons can be used for intra-mammary synthesis of AA or other nutrients. Indeed, the excess N from Lys contributed to the synthesis of Glx, Asx, Ser and Ala used for milk protein synthesis. With Lys depletion, the N-transfers from Lys to other AA within the MG were still present, but rates were considerably lower (Lapierre et al., 2009). Studies *in vitro* have reported that labelled C from Leu was incorporated into Glu used for milk protein synthesis by cow mammary tissue (Wohlt et al., 1977). Also recently, from *in vitro* studies, Bequette et al. (2006) estimated that as much as 12% of galactose synthesis was derived from EAA catabolism.

CONCLUSION

What do all these net flux measurements tell us in terms of improving the efficiency of transfer of protein intake into milk protein? Ultimately milk AA secretion will always equal AA absorption minus losses (oxidation, scurf, endogenous urinary and fecal secretions) plus/minus any tissue retention! First, these data clearly demonstrate that EAA have different fates in different organs and that removal/oxidation of EAA occurs across a wide variety of tissues. Briefly, on a net basis, the liver mainly removes His, Met, Phe, Trp and Thr whereas the BCAA, Lys and Thr are mainly removed by the PDV and peripheral tissues, including the MG. For those AA removed by a tissue, increased concentrations yield increased oxidation. In parallel, increased MP supply (under the same conditions) also means increased blood concentrations. This explains why, at higher supply, although the total return in terms of milk yield is larger, the efficiency of conversion of the absorbed EAA always decreases compared with lower protein intake. In practical terms, this means that in predictive schemes for milk protein production, once the supply is estimated, we must stop using a fixed factor of conversion and introduce variable efficiency factors depending on the interaction among individual AA and between N and energy supply.

Based on the uneven removal amongst AA by the different tissues, the utilization of different efficiency factors for maintenance and lactation is questioned. For the same reason, the simple ratio of mammary uptake to milk output is not an adequate representation of the efficiency of utilization of AA supply towards “lactation”. If increasing the efficiency of transfer of N can be better achieved by reducing total N intake, this implies a reduced margin of safety. In such conditions, imbalances in the AA supply will have a more rapid negative effect on milk protein output, and hence, our need to properly balance the supply of AA with demand increases. The positive outcomes of meeting this challenge will be a decreased N excretion in the environment and a reduced feed cost for the producer, both inescapable targets to satisfy market forces and consumer perceptions.

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CHROMIUM IN DAIRY CATTLE NUTRITION

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SUMMARY

Chromium functions in the trivalent form to enhance insulin sensitivity. Requirements for chromium are low, and it has generally been assumed that practical livestock diets contain sufficient chromium to meet animal requirements. However, over the past 15 years considerable research has suggested that cattle diets often may contain inadequate amounts of bioavailable chromium to maximize animal productivity. The FDA CVM issued a regulatory discretion letter in 2009 which permitted the use of chromium propionate as a source of chromium in cattle diets at a level up to 0.5 mg Cr/kg of complete feed. Addition of chromium to cattle diets has increased insulin sensitivity following intravenous administration of glucose. Supplementing high producing dairy cows with chromium during the transition period has increased feed intake and milk production during early lactation. Limited research has indicated that chromium supplementation may improve reproductive performance. A number of studies have also demonstrated that chromium supplementation can affect cell-mediated and humoral immune responses. Little is know regarding chromium concentrations in feedstuffs or bioavailability of chromium from animal feeds.

INTRODUCTION

In the late 1950's Schwartz and Mertz (1959) reported that trivalent chromium (Cr) was an essential component of a factor in brewers yeast that corrected impaired glucose metabolism in rats fed certain diets. Subsequent studies demonstrated that Cr functioned as a potentiator of insulin action (Vincent, 2001). Considerable research has been conducted with Cr in human nutrition and an adequate intake of Cr has been established for humans by the Institute of Medicine (DRI, 2001).

Chromium requirements for cattle have not been estimated by the NRC. Traditionally, practical diets fed to domestic animals were assumed to provide sufficient Cr to meet animal requirements. However, in the past 15 years a number of studies in cattle and other species have indicated that Cr supplementation of diets can affect animal metabolism and production criteria.

Although considerable research has been conducted with Cr in cattle, only recently has Cr supplementation been allowed in cattle diets. The FDA CVM issued a regulatory discretion letter in July of 2009 which permitted the use of Cr propionate as a source of Cr in cattle diets. Chromium propionate is the only Cr source permitted for supplementation to cattle diets in the U.S. It can be added at levels up to 0.50 mg Cr/kg of complete diet. The safety of Cr propionate has been thoroughly investigated, and

supplementation with Cr propionate for 120 days at 4 times (2 mg/kg) the permitted level did not increase Cr concentrations in milk, muscle or fat (Lloyd et al., 2010). However, supplementation with 2 mg Cr/kg diet did increase Cr concentrations in liver and kidney. This paper will discuss responses that have been observed to Cr supplementation of dairy cattle diets.

CHROMIUM AND INSULIN ACTION

Glucose tolerance tests have been conducted in cattle to evaluate the effects of Cr on glucose and insulin metabolism. In these studies a glucose solution has been infused intravenously (iv) and circulating concentrations of glucose and insulin measured frequently until they returned to baseline values. The addition of Cr to diets of growing calves has increased glucose clearance rates following glucose infusion without affecting serum insulin concentrations in some studies (Bunting et al., 1994; Sumner et al., 2007). Supplementing a milk replacer diet with 0.4 mg Cr/kg DM did not affect glucose clearance rate following a glucose infusion in young calves with undeveloped rumens (Kegley et al., 1997). However, insulin concentrations were lower following glucose administration in calves supplemented with Cr, suggesting increased insulin sensitivity in this group.

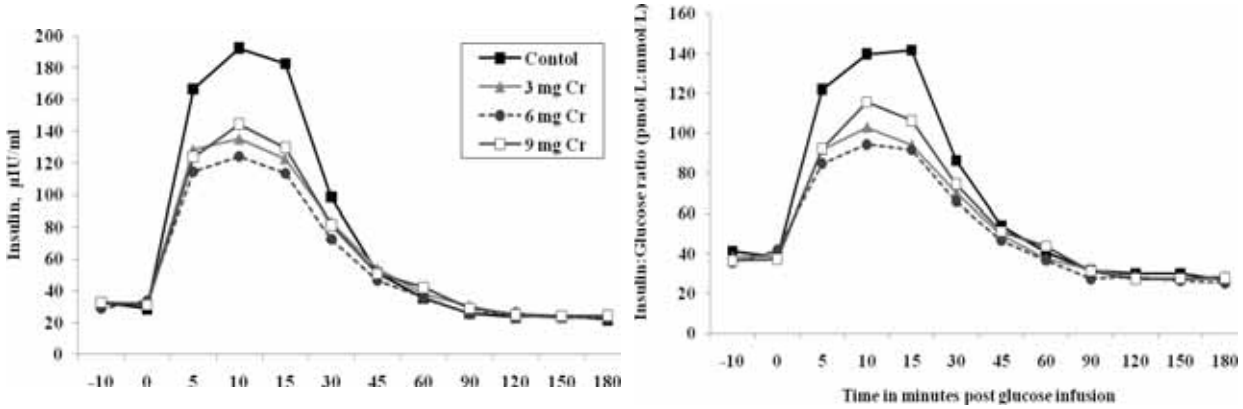
We recently examined the effect of level of supplemental Cr from Cr propionate on glucose metabolism in growing heifers (Spears et al., 2010). Chromium was supplemented at 0, 3, 6, or 9 mg Cr/head/day. These daily levels corresponded to 0, 0.47, 0.94, and 1.42 mg Cr supplemented/kg diet DM. Serum insulin concentrations and insulin:glucose ratios were much lower in all Cr-supplemented groups the first 15 minutes following glucose infusion (Figure 1). The lower release of insulin and decreased insulin:glucose ratio in Cr-supplemented heifers indicates that their tissues were more sensitive to insulin. Insulin concentrations and insulin:glucose ratios did not differ among heifers supplemented with 0.47, 0.94, and 1.42 mg Cr/kg DM. This suggests that Cr requirements of growing heifers do not exceed 0.47 mg Cr/kg DM.

Insulin increases glucose uptake in muscle and adipose tissue primarily by stimulating a specific glucose transporter (GLUT-4). It is well documented that insulin resistance occurs in late gestation and early lactation in dairy cows (Sano et al., 1993). The insulin resistance that occurs in early lactation spares glucose for lactose synthesis in the mammary gland but also increases mobilization of non-esterified fatty acids (NEFA) from adipose tissue (Bell, 1995). Glucose uptake by the mammary gland is not insulin dependent and involves GLUT-1 and other glucose transporters (Zhao and Keating, 2007). Research in dairy (Subiyatno et al., 1996) and beef cows (Stahlhut et al., 2006) suggests that Cr supplementation may increase insulin sensitivity in late gestation and early lactation. Adipose tissue from dairy cows supplemented with Cr propionate also had a greater rate of lipid synthesis (lipogenesis) and a tendency for decreased lipolysis compared to control cows (McNamara and Valdez, 2004). These changes in lipid metabolism of dairy cows in early lactation are consistent with increased sensitivity of adipose tissue to insulin.

FEED INTAKE AND MILK PRODUCTION

It is well documented that the transition period from 21 days prepartum to approximately 21 days postpartum is a critical period in regard to health and subsequent milk production of high producing dairy cows (Drackley, 1999). Most of the Cr supplementation studies with dairy cows have involved supplementation during the transition period. Chromium supplementation has tended to increase prepartum intake in some studies (Hayirli et al., 2001; McNamara and Valdez, 2005; Sadri et al., 2009), but not in others (Yang et al., 1996; Besong, 1996; Smith et al., 2005). Supplementation of 0, 3.9, 8.3 and 16.5 mg Cr/day resulted in a linear increase in prepartum DM intake (Hayirli et al., 2001). Sadri et al. (2009) reported that supplementation with Cr (approximately 10 mg Cr/day) increased prepartum DM intake when barley was used as the grain source but not when corn served as the prepartum grain source.

Figure 1. Effects of dietary chromium propionate on serum insulin concentrations and insulin:glucose ratios in growing heifers following a glucose tolerance test.



Supplementation of 0.5 mg Cr/kg diet increased milk yield in primiparous dairy cows in two separate experiments (Table 1; Yang et al., 1996). Chromium was supplemented during both experiments from 6 weeks prepartum until 16 weeks postpartum.

Besong (1996) supplemented multi and primiparous cows with 0 or 0.8 mg Cr/kg diet from 30 days prepartum until 8 weeks postpartum. Performance results were not presented by parity in this study but parity was included in the statistical model. Chromium supplementation increased average milk yield from 31.1 to 33.4 kg/day. Feed intake was higher in Cr- supplemented cows during weeks 2, 3, 4, 5, and 6 of lactation.

A summary of studies evaluating the effects of Cr supplementation on milk production and DM intake in multiparous dairy cows is presented in Table 2. In most studies with multiparous cows Cr supplementation has increased or at least tended to increase DM intake and milk yield. Yang et al. (1996) observed no DM intake or milk production response to Cr supplementation in multiparous cows. However, in these same experiments Cr supplementation improved milk yield in primiparous cows.

Table 1. Effect of chromium supplementation on feed intake and milk production of primiparous cows[a,b]

	Control	+Cr	P-value
Exp 1			
n	6	6	
Milk, kg/d	24.3	27.5	0.06
DMI, kg/d	16.4	16.8	0.76
Cows open	3/6	0/6	0.05
Exp 2			
n	9	9	
Milk, kg/d	24.1	25.7	0.03
DMI, kg/d	15.1	15.5	0.43
Cows open	2/9	1/9	0.53

aAdapted from Yang et al., (1996).

bChromium was supplemented from 6 weeks prepartum until 16 weeks postpartum in both experiments. In experiment 1, chromium was supplemented at 5.5 mg Cr/cow prepartum and 10 mg Cr/cow postpartum. In experiment 2, cows were supplemented with 4.25 mg Cr/cow prepartum and 7.75 mg Cr/cow postpartum.

Estimated NE_L (1.59 Mcal/kg DM) was lower in the lactation diets used by Yang et al. (1996) compared to the other studies (1.67 to 1.74 Mcal/kg DM) summarized in Table 2. It is unclear if feed intake and milk production responses to supplemental Cr are affected by dietary energy level. However, Cr supplementation has not affected milk production in grazing dairy cows where forage was the major source of energy Peterson, 2000; Bryan et al., 2004. High energy diets provide more gluconeogenic substrates and an adequate supply of precursors for glucose synthesis may be necessary to achieve a milk production response to supplemental Cr.

Cows supplemented with Cr from 21 days prepartum through early lactation had higher DM intake and milk production during the first 28 days in milk (Hayirli et al., 2001; Smith et al., 2005). Sadri et al. (2009) reported that grain source used in the pre and postpartum diets affected responses to supplemental Cr. Chromium supplementation, at a level of approximately 10 mg/day, increased DM intake and milk production during the first 28 days in milk when barley was used as the grain source in the TMR. Feed intake and milk production were not affected by Cr addition when corn was used in the TMR.

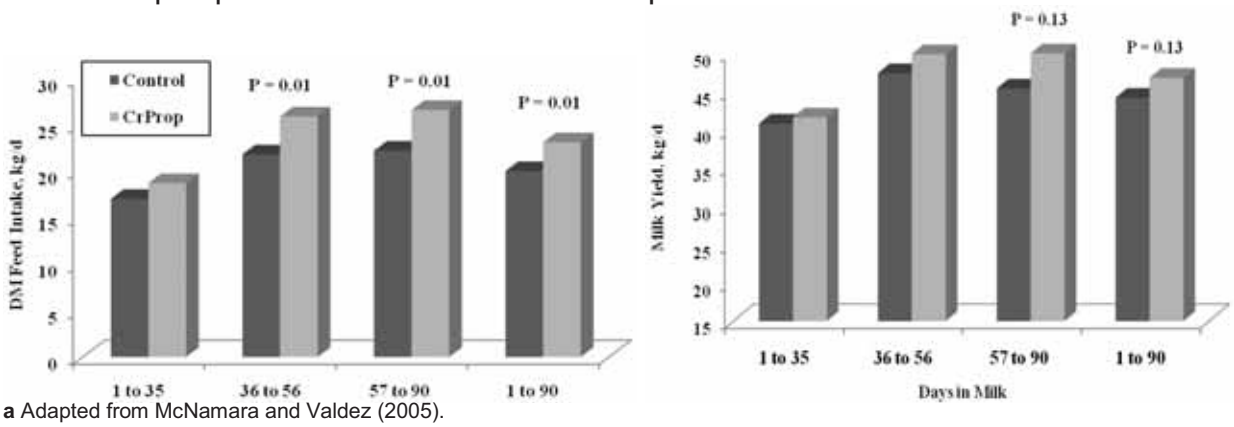
Supplementing with Cr during the transition period may increase feed intake and milk production later in lactation even if Cr supplementation is discontinued. McNamara and Valdez (2005) supplemented dairy cows with Cr propionate from 21 days prepartum until 35 days postpartum. After Cr was removed from the diet on day 35, DM intake and milk production continued to be monitored through 90 days in milk (Figure 2). Numerical increases in DM intake and milk yield were observed in Cr-supplemented cows the first 35 days of lactation. However, differences in intake and milk production

Table 2 . Summary of feed intake and milk production of multiparous dairy cows supplemented with chromium.

Reference	Supplementation period	Cr, mg/cow/day				
		Control	Level 1	Level 2	Level 3	
Yang, et al., 1996	-42 to 112 d postpartum					
Exp 1						
n		11	11			
DMI, kg/d		22.2	22.5			
Milk, kg/d		36.4	36.6			
Exp 2						
n		11	11			
DMI, kg/d		20.8	20.7			
Milk, kg/d		36.8	36.3			
Hayirli et al., 2001	-21 to 28 d postpartum					
n		10	10	10	11	
DMI, kg/d		13.8	14.9	17.2	16.3	Quadratic P = 0.01
Milk, kg/d		33.5	34.0	38.5	31.8	Quadratic P = 0.02
Al-Saiady et al, 2004	120-190 d postpartum					
n		80	80			
DMI, kg//d		19.6	21.2			P = 0.01
Milk, kg/d		29.9	33.2			P = 0.01
McNamara and Valdez, 2005	-21 to 35 d postpartum					
n		10	10			
DMI, kg/d		17.0	18.7			
Milk, kg/d		40.8	41.6			
Smith et al., 2005	-21 to 28 d postpartum					
n		22	25	25		
DMI, kg/d		18.2	18.9	19.7		Linear P = 0.01
Milk, kg/d		40.3	40.5	42.8		Linear P = 0.03
Sadri et al., 2009	-21 to 28 d postpartum					
Barley						
n		8	8			
DMI, kg/d		16.9	18.4			Cr x grain source P = 0.10
Milk, kg/d		34.3	37.7			P = 0.08
Corn						
n		8	8			
DMI, kg/d		18.3	17.8			
Milk, kg/d		34.9	35.2			
An-Qiang et al., 2009	21 to 84 d postpartum					
n		6	6	6	6	
DMI, kg/d		17.6	18.0	18.2	18.2	Linear P = 0.01
Milk, kg/d		24.3	25.3	25.6	25.5	Linear P = 0.01

between control and Cr-supplemented cows were greater from days 36-90 of lactation even though Cr was no longer being supplemented.

Figure 2. Effects of supplementing chromium propionate from 21 days pre until 35 days postpartum on DM intake and milk production[a].



a Adapted from McNamara and Valdez (2005).

The greater feed intake of Cr-supplemented cows in early lactation may relate adipose tissue being more sensitive to insulin. Increasing insulin sensitivity would be expected to reduce release of NEFA from adipose tissue. In turn, blood NEFA concentrations and DM intake are generally inversely related (Overton and Waldron, 2004). Supplementation of dairy cows with Cr has reduced circulating NEFA concentrations at 7 to 10 days prepartum in some studies (Bryan et al., 2004; Hayirli et al., 2001) but not in others (Smith et al., 2008)

Studies in humans and rodents suggest that stress increases Cr requirements. Recently, Cr has been evaluated in lactating dairy cows under heat stress conditions. In Saudi Arabia, supplementation of dairy cows in mid lactation with Cr (4 mg Cr/day) increased DM intake by 1.6 kg/day and milk production by 3.3 kg/day (Al-Sarady et al., 2004). Supplementation of heat-stressed dairy cows with Cr during early lactation in China also increased DM intake and milk production (AnQiang et al., 2009).

REPRODUCTION

Limited research indicates that Cr supplementation may improve reproduction in cattle. Chromium supplementation reduced the number of open cows in one of two experiments with primiparous dairy cows (Table 1) but not in multiparous cows (Yang et al., 1996). Pregnancy rate tended to be higher in intensively grazed dairy cows supplemented with Cr than in controls (Bryan et al., 2004).

Chromium has also affected reproduction in beef cows grazing pastures. Providing Cr in a free choice mineral improved pregnancy rate in beef cows (Stahlhut et al., 2006b). The improvement in reproduction was due to increased pregnancy rate in cows 5 years of age or younger. Chromium did not affect pregnancy rate in beef cows 6 years of age or older. The improved pregnancy rate was associated with much lower plasma NEFA concentrations at approximately 21 and 79 days postpartum in Cr-

supplemented cows (Stahlhut et al., 2006a). Chromium supplementation reduced postpartum body weight loss in 2 and 3-year old cows but not in older cows (Stahlhut et al., 2006b). Supplementation of Cr in a free choice mineral reduced the interval from calving to first estrus and tended to improve pregnancy rate in primiparous Zebu beef cows in Brazil (Aragon et al., 2001). Body weight gain was also greater in Cr-supplemented cows from parturition until their calves were weaned (Aragon et al., 2001). Reproductive responses to Cr may relate to its ability to increase insulin sensitivity. Insulin administration improved ovulation rate in energy-deprived heifers (Harrison and Randel, 1986).

IMMUNITY AND HEALTH

Studies in periparturient dairy cows indicate that Cr supplementation of practical diets may affect cell-mediated and humoral immune responses. Lymphocytes from cows supplemented with 0.5 mg Cr/kg diet had increased blastogenic responses to Con A stimulation (Burton et al., 1993). Furthermore, Cr supplementation prevented the decrease in blastogenic response that was observed in control cows 2 weeks prepartum. Chromium supplementation also improved primary and secondary antibody response to ovalbumin administration but not antibody response to human erythrocytes (Burton et al., 1993). The primary injection of ovalbumin and human erythrocytes was given 2 weeks prepartum and the secondary injection was administered 2 week postpartum. Supplementation with 5 mg Cr/day increased antibody responses following vaccination with tetanus toxin in dairy cows (Faldyna et al., 2003). Neutrophil function has not been affected by dietary Cr (Chang et al., 1996; Faldyna et al., 2003).

Studies examining the effects of dietary Cr on health in dairy cows are limited. Supplementing 3.5 mg Cr/day during the last 9 weeks of pregnancy reduced the incidence of retained placenta in dairy cows from 56 to 16% (Villalobos-F et al., 1997). Chromium supplementation prepartum and during the first 16 weeks of lactation did not affect mammary gland health status (Chang et al., 1996).

Chromium may affect incidence of ketosis by enhancing insulin sensitivity. Insulin is an anabolic hormone that promotes lipogenesis and inhibits lipolysis. Dairy cows supplemented with Cr had lower plasma concentrations of β- hydroxybutyate than controls at 3 and 30 days postpartum (Besong, 1996). Liver triglyceride concentration were also lower in Cr-supplemented cows at 30 days postpartum (Besong, 1996). Chromium supplementation has not affected clinical cases of ketosis in lactation studies that have reported health-related disorders (Chang et al., 1996; Yang et al., 1996; Smith et al., 2005).

CHROMIUM IN FEEDSTUFFS

Variation among studies in response to Cr supplementation may relate to differences in Cr content or bioavailability from feedstuffs. Little is known regarding Cr concentrations in practical feedstuffs and even less is known regarding bioavailability of Cr from common feedstuffs. In most Cr studies with lactating dairy cows the Cr content

of the control diets have not been reported. Chromium analysis of diets is challenging due to the low levels of Cr normally present and problems with Cr contamination of feed samples during collection and preparation of samples for analysis (NRC, 2005). Li et al. (2005) reported Cr concentrations in homegrown and imported feeds from 54 dairy farms in Wisconsin. Mean Cr concentrations in homegrown feedstuffs ranged from 0.33 mg/kg DM for corn grain to 0.91 mg/kg DM for alfalfa haylage. Of the imported feed ingredients, mineral supplements contained by far the highest concentrations of Cr (69 mg Cr/kg). Chromium would be expected to occur as a contaminant in most mineral ingredients and has been found to be particularly high in phosphate supplements (Sullivan et al., 1994). It is unclear if any of the Cr present in phosphate or other mineral sources is present in a form that is bioavailable to cattle. From a total diet perspective, in a recent controlled study prepartum and postpartum TMRs analyzed 0.48 and 0.38 mg Cr/kg DM (Lloyd et al., 2010).

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CLEARING THE AIR ON 'LIVESTOCK AND CLIMATE CHANGE'

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With global meat production projected to more than double the current rate by 2050 (Smith et al., 2007) and the majority of this livestock production growth occurring in the developing world (Wood et al., 2006), assessment of the holistic impacts of food animals in the context of global and regional environmental policy and food security becomes imperative. Much of the growth in the global livestock sector will occur in areas that are currently forested (i.e., parts of South America and South East Asia). It has been well established that significant reductions of carbon sequestering forests will have large effects on global climate change.

The United Nation's Food and Agriculture Organization's (FAO) publication titled 'Livestock's Long Shadow' (LLS; FAO et al., 2006) has been most instrumental in pointing the public attention to the kinds of environmental consequences in which livestock production can potentially result, with special emphasis on climate change. Unfortunately, some of the report's key conclusions (i.e., livestock produces more greenhouse gases (GHG) than transportation) have been applied regionally and out of their intended context, leading to significant consequences on major public policy affairs. For example, the statement that 18% of anthropogenic global GHGs is caused by livestock production and that livestock produces more GHG than transportation (FAO et al., 2006) is based on inappropriate or inaccurate scaling of predictions, and thus is open to intensive debate throughout the scientific community.

Livestock production in most countries of the developed world (e.g., United States and Europe) has a relatively small GHG contribution within the overall carbon portfolios, dwarfed by large transportation, energy, and other industry sectors. In contrast, livestock production in the developing world can be a dominant contributor to a country's GHG portfolio, due to the developing world's significantly smaller transportation and energy sectors. In the United States, transportation accounts for at least 26% and electricity production and use 31% of total anthropogenic GHG emissions compared to roughly 5.8% for all of agriculture, which includes less approx. 3% associated with livestock production. However, in countries like Paraguay, the trend is likely reversed because of Paraguay's much smaller transportation and energy sectors, and a relatively large livestock sector, which might contribute to more than 50% of that county's carbon footprint.

The fact that land-use changes associated with livestock (i.e., forested land converted to pasture or cropland used for feed production) are a significant source of anthropogenic GHGs in Latin America and other parts of the developing world is apparent. However, it is likely that any kind of land-use change from the original

forestland will lead to great increases in global warming. LLS (FAO et al., 2006) attributes almost 1/3 to 1/2 of the climate-change impact associated with livestock to the change of land-use patterns. Latin America has the greatest pool of 'unused but suitable' land that is currently covered by forests but could be turned into agricultural crop or livestock production (Bruinsma, 2003). In 2000, Latin America had 203 million hectares arable land in use and 863 million hectares of unused land suitable for cropland (19% in use) (Bruinsma, 2003). Over the same time span, developed countries had 387 million hectares arable land in use and 487 million hectares of unused land suitable for cropland and livestock (44% in use) (Bruinsma, 2003). Transformation of land from forest to agriculture has occurred in the developed countries centuries ago to make way for industrialization and general societal wealth. Not surprisingly, numerous developing countries are currently attempting to develop their economies by turning economically marginal land into production.

The United States and most other developed countries have not experienced significant land-use change practices around livestock production within the last few decades. Instead, over the last 25 years forestland has increased by approximately 25% in the United States and livestock production has been intensified (concentrated geographically), thus reducing its geographical footprint. Modern livestock production has experienced a marked improvement of efficiencies, leading to significantly decreased numbers of animals to produce a given amount product that satisfies the nutritional demands by society (Capper et al., 2009). According to LLS, intensification of livestock production provides large opportunities for climate change mitigation and can reduce greenhouse gas emissions from deforestation, thus becoming a long-term solution to a more sustainable livestock production.

When comparing GHG portfolio sectors such as livestock versus transportation, comparable assessment tools should be used. For example, the transportation figures used in LLS are 'direct emissions' associated mainly with combustion during transportation and do not include indirect emissions associated with the transportation or oil industries (i.e., manufacturing of vehicles, resource extraction, etc.). On the other hand, the report assesses livestock holistically from a direct and indirect perspective. A comparison between livestock production versus transportation, with one (livestock) assessment based on a complex life cycle assessment (LCA) and the other (transportation) without LCA, is generally questionable.

Comparing LLS (FAO et al., 2006) with several regional reports (CEC, 2005; EPA et al., 2006) shows large agreement with respect to emission predictions from most livestock related categories. There is general consensus that as a direct GHG category, enteric fermentation in ruminants and manure management are the most important categories within livestock production. Categories like on-farm fuel use or feed production are dwarfed by emissions coming from the animals and their manure.

Many investigators use the international standard (ISO 14040) for LCAs that are often rigid, impractical, and not sufficiently transparent. One means of improvement would be the use of a ''numerical suffix system'' indicating the ''degrees of separation''

between the product (e.g., animal protein) and the indirect emissions source input (i.e., the greater suffix number, the more complete the LCA). Furthermore, all current and future assessments of GHG impacts should include mass-balance accounting of energy per GHG unit basis to assess the true environmental impact of direct and indirect emissions. Examples include GHGs associated with displaced fertilizer production through use of animal manure. LLS does not currently account for fertilizer that is not produced because animal manure is present.

LLS (FAO et al., 2006) does not account for 'default' emissions. Specifically, if domesticated livestock were reduced or even eliminated regionally, the question of what 'substitute' GHGs world be produced in their place has never been estimated. While never explicitly stated in any publication, the idea that if livestock were simply eliminated, 18% of anthropogenic GHGs would also be eliminated as well, is unrealistic. In fact, many of the resources previously dedicated to domesticated livestock would be utilized by other human activities, many of which produce much greater climate change impacts. It is also important to realize that livestock provides not only meat, dairy products and eggs, but also wool, hides, and many other value-added goods and services. Livestock are often closely integrated into mixed and some landless (e.g., landless dairy) farming systems as consumers of crop by-products and sources of organic fertilizer, while larger animals also provide power for plowing and transport. Therefore, to estimate accurately the 'footprint' of all livestock, 'default' emissions for non-livestock substitutes need to be estimated and compared to livestock emissions (e.g., manure versus fertilizer, leather versus vinyl, wool versus microfiber, etc.). The net GHG differences between livestock and other land-use forms can then be used to estimate a more accurate GHG 'footprint' of livestock's impact.

Overall, growing demands for animal protein could strongly increase GHG emissions from agriculture. However, knowledge exists to improve efficiencies in livestock production, which dramatically reduces GHG per unit of production. What is called for is a global green revolution in animal agriculture, coupled with technology transfers from developed to developing countries, to supply a growing demand for animal protein using sustainable and modern production practices.

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Development of a mathematical model to predict sizes and rates of digestion of a fast and slow degrading pool and the indigestible NDF fraction

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INTRODUCTION

Neutral detergent fiber (NDF) is the most common measure of fiber used for animal feed analysis, but it does not represent a unique or homogenous class of chemical components. Heterogeneity of the NDF fraction of a plant can be demonstrated by the Lucas test (Lucas, 1964; Van Soest, 1994). The purpose of the Lucas test is to identify ideal nutrition entities that have uniform digestibility over a wide range of feedstuff, by plotting the digestible nutrient concentration in DM against the nutrient concentration in DM. The slope of regression estimates the true digestibility and the intercept is an estimate of the metabolic and endogenous fecal matter. The neutral detergent divides the feed into a soluble fraction that is rapidly and almost completely available and a fiber fraction that is slowly and incompletely degraded by microbial enzymes (Huhtanen et al., 2006b). Furthermore, NDF is also characterized by the presence of a fraction that is unavailable to microbial digestion in ruminants (i.e., indigestible NDF = iNDF) even if total tract residence time of fiber could be extended to infinite time (Allen and Mertens, 1988; Van Soest, 1994). Thus by definition, iNDF represents a uniform feed fraction with zero true digestibility according to the Lucas test (Lucas, 1964). The potentially digestible NDF (pdNDF) will then result from the difference NDF- iNDF.

Although dietary concentration of NDF is related positively to bulk density of feeds and affects feed intake potential (Karkalas, 1985) forage NDF greatly varies in its digestibility in the rumen (Allen and Mertens, 1998) and in vitro (Huhtanen et al., 2006a). Digestibility of NDF influences animal performance independent of dietary NDF concentration. Animal trials where forages of different in vitro digestibility but similar NDF concentration have been fed, reported significant increases in DMI and milk production (Grant et al., 1995; Dado and Allen, 1996; Oba and Allen, 2000). A faster disappearance of the NDF fraction from the rumen because of increased rate of digestion or passage might reduce physical fill in the rumen over time and allow greater voluntary feed intake (Mertens, 1994; Van Soest, 1994).

Accurate and precise predictions of the intrinsic digestion kinetic parameters are critical to the prediction of NDF rumen digestibility and intake. The importance of the fractional rate (k_d) and extent of NDF digestion on total tract OM and NDF digestibility can be demonstrated by simulation with the CNCPS model (Fox et al., 2004; Tylutki et al., 2008) or with the Nordic model of cow metabolism, “Karoline” (Danfær et al., 2006a; Danfær et al., 2006b). Simulation results clearly demonstrate profound effects of these parameters on digestibility and therefore on the supply of energy and microbial protein.

Digestion rates can be highly variable between feeds and even within the same species (Van Soest, 1994).

One of the main problems in describing digestion kinetics is that residues remaining at any digestion time are a mixture of undigested and indigestible matter (Mertens, 1993). Furthermore, Mertens has indicated (Mertens, 1973; Mertens, 1977; Mertens and Ely, 1979) that overall digestion is better predicted when assuming that the pdNDF fraction is the sum of two digestible fractions each of which are first order but with different rate constants. According to Van Milgen et al. (1991) the assumption of a single fractional digestion rate constant is also untenable because of the chemical and morphological diversity of forages fed to livestock. More recently Ellis et al. (2005) demonstrated an improved fit of two-pool pdNDF models that conformed to expectations of a composite of lifetimes of two concurrently degrading sub-entities of pdNDF with different degradation rates. Also for in-vitro gas production and NDF digestion, Huhtanen et al. (2008b) has recently shown a marked improvement of the model when pdNDF was assumed to be comprised of rapidly and slowly degradable fractions. Rate of digestion of NDF is an input in ration analyzers and models (Fox et al., 2004; Tylutki et al, 2008). However, incorporation of digestion rates as a standard procedure to define the nutritive quality of specific feeds and diets has been achieved only recently, in part, because of lengthy laboratory analyses and statistical interpretation of fiber digestion rates. The mathematical approach by Van Amburgh et al. (2004) described a method for determining rates of digestion for a single pool of pdNDF with one time point assuming first order behavior and a fixed iNDF pool. The indigestible fraction was in that case estimated using the formula (ADL × 2.4)/NDF where the 2.4 was the factor obtained by Chandler et al. (1980).

In a previous paper we demonstrated, through improved recoveries and definitions of both ADL and iNDF (obtained after 240 hours of fermentation), the relationship between iNDF and ADL is more dynamic and can be predicted using forage group-specific ranges (Raffrenato et al., 2009; Raffrenato et al., 2010; submitted) according to the ADL/NDF ratio of the specific forage. Our hypothesis is that the improved methodology for measuring ADL and the dynamic estimation of iNDF can result in a better description of pdNDF, with the least number of fermentation points. Therefore, our objective was to describe fast and slow degrading pools of NDF, and the respective fractional digestion rates using 240 hours of fermentation as the endpoint for iNDF with a minimum of time points for application by commercial laboratories. A secondary objective was to assess the accuracy and precision of predicting the same parameters when an end point is not available and a forage-group-specific range for the ratio iNDF/(ADL/NDF), implemented as a proxy for iNDF was used instead. Finally, we wanted to demonstrate how a single weighted k_d for the whole pdNDF fraction, from the fast and slow fractions, can be obtained and used when a fast and slow pool of NDF fermentation is not yet implemented in ration balancing software. The approach used was to develop composite decay models to describe the heterogeneous behavior of NDF digestion. Composite decay models are formed by the sum of several exponential functions and have been used to describe various physical phenomena and the non-linear least-

squares-fit is the most common method in use to solve them (Villuendas and Pelayo, 1987).

MATERIALS AND METHODS

Thirty five forages, including grasses, conventional and bmr corn silages and alfalfas, were analyzed in duplicates for NDF, ADL and 0.75 g of each sample fermented, and residues analyzed for NDF, from 0 to 240 hours according to Goering and Van Soest (1970). Rumen fluid was harvested from two cows being fed a TMR and housed at the Cornell University research farm. Residual NDF of the fermented samples was obtained at 0, 6, 12, 24, 30, 36, 48, 72, 96, 120, 144, 216 and 240 hours based on the procedure of Mertens (2002). The samples that fermented longer than 120 hours were re-inoculated at 120 hours with the same amount of the initial rumen liquor/medium mix. A glass microfiber filter (934-AH, Whatman) was used in all analyses as suggested by Raffrenato and Van Amburgh (2010a and 2010b), to avoid particle loss and increase recovery. A composite decay model was used to estimate all parameters, pdNDF1, pdNDF2, and the respective fractional rates of digestion (k_d1 and k_d2), and iNDF. Therefore the residual NDF at time t was described by:

$$(1) \qquad NDF_t = pdNDF1 * e^{-k1(t-L)} + pdNDF2 * e^{-k2(t-L)} + iNDF$$

where pdNDF1, and k1 are the size and the fractional rate of the fast pool; pdNDF2, and k2 are the size and the fractional rate of the slow pool, respectively, L is the lag and iNDF is the indigestible NDF. Simultaneous estimations of the parameters were initially obtained using PROC NLIN of SAS (SAS Institute, Inc., Cary, NC) and the Marquardt algorithm. Since non-linear regression methods assume equal error at each observation, simultaneously fitting all parameters to the data results in the smallest residual sums of squared deviations and no assumptions are required, thus they were chosen to be the standard procedure. For non-linear estimations, there are problems establishing the initial parameters, so a linear approach was used to seed the non-linear estimation as done by Grant and Mertens (1992). We used the log-linear approach of Van Soest et al. (2005) to generate the initial values, for each sample, to parameterize the composite decay model, including a fast, a slow, and an indigestible pool for model (1) described previously. This results in reasonable confidence that the global solution was obtained for each set of data. Furthermore, our mathematical approach described here is derived from the linearization of the non-linear first order composite decay and it therefore seems reasonable to make comparisons to this procedure and provides a simple cross-check of procedures.

Assuming there is more than one fraction of pdNDF that can be described mathematically (Van Soest et al., 2005) a prediction of three NDF fractions with the least possible number of fermentation points for use in commercial laboratories was an objective. Vensim® (Ventana Simulation Environment; Ventana Systems Inc., Belmont, MA, 2005) is a visual modeling tool that allows the user to optimize models with dynamic components or behavior. In our case, optimization can be used to validate parameters of a model. In order to use the optimization we need to define a “payoff”,

which is a single number that summarizes the simulation, reported at the end of each simulation. In this procedure, an optimization was run using a different combination of fermentation endpoints and the choice of the fermentation endpoints was made by evaluating the time points that best represented the three fractions with the least amount of error. Previous work by Van Soest et al. (2005) showed that the fast pool was exhausted by 48 hours, so the choice was made for a time point up to 48 hours to represent the fast pool, a point between 48 and 216 to represent the slow pool, and the 240 hours to represent the iNDF fraction. All combinations of the points available (6, 12, 24, 30, 36 and 48 for the fast pool; 72, 96, 120, 144 and 216 for the slow pool) resulted in 30 possible combinations to be optimized.

The model was defined in Vensim 5.5 (Ventana Simulation Environment; Ventana Systems Inc., Belmont, MA, 2005) and a modified Powell hill-climbing algorithm (Powell, 1964) was used to perform the parameter optimization as defined in Vensim 5 Reference Manual (Ventana Systems Inc., Belmont, MA, 2006). The payoff for a model can be defined in terms of comparison of model variables with actual data, or as combination of model variables. The residual NDF from zero to 240 hours was chosen as payoff because this was the combination of all the parameters that need to be estimated (i.e.: pdNDF1, pdNDF2, k1, k2 and iNDF). The NDF residue at each fermentation time available (time step) from the data was subtracted from the corresponding value of the model at the same time step. The difference between the data and the model variable was then squared, and all values from all time points available were added. This number was then subtracted from the model payoff. Maximizing the payoff means reaching a value as close to zero for the squared difference. Constants of the model that were optimized for were: L, pdNDF1, pdNDF2, k1, k2, and iNDF, meaning the lag for the fast and the slow pool, the fast and slow pool and their rates and the indigestible fraction, respectively.

For the non-linear optimization in Vensim, the iNDF was constrained to be between 0 and the respective 240 hours NDF residual value. Lag was also constrained based on previous work from our laboratory (Van Amburgh et al., 2004) and based on the results from the non-linear estimation in SAS (SAS Institute, Inc., Cary, NC). Based on our previous and contemporary work, L was constrained to be between 1.5 and 4.5 hours for all forage groups. The sum of the three pools was also constrained to be 1 to avoid negative results for the pools estimations.

To determine the minimum number of values required from the fermentation to establish the pool size and rates, constants were obtained from the optimization with Vensim and were then compared to the values obtained with the non-linear estimation from equation (1) from PROC NLIN in SAS (SAS Institute, Inc., Cary, NC). To evaluate the performance of the optimizations in Vensim, the goodness of fit was compared using the variance accounted for (R^2) and the residual mean squares (RMS) at convergence for ranking purposes, using the data pooling all forages analyzed and then by forage group, for each combination of time points, as in Ellis et al. (2005) and Huhtanen et al. (2008). For each combination, the evaluation was then made separately for each parameter (pdNDF1, pdNDF2, k1, k2, L and iNDF) predicted by the

optimization and computations were made as suggested by Piñeiro et al. (2008), with the non-linear estimation results considered the "observed" values. The root mean square error (RMSE) between the observed parameters and the predicted values using the least number of fermentation endpoints, were calculated as follows: RMSE = $\sqrt{\sum}$ (observed – predicted) ^{2}/n, where n is the number of forages. The mean square prediction error (MSPE) was divided into components resulting from mean bias, slope bias, and random or unexplained variation around the regression line according to analysis of Theil (1966) and Bibby and Toutenburg (1977). Significance of the deviation of the intercept from 0 and the slope from 1 was analyzed by *t*-test. The same evaluation was made by forage group to highlight possible dynamic behavior of pools and rates across forage groups.

Finally, to make this approach applicable to commercial laboratories, an analyses and estimation of extent of NDF digestion and iNDF was conducted using forage-family specific endpoints for iNDF as a starting point, knowing there is a range within the iNDF value within and among forage families and there is a possibility that a laboratory might not have 240 iNDF values. Therefore, assuming that the residue at 240 hours represents the true iNDF fraction, we defined specific ranges of the ratios iNDF/(ADL/NDF) for each forage group (conventional and bmr corn silages, alfalfas and grasses) and determined the range and variance associated with the iNDF value within our dataset. The same evaluation described above was then performed using these specific ranges to determine the absolute minimum of points needed to estimate pdNDF and kd. The iNDF was in then constrained to fall within the range defined for each forage group, based on the ADL/NDF ratio. The ratios iNDF/(ADL/NDF) were constrained in the following manner: 3.0-5.5 for conventional corn silages, 2.0-6.0 for bmr corn silages, 2.0-7.0 for grasses, 2.0-5.0 for straws and hays and 2.0-3.0 for alfalfas (Raffrenato and Van Amburgh, submitted). The other constraints were as previously described. Goodness of fit was evaluated as mentioned previously, using the 240 hours as the observed iNDF.

RESULTS AND DISCUSSION

Descriptive values of the forages by group are found in Table 1. The non-linear model resulted in a high R^2 (>0.98) for all forages. Average values of pools sizes and fractional rates obtained from the non-linear estimation are shown in Table 2 ("observed values"). A rate of digestion developed from the weighted average of the pdNDF was obtained. The pools sizes and rates obtained from the non-linear estimation (Table 2) allowed the extrapolation of the residual pools (example in Figure 1) for the whole curve and it was observed that residues of pdNDF1 became in most cases negative at 48 hours and always at 72 hours, meaning that the fast pool (pdNDF1) was exhausted between 48 and 72 hours for all forages analyzed, confirming the previous data (Van Soest et al., 2005). Forages such as bmr corn silages and alfalfas exhausted their fast pool earlier (36 to 48 hours) in the fermentation curve, when compared to conventional corn silages and grasses.

The R^2 and the RMS among forages resulted each in one value for each forage and combination of time points. For ranking purposes we used the average values among all forages, within each time point combination. This resulted in a ranking of goodness of fit of all possible combinations from Vensim, when pooling all forages. The best combinations were found to be from the following time points: 36-120, 48-120 and 48-96 hours. Although these combinations ranked the highest in order of goodness of fit, there were small differences in RMSE and MSPE, thus a decision was made to use only the 36 and 120 hours time step since that combination resulted in the best average R^2 and RMS. For this specific combination, the R^2 obtained regressing observed on predicted parameters, ranged between 0.77 and 0.98, among all the parameters, except for the lag. Other combinations will be addressed on a forage specific basis later in the paper.

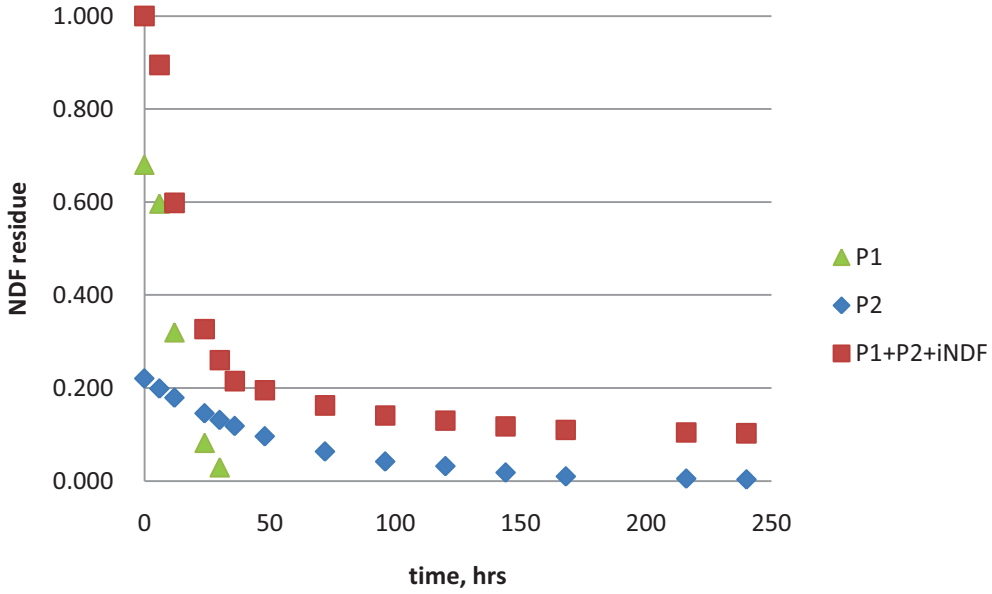
Table 1. Neutral detergent fiber (NDF) (% of DM), acid detergent lignin (ADL, g/kg NDF), indigestible NDF (iNDF, g/kg NDF) and calculated ratios of iNDF/(ADL/NDF) and respective ranges of the forages used in the analyses per group of conventional and bmr corn silages (C.S.), grasses and alfalfas(ranges in parentheses).

Group	n	NDF	ADL	iNDF	ratio
		% of DM	-----g/kg NDF------		
Conventional C.S.	7	44.04	68.6	232.4	3.38 (3.23-5.46)
BMR C.S.	6	39.06	34.2	123.2	3.60 (2.14-5.78)
Grasses	6	64.03	80.9	286.3	3.53 (2.59-6.53)
Straws and hays	4	77.25	90.0	343.2	3.45 (2.60-4.39)
Alfalfas	7	36.64	169.1	428.2	2.53 (2.43-2.95)

Table 2: Pools sizes and rates obtained from the simultaneous non-linear estimation using equation (1) and the non-linear procedure in SAS, averaged by forage group. The pdNDF1 represents the percentage of NDF in the potentially digestible NDF pool 1, pdNDF2 is the percentage of NDF in the potentially digestible NDF pool 2 and the k1 and k2 are the associated rates. The k_d represents a weighted average rate of pdNDF1 and pdNDF2.

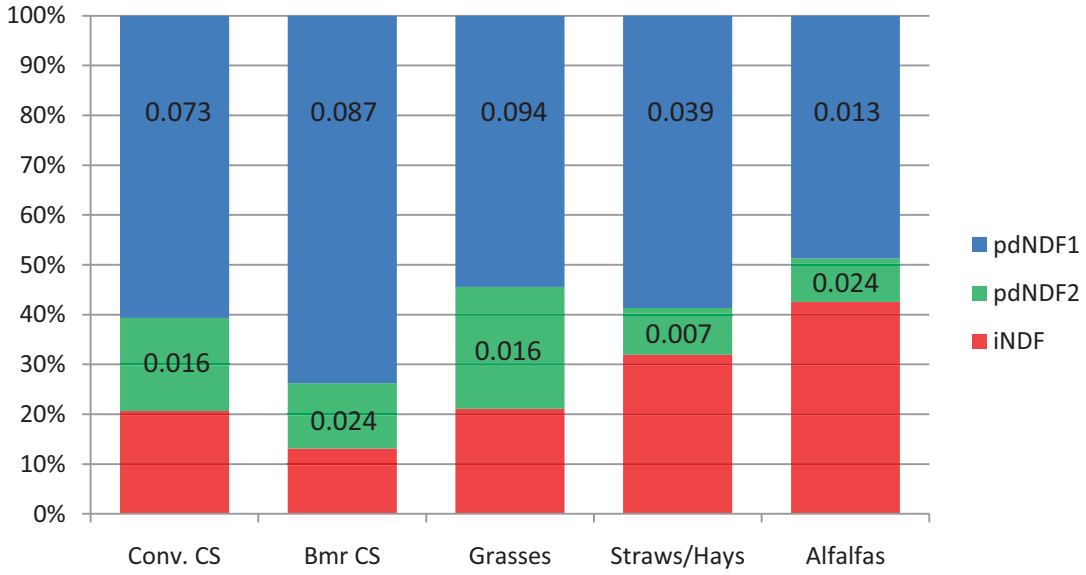
Group	n	pdNDF1	pdNDF2	iNDF	k1	k2	k_d
		----------% of NDF---------			----------%/h----------		
Conventional C.S.	7	60.66	18.71	20.63	0.0729	0.0162	0.0597
BMR C.S.	6	73.76	13.14	13.11	0.0873	0.0239	0.0781
Grasses	6	54.45	24.45	21.10	0.0941	0.0162	0.0669
Straws and hays	4	58.68	10.35	32.27	0.0399	0.0069	0.0351
Alfalfas	7	48.75	8.73	42.53	0.1344	0.0238	0.1128

Figure 1: Residual NDF during the in-vitro fermentation, from 0 to 240 hours, and the extrapolated amounts of the fast (P1) and the slow pool (P2) for a conventional corn silage.



and

Figure 2: Average distribution of fast and slow pool and indigestible fractions in the forages analyzed, averaged by forage group. Numbers within pools represent the respective average fractional rates (1/h).



Using 36 and 120 hours time points and the measured iNDF fraction provided the highest correlations between the optimization in Vensim and the values from the non-linear estimation ($P < 0.01$), with ranges in correlation between 0.88 and 0.99. However, even if the overall equations tended to describe the data equally well, our objective was the development of a mathematical approach that would allow a

commercial forage laboratory to generate this data for field application, therefore the analysis of the bias in the actual values of the parameters obtained due to calculation method was important. Pools sizes and rates of digestion were evaluated for their prediction accuracy and biological relevance, with the preferred prediction having a small regression bias and minimal unexplained variation.

In our current approach, the prediction of the lag resulted in very low correlation with the observed values ($P < 0.05$), a consistently high RMSE (>50%) and the prediction is omitted from the tables. This was most likely due to the lack of time points early enough in the fermentation to allow the optimizer to estimate the lag. However, results show that the lag does not affect the prediction of the pool size parameters, if the lag is constrained within a reasonable range of observed values during the optimization (Raffrenato et al., 2009). In our laboratory procedure, the lag is generally less than 3 hours and can average less than 1.5 hours depending on the forage and rate of hydration at inoculation. The prediction of the pool sizes resulted in RMSE lower than 5% of the total NDF pool size. In addition, the prediction of the rates resulted in RMSE lower than 0.5% of k1 and k2. Finally, the prediction of the iNDF was characterized by a very low RMSE, (1.4% of the total NDF pool), confirming that the 240 hours NDF residual represents the true indigestible fraction.

The relationships among the predicted and observed parameter values for the 36-120 hour time points are shown in Table 3. All R^2 were consistently higher than 0.77. The optimizations slightly over-predicted the size of pdNDF1, however slopes were not statistically different from 1 ($P > 0.05$). In most cases the random variation had the greatest contribution to the total MSPE, especially for pool size predictions.

To test the effect of not using the measured iNDF, the optimizer was constrained to forage-group-specific ranges for iNDF and this procedure resulted in an overall lower RMS and higher R^2 for the 30-120 hours combination. Under these conditions, the 36 hour time point did not provide the best solution. The outcome demonstrated that he indigestible portion was efficiently estimated, and the optimization was able to explain 97% of the variation in iNDF (Table 4). A gain, RMSE and MSPE values were mainly the result of unexplained variation. This suggests that once we understand the range in iNDF within a forage type, we can estimate the iNDF through non-linear approaches and that would preclude the need to conduct the long-term fermentations. However, we are going to strongly suggest that a large data set of 240 hr fermentations are needed to truly establish the relationships and then an NIR approach can be used.

Again, the optimization of the lag values resulted in the lowest correlations, and the highest values of MSPE and RMSE indicating a lack of information for the optimization. As previously shown (Raffrenato et al., 2009), the estimation of iNDF was therefore more important than the estimation of the lag in the prediction of the other parameters of a decay model. All other parameters resulted in very low bias, slope and random variation and the optimization in Vensim can become a routine tool to better define fiber fractions in forages for a better decision making process for nutritionists.

Table 3: Relationship between the predicted and observed model parameters when using 36 and 120 hours as fermentation time points and pooling all forages.

Parameter	Intercept	Slope	R^2
pdNDF1	0.1199	0.8483	0.83
pdNDF2	0.0108	0.7499	0.84
k1	0.0022	0.9240	0.87
k2	0.0009	0.7551	0.77
iNDF	-0.0128	1.0228	0.98

[a]: Intercept significantly ($P < 0.05$) different from 0.
[b]: Slope significantly ($P < 0.05$) different from 1.

Table 4: Relationship between the predicted and observed model parameters when using 30 and 120 hours as fermentation time points, and constraining iNDF to forage-family-specific ranges, when pooling all forages and not using the measured iNDF.

Parameter	Intercept	Slope	R^2
pdNDF1	0.0944	0.8562	0.77
pdNDF2	0.0098	0.8445	0.73
k1	0.0101	0.9010	0.76
k2	0.0023	0.8485	0.75
iNDF	0.0157	0.9590	0.97

After evaluating the global data set, it became apparent that due to the more dynamic nature of the iNDF and the apparent differences in the size and rate of the pdNDF1 pool, it would be more efficient and practical to run forage specific solutions. Therefore, we optimized all the same combinations of time points for the corn silages as an example of the improvements in efficiency and accuracy that could be obtained. We recognize the small data set, but the solutions appear to be very robust. Using the same evaluation for goodness of fit, the results clearly demonstrated that different time points might be necessary to reach the “best possible estimation” of pools and rates for a specific forage group. The relationship between predicted and observed values was quite good, with intercepts not different than zero and slopes not different than one (Table 5). Specifically, conventional corn silages showed the lowest RMS and highest R^2 for the 36-120 hours combination (with similar values for the 48-120 hours), when using 240 hours for iNDF. The parameter prediction was better when conducted on an individual forage family basis than when pooling all forages among groups, with lower RMSE and MSPE and higher R^2.

Table 5: Relationship between the predicted and observed model parameters when using 36 and 120 hours and the measured iNDF (240 hours) as fermentation time points for conventional corn silages.

Parameter	Intercept	Slope	R^2
pdNDF1	0.0399	0.9213	0.91
pdNDF2	0.0208	0.8563	0.88
K1	0.0032	1.0112	0.89
K2	0.0019	0.9653	0.81
iNDF	0.0019	0.9865	0.98

Constraining the optimizer to the range for iNDF specific for conventional corn silages and not including the 240 hours as time point, resulted in overall lower RMS and higher R^2 for the 36-120 hours combination (Table 6). There was an improvement in the slope and intercept parameters compared to pooling all forages and the iNDF was again efficiently estimated. The optimization was able to explain 98% of the variation in iNDF using the ranges to seed the non-linear process and the RMSE and MSPE values were primarily result of unexplained variation.

Other forage groups are not shown, however overall results show 36 (or 48) and 120 hours being the optimal combination of time points. Both bmr corn silages and alfalfas resulted in better goodness of fit when using 24-96 hours and 30-120 hours, respectively most likely because of the time point where the pdNDF1 is exhausted. Grass hays and straws instead resulted in better estimations of pools and rates when residual at 36-96 or 48-96 hours were used for the optimization, respectively. The prediction results appear to be dependent on when the fast pool is exhausted. More forage analyses will be necessary to confirm this concept and to better explain the variation within forage group.

The calculation of k1 and k2 using the Vensim optimization has the advantage of requiring the least amount of fermentation information that could be generated by commercial laboratories. Since the calculations are direct and use no statistical regression procedures, they are simpler to implement because many observations are not required. If a number of time point digestions are available, means and standard deviations of the respective lag and rate values can be calculated and their uniformity examined. An improvement of the lag estimation is possible using another time-point in early fermentation, to be used in the Vensim optimization as was done in the work by Van Amburgh et al. (2003). However the average value of the lag estimated by the non-

Table 6: Relationship between the predicted and observed model parameters when using 36 and 120 hours as fermentation time points, and constraining iNDF to forage-family-specific ranges, when pooling all forages.

Parameter	Intercept	Slope	R^2
pdNDF1	0.0532	0.9312	0.79
pdNDF2	0.0122	0.8923	0.75
k1	0.0098	0.9101	0.79
k2	0.0032	0.8332	0.75
iNDF	0.0034	1.0012	0.98

linear composite decay, per group, was between 1.7 and 3.1 hours, with the lowest values for alfalfas and the highest values for straws and hays, respectively. Constraining the lag to be the average per forage group during the optimization will improve the optimization. Ranges can however vary within and among laboratories, depending on the in-vitro procedure and rumen fluid handling. However, the results show that an unknown lag (within the normal range) will not bias the final estimation of pools sizes and rates.

According to Ellis et al. (1999) determination of iNDF should be included in all basic feedstuff analysis because it has a predictable digestibility; it can be used for the estimation of the pdNDF as NDF-iNDF and it has an important role in contributing to rumen digesta load. Furthermore, a close empirical relationship between silage iNDF and OM digestibility (Nousiainen et al., 2003) indicates that iNDF is a useful entity for the prediction of the nutritive value of forages. We demonstrated that prediction of the indigestible fraction is possible if longer time points are not available, by using forage-group specific ratios of iNDF/(ADL/NDF) (Tables 4 and 6). However, better results will be guaranteed by longer fermentations (up to 240 hours) to estimate the indigestible fraction. A larger data set of long-term fermentations (240 hours) with intermediate time points are needed to build a data set able to explain the variation in NDF pool size and within forage group and this should be linked to the agronomic conditions the plants were grown under.

SUMMARY

- NDF is confirmed to be a non-uniform fraction with multiple pools.
- Later points in the fermentation curve, even if not biologically relevant for the cow, explain the non-uniformity and result in a more accurate and precise single weighted rate of NDF digestibility.
- The fast, slow and indigestible pools and respective rates can be estimated using a minimum of time points of in vitro NDFD data and a forage-group specific ratio iNDF/(ADL/NDF) to obtain the indigestible portion.

- Measures of pools and rates can be used to help explain cow intake behavior or to compare forages, especially when chemical analyses show forages to be similar.
- Estimations of the slow pool and its rate along with the size of the iNDF fraction can theoretically be linked to effects on dry matter intake and passage by increasing the bulk of the diet (rumen load).

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FOOD CHAIN MYCOTOXINS 2010: THREATS AND SOLUTIONS

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Mycotoxins are produced by fungi found in both animal feedstuffs and human foods. These naturally-occurring poisons can cause kidney and liver damage, cancer, suppress the immune systems, induce malnutrition and interfere with the reproductive system among other acute and chronic disease states The reproductive effects include malformation of the genitals, infertility, feminization of males and early puberty and breast development in a variety of mammals, including humans.

Aflatoxins (found mostly in corn, peanuts, soy, cottonseed and nuts) are the best known mycotoxins and cause liver damage and liver cancer along with immune suppression and disruption of absorption and metabolism of essential nutrients.

Aflatoxins are produced by Aspergillus flavus and A. parasiticus which grows at 14-30% moisture, grows best around 25^{o}C and doesn't grow much <12^{o}C or >41^{o}C. Since only 20ppb total aflatoxins are allowed in US human food and dairy feeds and US milk must be less than 0.5ppb, aflatoxin is well-monitored by most feed companies. Those that produce feed for use on their own stock farms often lack the resources and motivation to test each bin, tank or silo for this known carcinogen and immunosupressant. And there is no widespread systematic monitoring of US dairy products for the M1 form of aflatoxin produced by animals fed mycotoxin-contaminated feed.

Zearalenone, found in grains (primarily corn), is one of the most powerful environmental estrogens known and, in contrast to aflatoxin, is not as frequently monitored at any step of the food chain, except in the case of some hog feeds. DON (vomitoxin) is produced by fusarium mold and causes reduced feed intake and a range of adverse symptoms in infected corn as well.

These bad actors have been followed by emerging mycotoxins such as citrinin, ochratoxin, fumonisins, and others, with various effects including severe damage to the kidney, the brain and give dairy producers false positive field tests for antibiotics in the milk. The purpose of this presentation is to discuss the threat, the detection and the means to stop the penetration of these mycotoxins into the human food at the retail, wholesale and production levels. This would include feeds and foods offered in commerce, as well as those produced, stored and processed on the farm.

Pre-emptive systematic characterization of what is going on throughout the food chain should help prevent outbreaks of animal and human disease rather than merely explain them in a post-disaster assessment.

AFLATOXINS SUPPRESS THE IMMUNE SYSTEM

Gambian children with detectible circulating aflatoxin adducts had lower IgA concentrations than those that did not and also had weaker responses to challenges by pneumococcal antigen (Turner et al. 2003). In their recent review of aflatoxicosis, Williams, et al. (2004) anticipated publication of results showing suppression of a variety of immune cell types and functions for Ghanaians with above average aflatoxin albumin adducts. Williams et al (2004) also infer that more rapidly turning over biomarkers, such as urinary AFM1, might be better matched to the pace of immune system modulation. Cusumano et al. (1996) found human monocytes were functionally impaired by AFB1.

In animal models, where prospective controlled dosage experiments are possible, the full extent and nature of damage to the immune system is better characterized. Hatori et al. (1991) found decreased CD4 cell numbers and associated drops in IL-2 when mice were dosed with AFB1. And a wide variety of animal models (mice, chickens, rats and swine) have shown that aflatoxin not only wrecks havoc on thymic and splenic T-lymphocytes (Pier at al. 1986, Ali et al. 1994), but also compromises the macrophages that envelope and present challenges to the lymphocytes (Richard and Thurston, 1975, Neldon-Ortiz 1991, Moon 1999).

It is little wonder that the most common acquired immune deficiencies studied in our food animals are caused by mycotoxins. Preliminary work in Haiti and Kenya has given us reason to believe that Haitian citizens have more than HIV to worry about in regards to immune competence, as well.

Stunts growth, anti-nutritive

Long known to impede growth in farm animals (Shane 1993) in the range of acute exposures reported for humans, aflatoxins have recently been implicated in stunting of children in Benin and Togo (Gong et al 2002, 2003, 2004) and perhaps causing kwashiorkor but certainly delaying recovery from the that condition (Adhikari, et al. 1994). Zinc, selenium, and vitamin A and levels are cut by half or more in animals fed aflatoxin, but less information is available for humans. None of this is surprising since aflatoxin binds the DNA responsible for the synthesis of proteins that represent the growth of young animals and the proteins responsible for the absorption of minerals and the binding and transport of vitamins.

Cirrhosis, liver cancer

These conditions are the usual focus of discussions of aflatoxin toxicology, but not here. Suffice to say that the livers of Third World peoples are no less susceptible to a given dose of aflatoxin (Gorelick et. al, 1993) and the connections among hepatitis B, aflatoxin and both cirrhosis and liver cancer are well documented for children and adults (Egner et. al 2001) in developing countries .

Role of mycotoxins in enhanced HIV infection.

Williams et al. (2010) have discovered strong associations between infection with HIV, the incidence of AIDS developing from those HIV infections and the inclusion of maize in the diets of Africans. Although the role of aflatoxin (commonly found in maize) in the suppression of the immune system is well known, the newly elucidated association seems to be more closely related to the presence of fumonisin. So in addition to checking horse feeds for the fumonisin that causes brain damage in horses, the time has arrived to assess the human food chain as well.

Mycotoxins as endocrine disruptors

Zearalenone (and related compounds and isomers) is produced by Fusarium molds that grow best at 20-25^{o}C at an optimum moisture of 45% but can grow at anything above 25%. Optimal toxin production requires a cool (15^{o}C) period after the fungi has established itself. These toxins are powerful environmental estrogens and reproductive toxins. These reproductive effects include malformation of the genitals, infertility, feminization of males and early puberty and breast development in a variety of mammals, including humans. Tomaszewski and others reported that women had elevated circulating zearalenone associated with hyperplastic endometria (47.8 ng/ml) and actual endometrial cancer (167.0 ng/ml) compared to women with normal uteri (below detection). Szuetz found early thelarche in Hungarian girls was associated with elevated zearalenone in the sera and food of these patients. Similar suggestions were made concerning an outbreak of precocious puberty in Puerto Rico (Saenz 1985), but in the latter case, no serum measurements were taken.

Improvements in animal nutrition have permitted US livestock to be bred earlier than was possible 50 years ago. Increased knowledge and application of nutritional sciences coupled with higher intakes of calories and reductions in physical activity have been accompanied by increased growth, increased obesity and decreased age at thelarche and first menstruation for US girls as well.

But, the dramatic increase among American girls for early puberty, early breast development and early development of adult secondary sex characteristics signal the presence of estrogen in these children's environment. A powerful estrogen like zearalenone bears examination as a contributor to this problem. New York's climate is ideal for producing zearalenone, and although many commercial swine feeds have been tested privately for this powerful environmental estrogen (commonly disrupts reproduction in both male and female pigs), most of our other livestock feeds and human foods are not.

DETECTION OF MYCOTOXINS

Dramatically increased awareness of the hazards of mycotoxins has led to the development and marketing of a wide variety of rapid detection methods, although the

quality varies. Part of the ongoing work in Cornell Animal Science Department is evaluating the various methods.

Visual examination of samples is the oldest and, for some commodities (e.g. peanuts) can be surprisingly effective. Counting the proportion of broken, soft, light, insect infested and (of course) moldy peanuts in the shell is quite predictive of the presence of aflatoxin, but is fairly subjective. This can be done with other commodities, but not as well: corn, cottonseed meal, etc.

Ultraviolet light can be used to look for aflatoxin, since it glows blue or green under such a lamp. Unfortunately, kojic acid, a common mold product, glows even brighter blue than aflatoxin, resulting in false positive tests for aflatoxin that frustrated many in the feed production and processing industry.

While HPLC, TLC and LC/MS methods are useful for precise, sensitive research and commercial lab services, they are not really viable on-farm methods. Fortunately, several companies have developed lateral diffusion immunoassays (dip sticks) and small ELISA columns available to pull a wide variety of mycotoxins from sample extracts for inexpensive fluorometry. The UN began work on well plate immunoassays which are evolving into useful techniques for commercial use.

ENTEROABSORBANTS

A recurring vision for those working in feed protection is an additive that can bind to mycotoxins and prevent their absorption by the animals fed contaminated feed. Unfortunately, there have been few successes in this area, and they tend to be of rather narrow application. For example there are hydrated sodium calcium alumino-silicates (HSCAS) that can selectively bind aflatoxin B1 without depleting micronutrients and are widely used in animal feeds (Williams et al. 2004). A few other clays of similar chemistry and mineral lattice architecture have some efficacy as well.

CHEMICAL TREATMENT

Once mycotoxins are formed in feed, there is not much one can do to get rid of them. In theory, a combination of heat with ammonia can irreversibly detoxify aflatoxins. Although what that does to feed texture and palatability is another story. Just heat or just a base other than ammonia can make the blue glow go away, but the feed is not protected. Ammonia can help prevent mold growth some, but not as well as propionic acid.

Propionic acid can help inhibit mold growth (and thereby prevent the production of mycotoxins). So if one finds themselves having to transfer high moisture corn or other fermented material from one place to another for subsequent storage or remote and delayed feeding, adding this silage preservative can prevent the mold growth that often happens under those circumstances.

Propionic acid is also the rare exception to the general lack of post synthesis destruction of mycotoxins: it can destroy citrinin at propionate concentrations used for general silage preservation.

BLENDING

Diluting an adulterated feed with clean feed to bring the total level below regulatory or toxic thresholds is tempting and often practiced. But the FDA frowns on this practice except in dire regional emergencies. In the view of some, mixing an adulterated feed with a clean feed produces a larger amount of contaminated feed. While that view is a non-quantitative way of looking at the world, giving problems inherent in getting representative samples of feeds for mycotoxins, it may have more merit than it seems.

Currently, to legally blend feeds contaminated with aflatoxin, one must get the permission of the regional FDA authorities and if clean feed is hard to find in your region, then one will usually be allowed to blend down anything with less than 500ppb total aflatoxins. So if you plan to meet the 300ppb standard for finishing beef cattle and your contaminated elevator is at 700ppb, you are out of luck. Similarly, if you are in a region where blending is OK'd by the FDA because of an emergency situation and you are trying to make dairy cattle feed (20ppb) by blending down a tank of 300ppb feed, then you will need a lot of clean feed.

Table 1. FDA action levels for aflatoxins (Food and Drug Administration, 2000)

Commodity	**Action Level ppb**
Corn and peanut products intended for finishing (i.e., feedlot) beef cattle	300
Cottonseed meal intended for beef, cattle, swine, or poultry (regardless of age or breeding status)	300
Corn and peanut products intended for finishing swine of 100 pounds or greater	200
Corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry	100
Corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals	20
Corn, peanut products, cottonseed meal, and other animal feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known	20
Brazil nuts	20
Foods	20
Milk	0.5 (aflatoxin M1)
Peanuts and Peanut products	20
Pistachio nuts	20

The most current FDA DON advisory levels were updated on July 7, 2010 and are as follows:

1. "1 ppm DON on finished wheat products, e.g. flour, bran, and germ, that may potentially be consumed by humans. FDA is not stating an advisory level for

wheat intended for milling because normal manufacturing practices and additional technology available to millers can substantially reduce DON levels in the finished wheat product from those found in the original raw wheat. Because there is significant variability in manufacturing processes, an advisory level for raw wheat is not practical.

2. 10 ppm DON on grains and grain by-products (on an 88% dry matter basis) and 30 ppm in distillers grains, brewers grains, and gluten feeds and gluten meals derived from grains (on an 88% dry matter basis) destined for ruminating beef and feedlot cattle older than 4 months and ruminating dairy cattle older than 4 months, with the added recommendations that the total ration[2] for ruminating beef and feedlot cattle older than 4 months not exceed 10 ppm DON, and the total ration for ruminating dairy cattle older than 4 months not exceed 5 ppm DON. For chickens, 10 ppm DON on grains and grain by-products with the added recommendation that these ingredients not exceed 50% of the diet of chickens.
3. 5 ppm DON on grains and grain by-products destined for swine with the added recommendation that these ingredients not exceed 20% of their diet.
4. 5 ppm DON on grains and grain by-products destined for all other animals with the added recommendation that these ingredients not exceed 40% of their diet.

[2] The total ration includes grains, all grain by-products including distillers and brewers grains, hay, silage, and roughage."

Table 2. FDA Guidance for Fumonisins (June 18, 2009)

Class of Animal	Feed Ingredients & Portion of Diet[1]	Levels in Corn & Corn By-products[1]	Levels in Finished Feeds
Equids and Rabbits	Corn and corn by-products not to exceed 20% of the diet **	5 ppm	1 ppm
Swine and Catfish	Corn and corn by-products not to exceed 50% of the diet**	20 ppm	10 ppm
Breeding Ruminants, Breeding Poultry and Breeding Mink*	Corn and corn by-products not to exceed 50% of the diet**	30 ppm	15 ppm
Ruminants >=3 Months Old being Raised for Slaughter and Mink being Raised for Pelt Production	Corn and corn by-products not to exceed 50% of the diet**	60 ppm	30 ppm
Poultry being Raised for Slaughter	Corn and corn by-products not to exceed 50% of the diet**	100 ppm	50 ppm
All Other Species or Classes of Livestock and Pet Animals	Corn and corn by-products not to exceed 50% of the diet**	10 ppm	5 ppm

[1] Food and Drug Administration, 2009

FDA has not yet committed to advisory, guidance or action levels for citrinin, ochratoxins, or zearalenone.

INCINERATION

Burning corn directly for to generate electricity to power vehicles is far more efficient than converting it to ethanol and burning it in a stove for heat is even more efficient. Unlike conversion of contaminated corn to ethanol, burning it does not leave a toxic byproduct feed behind. And burning “red-tagged” corn does not remove food from the economy, if it is too toxic to feed or eat anyway.

RECENT MYCOTXIN WORK AT CORNELL ANIMAL SCIENCE

In 2006, Dr. Patricia Wolf from Meds and Food for Kids (NGO active in Haiti) sent samples to our laboratory and the initial levels we found in Haitian peanuts were alarming: 380-1567ppb total aflatoxins with an average of 797.5 +/- 218.5ppb by ELISA and fluorometry. HPLC analysis showed that January harvest samples were 88.5% B1, 11.5% B2 and May samples were 77.9% B1, 11.4 %B2, 8.9 % G1, and 1.7% G2, indicating that in May there were probably two species of Aspergillus (both *Aspergillus flavus* and *Aspergillus parasiticus*) making the toxin in Haiti.

On our advice, MFK began sorting the peanuts visually and removing kernels that floated in water. In September 2006, MFK found market peanuts which we analyzed at 412.5 +/- 32.1ppb. In November 2006 some farmer-stored, were found that had 125 +/- 7.1 ppb aflatoxin. The same month after stringent bulk selection on a farm in Port Margot, MFK found peanuts with 26.8 +/- 7.0 ppb. Still above US standards but much better. In January 2007, the PI directed some trials of sorting and floating procedure that resulted in a peanut supply that tested at 0.20 +/- 0.10 ppb, showing that Haitian manufacturers can eliminate most of the threat of aflatoxin by manual methods.

We have been successful in receiving long term funding for similar work in Haiti and Kenya and anticipate beginning to apply much of what we have learned overseas to the intake, metabolism and transmission of mycotoxins by New York livestock this month (October 2010).

The 2009 harvest year has been wet, cool, and delayed in many places and provided us with some moldy grains and byproducts to practice on and we are engaged in making sure we know, before contaminated foods arrive at the market, what is going on in the mycotoxin world on New York farms.

It is hoped that programs such as this will help producers regain control of food chain mycotoxin levels.

OBJECTIVES FOR CORNELL FEED MYCOTOXIN RESEARCH

To create a useful, annotated database that provides planners and extension personnel with the following:

1) Measurements of the incidence of aflatoxin and zearalenone incidence in corn produced and stored for use on 30-100 New York State animal production units per year for three years.
2) Measurements of the incidence of aflatoxin and zearalenone in commercially available animal feed from 30-100 companies of various sizes.
3) Measurements of citrinin, DON and ochratoxin in those above feeds destined for dairy stock and fumonisin in those destined for horses and human populations.
4) Published summary of practices and seasonal climate variables associated with these findings.
5) Measurements of the incidence of aflatoxin and zearalenone incidence in snack foods, milled grains, dairy and meat products produced in New York from 30-100 retail outlets across the state.
6) Investigation of how much of the mycotoxin that reaches livestock is transferred to food products. We plan to apply Wang's blood aflatoxin-lysine methods to dairy cattle so they can be used to sample feed over time, avoiding the problems of mold infection heterogeneity.

The following three populations will benefit from this work:

1. Consumers of New York State foods made from crops susceptible to aflatoxin contamination.
2. Animals and their owners that consume feeds grown and processed in New York.
3. The feed and food producers themselves that will find themselves ahead of the when public concern demands low mycotoxin foodstuffs.

In particular, there are sub-populations of New York citizens that will benefit from this work. Individual farmers, small processors and consumers lack the resources to fully and frequently test their foods for these contaminants. With exception of aflatoxins, Federal and State requirements for routine testing of these mycotoxins are entirely inadequate to follow these poisons through the food chain. Even in the case of aflatoxins, on-farm testing is rarely applied as regulatory focus falls on feeds and foods offered for sale after off-farm storage and processing. For this reason, public funding is needed to establish ways to fix this problem that can later be shared with private mycotoxin monitoring enterprises. We expect that systematic surveys of grains produced and processed in New York and the products made from them by both factories and livestock will show us where the critical points can be found to turn off the flow of mycotoxins that would otherwise harm our domestic animals and ourselves. We can't test all foods produced here, but examining a few representative product pathways can tell us a great deal about the rest of them. Because New York has both the warm humid summers molds need to establish themselves and the cool storage periods that Fusarium molds need to manufacture zearalenone and fumonisin, its small farms and

moderate sized feed and food processors are particularly vulnerable to this kind of threat.

We expect to have a database of information concerning the incidence of aflatoxin and zearalenone in a large sample (30+ enterprises at each level) of homegrown feeds, commercially sold feeds, and retail stock feed and New York food grain products including snack foods, dairy and meat. (and possibly a smaller base for citrinin and ochratoxin in dairy feeds and fumonisin in feeds destined for horses and humans). When they go on line, we plan to sample distillers' grains from ethanol producers, as well.

With this information, we can trace contamination forward and back through the food chain (in full consultation of actors at each level). The first impact will be awareness on the part of the feed and food industry, the second will be reductions based on recommendations of known steps to reduce contamination and the final step will be experimental implementation of new techniques.

Additional updates regarding approved detection methods and allowed strategies for reducing the impacts of mycotoxins will be presented at the oral presentations. FDA mycotoxin guidance and variance policies change regionally and with each harvest season.

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MEASURING THE EFFECT OF STRESS DURING THE TRANSITION PERIOD ON SUBSEQUENT HEALTH AND PERFORMANCE OF DAIRY CATTLE

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METABOLITES ASSOCIATED WITH DISEASE OUTCOMES

Metabolite testing may be a promising tool that producers can use to identify opportunities for improving dairy cow management (Kaneene et al., 1997; LeBlanc et al. 2005; Oetzel 2004; Ospina et al., 2010a). Work in this area has primarily focused on the relationship between health and metabolites related to energy status (e.g. NEFA and BHBA). For example, Ospina et al. (2010a) reported that cows with circulating NEFA concentrations > 0.29 mEq/L during the 2 wk period before calving had twice the risk for postpartum disorders (displaced abomasum, clinical ketosis, metritis, or retained placenta). Furthermore, cows with circulating NEFA concentrations > 0.57 mEq/L during the 2 wk period after calving, had 4 times the risk for these disorders compared to cows with NEFA concentrations below this cutpoint (Ospina et al., 2010a).

Little work has explored whether physiological indicators of stress may also be used to predict disease. The transition period is marked by numerous environmental and management changes including social regroupings, mixing of heifers and cows, and changes in diet formulation (Grant and Albright, 1995; Cook and Nordlund, 2004) which may represent stressors capable of influencing health and performance. Physiological indicators of stress particularly during the period before calving may reveal opportunities for improvements in transition cow management.

Changes in the activity and functioning of the hypothalamic-pituitary-adrenal (HPA) axis are often used to quantify an animal's response to a potential stressor (Mormède et al., 2007). Increased plasma cortisol concentrations have been associated with management and environmental factors thought to be stressful. Cortisol concentrations in cattle increase in response to overstocking (Friend et al., 1979), transportation (Lay et al., 1996), and re-penning or re-grouping (Friend et al., 1977; Gupta et al., 2005), however plasma cortisol may be a poor candidate for metabolite testing and field diagnostics for several reasons. Restraint and handling, which are required during blood sampling, can activate the HPA axis and raise circulating cortisol concentrations quickly (Cook et al., 2000). Further, the release of cortisol from the adrenal gland throughout the day is pulsatile, has a diurnal cycle and is subject to substantial individual variation (Thun et al., 1981), therefore in order to obtain an accurate assessment of HPA axis activity, multiple plasma cortisol samples would have to be evaluated. Since field metabolite testing generally involves collecting a single blood sample from a representative population of animals, plasma cortisol measurements may not provide useful information. Fecal cortisol metabolites may be an alternative to plasma cortisol as

a measure of the stress response in cattle, due to the feedback-free nature of the sampling method (Palme et al., 1999). The correlation between fecal cortisol metabolites (11,17-dioxoandrostanes) and plasma cortisol has been validated in ruminants by ACTH and dexamethasone tests (Palme et al., 1999); it was found that fecal cortisol metabolites paralleled those of cortisol in the blood with a delay time of 10-12 hours.

Environmental stressors also have been shown to induce an acute phase response in cattle. Transportation and regrouping in cattle increase acute phase proteins such as haptoglobin (**Hp**) and serum amyloid A (Arthington et al., 2003; Lomborg et al., 2008). Acute phase proteins are produced by the liver during periods of inflammation, tissue damage, and infection. Although there are many acute phase proteins, Hp has been of particular interest for the detection of sick animals due to its very low concentrations in the blood of healthy animals (Eckersall, 2000). Therefore in addition to cortisol, Hp was also evaluated as a potential metabolic predictor of disease.

To evaluate whether prepartum physiological indicators of stress and inflammation were associated with the occurrence of health disorders after calving data were collected from 412 cows on two commercial dairies in New York State. Farms were visited weekly to collect blood, fecal samples, and BCS. Sampling began approximately 4 wk prior to each cow's expected calving date. One blood and fecal sample per cow was collected between d -21 to -15 relative to the actual calving date to represent wk -3, d -14 to -8 (wk -2), and d -7 to -2 (wk -1). Prepartum plasma was analyzed for NEFA, Hp, and cortisol and fecal cortisol metabolite concentrations (11,17-dioxoandrostanes) were determined from fecal samples.

Health events occurring within 30 DIM, including retained placenta (**RP**), displaced abomasum (**DA**), and death (not including voluntary culls) were collected from DairyCOMP 305. A postpartum blood sample was collected within 3 to 10 d after calving and sub-clinical ketosis (**SCK**) was diagnosed when plasma BHBA concentration was greater than 10 mg/dl (Ospina et al., 2010). Cows were divided into three health categories for statistical analysis: 1) "Healthy", included cows that did not have RP, DA, SCK, or die by 30 DIM; 2) "One Event", included cows that developed only one health event (RP, DA, or SCK); and 3) " > One or Death", included cows that developed 2 or more health events (RP, DA, SCK) or died (Table 1).

As expected prepartum plasma NEFA was a strong predictor of postpartum health, however, this relationship was dependant on the degree of illness after calving. Prepartum NEFA concentration was only higher in those animals that had multiple disorders or died (Table 2). Parity also influenced this relationship. During wk -3 there was no relationship between high NEFA concentration and postpartum health in heifers. In multiparous animals however, for every 150 µEq/L increase in NEFA during wk -3 the odds of having more then one disorder or dying were 1.9 times greater than the odds of illness with out this increase ($P < 0.001$; CI_{95}: 1.3 - 2.8). During wk -2 and -1 the odds of having more than one health event or dying increased by 1.6 (CI_{95}: 1.2 - 2.1) and 1.5 (CI_{95}: 1.2 - 1.8) times respectively in both heifers and multiparous cows.

Table 1. Number of health events [1] during the first 30 DIM by farm (A, n = 212 vs. B,n =202) and parity (primiparous (PP), n =182 vs. multiparous (MP), n =230) including overall incidence (n = 412).

	Farm		Parity		Overall	
Event	A	B	PP	MP	n	%
Retained Placenta (RP)	28	17	9	36	45	10.9
Displaced Abomasum (DA)	11	14	6	19	25	6.1
Sub-clinical Ketosis (SCK)	74	50	46	78	124	30.1
Died	7	13	3	15	20	6.3
Healthy	117	132	125	124	249	60.4
One Event	55	55	47	63	110	26.7
> One Event or Death	30	23	10	43	53	12.9

[1] Healthy = cows that did not develop RP, DA, SCK or die by 30 DIM; One Event = cows that developed only one disorder (RP, DA, or SCK); > One Event or Death = cows that developed more than one disorder or that died by 30 DIM

There was no association between plasma cortisol at any period before calving and postpartum health status confirming our predictions. There was a tendency for fecal cortisol metabolite (FCORT) concentrations to be greater during wk -2 and -1 in cows that developed multiple disorders or that died after calving (Table 2). Similar to NEFA, there was no difference in FCORT concentrations between cows that developed only one post-partum disorder and those that remained healthy. After accounting for parity and twins, for every 50 unit (ng steroid/g fecal DM) increase in FCORT during wk -2, the odds of developing more then one disease or death by 30 DIM increased 1.2 times (CI_{95}: 1.01 – 1.43).

Prepartum concentrations of Hp were numerically higher during wk -2 and -1 in cows that developed health problems after calving, compared to healthy cows however these differences were not significant (Table 2). Elevations in plasma Hp have been associated with excessive negative energy balance and fatty infiltration of the liver (Katoh, 2002). The numerically higher prepartum Hp concentrations observed in cows that went on to develop postpartum health complications may have been associated with an increase in fatty acid metabolism in the liver as prepartum NEFA concentrations were also highest in these cows.

The major outcome of this study was the observation that there is a relationship between prepartum fecal cortisol metabolites and postpartum health outcomes. The data also clearly showed that for field application, producers who want a snapshot evaluation of cortisol production in their animals should consider analyzing fecal cortisol metabolites rather than plasma cortisol. Understanding why prepartum fecal cortisol metabolite concentrations are higher in cows that go on to develop postpartum health complications should be a focus of future research. Individual differences in glucocorticoid production could be a consequence of differences in individual's ability to cope with the environmental stressors associated with the transition period.

Table 2. Least square means (± SE) for plasma NEFA, haptoglobin (Hp) and fecal cortisol metabolites (FCORT) for cows in three different post-partum health categories[1] during 3 wk before calving.

		Period		
Metabolite	**n**	**wk -3**	**wk -2**	**wk -1**
NEFA (µEq/L)				
No Event	249	245.4 ± 10.3	294.2 ± 11.9	361.0 ± 18.0
One Event	110	228.6 ± 16.1	326.6 ± 18.2	379.5 ± 27.1
> One Event or Death	53	315.1 ± 28.3*	450.3 ± 33.0***	662.1 ± 48.6***
Hp (g/L)				
No Event	249	0.26 ± 0.02	0.28 ± 0.02	0.24 ± 0.03
One Event	110	0.22 ± 0.03	0.29 ± 0.04	0.29 ± 0.04
> One Event or Death	53	0.17 ± 0.06	0.31 ± 0.06	0.34 ± 0.08
FCORT (ng/g fecal DM)				
No Event	249	166.1 ± 5.1	175.8 ± 5.8	201.4 ± 8.4
One Event	110	167.1 ± 8.0	170.7 ± 8.8	178.7 ± 13.0
> One Event or Death	53	177.3 ± 14.5	207.8 ± 15.9†	246.0 ± 24.2†

Significance level for the contrast analysis of the diseased and non-diseased health categories ("one disorder" or " > one or death" contrasted with "none"): †P < 0.1; * P <0.05; ** P < 0.01; *** P < 0.001.

[1] No Event = cows that did not develop RP, DA, SCK or died by 30 DIM; One Event = cows that developed one disorder only, by 30 DIM (RP, DA or SCK); > One Event or Death = cows that developed more then one disorder (RP, DA or SCK) or that died by 30 DIM

METABOLITES ASSOCIATED WITH PRODUCTION OUTCOMES

A secondary objective of the study described above was to evaluate the relationship between physiological indicators of stress and milk yield. Plasma NEFA, Hp, cortisol, and FCORT concentrations from the three prepartum periods were considered (wk -3, -2 and -1) and a postpartum blood and fecal sample (wk +1 = collected between d 3 - 10 post-partum) was also included in this analysis. Herd DC305 records were used to collect information on each cows predicted 305ME from the 2nd test day (approximately 62 DIM). A range of metabolic cutpoints were evaluated for each period and the effect of being above or below the cutpoint on predicted 305ME was then evaluated.

Table 3 presents the metabolic cutpoints used for this analysis and the proportion of animals that were above the cutpoints at each period relative to calving. These cutpoints were selected based on the magnitude of the difference in 305ME between the categorized cows (those above the cutpoint and those below the cutpoint) and also on the proportion of animals in each category. In other words a higher cutpoint may have revealed greater differences in 305ME between the two categories but resulted in

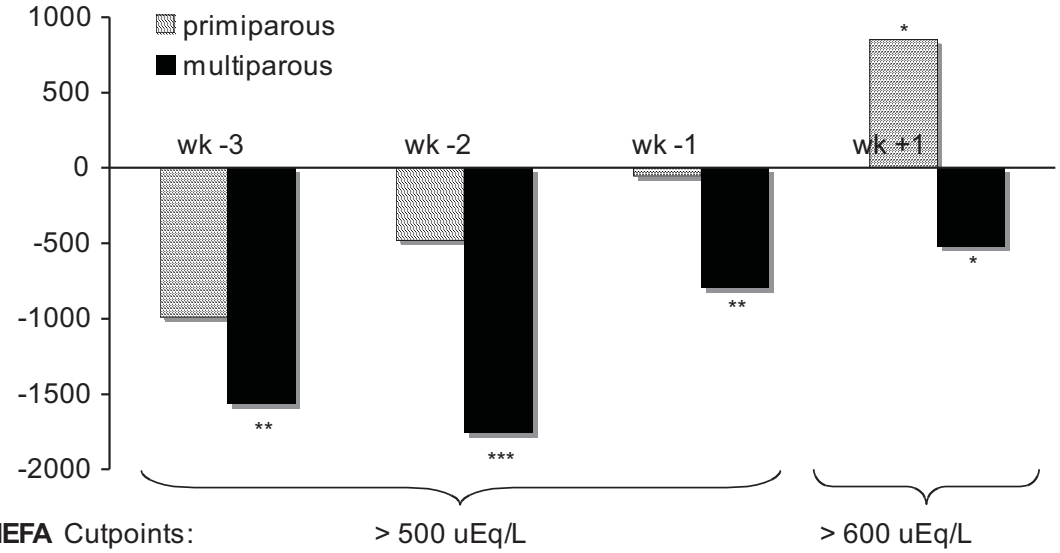
so few animals in one category that the data was not practically useful; consequently a lower cutpoint was selected.

Table 3. Percent of cows above the metabolite cutpoints

	Primiparous (n=182)				Multiparous (n=230)			
Cutpoint	wk -3	wk -2	wk -1	wk +1	wk -3	wk -2	wk -1	wk +1
NEFA > 500 uEq/L	18.1	24.7	33.0	-	5.7	9.1	12.6	-
NEFA > 600 uEq/L	-	-	-	12.1	-	-	-	14.3
Hp > 1.1 g/L	4.9	7.7	6.0	39.0	3.0	4.8	3.0	27.4
FCORT > 250 ng/g DM	8.2	17.0	25.3	-	6.1	13.0	27.0	-
FCORT > 70 ng/g DM	-	-	-	20.3	-	-	-	35.2

Analysis was stratified by parity since the relationships between the metabolites and 305ME at various periods relative to calving were not consistent between cows and heifers. There was no association between prepartum NEFA and 305ME in primiparous cows, however, heifers with NEFA > 600 mEq/L during wk +1 had a 851 kg greater projected 305ME (Figure 1). Multiparous cows with NEFA > 500 mEq/L during wk -3, -2, or -1 had on average a 1371 kg lower projected 305ME (Figure 1). During wk +1, multiparous cows with NEFA > 600 mEq/L had a 517 kg lower projected 305ME.

Figure 1. Difference[1] in predicted 305ME for cows above the indicated NEFA cutpoints relative to cows that are below these cutpoints

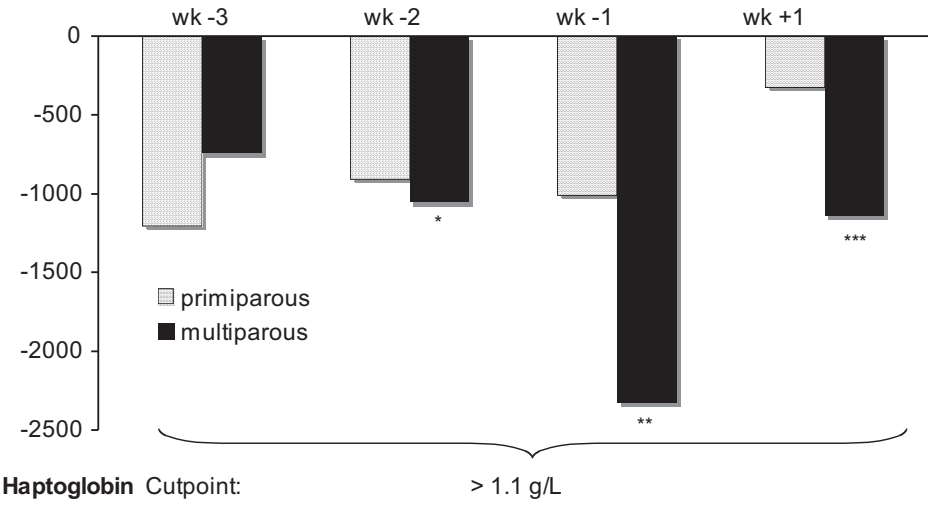


[1]Difference = (average 305ME for cows above cutpoint) – (average 305ME for cows below cutpoint)
Level of significant for the difference: †P=0.01; *P=0.05; **P=0.01; ***P=0.001

Projected 305ME tended to be 1204 kg lower in heifers with Hp > 1.1 g/L during wk -3 and -2. Multiparous cows with Hp > 1.1 g/L during wk -2, -1 or +1 had on average a 1504 kg lower projected 305ME (Figure 2).

There was no association between plasma cortisol and milk yield at any period relative to calving for either multiparous or primiparous cows. FCORT were not associated with 305ME in primiparous cows. Multiparous cows with FCORT > 250 ng steroid/g fecal DM during wk -3 or -2 relative to calving had on average a 1102 kg lower 305ME relative to cows below this cutpoint. Projected 305ME was 1327 kg lower among MP cows with FCORT > 70 ng steroid/g fecal DM during wk +1 (Figure 3).

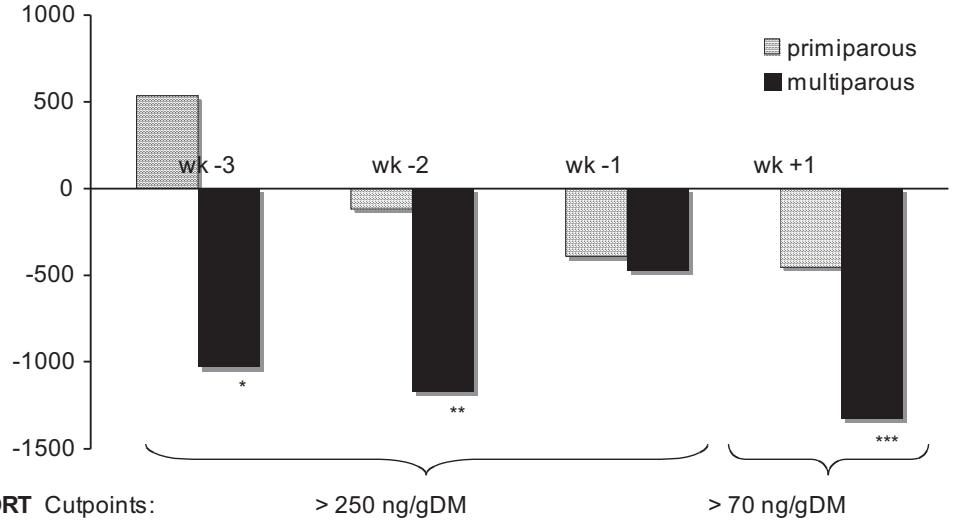
Figure 2. Difference[1] in predicted 305ME for cows above the indicated Haptoglobin cutpoint relativeto cows that are below the cutpoint



[1]Difference = (average 305ME for cows above cutpoint) – (average 305ME for cows below cutpoint)
Level of significant for the difference: †P=0.01; *P=0.05; **P=0.01; ***P=0.001

In summary, cows with higher concentrations of NEFA (with the exception of heifers during wk +1), Hp or fecal cortisol metabolites around calving have lower projected 305ME at the 2nd test day and these animals can be identified up to 3 wk prior to the onset of lactation. While higher concentrations of NEFA, Hp and FCORT were associated with lower predicted milk yields, the significance of these relationships was stronger for Hp and FCORT compared to NEFA. The reduction in predicted milk yield was also greater for cows above the Hp and FCORT cutpoints then it was for those above the NEFA cutpoint. Finally, a greater proportion of animals were above the Hp and FCORT cutpoints during wk +1 then were above the NEFA cutpoint. These results suggest that Hp or FCORT may be alternative and perhaps more effective metabolites, compared to NEFA, for detecting cows at risk for reduced milk yield.

Figure 3. Difference[1] in predicted 305ME for cows above the indicated fecal cortisol metabolite (FCORT) cutpoints relative to cows that are below the cutpoints



[1]Difference = (average 305ME for cows above cutpoint) – (average 305ME for cows below cutpoint)
Level of significant for the difference: †P=0.01; *P=0.05; **P=0.01; ***P=0.001

TRANSITION COW MANAGEMENT AND HEALTH

As previously discussed there are numerous events and situations that can occur during the transition period that may be perceived as being stressful. For the next phase of this research we were interested in exploring the effect of overcrowding on physiology and behavior.

The behavioral consequence of overstocking have been well documented and generally include reductions in feeding time, increased inactive standing, increased competition for the resource being overstocked (e.g., lying stalls or feed bunk space), increased feeding rate, and changes in daily feeding pattern (Huzzey et al., 2006; DeVries et al., 2004; Olofsson, 1999). Whether overstocking has any consequences on metabolic health is less understood. Overstocking is a management practice that has been shown to influence components of stress physiology such as increasing glucocorticoid production (Friend et al. 1977). Since glucocorticoids play a strong role in regulating energy metabolism it is possible that chronic or severe situations of overstocking may influence stress physiology to the point where it could interfere with energy metabolism and thus overall health. Behavioral adaptations to crowded environments may further influence these responses. The objective of this study was to evaluate the effects of overstocking on energy metabolism and stress physiology and also to determine whether behavioral adaptations to overstocking could influence these physiological effects.

Two stocking density treatments were evaluated in this study: 1) Control: 1 lying stall per cow and 0.67 m (26.4 inches) linear feed bunk (FB) space per cow, and 2) Overstocked: 0.5 lying stalls per cow and 0.34 m (13.4 inches) linear FB space per cow. Two groups of 10 cows each were exposed to the treatments in a 2x2 crossover design.

Groups were balanced based on parity (4 primiparous and 6 multiparous animals per group) and previous 305ME among multiparous animals. Treatments lasted 14 d each and were separated by a 3-d washout period during which both groups were housed at the control stocking density. After each of the two groups received both treatments, an additional two groups were formed and the study design repeated. During each treatment, blood and fecal samples were collected on d 1, 3, 5, 7, 9, and 11 and analyzed for NEFA, glucose and FCORT, video cameras recorded behavior at the FB on d 7-10 and on d 13 all cows were given a glucose tolerance test (GTT: 0.25 g dextrose/kg BW).

There was no difference in the average daily feeding time for groups housed at the control stocking density or those that were overstocked however DMI was greater in the crowded cows suggesting an overall increase in feeding rate for cows in this treatment (Table 4). In the crowded groups, NEFA and glucose concentrations were higher and FCORT tended to be higher across the treatment, compared to the control groups (Table 4). There was a negative correlation ($P \leq 0.003$) between daily feeding time and NEFA (r = -0.54), glucose (r = -0.32) and FCORT (r = -0.60) and a positive correlation between the time it took cows to approach the FB following fresh feed delivery and NEFA or glucose (r = 0.30 or 0.24 respectively, $P \leq 0.02$). This suggests metabolism may be affected by individual feeding strategies in groups.

Table 4. Average daily LSMEANS (± SE) for behavioral and metabolic measures collected from 4 groups housed at either a control stocking density or a crowded stocking density[1].

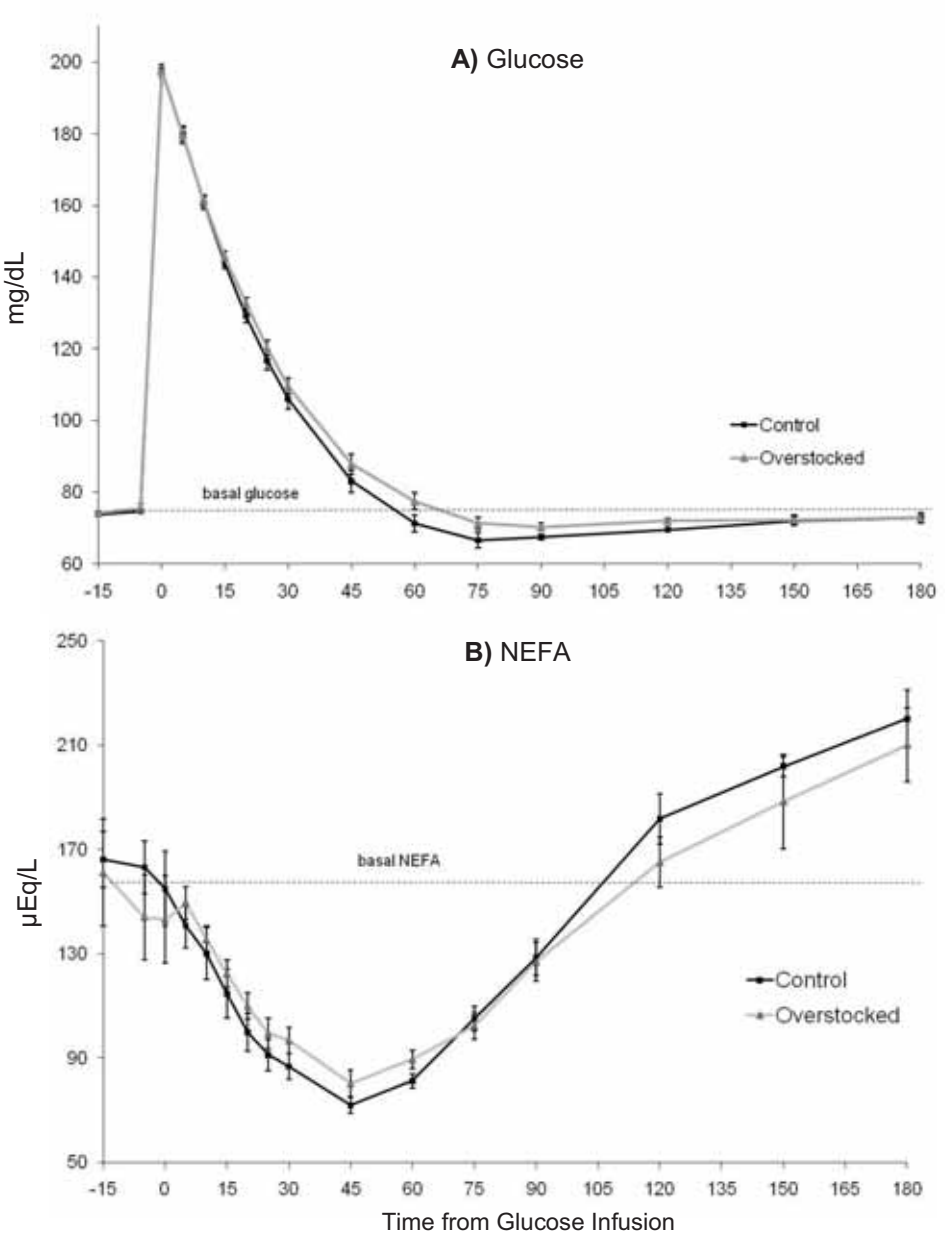
	Treatment			
Variable	**Control**	**Treatment**	**SEM**	**P**
Feeding Time (min/d)	240.9	242.1	3.1	0.77
Time to feed bunk post fresh feed delivery (min)	35.2	68.0	7.4	0.005
Dry Matter Intake (kg DM/cow/d)	14.9	15.9	0.1	<0.001
Daily NEFA (uEq/L)	90.6	106.0	32.	0.002
Daily Glucose (mg/dL)	64.2	65.3	0.4	0.05
Daily Fecal Cortisol Metabolites (ng/g DM)	16.4	18.7	0.9	0.103

[1]Control Stocking Density = 1 lying stall/ cow and 0.64 m linear feed bunk space/cow; Crowded Stocking Density = 0.5 lying stalls/cow and 0.35 m linear bunk space/cow

There were no differences between treatments in the NEFA area under the curve (AUC) response to the GTT; however, the rate of NEFA clearance during the first 30 min of the challenge was lower for crowded cows (1.4 vs. 1.9 μEq/L per min, $P = 0.04$). Following glucose infusion the overstocked group tended to take longer to return to basal glucose concentration and had a greater positive AUC (when glucose values were above basal concentration, possibly suggesting changes in insulin sensitivity or responsiveness. The glucose and NEFA response curves to GTT are presented in Figures 4. These results suggest that overstocking can influence metabolic status by

increasing the concentrations of circulating NEFA and slowing glucose clearance. These differences suggest that overstocking dry cows may increase insulin resistance and thus alter the regulation of energy metabolism during the transition period.

Figure 4. Glucose (**A**) and NEFA (**B**) (arithmetic mean ± SE) response to the intravenous glucose tolerance test for cows housed at the control and crowded stocking densities[1].



[1] Control stocking density = 1 lying stall/cow, 0.67 m linear feed bunk space/cow
Crowded stocking density = 0.5 lying stalls/cow, 0.34 m linear feed bunk space/cow

SUMMARY

This data shows that higher concentrations of metabolites associated with stress during the period before calving, including fecal cortisol metabolites and plasma haptoglobin may increase a cow's risk for postpartum health complications and reduced milk production. Overstocking during the dry period represents an environmental stressor that appears to alter the regulation of energy metabolism, possibly by increasing insulin resistance. Future work in this area should focus on determining if other management practices such as commingling heifers and cows or frequent group changes also have the capacity to affect health by altering stress physiology and energy metabolism.

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HOW WELL DO WE REALLY UNDERSTAND SILAGE FERMENTATION?

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INTRODUCTION

The primary goal of ensiling forages has been to conserve the maximum amount of original dry matter, nutrients and energy in the crop for feeding at a later time. There are four general phases of silage fermentation: 1) the initial aerobic phase, 2) the active fermentation phase, 3) the stable phase and 4) the feedout phase (Barnett (1954). In brief, the production of organic acids and the attainment of a low pH under anaerobic conditions result in the end product inhibition of plant and microbial processes to attain the "stable phase". Without silage additives, the general consensus has been that silage fermentation cannot make forage quality better than what was in the original crop. This paper asks the question, "how well do we understand silage fermentation" relative to the stable phase.

CHANGES IN FERMENTATION END PRODUCTS AND MICROBIAL POPULATIONS

A period of 3 to 4 weeks has generally been accepted as adequate time for active silage fermentations to cease and reach a stable phase, but a variety of factors may affect this. However, Ward and de Ondarza (2008) suggested that based on changes in silage fermentation samples submitted to Cumberland Valley Analytical, corn silage requires at least four months for a full fermentation.

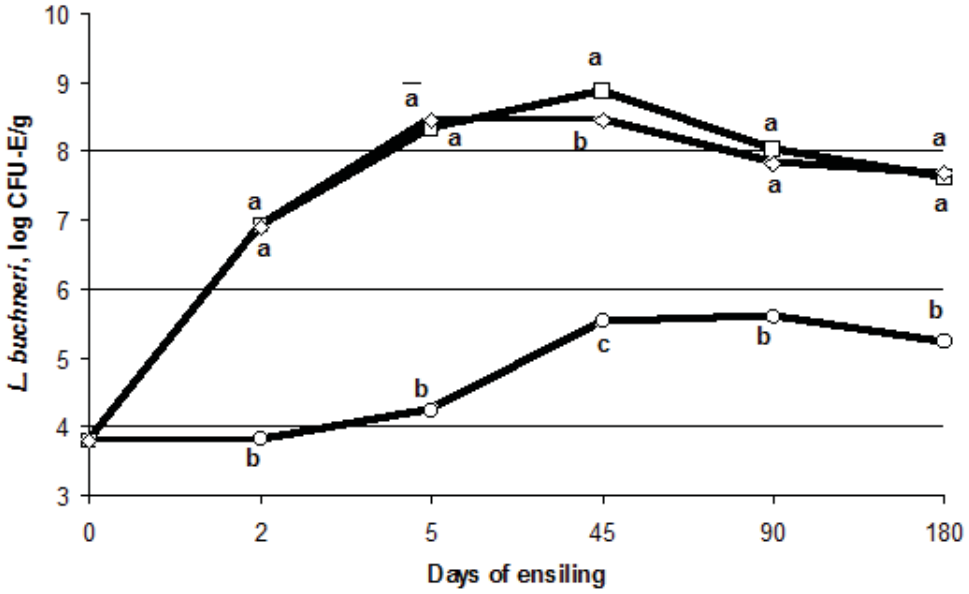
Typically, silages with high moisture content ferment more rapidly then those with low moisture contents because in the latter, the availability of metabolic water for microbial growth decreases as moisture declines (Whiter and Kung, 2001). Extremely cold temperatures at harvest may cause a slow and prolonged fermentation period. In fact, Periera et al. (2009) showed that alfalfa forage can be frozen for several months, and then thawed and the material will then ensile normally. During the classical stable phase, many microbes are in a dormant state but still culturable. For example, lactic acid bacteria have been detected in relatively high numbers (> 5-6 log cfu/g of wet silage) in silages stored for up to a year (unpublished data, University of Delaware). Schmidt et al. (2008) reported that lactic acid bacteria in untreated alfalfa silage peaked between days 5 and 45 (> 9 log cfu/g) of ensiling and was still greater than 6-log cfu/g after 180 d of ensiling. Yeasts can withstand very low pH in silages and Kleinschmit et al. (2006) were able to enumerate them from corn silage after a year of storage. In samples of corn silage that had been stored under anaerobic conditions for 5 years we could culture yeasts in 1 of 9 and lactic acid bacteria in 2 of 9 corn silage samples (unpublished data, University of Delaware). Spores of clostridia and bacilli can also persist for prolonged periods in silages.

Changes in fermentation end products even after several months of ensiling suggest that microbial activity persists even when the pH is low. For example, some strains of *Lactobacillus plantarum* have the capability of converting lactic to acetic acid in the absence of fermentable sugars via a pyruvate-formate lyase system (Lindgren et al., 1990). The result of this is a silage with a slightly higher pH, lower lactic acid concentration and higher acetic acid concentration at a later date when compared to an analysis of the same silage from an earlier date. Lactobacillus *buchneri* also appears to be fairly active for long period of time in corn silage and probably also contributes to this finding. Under anaerobic conditions and low pH, this organism is able to convert lactic acid to acetic acid, ethanol and 1,2 propanediol (Oude-Elferink et al., 2001). Kleinschmit et al. (2006) reported on changes in the fermentation end products of corn silage ensiled in mini silos for up to 361 d. In that study, silage was untreated or treated with a combination of *L. buchneri* and *Pediococcus pentosaceus.* Across both treatments the concentrations of lactic acid decreased by about 15% between day 14 and a year of ensiling. Both treatments showed more than an 80% increase in acetic acid over the same period of time. However, the major increase in acetic acid in untreated silage occurred between 282 and 361 days whereas this increase was observed as early as day 56 for silage treated with *L. buchneri.* Evidence suggesting that the change in concentration in acetic acid for both silages was most likely due to *L. buchneri* (either natural or added populations) was the fact that 1,2 propanediol was only detected in untreated silage at day 361 whereas substantial amounts of this compound were detected in treated silages as early as 42 d of ensiling. Using a quantitative-polymerase chain reaction method, Schmidt et al. (2009) showed large increases in the numbers of *L. buchneri* in treated alfalfa silage with smaller increases (from the natural epiphytic population) in untreated forage (Figure 1). The population of *L. buchneri* in treated silage was greater than 7 log cfu/g of wet silage after 180 d of ensiling documenting that this organism is relatively acid tolerant and can survive for long periods of time in fermented silage.

CHANGES IN NITROGENOUS COMPOUNDS

There are considerable changes in the nitrogenous fractions of forages during ensiling. Proteases from plant sources are quickly inactivated with a drop in pH, thus their contribution to nitrogenous changes during prolonged storage is doubtful. However, microbial proteases appear to be relatively active throughout a prolonged period of fermentation. Schaadt and Johnson (1969) reported a loss of true protein in corn silage presumably due to proteolysis up to 180 d of ensiling but it was unclear when or if there was a plateau for proteolysis because there was a large gap between the day 8 and 180 sampling times. Filya (2002) reported increasing amounts of NH3-N in corn and sorghum silages through 90 d of ensiling. Kleinschmit et al. (2006) reported a steady increase in NH3-N in corn silage through 361 d of ensiling without reaching a plateau. Newbold et al. (2006) ensiled 15 corn silages and reported increasing amounts of degradable CP with time through 10 months of sampling. Changes in the amino acid content of silages with prolonged storage have not been quantified to our knowledge.

Figure 1. Changes in populations of *L. buchneri* as measured via Q-PCR in alfalfa silage. The top two lines show changes in populations from silages treated with *L. buchneri 40788*. The bottom line shows changes from untreated silage. Points within a sampling day with unlike letters differ, $P < 0.05$.



CHANGES IN CARBOHYDRATE FRACTIONS OF SILAGES

Ensiling also causes a number of changes in the carbohydrate fraction of forages. Water-soluble carbohydrates are used by lactic acid bacteria in the production of organic acids. Morrison (1979) showed that the core lignin concentration of forage did not change and cellulose decreased up to 5% during ensiling after 150 d of storage. However, there were substantial decreases in hemicellulose, up to 10-20%, which may explain the acidic conditions and microbial activity in the silage. Forage treated with exogenous acid additives lost the most carbohydrates during ensiling. Yahaya et al. (2001) confirmed that considerable loss of the hemicellulose and pectin fractions occurs in alfalfa and orchard grass silage between fresh forage and material ensiled. That study reported that hemicellulose digestion decreased with time of ensiling for both forages whereas digestion of cellulose actually increased with time of storage for alfalfa. One limitation of that study was that sampling only occurred up to 56 d in the silo. We are aware of only a few studies where the effects of prolonged ensiling (>150 d) on the digestibility of fiber in silages have been reported. Hallada et al. (2008) showed a steady increase in in situ NDF-D of corn silages of about 1.2% units per month between samples from day 1 through 330 d of ensiling without appearing to reach a plateau. In contrast, we (Der Bedrosian et al., 2010) found that time of ensiling did not affect the in vitro NDF-D of two corn silage hybrids between 45 and 315 d of ensiling. Cone et al. (2008) also reported that length of storage (up to 180 d) did not affect the digestibility of cell walls as evaluated by in vitro gas production. The reasons for the discrepancies between studies is unknown but may be related to the methods used for assessing fiber digestion, the silage hybrids used and other silo management factors.

Prolonged ensiling has been shown to improve the digestibility of DM and starch in corn crops. Benton et al. (2005) ensiled HMC for up to 298 d at various moistures and sampled the material every month. In all cases, in situ digestion of DM increased as days of storage increased. Of particular note was the fact that the increase in digestion was greater for HMC with 30% (less mature) than 24% (more mature) moisture content. Hoffman et al. (2010a) also reported that the fractional degradation (via in vitro gas production) of HMC increased moderately with time in the silo. In corn silage, European researchers reported increases in in sacco starch digestion of corn silages (Newbold et al., 2006) and Hallada et al. (2008) reported improvements in laboratory estimates of total tract starch digestion with increasing time in the silo as well. Recently, our group (Der Bedrosian et al., 2010) reported increased in vitro digestion of starch in normal and BMR corn silage hybrids through 270 d of storage. In contrast, Cone et al. (2008) reported that increasing the time of storage did not affect estimates of starch digestion when evaluated through an in vitro gas production system. Increased starch digestion with prolonged storage was hypothesized to be due to solubilization of prolamins by acids and alcohols, but recent evidence suggests that proteolytic activity may explain this phenomena (Hoffman et al., 2010b). The protein-starch matrix in starches is somewhat analogous to the lignin-fiber matrix in forages, thus degradation of prolamins in the matrix improves the availability of starch.

CHANGES IN LIPID COMPOSITION

The ensiling process can affect the lipid composition of forages. Van Ranst et al. (2009) suggested that lipolysis in the silo was greater in silages that had undergone extensive fermentation. To our knowledge, a change in the lipid composition of forages due to prolonged storage in the silo has not been studied. A joint research effort between the University of Delaware and Michigan State University (A. Lock) is currently addressing this question in corn silage.

PRACTICAL IMPLICATIONS AND QUESTIONS

What do these findings mean for the average dairy farmer?

Although increasing the storage time of corn silage and HMC appears to improve the rumen availability of starch, not all producers have the capability to increase inventory to achieve this because of a limited land base and (or) fixed silo inventory. The added cost of carry over has also not been thoroughly evaluated. Furthermore, when modeling forage changes in the silo, Buckmaster et al. (1989) reported that emptying a silo in 120 d vs. 360 d reduced DM loss by 6%. They concluded that increasing time in the silo results in greater DM loss because of infiltration of air in to the mass. Thus, this factor should be considered when making a decision about storing silage for longer periods of time.

For those producers that can store silage for longer periods of time before feeding, extra precautions must be taken in silos where plastic is used to maintain anaerobic

conditions (bunks, pile, and bags). Chances of damage to the plastic increase with time of exposure to UV radiation, animals and general degradation of the plastic. Because silo plastic is permeable to oxygen, prolonged storage ultimately increases the exposure of surface silage to more air. Prolonged storage also means that silages will be fed in warmer weather and be prone to more aerobic deterioration. The need for excellent silo management in terms of correct moisture, chop length, density and sealing will also be critical to maintaining silage quality. Use of plastic with low oxygen permeability and the use of additives to improve aerobic stability (e.g. *Lactobacillus buchneri)* should be considered.

Changes in starch digestion with prolonged storage may explain two phenomena that are commonly observed with lactating cows. First, it is common to hear reports of cows dropping in milk production when switched from “last season’s” corn silage to freshly ensiled corn. Some of this “slump” may be attributable to the consumption of large quantities of unfermented sugars, but it may also be due to the fact that freshly ensiled corn silage is much lower in starch digestion than silage fermented for a longer period of time. Second, it is also common to receive reports of cows with laminitis and low fat tests in the spring. This may be a result of the increased availability of starch in corn silage and HMC. Thus, although prolonged storage of silage improves the digestibility of starch, many may find it difficult to adjust diets to compensate for these highly digestible feeds. If this is the case, one suggestion might be to process corn silage and grind corn less for those feeds that will be stored for longer than 8-9 months.

CONCLUSIONS

To date, there is mounting evidence that prolonged storage time of corn silage improves starch digestion and increases protein solubility. These factors should be taken into consideration when balancing diets for dairy cows. In addition, more research is warranted in this area because some management factors may affect the degree of changes one might obtain with prolonged storage. For example, the effects of prolonged storage on poorly processed corn may have less of an effect because there would be less surface area in contact with microbial proteases. Packing density may have an effect on effects of storage time because of pressure and distribution of fluids throughout the mass. We (University of Delaware) are currently investigating the effects of prolonged storage on alfalfa silage. Future studies in this area will help us to better utilize silages in diets for ruminants.

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DEVELOPING BIOTECHNOLOGY TO CONVERT POULTRY FEATHERS INTO A HIGH-QUALITY FEED PROTEIN SUPPLEMENT

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The 8 billion chickens raised and processed annually in the US generate over one million metric tons of feathers per year. Although feathers contain > 90% crude protein, only a small fraction of feathers is processed as feed supplements. Feathers consist of almost pure β-keratin. With extensive disulfide bonds and cross-linkages, β-keratin is made of β-sheets and twisted in a parallel manner to form stable fibrils. This highly rigid structure of keratin renders it insoluble and resistant to commonly-known proteases such as pepsin or trypsin. Because animals do not secrete enzymes that can break down disulfide bonds or cross-linkages of keratin, they are unable to digest feather protein. Consequently, poultry feathers are dumped or land-filled, not only wasting voluminous amounts of potentially valuable animal protein but also adding 150,000 metric tons of nitrogen as environmental pollutants into land and water per annum. Meanwhile, high-quality proteins such as soybean meal are supplemented in diets for animals to meet their nutrient requirements. This supplementation accounts for a substantial portion of animal feed cost, and directly competes with human consumption for a limited world supply of edible food protein.

Although autoclaving, pressure-cooking, or alkali treatments may enhance protein digestibility of feathers, those methods require considerable energy and often decrease nutritional value of the final products. While a number of bacteria or fungi can hydrolyze up to 60% of feather content, direct applications of these microbes remain problematic. These microorganisms secrete very little feather-degrading enzymes for large-scale production. Fermenting feathers with them will end up mainly as a microbial biomass of low nutritional quality.

Enzymatic hydrolysis is a promising method to improve protein digestibility of feathers. In fact, we have tested two keratinolytic enzymes isolated from *Bacillus licheniformis* PWD-1 and *Thermomonospora fusca*. However, neither of these enzymes exhibits sufficient efficiency for industrial applications. To tackle this problem, we obtained a bacterial strain that can completely dissolve feathers within 60 h. With the generous grant funding by the Institutes of Biotechnology and Life Science Technologies at Cornell and the strong technical support by three Cornell Core Facilities, we have sequenced the genome of this strain and initiated proteomics analysis of key enzymes for feather protein hydrolysis. Our long-term goal is to develop novel enzymes or complexes (keratinases) to convert poultry feathers into high-quality feed protein supplements.

Accomplishing our long-term goal will help in developing commercial enzyme complexes for feather degrading. The estimated world market for the enzyme complex will be approximately $500 million per year for the poultry and swine feed industries.

Meanwhile, converting one million tons of feather waste into soluble protein supplements will create a new product potentially worth $800 million per annum in the US alone. Equally important, this conversion will prevent 150,000 metric tons of nitrogen from entering the environment annually, and enable animal producers in New York State and elsewhere to comply with the Clean Water Act. The use of feather protein will spare high-quality protein such as soybean for human consumption. In addition, novel keratinases will have tremendous potential in the meat processing, leather-manufacturing, detergent, and cosmetic industries.

"THE RUSSELL CNCPS LEGACY"
THE MICROBIAL SUBMODEL

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INTRODUCTION

The CNCPS system started at quite a simple level with a Radio Shack Basic program. It was quickly realized that we needed to go beyond that. A spreadsheet was developed which had very simple equations with minimal dynamics involved. It was at this point that we decided that we needed to go beyond the simplistic approach. We started to think in terms of submodels. This brought us to the realization for the need to link our latest thinking in the analyses of feedstuffs with the different submodels that were being developed. It was logical that we needed to develop a dynamic rumen submodel utilizing the carbohydrate and protein pools identified in the feedstuffs. The basic premise in the rumen submodel is that the ingredients consumed by the animal would be influenced by rates of passage and rates of digestion. It was also assumed that each of the carbohydrate and protein pools within an ingredient would have different rates of digestion. It was assumed that each ingredient consumed by an animal would have a unique rate of passage, based initially on its particle size and its density. This was later modified to just a particle size consideration. This part of the submodel then needed to be linked to a microbial submodel.

Dr. Russell made this statement in his paper describing the model (Russell et al, 1992). "The Cornell Net Carbohydrate and Protein System (CNCPS) has a mechanistic submodel that provides quantitative estimates of fermentation end products (the ME from VFA production, microbial protein and ammonia) and materials that escape ruminal degradation (carbohydrates, protein, and undegraded peptides). The CNCPS can serve as a research tool or a guide for practical ration balancing." The statement that it could serves as a research tool and had the potential to be included in nutrition platforms was indeed a visionary statement. The CNCPS model has been the basis for the conduct of research, providing a platform for the design of many experiments over the last 18 years. It has also been incorporated in several nutrition platforms since 1991, allowing the field evaluation of the model.

THE MICROBIAL SUBMODEL

The rumen microbial ecology is a complex system. The challenge Dr. Russell faced was to aggregate this complexity (Hungate, 1966; Russell and Hespell, 1981), into an operating dynamic model using microbial kinetics linked with the various nutrient fractions presented from the feedstuffs.

He noted the different microbial models that had been developed. Reichel and Baldwin (1976) had developed a microbial 8 group model which is quite complex and

then in subsequent years, Baldwin and colleagues developed a one group model (Baldwin et al, 1977). This model eliminated the diversity modeled originally.

For the CNCPS system, it was decided to develop a two microbial pool system (Russell et al, 1992). The first group was those bacteria digesting the cell wall in the feedstuffs (SC). The second group digested the non structural carbohydrates (NSC) in the feedstuffs. He stated, "This segregation reflects differences in N utilization and growth efficiency as well as an almost exclusive partition of energy source utilization". This aggregation, based on differences in nutrient utilization made sense as a first step with the recognition that as we gained more knowledge we could expand the model to a lower level of aggregation with an increase in sensitivity.

The SC bacteria in the model only ferment fiber and only utilize NH_3 (Bryant, 1973). It was recognized that there is also a requirement for isoacids produced from the fermentation of branch chain AA. This needed to be incorporated into the model (Russell and Sniffen, 1984). The NSC bacteria ferment the sugars, starch, and soluble fiber. They use NH_3, peptides and amino acids for N sources. At the time of the development of the model the amino acid submodel had not yet been developed.

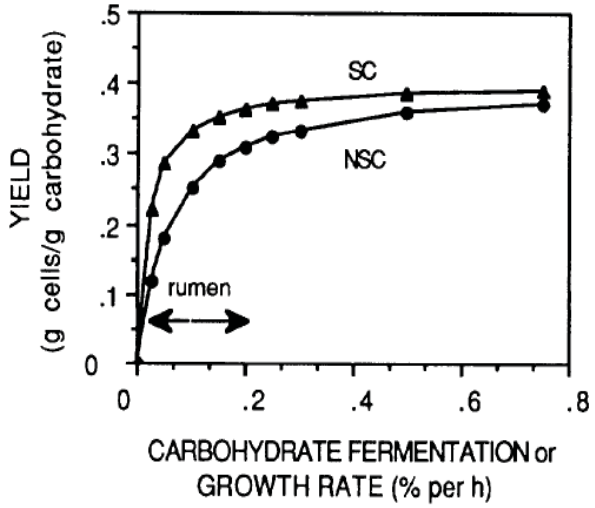
The major point that was made in the development of the model from a ration formulation perspective was that we should be formulating rations to optimize microbial growth and efficiency. This will result in excellent microbial amino acid flow to the small intestine and will result in the optimization of providing metabolizable energy to the cow.

Dr. Russell introduced a different approach to the prediction of microbial growth. He argued that predicting microbial growth from whole tract digestibility of TDN or organic matter digestion was inappropriate and lacked the sensitivity needed for prediction of the ME derived from the digestion of CHO's in the rumen and the prediction of flow to the small intestine.

He further argued that we needed increased sensitivity in the prediction of the delivery of MP from microbial growth in the rumen and the subsequent net flow of microbial dry matter flow to the small intestine. This meant a more mechanistic approach to predicting the growth of the microbial mass in the rumen with a refinement of the prediction of the requirements of this microbial mass.

The approach was based on the work of Pirt, 1965. This approach recognized that organisms have a maintenance requirement (Russell and Baldwin, 1981) as well as potential maximum yield (Hespell and Bryant, 1979). It was recognized that there was a variable maintenance requirement between the two microbial types.

Figure 1. The effect of carbohydrate fermentation rate (growth rate) on the yield of ruminal bacteria that ferment structural carbohydrate (SC) and nonstructural carbohydrate (NSC). The theoretical maximum growth yield is 0.4 g of cells per gram of carbohydrate, and the maintenance energy requirements for SC and NSC bacteria are 0.05 and 0.150 g of carbohydrate per gram of bacteria per hour, respectively. Growth rates in the rumen usually range from 0.05 to 0.2 h^{-1}.



The general equation:

1/Y = (km/kd) + (1/Yg)

Where:

Y = yield efficiency (g of bacteria/g of carbohydrate fermented)
km = maintenance rate (g of carbohydrate fermented/g of bacteria/h)
kd = growth rate of bacteria = degradation rate of carbohydrate (%/h)
Yg = theoretical maximum yield of bacteria (g of bacteria/g of carbohydrate fermented

Total bacteria (g/d) = Y * g of carbohydrate fermented

In vitro work of Isaacson (Isaacson et al, 1975) with mixed rumen culture show a maximum yield of 0.5 g of cell dry weight/g of carbohydrate fermented. However, there were not protozoa in these fermentation studies. The figure above presumes a reduction in maximum yield and flow to the small intestine due to protozoal predation from 0.5 down to 0.4 g of cell dry weight/g carbohydrate fermented. This is a prediction of the net flow to the small intestine of microbial matter from the two pools.

This is an elegant model with an aggregation that represented a departure from the less sensitive models that were in use at the time in which he expanded on in 1995

(Russell & Cook, 1995). Dr. Russell then built on this basic model with some excellent studies to modify microbial growth.

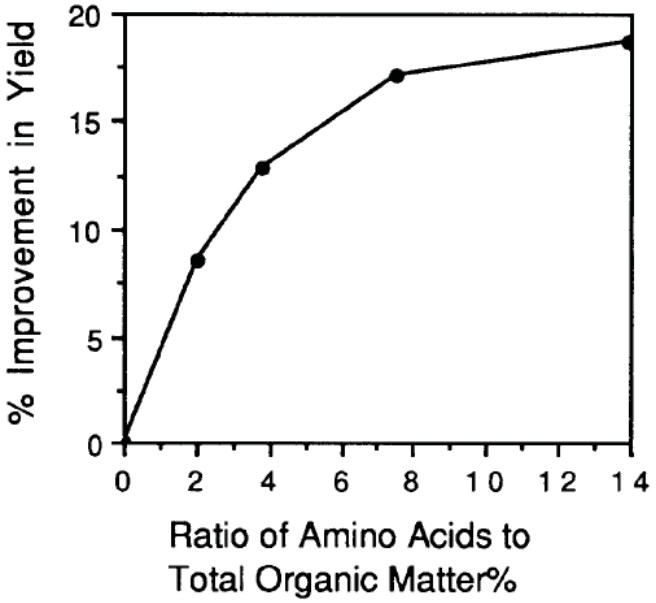
The fiber bacteria (SC) required just NH_3 for the nitrogen source (Winters et al, 1972). The fiber bacteria are sensitive to rumen pH so the maintenance requirement is increased and maximum yield decreased as non-linear functions of the predicted rumen pH. It was observed (Strobel and Russell, 1986) that there was a 50% decline in microbial yield at a pH 5.7 vs. 6.7. The suggested change in yield was a 2.5% decrease in yield for every 1% decline in ration NDF. This was modified in the model to use peNDF rather than NDF to account for particle size differences, which he pointed out, could have an effect.

There was recognition of the need to enhance the SC model to meet the requirement for the branch change amino acids (Russell and Sniffen, 1985). He discussed the need to generate isoacids and pointed out that it should be relatively easy to do through the identification of the rumen degraded branch chain amino acids in feedstuffs.

The non-fiber bacteria (NSC) growth is modified by the amount of available peptide relative to the available rumen fermentable CHO (Chen et al, 1987a, b). When the peptide available is 14% of the available NSC there can be a maximum %improvement in yield of 18.7% as can be seen in figure 2. The NSC bacteria are represented by 2 equations: sugar and starch. In the original model, the sugar was an aggregation of sugar and fermentation acids from ensiled feeds (Sniffen et al, 1992). The starch represented the starch and soluble fiber (a residual CHO fraction by calculation, including a variable mix of pectins, fructans, and other oligosaccharides). These two fractions were later divided into 4 fractions: fermentation acids, sugars, starch, and soluble fiber in CPM 3.0. These were again subdivided into lactic acid, other fermentation acids, sugars, starch, organic plant acids and soluble fiber + fructans.

Note the non linear relationship with this ratio. The application of this is that in balancing rations we need to have a source of peptides as well as ammonia if we are going to enhance microbial yield. Of interest is that the peptide uptake rate is limited to 0.07/h (Hino and Russell, 1985). This is reduced 34% if an ionophore is being fed (Russell et al, 1992). It was also observed that certain peptides with higher proline content were taken up slower that those peptides without proline. This is coupled with a liquid turnover rate (Chalupa et al, 1991) which decreases the surplus of peptides (Mangan, 1972,). It should be noted that in CNCPS 6.1 the rate of degradation of the protein pool (B1) rich in potential peptide supply has been decreased 10 fold. This has been coupled with a higher liquid turnover rate, potentially limiting the supply of peptides. It should also be observed that with ionophores in this model more peptide will escape fermentation increasing the supply of metabolizable protein. It is assumed, based on the research (Russell et al, 1983) done that the NSC bacteria have an N requirement of 66% of the N coming from peptides. Dr. Russell noted however that there would be a supply from recycled N.

Figure 2. Effect of amino acid nitrogen on the yield of ruminal microbial protein in vitro. Redrawn from Russell and Sniffer (1984).



At the time of the development of the model, Dr. Russell acknowledged that the N recycled could make up a large % of the total N available to the bacteria. We are beginning to appreciate that this supply might be greater than we thought. The challenge for us is what the nature of the N recycled is. This could be in the form of peptides, amino acids, and NH_3.

RUMINAL BACTERIAL COMPOSITION

There have been several papers published in this area over the years. It is recognized that the composition of the bacterial protein washed out with the liquid could be different from the bacteria associated with the solids. It also could be recognized that about 20% of the microbial flow to the small intestine could be protozoal. Dr. Russell chose to, at the time of the publication of the model, to aggregate to the analyses published by Hespell and Bryant (1979).

Bacterial Composition

Parameter	Units	Value
Bacterial Protein	%DM	62.5
Bacterial Nucleic Acid	% Bact CP	15.0
Bacterial True Protein	% Bact CP	60.0
Bacterial Cell Wall Protein	% Bact CP	25.0
Bacterial Carbohydrate	% DM	21.1
Bacterial Fat	% DM	12.0
Bacterial Ash	% DM	4.4

The model assumes that the cell wall protein is not available (Ling and Buttery,1978, Van Soest, 1987). The microbial true protein is assumed to have a 100% intestinal digestibility. This is a unique approach, allowing for the expansion in the future of microbial niches as our knowledge expands.

FERMENTATION MODEL

With the basic model as outlined above developed, it was necessary to link the model to the fermentation system in CNCPS (Sniffen et al, 1992). Briefly, the CNCPS system is based on identifying fractions within the major nutrients of protein and carbohydrates that will behave similarly in the rumen and the cow. The microbial submodel aggregation fits reasonably closely with this approach with the CHO being divided into two major fractions of fiber and NSC. Each pool within the protein and CHO nutrient fractions has a variable degradability based on rates of digestion and rates of passage for each pool utilizing the general equations:

Ruminally degraded = $(A_n \text{ or } B_n)*(Kd/(Kd + Kp))$

Rumen escape = $(A_n \text{ or } B_n)*(Kp/(Kp + Kd))$

Where

A_n and B_n are nutrient pools
Kd = rate of degradation
Kp = rate of passage

There are 4 protein pools and 4 CHO pools in the original model. Each pool had a range of degradability that is orders of magnitude different. These pools have been modified over time as the model has evolved with new information.

The model was tested with data from steer and dairy data.

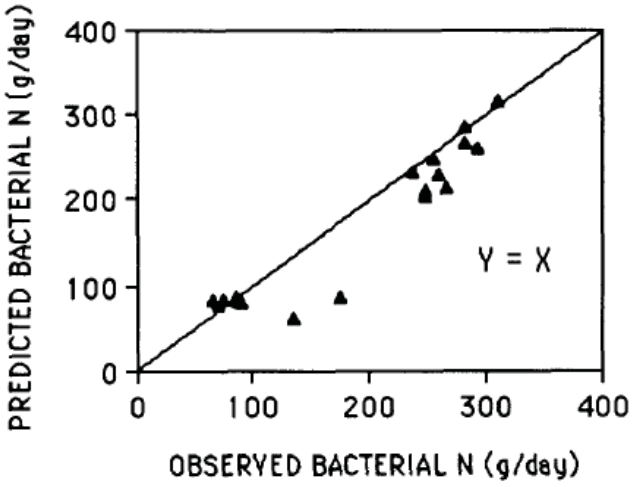
The model exhibited an increased sensitivity over a large range of data when compared to other models. It must be remembered that this model was with aggregated NSC CHO fractions. This part of the model is much less aggregated today and should exhibit an increased sensitivity.

SUMMARY

The microbial submodel was a significant step forward in the prediction of microbial flow to the small intestine. This model was a significant departure from the empirical models used previously and established a base for a more mechanistic approach that could be linked with a nutrition model that was useable in the field. This, perhaps, was the biggest contribution. There have been microbial models that have been developed which are quite sophisticated but were not linked to a field nutrition model. This effort allowed us to take a significant step forward in ration formulation. The additional power of this model is that the development allows us to increase the sensitivity through the addition of new nutrient fractions as well as new microbial niches as we gain knowledge

of the rumen ecological system. Much of his concepts and ideas are compiled in a teaching laboratory manual on rumen microbiology (Russell, 2002) which will be of great value to many students and scientists in the years to come.

Figure 3. The relationship between observed flows of microbial nitrogen from the rumen and those predicted by the Cornell Net Carbohydrate and Protein System. Data were taken from Robinson and Sniffen (1985), Garret et al. (1987), and Song and Kennelly (1989). The regression line (not shown) had an r^2=0.88, a slope of 0.94 and an intercept of -12 g of N/d. The high N flows were observed in lactating dairy cow, whereas the low N flows were from trials with steers.



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JAMES BERNARD RUSSELL: SCHOLAR, COLLABORATOR, MENTOR

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Dr. James B. Russell was a rumen microbiologist who became a legend in the field of animal and dairy science, both for his towering intellect and his unique personality. The latter has provided fodder for many legendary (even apocryphal) stories in the scientific community, and most of the stories are probably true. In this presentation, we hope to give you a sense of Jim both as a scientist and as a complex person, and why he has left such a mark on the fields of ruminant nutrition and rumen microbiology.

Jim grew up on a dairy farm in upstate New York. He received a B.S. degree from Cornell University in 1973 and M.S. and Ph. D. degrees from the University of California at Davis in 1974 and 1978, respectively. He began his professional career as an Assistant Professor at the University of Illinois in 1978. Jim joined the Agricultural Research Service of the USDA in 1981 and returned to Ithaca, NY where he served as a microbiologist in the Plant, Soil, and Nutrition Laboratory until his untimely death on September 20, 2009. For over 25 years, in addition to his federal duties, Jim held a courtesy appointment as Professor of Animal Science and Microbiology at Cornell University. He trained over 40 undergraduate, graduate and post-doctoral students. He received numerous ARS outstanding performance awards, including the North Atlantic Area Scientist of the Year, and the 1994 American Dairy Science Association/American Feed Industry Association (AFIA) Award. In 1999, he was elected to the American Academy of Microbiology via a "highly selective, peer review process, based on scientific achievement and original contributions that have advanced microbiology." In 2001, the American Society of Information Science and Technology named him to their list of World's Most Productive Scientists (an elite group of the top 0.5% scientists based on publication output). In 2002, Jim self-published his book, “Rumen Microbiology and Its Role in Ruminant Nutrition”, which was aimed as an overview to provide a service to the broadest possible audience, and today is one of the most concise approaches to analyzing the relationship between the ruminant and its microbial consortium. In 2004, the USDA/ARS Grade Evaluation System promoted him from GS-15 to ST (super-grade), an honor bestowed upon fewer than 1% of its career scientists. In 2005, Jim received the AFIA Award in Ruminant Nutrition Research through the American Society of Animal Science (ASAS). In 2008, Jim was the recipient of the Morrison Award from the ASAS, a professional capstone award in recognition of his outstanding contributions to the field of animal production, Jim’s stature was further demonstrated by the fact that numerous foreign scientists selected his laboratory as a site for their sabbaticals. Jim served on the editorial boards of *Applied and Environmental Microbiology*, *Microbiology* and the *Journal of Dairy Science*. He chaired or co-chaired numerous scientific meetings, received numerous extramural grants, and was a syndicated columnist for

Farm Progress Magazines. An objective measure of the breadth of Jim's impact is demonstrated by a SCOPUS search, which displays more than 6,000 citations (excluding self-citations) over the past 15 years.

Even after all his awards and world travel, at heart, Jim would always be a "dairy kid". He grew up on a New York dairy, but his early experience (and his father's) in California also influenced his personality. The main trait this upbringing provided him was the ability to focus on the "big picture" – how everything fit together. One of his cardinal rules was to always be able to explain your research to the guy driving a tractor on a dairy farm, or to a pen rider in a feedlot. If you could not get across to that person why your research was important, then you needed to rethink what research you were performing. He applied this explanatory approach to such seemingly esoteric concepts as the electrochemistry behind ionophore function (Russell, 1987; Kajikawa and Russell, 1992), energy utilization, enzymes and metabolism of bacteria (Van Kessel and Russell, 1992; Bond and Russell, 1996, 1998), as well as how to feed cattle more efficiently (Fron et al., 1996; Diez-Gonzalez et al., 1998; Russell, 1999; Russell and Rychlik, 2001).

Jim's mind worked in ways unlike other peoples' minds. He had a special ability to understand mechanistic details at small scale, without losing sight of the big picture of feeding the animal or the economics of dairy production. This is probably what made him such an asset to a modeling effort like the CNCPS, which required breaking down complex phenomena into individual equations that, when combined, were still relevant to the animal. This skill in thinking mathematically and biologically at multiple levels, directions, scales while still maintaining a focus on the big picture also allowed him to be a successful collaborator with his fellow microbiologists. One of us (PJW) recalls a personal experience, relating Jim's contribution to our understanding of the crossfeeding of nutrients between ruminal bacteria (Wells et al., 1995). In the course of determining some fundamental growth parameters of ruminal cellulolytic bacteria, I was attempting to determine the kinetic constants for growth of individual cellulolytic species on each of the compounds in the oligomeric series of cellulose hydrolysis products. This was quite simple for glucose and for cellobiose, as both are readily available and relatively inexpensive. But the individual pure cellodextrins (cellotriose, cellotetraose, etc.) are extremely expensive --- several dollars per milligram, because they are very difficult to isolate from cellulose hydrolysis mixtures at a preparative scale. To get around this, I had this idea to grow the bacteria in continuous culture on a cellodextrin mixture prepared by partial acid hydrolysis of cellulose. By measuring concentrations of each component of the mixture in the inflow and outflow of the chemostat, I hoped to calculate uptake rates for each of the individual cellodextrins. To test the idea, I had my graduate student, Yan Shi, first grow *Fibrobacter succinogenes* on cellobiose, and measure cellobiose concentrations in the inflow and outflow of the chemostat. She observed, as we expected, that cellobiose was consumed in the chemostat, but to our surprise, she observed that the chemostat effluent contained substantial amounts of the longer oligomer, cellotriose. I happened to mention this to Jim. He jumped on it immediately, proposing that cellotriose was synthesized exergonically by an intracellular cellodextrin phosphorylase and then effluxed from the cells to maintain the equilibrium

in the direction of cellodextrin synthesis. In addition, he recognized that this “cellodextrin efflux” can serve as a means of cross-feeding cellodextrins to both noncellulolytic and planktonic cellulolytic bacteria (both of which can sometimes grow better on cellodextrins than on glucose). A few weeks of collaborative experiments proved the concept. Thus, the idea of using the chemostat to characterize fermentation of individual cellodextrins had to be abandoned, but in its place we had a more general, “bigger-picture” cross-feeding story to tell.

Another facet of Jim’s persona was molded on the dairy farm through the 1950’s and 60’s: An insecurity that manifested itself in a tremendous drive and work ethic, and a scientific restlessness that is a hallmark of the “great ones”. But Jim’s insecurity also made him feel that he had to prove himself, at any cost ---- which was often his undoing in his personal relationships. Jim revered Bob Hungate and Marvin Bryant, who together truly founded the study of the microbiology of the rumen, and Jim always gave them their due credit and recognition. However, he craved to be on the Mount Rushmore of Rumen Microbiology with them. He had a need to be esteemed for his science and his science alone, because for him respect could only be based on scientific abilities and accomplishments; no other criterion for greatness was acceptable. This peculiar naivety with regard to basic social skills stands in stark contrast to Jim’s scientific brilliance (discussed in other presentations herein). Jim could never understand what motivated or rewarded other people. He only understood what motivated him. This led to many conflicts and misunderstandings throughout his professional career. Ironically, the unusual combination of personality traits that drove him to scientific heights prevented him from forming and/or maintaining many long-term close collaborative relationships, and from being broadly admired in the same way his mentors (Baldwin, Hungate, and Bryant) were. Jim himself would occasionally reflect unfavorably on his own brusque manner and emotional distance. He once remarked that all the while he was at UC-Davis he envied fellow grad student Bob Stack, who apparently had a warm personal relationship with Hungate, his mentor. Jim said, “Stack would come into the lab and playfully remark, ‘So what’s the ‘Gater been up to?’ I could never seem to do that.” Jim regarded Hungate, his microbiological mentor, with such awe that he would never presume to speak of him so casually, and he expected the same of his own students. Thus, throughout his career, even with all the professional accolades, Jim always felt himself on the outside looking in. Interestingly, in spite of that outsider status, Jim always regarded his time at Davis as a highlight of his life and he maintained that connection for years by bringing graduate students from Davis (Cotta and Russell, 1982; Martin and Russell, 1988) into his own lab.

Despite Jim’s personality quirks, one of his unsung contributions to rumen microbiology was his role as a sounding board for other people’s work. His natural skepticism pushed his colleagues to prove their particular cases more rigorously, and sometimes he could be a hard nut to crack. One of us (PJW) recalls one case in particular (Mouriño et al., 2001). There was an opinion among many that ruminal cellulose degradation slows dramatically at pH values below 6.0, the minimum growth pH of most cellulolytic bacteria. My students and I did some experiments which showed that the first-order rate constant of cellulose degradation by mixed ruminal microflora in

vitro varied with the initial pH of the culture, but that this value actually remained constant as pH declined, until pH reached nearly 5. I showed the data to Jim. He was not convinced, and wanted to see the phenomenon demonstrated in binary defined cultures (perhaps to help put it on a more mechanistic footing). So I demonstrated the same effect with pure cultures of three different cellulolytic species, when each was combined with a non-cellulolytic, cellodextrin-fermenting species (like *Selenomonas ruminantium* or Jim's favorite, *Streptococcus bovis* JB1 [a strain initialed after Jim himself]). As long as the noncellulolytic partner was present to consume cellodextrins produced by the cellulolytic partner, cellulose degradation continued unabated, until the pH reached around 5. I presented the new data to a still-skeptical Jim, and was exasperated by his response: "Are you sure you know how to measure pH?"

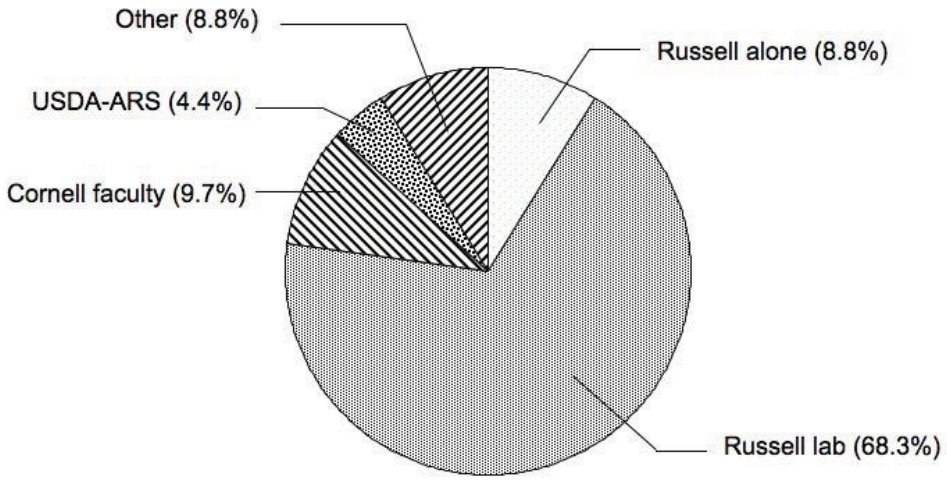
Jim's combination of natural skepticism and less-than-tactful expression was on full display whenever he was surrounded by his colleagues. For despite his aversion to travel, he was a frequent attendee and imposing presence at scientific meetings -- especially the biennial Conference on Rumen Function and the Cornell Nutrition Conference. Jim in particular hated flying, to the point that he often drove long distances to meetings, usually with lab members cowering or feigning sleep in the back seat as he motored nonstop from Ithaca to Chicago, or Indianapolis, or Maine, or wherever (Sample quote: "There's no need for a bathroom stop if you don't drink anything while we are driving"). At the meetings, Jim would drift from session to session, occasionally – if his scientific mind was sufficiently affronted – bestowing a question on a not-quite-unsuspecting colleague up on the podium. If Jim thought the response inadequate, he would often stretch out his arm at waist level, roll his palm upward ("Here it comes!" we all thought), sigh "Well…", and deliver some withering comment. Although this inability to "suffer fools gladly" would cause problems for Jim professionally, he maintained a deep and abiding respect for that surprisingly large number of colleagues whom he held in very high esteem, such as Milt Allison, for whom Jim named the genus *Allisonella* (Garner et al., 2004). Bob Hungate's passing in 2005 was a significant personal and professional blow to Jim because of the high esteem with which he regarded Dr. Hungate.

In spite of this sometimes-insensitive treatment of his colleagues, Jim recognized the need to bring new blood into our field, and he would never publicly embarrass graduate students from other labs. This generally supportive attitude came much to their mentor's surprise. Jim often welcomed students from various labs into his own lab for collaboration on a variety of projects. Additionally, having himself come to Cornell as an undergraduate from a farm background, there was always a position for undergraduate students to work in the Russell lab. If an undergraduate showed research promise, he or she was able to eventually get their own laboratory project and be co-author on manuscripts, if they could also get past Jim's unintentionally intimidating nature. When one undergraduate working on a project (Callaway et al., 1999) was called into Jim's office for "wisdom hour", she was struck mute with fear, unable to answer any questions due to her catatonic state. After about 2 months of this, Jim remarked, "She's really smart, she knows when to keep her mouth shut". Many of these research undergraduates went on to obtain advanced degrees in other laboratories (Bond et al.,

1999; Jarvis et al., 2000; Kurtovic et al., 2003).

Jim was a prolific writer (by our count, 227 journal articles, book chapters and review papers). Many of these papers were sole-author works of his own hand (Fig.1). As his co-authors soon found out, the world of Russell was full of

Figure. 1. Distribution of Dr. James B. Russell's 227 peer-reviewed journal publications, review articles and book chapters, based on co-authorship.



rules that put Jim in absolute control: Simple sentences (every sentence has a subject and a verb), direct wording (no need to be flowery), focused introductions, only three to five sentences in a paragraph. Tell a simple story so anyone can understand it. Keep your audience in mind at all times. Use gerunds often. Jim would frequently complain that one of us (TRC) did not know how to properly use gerunds, but educators in the deep South never discussed them, and to this day, I still don't know what a gerund is (Sorry, Jim). Jim's control of the writing process extended well beyond his own students, and seeped into his various outside collaborations. Regardless of how the dynamics of an experimental collaboration played out, there was never any doubt when it came to the writing: Jim was the boss, and writing with him, especially over the phone, often seemed like an endless, even Sisyphean, task. Over the course of hours, each sentence was evaluated, dissected, rearranged, discarded, resurrected and rehabilitated before it would meet his standards, Jim quickly abandoned the "Track Changes" option in Microsoft Word, probably because the result – an occasional word in black text bobbing up mournfully in a sea of red, struck-out text – was simply too dispiriting to his co-authors. He spent tremendous amounts of time in search of the perfect word, the perfect phrase, the perfect sentence. Here again, economy of language was paramount, and woe to the collaborator that brought him a sentence more than two lines long! Jim often quoted Blaise Pascal's famous line from 1657, "I have made this letter longer than usual, only because I have not had the time to make it shorter". With Jim Russell, you always took the time to make it shorter.

Despite this apparent rigidity, Jim's ability to teach scientific writing was unparalleled, even if the lessons were painful. Writing was a one-on-one effort. Every manuscript was a product of several weeks of writing, with slow progress made daily. When beginning as a graduate student, the retention of a word in a sentence was cause for celebration, but as one progressed, the deletions *en masse* were reduced. By completion of the time in Russell's workshop, writing had become part of a highly disciplined and organized process, an art rather than a science. Oddly enough, some of Jim's best creative writing emerged in the titles of his papers, where words like "spiraling" crept in to replace the simpler, utilitarian language to which he normally adhered (Russell and Hino, 1985).

Jim was convinced that his writing style was a big part of his ability to effectively use his experimental results to tie up the loose threads hanging in the existing literature. But, to us, the key to this success was that he seemed to know where all those loose threads were. One of the little known attributes that aided Jim in his scholarship and his quest to be known as the best rumen microbiologist of his generation was the fact that he had a pure eidetic (photographic) memory. This allowed him to have mastery of all of the past literature, which helped him see how it all fit together. Once, while we were listening to a talk on microbial degradation of protein in the rumen, Jim leaned over to one of us, pointed to a data slide on the screen, and whispered, "There's an almost identical figure on page 300 of Hungate's book!" Indeed it was so, and this was no fluke: Jim had a collection of more than 2,900 reprints, and could draw a figure from memory out of nearly any one of them. Nearly anytime that one of us (TRC) would come up with a "great experiment" or "what would happen if" question, Jim would say something like, "Jones did that in 1973 and showed…", and he would draw a good approximation of a graph; when you went and pulled the paper out and looked at it, the graph was nearly identical. This skill allowed Jim to appear to be a "witch" when discussing experiments. He could predict in advance how they would turn out, because he truly had seen it all before, and he kept those images in his mind always.

Jim believed that the big picture stories were the most crucial to the animal, but most especially to the farmer. One of these big stories that Jim was most proud of was the isolation of the obligate amino acid fermenting bacteria. The rate of ammonia production in the rumen was known to be greater than the individual rates of ammonia production of the known important ruminal bacterial species (e.g., *Prevotella*). The ionophore monensin primarily inhibits Gram positive organisms (Russell, 1987; Russell and Strobel, 1988; Russell and Strobel, 1989) yet most of the known ammonia-producing bacteria were Gram-negative, and the addition of monensin to cattle rations decreased ammonia production by nearly 50%. This was never effectively explained until Jim isolated the obligate amino acid fermenting bacteria that were monensin-sensitive and produced 50-fold more ammonia on a specific activity basis than did the more well-known ruminal species (Chen and Russell, 1989, 1990; Yang and Russell, 1993). This discovery led to recognition of the mode of action of monensin in cattle, and an appreciation of the potential role of these so-called " hyper-ammonia producing bacteria" in ruminal protein degradation (Attwood et al., 1998).

The passing of Jim Russell marks the end of an era in the field of rumen microbiology. Jim combined a rock-solid dairy background, a superb intellect and a single-minded obsession with rumen microbes to become the unquestioned leader in the field, a fitting inheritor of the mantle of Hungate and Bryant. Over the years rumen microbiology, like other disciplines of science, has changed immensely. “Individual investigator” science, at which Jim excelled, is giving way to grand-scale collaborations conducted among scientists of increasingly extreme specialization, using outlandishly expensive equipment. Microbial ecologists have exposed the limitations of culture-based studies, and molecular approaches are now *de rigueur* for obtaining the funding that will drive the acquisition of new scientific knowledge. Despite this, the “old-school” thinking of Jim Russell still has a place in the new molecular world: It is critical that we understand concepts such as kinetic order, specific activities and reaction rates and apply them to the torrent of new information that is being unleashed on rumen microbiologists through the development of pyrosequencing and the advanced techniques yet to come. Without a basic understanding of the mechanics, roles and even mathematics behind the ecology of the ruminant, further advances and understanding of the microecology and nutritional impacts of the microbial population will be delayed. Time, and science, march on. But none of these new realities will minimize the many contributions of Jim Russell, and of the students and colleagues he inspired.

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JAMES B. RUSSELL: CONTRIBUTIONS TO RUMINAL MICROBIOLOGY

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James B. Russell (1951-2009) was born in California and moved with his family to New York in 1953. His parents purchased a dairy farm in 1954 and the family has operated it ever since. The farm meant everything to Jim and I was fortunate to visit the farm on a visit to Cornell from South Africa in 1984. It was here that Jim gained his lifelong interest and passion for work with dairy cows. Initially Jim had planned to become a veterinarian but after taking a course in introductory microbiology he changed his major. After completing his BS degree at Cornell University, he completed MS and PhD degrees at the University of California, Davis. His PhD advisor and mentor Professor R. Lee Baldwin had a strong interest in metabolism both in the rumen and the host animal and he integrated this knowledge of ruminant digestion and metabolism using mathematical models. In fact, over the last few years of his life Jim continued with contributions to rumen modeling efforts. This background of graduate studies made Jim uniquely qualified to contribute to rumen models and make major contributions to the rumen sub-model of the Cornell Net Protein and Carbohydrate and Protein System. He interacted with Professor Robert E. Hungate in the Department of Bacteriology at Davis during the course of his PhD research and published a series of seminal papers on substrate preferences and affinities, maintenance energy requirements and effects of pH on growth rates and efficiency using chemostat approaches. This technique and approach made another strong and lasting contribution to his research portfolio. His first position was an Assistant Professor in the Department of Animal Sciences at the University of Illinois in 1978 where I first met Jim and established a lifelong relationship and respect for his research. He taught courses on intermediary metabolism in ruminant animals and microbiology of the gastrointestinal tract. These teaching themes continued at Cornell and he was consistently listed as an excellent and innovative teacher. He brought Mike Cotta from California with him as his first graduate student. At the time, I was a Postdoctoral Research Associate in the laboratory of Professor Marvin. P. Bryant in the Department of Dairy Science. Also, at that time, the Department of Dairy Science had also recently hired Prof Robert B. Hespell to replace Mike Wolin. We were therefore in the fortunate but unusual position of having three top anaerobic microbiologists in one building together with a group of prominent microbiologists up campus in the Department of Microbiology namely Professors Ralph Wolfe, Carl Woese and Abigail Salyers. This was a glorious time and rumen microbiologists were in the vanguard with respect to new developments and concepts relating to anaerobic microbiology and especially rumen microbiology. In 1981, the USDA established a new position at Cornell University and Jim returned to his alma mater and worked as a rumen microbiologist initially in the Department of Animal Sciences and in 1991 he moved to the Department of Microbiology.

James B. Russell was a prolific scientist publishing over 220 peer reviewed scientific publications during his career mainly in the field of rumen microbiology. Any attempt to summarize all of these contributions would be lengthy and difficult and in the following sections we have chosen to highlight a few areas where he made significant contributions to our knowledge and understanding of rumen microbiology.

FACTORS INFLUENCING COMPETITION AND COMPOSITION OF THE RUMEN BACTERIAL MICROBIOTA

This area of research constitutes Jim's first series of publications and launched him on a long and successful career as a rumen microbiologist. In fact, this is the topic that I asked him to present at the First International Symposium on Herbivore Nutrition held in 1983 in Pretoria since in my interactions with him at Illinois it was clear that this subject was significant to the study of bacterial growth in the rumen (Russell 1984). Incidentally, I also invited Bob Hespell to present a paper on ammonia assimilation pathways and survival strategy on rumen microbial growth at the same symposium (Hespell 1984). They travelled together to South Africa and spent the first few days at my home in Pretoria and never stopped talking once about science and rumen microbiology. Also of note, the Chairs for the two sessions on Limitations of Rumen Fermentation were Bob Hungate and Marv Bryant. In 1981, Russell and Hespell were invited to write a review article celebrating the 75th Anniversary of the Journal of Dairy Science. This paper entitled "Microbial rumen fermentation" summarized the previous 25 years of progress in rumen microbiology (Russell and Hespell, 1981).

The quality and quantity of rumen fermentation products is dependent on the types and activities of microbes in the rumen. Diversity within the rumen microbial ecosystem is high and nutritionists are concerned about the changes that occur in these populations and their effects on fermentation end-products. Understanding the quantitative and dynamic interrelationships between individual bacterial species is critical to understanding rumen ecology and Russell set out to study factors that influence the competition and composition of the rumen bacterial population. Thus, when soluble nutrients are in excess an important determinant of relative microbial success is maximum specific growth rate and those organisms with higher maximum specific growth rates are able to outcompete slower growing organisms. When maximum growth rates were compared between several rumen bacteria differences were large and growth rate was dependent on both energy source (Russell and Baldwin, 1978) and pH (Russell and Dombrowski, 1980). However, during much of the feeding cycle soluble substrate concentrations are limiting and increments in substrate concentration result in increased growth rate following saturation kinetics typical of enzyme systems. The affinity constant K_s is defined as the substrate concentration that yields one-half maximum growth rate. Russell demonstrated, using chemostat cultures, that affinities for the same substrate varied greatly between species and that single species also have higher affinities for some substrates than other (Russell and Baldwin, 1978). He also showed that although maintenance energy requirements of rumen bacteria are low compared to other bacteria, maintenance energy for individual rumen

bacteria varied greatly (Russell and Baldwin, 1979). These results were consistent with the observation that organisms with low maintenance energies would be able to grow faster and dominate the rumen population when substrate availability and growth rates were low. These data were consistent with *in vivo* observations and explain how some species such as *Butyrivibrio fibrisolvens* could predominate on poor forage diets. When Russell grew pure cultures of rumen bacteria in batch culture some substrates were not utilized until others were depleted and the more preferred substrates inhibited utilization of less preferred substrates (Russell and Baldwin, 1978). Importantly, bacteria had different substrate preference patterns and that these differences together with substrate affinities suggested that rumen bacteria have evolved different growth strategies and these physiological factors affect competition among rumen bacteria (Russell and Hespell, 1981). Russell explored these relationships later in his career and studied coupling of growth and energy utilization by rumen bacteria as well as the bioenergetic role of chemical agents such as monensin.

BACTERIAL NUTRIENT TRANSPORT AND BIOENERGETICS

Bacterial growth is dependent on the availability of a suitable carbon and energy source and the success of most ruminal bacteria is related to their ability to degrade and ferment substrates. Since many bacteria utilize sugars preferentially and these substrate preferences are generally mediated by regulated transport systems, Russell and his lab described the transport mechanisms of predominant ruminal bacteria. Their research showed that predominant ruminal bacteria are capable of transporting soluble nutrients by several mechanisms (Russell et al. 1990; incidentally I presented a review entitled Recent advances in rumen microbial ecology and metabolism: Potential impact on nutrient output at the same ADSA Symposium in 1990 soon after arriving at the University of Illinois. Bob Hespell presented a paper on Physiology and genetics of xylan degradation by gastrointestinal tract bacteria at the same meeting). *Megasphaera elsdenii*, *Selenomonas ruminantium*, and *Streptococcus bovis* transport glucose by the phosphoenolpyruvate phosphotransferase system (PEP-PTS), and *S. ruminantium* and *S. bovis* also possess PEP-PTS activity for disaccharides. Glucose PTS activity in *S. bovis* was highest at a growth pH of 5.0, low glucose concentrations, and a dilution rate of 0.10 h^{-1} accounting for its ability to overgrow other ruminal bacteria at low pH on high grain diets. The cellulolytic ruminal bacterium *Fibrobacter succinogenes* uses a Na^+ symport mechanism for glucose transport that is sensitive to low extracellular pH and ionophores. Sodium also stimulated cellobiose transport by *F. succinogenes*, and there is evidence for a proton symport in the transport of both arabinose and xylose by *S. ruminantium*. A better understanding of these nutrient transport systems and factors influencing their activity have enabled ruminant nutritionists to improve efficiency of feed utilization by beef and dairy cattle. Thus, many feed additives have either a direct or indirect effect on rumen bacterial transport. For instance, ionophores can inhibit transport by destroying, and sometimes even reversing, ion gradients, lowering intracellular pH, or causing excessive ATP hydrolysis.

Biological growth depends on the transfer of energy from catabolic to anabolic processes but the conversion or coupling is never complete and energy is dissipated

into the environment as heat. Russell was familiar with continuous culture theory and was aware of data that showed that cell yields were generally lower at slow growth rates and that energy could be used for non-growth functions such as maintenance energy. He inferred that maintenance energy would contribute to heat production and that few, if any, applications of microcalorimetry to the study of microbial growth efficiency had been carried out. Jim purchased an LKB bioactivity monitor that was equipped with Peltier elements as heat source and gold flow cells. The instrument was calibrated with an internal electric heat source and gave very stable digital readouts in units of uW. *Selenomonas ruminantium* and *Prevotella* (*Bacteroides*) *ruminicola* were grown in chemostat culture with glucose as the energy source and heat production was measured continuously with the microcalorimeter (Russell 1986). He was able to calculate complete energy balances for substrate utilization and product formation and showed that heat of fermentation i.e. maintenance declined as growth rate increased. These experiments indicated that bacterial maintenance energy was not necessarily constant and that accumulation of energy source was associated with an increase in heat production. This led to the idea, already articulated by Neijssel and Tempest, that bacteria have mechanisms to hydrolyze ATP when energy source is in excess and termed overflow metabolism, energy spilling reactions or futile cycles to explain this uncoupling of catabolism and anabolism. Russell thought that mechanisms involved in heat or energy spilling were related to the chemiosmotic hypothesis and the generation of proton motive force and he continued with research to test these postulates and the role of intracellular pH regulation and membrane physiology on this phenomenon.

It had long been recognized that fermentation acids were more inhibitory to some bacteria than others, but the mechanism was not understood. Jim and his graduate students were the first to show that the toxicity of fermentation acids was mediated by the transmembrane pH gradient and an intracellular accumulation of fermentation acid anions. Together with Greg Cook, a postdoc in his lab, he published a landmark review entitled “Energetics of bacterial growth: balance of anabolic and catabolic reactions” (Russell and Cook 1995) that is well worth studying today. They concluded that when bacteria are limited for energy sources, the free energy change in catabolic reactions is generally tightly coupled to the anabolic steps in cellular biosynthesis, and the total energy flux can be partitioned into growth and maintenance functions. If growth is limited by nutrients other than energy, such as nitrogen, bacteria can spill ATP in non-maintenance reactions. This finding greatly enhanced our understanding of ruminal ecology so it could be manipulated in a systematic fashion to increase the efficiency of bacterial growth in the rumen and decrease feed costs. The anion accumulation model of fermentation acids is now widely accepted and still used by food scientists.

Pathogenic *E. coli* must survive the low pH of the human gastric stomach before they can infect humans. In some of his most controversial and sensational research, Jim and his lab demonstrated that cattle fed grain had large numbers of acid-resistant *E. coli*, but these potentially deadly bacteria could be eliminated if cattle were switched to hay for only five days (Diez-Gonzalez et al. 1998). This practical scheme of combating *E. coli* was based on the observation that grain feeding causes an accumulation of fermentation acids in the colon and a subsequent induction of extreme

acid resistance in *E. coli* (Russell et al. 2000a, 2000b). The impact of this paper was huge and led to much debate, discussion and dissension. For Jim, it was all clear that pathogenic *E. coli* (e.g. O157:H7) cause more than 70,000 human infections each year and beef is the primary source of this infection although he did not use E. coli O157:H7 for his work using more general growth media for commensal *E. coli*. The work was subsequently confirmed at the University of Nebraska and other work at the Meat Animal Research Center who expanded on his work and showed that the diet shift from concentrate to hay also caused a large decrease in the number of cattle carrying *E. coli* O157:H7.

IONOPHORES

The manipulation of rumen fermentation using ionophores through alteration of microbial populations and activity and their role in animal growth promotion was, and remains, a topic of research interest and importance. Jim and his lab became interested in this research area as a result of his previous work on bioenergetics and growth efficiency. He published together with Herb Strobel a comprehensive mini review entitled “Effect of ionophores on rumen fermentation” (Russell and Strobel 1989) that is still widely cited. It includes an introduction on the general effects of ionophores on ruminal fermentations and has a classic diagram (Figure 1) that summarizes the possible ionophore effects in the rumen that few others could have conceived. Few studies had been published on the mechanism of ionophore action in ruminal bacteria. Monensin is an antiporter with high selectivity for Na but can also translocate K, so starting with an understanding of ruminal concentrations of Na (90-150mM) and K (4-5 fold lower but the predominant intracellular cation). Jim established that in *Streptococcus bovis* a large K gradient was driving influx of H^+ and published a manuscript with a schematic diagram showing the effects of monensin on ion flux in S*. bovis* (Russell 1987).

As part of his research with ionophores, Russell defined mechanisms by which monensin inhibited sensitive ruminal bacteria; isolated three previously unrecognized bacteria from the rumen; showed that the new bacteria were monensin-sensitive; demonstrated the importance of the isolates in wasteful amino nitrogen degradation; and devised potassium efflux experiments so that monensin activity could be monitored *in vivo* (Lana and Russell 1996). The new isolates were 20 times more active than previously studied ruminal bacteria, and accounted for as much as 80% of the ruminal ammonia production. In the interests of brevity and topic, we cover the hyper ammonia producing bacteria in the section on amino acid fermentation in the following section.

In the minireview, they also considered the concept of monensin resistance and concluded that after many years of extensive use, ionophores continued to improve the efficiency of animal performance and suggested that the sensitivity of ruminal microorganisms was relatively stable and that the pattern of resistance was due to fundamental differences between the nature of the cell wall and physiology between microbes (Russell and Strobel 1989). To account for observed species difference in ionophore sensitivity they concluded that individual species may have different abilities

to cope with a loss of active transport activity, a decline in intracellular pH, a change in intracellular ions or a drain in futile cycles (Russell and Strobel 1989). In a later paper, he revisited this topic based on arguments that ionophores posed the same threat to public health as conventional antibiotics (Russell and Houlihan 2003). His overall summary was that Gram-positive ruminal bacteria were in many cases more sensitive to ionophores than Gram-negative species, although this model of resistance was not always clear since some Gram-negative ruminal bacteria were initially ionophore-sensitive, and that even Gram-positive bacteria can adapt. Ionophore resistance appeared to be mediated in some cases by extracellular polysaccharides (glycocalyx) that exclude ionophores from the cell membrane. Because cattle not receiving ionophores have large populations of resistant bacteria, it appears that this trait was due to a physiological selection rather than a genetic mutation per se. He concluded that because genes responsible for ionophore resistance in ruminal bacteria had not been identified, and there was little evidence that ionophore resistance could be spread from one bacterium to another. Thus, use of ionophores in animal feed was unlikely to have a significant impact on the transfer of antibiotic resistance from animals to man.

Jim always managed to continue with a theme and this was evident in some of the last active research that he carried out in the lab. This theme was the role of bacteriocins in controlling population growth and survival in the rumen and proposed that ruminal bacteriocins, some as potent *in vitro* as nisin, could be used as an alternative to antibiotics in cattle rations. Naturally this research also featured his favorite bacterium *Streptococcus bovis*. The group published a paper on a bacteriocin mediated antagonism by ruminal lactobacilli against *S. bovis* (Wells et al. 1997). Early work indicated that some *S. bovis* strains produced bacteriocins. Approximately 50% of *S. bovis* strains isolated from the rumen had antimicrobial activity, but some strains were distinctly more active (Mantovani et al. 2001). *S. bovis* HC5 was the best strain, and its bacteriocin (bovicin HC5) had a broad spectrum of activity (Mantovani et al. 2002). Bovicin HC5 inhibited the methane production of mixed ruminal bacteria (Lee et al. 2002) and ammonia production by the amino acid-fermenting ruminal bacterium, *Clostridium aminophilum* (Mantovani et al. 2002). In some of his last published research, Jim and his group showed that bovicin HC5 was a broad spectrum antibiotic that catalyzed the efflux of K from *S. bovis* JB1, a sensitive strain, and described the mechanism for this to bind and transfer to other sensitive bacteria (Mantovani and Russell 2008; Xavier and Russell 2009). These results supported the idea that bacteriocins have the potential to be used as ruminal additives.

AMINO ACID FERMENTATION

Amino acid deamination by ruminal microorganisms is a nutritionally wasteful process that often yields more ammonia than can be used in microbial growth. Ionophores that inhibit Gram positive bacteria and protozoa also decreased deamination so Jim set out to study this process and provide a satisfactory explanation for these effects. He reasoned that earlier work concluding that *Bacteroides (Prevotella) ruminicola* was the most important ammonia producing bacterium in the rumen of cattle was incorrect based on analysis of specific rates of ammonia production and that mixed

bacterial cultures had activities significantly higher than those of the most active pure cultures available. He carried out enrichments using Trypticase (15 g/liter) as the only energy and nitrogen source, and demonstrated very high rates of ammonia production, and two obligate amino acid-fermenting, monensin-sensitive bacteria, a *Peptostreptococcus* species and a *Clostridium* species, were obtained in pure culture (Russell et al. 1998). He subsequently described two additional ammonia-producing, monensin-sensitive ruminal bacteria (strain SR and strain F) which grew rapidly with amino acids as their sole energy source (Chen and Russell 1989). Using 16S rRNA phylogeny together with Bruce Paster these isolates were taxonomically assigned to the genera and species *Peptostreptococcus anaerobius, Clostridium sticklandii* and a new species *Clostridium aminophilum* (Paster et al. 1993). This work resulted in considerable controversy and disagreement among rumen microbiologists but created a new research arena on ruminal hyper ammonia producing bacteria (HAP's or HAB's).

Jim was adept at growing and isolating anaerobic rumen bacteria. It was well known that bacteria decarboxylate amino acids and that decarboxylation of histidine produces histamine, an amine with potent biogenic properties. Previous work had shown that histamine could be produced in the rumen and that this was correlated with negative health effects with side effects such as laminitis in cattle and horses and a major cause of culling in lactating dairy cows. Russell established that high numbers of histamine producing bacteria could be enriched from the rumen of grain fed cattle and these enrichments were not stimulated by glucose ruling out Lactobacilli as the source. Instead the histamine producing bacteria used histidine decarboxylation as their sole mechanism for energy generation and that they produced histamine 3-fold faster than any other previously studied bacterium. Analysis of their 16S rRNA gene sequence showed they were novel and proposed a new taxon *Allisonella histaminiformans* for these isolates (Garner et al. 2002). Also, Russell showed that cows fed alfalfa silage had very high numbers of *A. histaminiformans* but those fed hay did not (Garner et al. 2004). Because extracts of alfalfa silage, containing high levels of peptides stimulated growth of *A. histaminiformans,* but not extracts from timothy silage, he suggested that this component of the diet is detrimental to lactating dairy cattle.

RECOMBINANT DNA IN THE RUMEN

Soon after arriving at Cornell, Jim established a collaboration with David Wilson in the Department of Molecular Biology and Genetics at Cornell to use recombinant DNA approaches to study metabolic activity and regulation in rumen bacteria. It was well established that when cereal grains are included in ruminant diets the rate of starch fermentation is rapid and ruminal pH falls inhibiting the growth of cellulolytic bacteria. So Jim had this idea that CMCases, which in vivo are likely to hydrolyze soluble glucans but not insoluble cellulose, could be converted into cellulases and that using an acid-resistant ruminal bacterium such as *Prevotella bryantii* B_14 he could create an acid-resistant cellulolytic bacterium. He had already established that a variety of non-cellulolytic ruminal bacteria can utilize cellodextrins using endoglucanases capable of cleaving CMC (Russell 1985). In an early publication, they described the production, isolation and activity of a reconstructed fusion cellulase formed from a CMCase gene

from *Prevotella bryantii* $B_1$4 with a cellulose binding domain from *Thermomonospora fusca*. This hybrid enzyme bound tightly to cellulose and had higher specific activities on CMC, amorphous cellulose and ball milled cellulose than the native CMCase. Also, the modified enzyme showed synergism with an exocellulase in the degradation of filter paper (Maglione et al. 1992). The next step was to construct an *E.coli-Bacteroides* shuttle vector that could be transferred into *P. ruminicola* $B_1$4. So they constructed a new shuttle vector (pTC-COW) to transfer the genetically constructed CMCase gene from *E.coli* through *Bacteroides uniformis* into *P. bryantii* $B_1$4 (Gardner et al. 1996). This and other work on 16S rRNA phylogeny led to an invitation from the Science journal to contribute a review (Russell and Rychlik, 2001).

CONCLUSIONS

In summary, much of what I have covered in this short review of Jim's scientific contributions to rumen microbiology are included in his self published book on "Rumen Microbiology and its Role in Ruminant Nutrition". I remember two things in particular about Jim and his scientific method and approach. The first was how efficient he was in using most of the research carried out at the lab bench ending up in the final manuscript. We discussed this and his conversion ratio was in the range of 0.8-0.9 while most other scientists are happy with a 0.3-0.5 ratio. This efficiency was made possible by his wide knowledge of what had already been done and what was required to advance the science and make a contribution in the field. The second was his amazing ability to link rumen microbiology to ruminant physiology in a practical and productive way based on his understanding of the physiology and digestion and metabolism in the ruminant animal. Jim was a prolific and productive scientist as well as a colorful character and passionate champion of Rumen Microbiology. He will be greatly missed by most scientists working in the field.

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IS THERE OPPORTUNITY TO BOOST MILK PROTEIN PRODUCTION?

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INTRODUCTION

Protein is the most valuable milk constituent in multiple-component pricing systems, receiving the largest dollar per unit price of all the milk components. At the end of 2009, milk protein was valued at $2.88/lb, compared to $1.55/lb for milk fat. Long-term projections are for 2 percent annual increases in demand for dairy products (FAO) because of increasing worldwide demand for milk protein and whey components. The financial incentive for milk with higher protein content as well as the growing consumer demand for milk protein highlights the need to gain a greater understanding of nitrogen efficiency within the cow and how milk protein is synthesized within the mammary gland.

The efficiency of converting dietary nitrogen into milk protein output is relatively poor in the lactating animal, between 25 to 30 percent (Bequette et al., 1998). This low level of efficiency also represents an area of opportunity for the dairy industry. Improving nitrogen efficiency within the cow will help the industry to avoid costly nitrogen loss to the environment.

The Dairy NRC (2001) summarized available information regarding dietary influences on milk protein content and yield, with primary focus on modulation of milk protein through amino acid supplementation. In general, the opportunity to increase milk protein content and yield through known dietary strategies appears to be less than 5 percent per day. However, if we gain further understanding of the process of milk protein synthesis and what regulates that process, we might be able to reach higher levels of milk protein production in dairy cows.

INSULIN AND MILK PROTEIN

Starting in the 1990s, a series of studies conducted by Dale Bauman's group at Cornell demonstrated that chronic elevation of circulating insulin concentrations through the use of hyperinsulinemic-euglycemic clamps can result in larger increases in milk protein content and yield than those described above. McGuire et al. (1995) reported a 0.07 kg/d increase in milk protein yield in post-peak lactating cows subjected to the hyperinsulinemic-euglycemic clamp with insulin concentrations elevated five times above baseline. Griinari et al. (1997a) observed increases in milk protein yield and concentration under the hyperinsulinemic-euglycemic clamp with and without abomasal casein infusion. In a study by Mackle et al. (1999), use of the hyperinsulinemic-euglycemic clamp increased milk protein concentration by 11 percent and total milk protein yield by 25 percent when cows were abomasally-infused with casein (500 g/d)

and branch-chained amino acids (88 g/d). These changes in milk protein output were observed 4 d after the start of the hyperinsulinemic-euglycemic clamp.

In cows treated in early lactation with recombinant bovine somatotropin (rbST) as well as insulin and glucose, during insulin infusion, milk protein yield was increased by 0.05 kg/d compared to saline-infused controls (Leonard and Block, 1997). In this study, glucose was infused alone in a separate treatment at a rate of 50 g/hour, and milk protein yield was decreased by glucose infusion by 0.05 kg/d compared to saline-infused controls.

All of the studies referenced above indicate that insulin influences mammary gland protein synthesis. However, interpretation of results from hyperinsulinemic-euglycemic clamps is not that simple. The confounding effect of co-infusion of both insulin and exogenous glucose make it impossible to determine if the results are due to the effects of insulin, glucose, or both. During hyperinsulinemic-euglycemic clamps, significant amounts of glucose, and thus energy, are being infused into the cow. In the study by Mackle (1999), the amount of glucose infused per day (3.336 kg) was the equivalent of 12.2 Mcal NE_L/d[1], which is a considerable amount of energy.

When administered by itself, insulin is commonly associated with reduced milk production and lowered blood glucose. Schmidt (1966) administered short-acting insulin subcutaneously in primiparous cows and observed an increase in milk protein percentage during insulin treatment, but no change in protein yield. Twice-daily injections of protamine zinc insulin reduced milk yield and blood glucose in a study by Kronfeld et al. (1963). When given alone, the effect of insulin is also confounded by the effect of reduced glycemia. So, regardless of how the effects of insulin are investigated, either infused glucose or hypoglycemia may confound the results and interpretation. Ideally, the effects of insulin should be evaluated in situations without significant changes in glycemia or provision of additional glucose.

INSULIN ANALOGUES AND INSULIN GLARGINE

The discovery and successful partial isolation of insulin extracts in the 1920s by Banting and Best was a scientific breakthrough that almost single-handedly took away the lethality of diabetes, which at the time had been an incurable, untreatable disease (Bliss, 1982). Through a transition from animal insulins to recombinantly-made insulins, human diabetes has become a very treatable disease. Today, numerous insulins and insulin analogues are readily available for patients to use depending on type and severity of diabetes, time of day or timing of last meal, and other varying factors (Sheldon et al., 2009). Commercially available insulins and insulin analogues come in rapid-acting, intermediate-acting, and long-acting formulations.

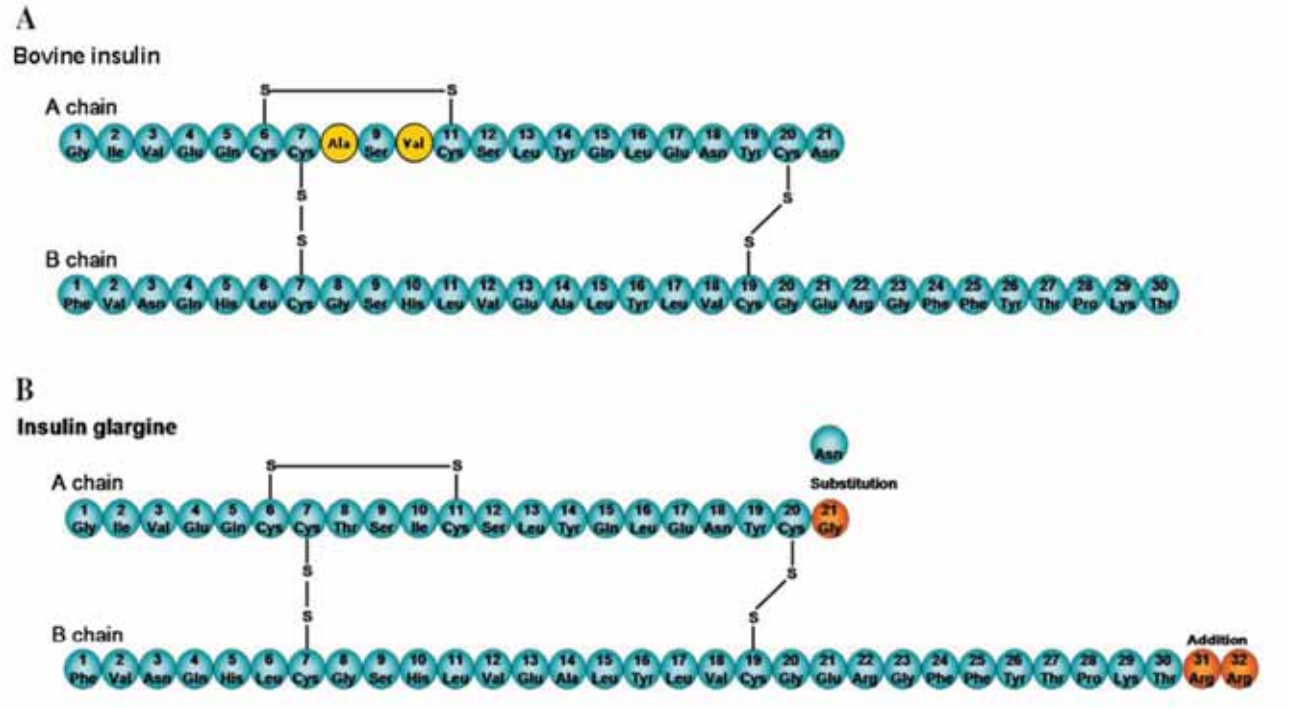
Among the long-acting insulin analogues commercially available for human use is an analogue called insulin glargine. Insulin glargine is a recombinant, human insulin

[1] The NE_L value used for glucose calculation was described by Leonard and Block (1997) as 3.66 Mcal NE_L/kg.

analogue that lasts up to 24 hours in duration (Goykhman et al., 2009). Glargine was approved for use in the United States by the FDA in April 2000. As opposed to other long-acting or intermediate-acting insulin formulations, such as Ultralente® or neutral protamine hagedorn (NPH) insulins, the pharmacokinetic profile of insulin glargine is relatively flat and without a pronounced peak, thus mimicking basal insulin secretion more closely. An advantage of using long-acting insulin analogues in humans, such as insulin glargine, is reduced risk of hypoglycemia in patients (Goykhman et al., 2009).

Glargine differs from native human insulin with a glycine substitution for an asparagine residue at position A21 and addition of two arginine residues on the carboxyl end of the B-chain at positions B31 and B32 (Bolli and Owens, 2000; Owens and Bolli, 2008) (Figure 1). The amino acid substitutions to this analogue shift the isoelectric point of the molecule from pH 5.4 to 6.8. The molecule is thus more soluble in a more acidic solution, but when given subcutaneously, it precipitates at the more neutral pH of the injection site, slowly dissipating and being absorbed over a prolonged period of time. Insulin glargine is available in the United States under the trade name Lantus® and is marketed by Sanofi-Aventis (Bridgewater, NJ).

Figure 1. Amino acid sequences and structures for (A) bovine insulin and (B) insulin glargine, adapted from Owens and Bolli (2008).



EFFECT OF INSULIN GLARGINE ON LACTATING COW METABOLISM

In an effort to avoid the confounding effect of severe hypoglycemia induced by insulin, we chose to use insulin glargine in lactating cow studies to investigate the role of insulin in milk protein production. With its long duration of action, insulin glargine was

an ideal choice to study the effect of insulin-action in lactating cows without intensive use of venous catheters, infusion pumps, and exogenously-supplied glucose.

Insulin glargine (Lantus®) was used in a dose response study to determine the response of lactating dairy cows to this insulin analogue. In the study, 16 multiparous cows (237 DIM ± 11 d) were divided into two groups of eight and randomly assigned to one of four treatments (control, 0.1 IU insulin glargine/kg BW, 0.2 IU/kg BW, and 0.4 IU/kg BW). Cows were fitted with jugular catheters on the day before the study. Subcutaneous (SQ) injections of insulin glargine or sterile water were given at 0900 h. Cows were fed hourly and milked at 1500, 2300, and 0700 h. Blood samples were taken hourly via jugular catheter for 24 h following administration of treatments.

Administration of insulin glargine resulted in a linear ($P<0.001$) decrease in plasma glucose concentrations with increasing dose of insulin glargine (66.0, 62.3, 61.0, 54.1 mg/dl glucose for control, 0.1 IU/kg, 0.2 IU/kg, and 0.4 IU/kg, respectively) (Figure 2). Endogenous insulin secretion[2] decreased linearly ($P=0.028$) with insulin glargine administration (1.04, 0.88, 0.79, 0.64 ng/ml for control, 0.1 IU/kg, 0.2 IU/kg, and 0.4 IU/kg, respectively)

Following the dose response study, two studies were conducted to determine the effects of insulin glargine on metabolism and production of lactating Holstein cows. A mammary metabolism study was conducted to determine the effect of insulin glargine on amino acid uptake and utilization in the mammary gland. In the mammary metabolism study, 3 multiparous cows (101 DIM ± 22 d) fitted with indwelling intercostal arterial and mammary vein catheters were used to determine the effect of insulin glargine in a two-period crossover design. Periods lasted 4 d with a 2 d washout occurring in between. In the first period, two cows received 0.15 IU/kg BW of insulin glargine via SQ injection 2x/d, while the remaining cow was a control. Treatments were reversed for the second period. On d 4 of each period, simultaneous blood samples were taken from the arterial and venous catheters at hourly intervals for 12 h.

Dry matter intake, milk yield, and all milk components, except lactose, were not significantly different between the control and insulin glargine treatments. Lactose content was reduced by 11 percent ($P=0.094$) and yield was reduced by 5 percent ($P=0.091$) by insulin glargine treatment. The significant decrease in lactose yield and content is likely related to the numerically lower milk yield for the glargine treatment.

Milk nitrogen fractions were also analyzed by Kjeldahl analysis. Casein content and yield were not different between treatments ($P>0.10$), but non-casein nitrogen content was 7 percent higher for insulin glargine ($P=0.006$). It should be noted, however, that this study used a very limited number of cows and one cow had a high somatic cell count while she was on the insulin glargine treatment. As high somatic cell counts can

[2]The insulin ELISA used in this experiment did not appear to exhibit cross-reactivity with insulin glargine and its metabolites. Therefore, we are presuming that the ELISA was measuring native, endogenous insulin concentrations.

increase proteolysis of casein in raw milk (Ma et al., 2000) these results should be interpreted with care.

Figure 2. Twenty-four hour plasma glucose profiles in lactating cows given one of four treatments: water (control), 0.1 IU insulin glargine/kg BW, 0.2 IU/kg BW, or 0.4 IU/kg BW.

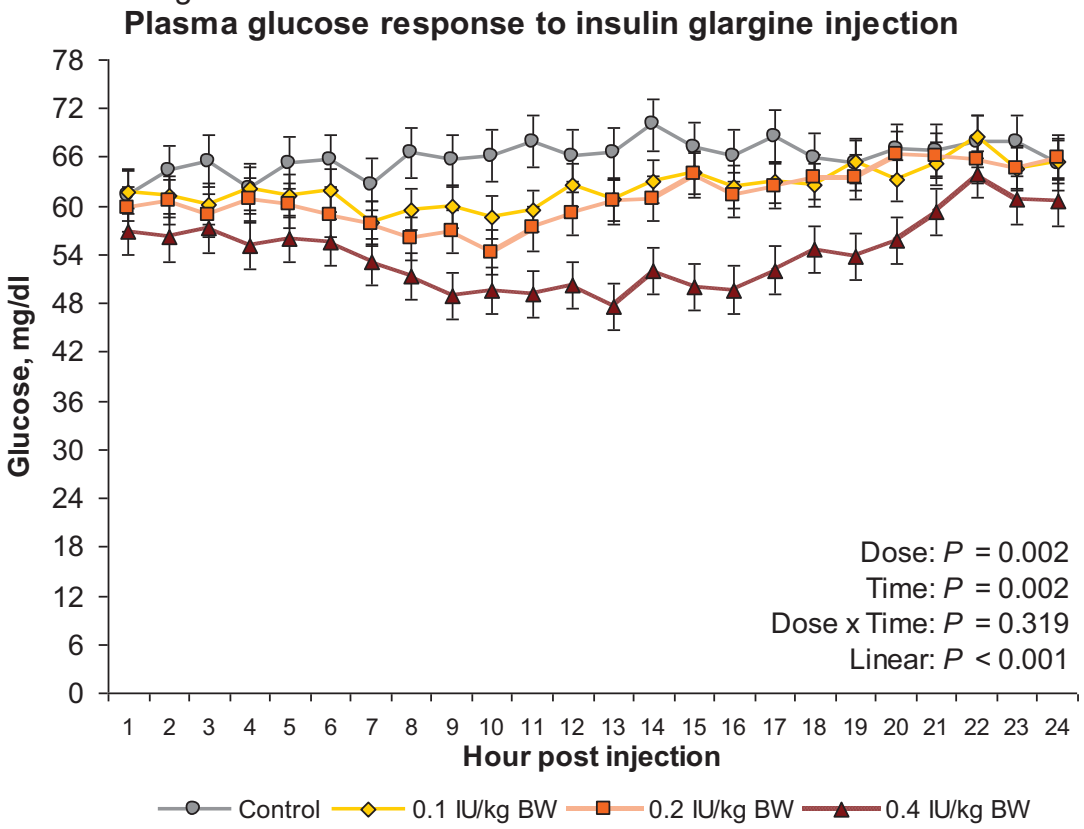


Table 1. Least square means for production variables of cows (n=3) used in a two period crossover experiment.

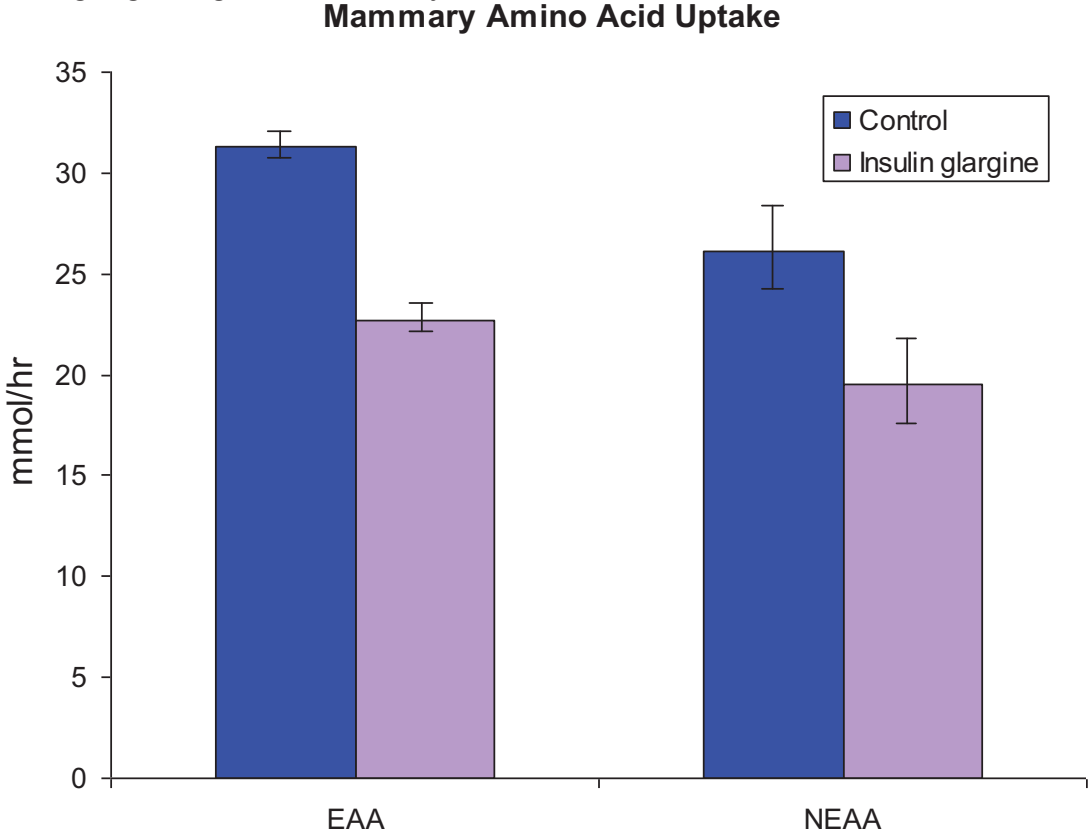
	Treatment			*P*-value
Variable	Control	Insulin glargine	SE	Trt
DMI, kg/d	25.2	26.0	2.2	0.333
Milk, kg/d	47.2	45.6	4.34	0.150
Fat, %	3.64	3.76	0.36	0.310
Fat yield, kg/d	1.73	1.73	0.33	0.879
Protein, %	2.94	3.11	0.19	0.388
Protein yield, kg/d	1.39	1.42	0.22	0.740
Lactose, %	4.94	4.83	0.03	0.094
Lactose yield, kg/d	2.33	2.21	0.21	0.091
Total solids, %	12.42	12.58	0.57	0.182
Total solids yield, kg/d	5.88	5.76	0.80	0.367
SCC, cells/ml	600	911	1160	0.672

Mammary blood flow, calculated with the Fick Principle (Cant et al., 1993), was not significantly different between control and insulin glargine treatments, though blood flow

was numerically lower for insulin glargine (725 and 627 ± 135 ml blood/ml milk for control vs. insulin glargine, respectively). Arterial and venous plasma glucose were both 10 percent lower (P<0.10) for insulin glargine (arterial: 63.1, 56.9 mg/dl for control vs. insulin glargine, respectively; venous: 45.4, and 40.7 mg/dl for control vs. insulin glargine, respectively). Glucose uptake across the mammary gland was lower for the insulin glargine treatment (P=0.023; 248 vs. 191 g/h for control and treatment, respectively). Arterial plasma urea nitrogen was not different between treatments.

Deproteinized plasma samples were also analyzed for amino acid content by HPLC. During insulin glargine treatment, mammary uptake of both essential (P=0.059) and nonessential (P=0.086) amino acid were reduced by more than 25 percent (Figure 3). Recall, however, that milk protein yield was not different between treatments (Table 1). The fact that the mammary gland had reduced amino acid uptake yet maintained similar milk protein yield suggests that the mammary gland increased the efficiency of use of amino acids during insulin glargine treatment. Bequette et al., (2001) suggested that insulin may reduce catabolism of amino acids in the mammary gland, thereby sparing them for milk protein synthesis.

Figure 3. Mammary uptake of essential (EAA) and nonessential amino acids (NEAA). During insulin glargine administration, cows were given 0.15 IU of insulin glargine/kg BW twice daily at 12-h intervals.



In a follow-up to the mammary metabolism study, 20 multiparous Holstein cows in early lactation (88 ± 25 DIM; range 52 to 130 DIM on d 1 of treatment) were used to test the effect of insulin glargine on milk protein production. Cows were balanced for DIM

and average daily milk production and randomly assigned to either control or insulin glargine treatments. A higher dose of insulin glargine was selected for this study and 0.2 IU insulin glargine/kg BW was administered twice daily at 12-h intervals for 10 d.

The goal of this study was to determine whether long-term insulin glargine administration would change milk protein production. Blood samples were taken twice daily, immediately before and 6 h after the morning insulin glargine injection, and analyzed for glucose and non-esterified fatty acids (NEFA). Cows were milked 2x/d and milk samples taken from both milkings on d 2, 4, 6, 8, and 10 were analyzed by Dairy One Cooperative Inc. (Ithaca, NY) for fat, true protein, lactose, SCC, and MUN.

Production and plasma variables from this study are reported in Table 2. As observed in the previously mentioned studies, insulin glargine administration reduced circulating plasma glucose concentrations (P=0.001). Cows on insulin glargine treatment had 16 percent lower plasma glucose. Plasma NEFA concentrations were not different between treatments.

Table 2. Production and plasma variables for control and insulin glargine treatments.

	Treatment			*P*-value		
Variable	Control	Insulin glargine	SE	Trt	Day	Trt x Day
DMI, kg/d	26.4	26.8	0.39	0.407	<0.001	0.194
Milk yield, kg/d	48.3	47.0	0.96	0.343	0.363	0.067
Fat, %	3.17	3.46	0.091	0.035	0.463	0.142
Fat yield, kg/d	1.49	1.62	0.057	0.103	0.355	0.459
Protein, %	3.05	3.33	0.047	0.002	0.008	0.186
Protein yield, kg/d	1.47	1.55	0.031	0.089	0.079	0.008
Lactose, %	4.85	4.71	0.021	<0.001	0.334	0.463
Lactose yield, kg/d	2.35	2.21	0.048	0.053	0.307	0.013
Total solids, %	11.96	12.37	0.110	0.020	0.125	0.108
Total solids yield, kg/d	5.75	5.78	0.126	0.876	0.145	0.033
SCC (x 1,000)	70	106	37.3	0.510	0.282	0.060
MUN, mg/dl	13.4	12.4	0.30	0.029	0.049	0.198
Plasma glucose, mg/dl	56.5	47.7	1.41	0.001	0.025	0.036
Plasma NEFA, µeq/L	166.1	178.9	8.28	0.281	<0.001	0.379

Insulin glargine had no effect on dry matter intake or milk yield. There was a treatment x day interaction for milk yield with control cows having higher milk yields on d 2 and 6. The difference on d 2 and 6 was roughly 3 kg of milk, but on d 10, there was no difference in milk yield. The non-significant 1 kg difference in overall milk yield is likely related to the 3 percent reduction in milk lactose content and 6 percent decrease in lactose yield for the insulin glargine treatment.

Cows given insulin glargine had 9 percent higher milk fat content and tended to have 9 percent higher milk fat yield (Table 2) than control cows. The response in milk fat was unexpected as experiments using hyperinsulinemic-euglycemic clamps have shown no change in milk fat (Griinari et al., 1997b; Mcguire et al., 1995). It is well understood that insulin inhibits lipolysis in adipose tissue, thus it would be hypothesized that provision of additional insulin activity would reduce lipolysis and thus reduce fatty acid precursors

available for milk fat synthesis. However, cows treated with insulin glargine did not have different plasma NEFA concentrations than control cows (Table 2), suggesting a change in mammary metabolism and fat synthesis within the gland.

In addition to changes in milk fat yield and content, insulin glargine-treated cows had 9 percent higher milk protein content and 5 percent higher protein yield when compared to control cows. Milk urea nitrogen was reduced by 7 percent in insulin glargine-treated cows. The increase in milk protein yield in the insulin glargine treatment for this study supports the proposed mechanism from the mammary metabolism study that insulin glargine enhances use of amino acids within the mammary gland.

Milk samples collected on d 10 were also analyzed by Kjeldahl analysis (Barbano et al., 1991; Lynch et al., 1998) to determine the nitrogen fractions of the milk. A covariate milk sample was also taken before treatments began. Results are shown in Table 3. True protein (TP), as measured by Kjeldahl, was similar to the values obtained from Dairy One (Table 2) and was 7 percent higher for TP content (P=0.005) and 10 percent higher for TP yield (P=0.018) for the insulin glargine treatment. Similarly, non-casein nitrogen content was 7 percent higher (P=0.003) and yield was 6 percent higher (P=0.059) during insulin glargine treatment, verifying the observation from our mammary metabolism study reported here. Additionally, casein content was 7 percent higher (P=0.024) and casein yield was increased by 9 percent (P=0.023) for insulin glargine-treated cows.

Table 3. Milk nitrogen fractions for samples taken on d 10.

	Treatment			*P*-value
Variable	Control	Insulin glargine	SE	Trt
CP[1] , %	3.22	3.45	0.152	0.006
CP, kg/d	1.55	1.68	0.107	0.025
True Protein[2], %	3.05	3.27	0.14	0.005
True Protein, kg/d	1.46	1.60	0.10	0.018
NCN[3], %	0.70	0.75	0.031	0.003
NCN, kg/d	0.34	0.36	0.022	0.059
NPN[3], %	0.18	0.18	0.01	0.840
NPN, kg/d	0.09	0.09	0.01	0.823
Casein[4], %	2.53	2.70	0.135	0.024
Casein, kg/d	1.21	1.32	0.088	0.023
Casein, % of True Protein	82.8	82.7	0.69	0.606

[1] Crude protein is equal to total nitrogen (TN) x 6.38.

[2] True protein (TP) calculated as (TN - NPN) x 6.38.

[3] Both NPN and NCN are multilplied by 6.38 to allow comparison with other protein fractions.

[4] Casein protein is calculated as (TN - NCN) x 6.38.

The studies described here are the first to use insulin glargine in high producing, lactating cows to elevate insulin activity in the cow without severe hypoglycemia. Though plasma glucose was significantly reduced in these experiments, which served as our proxy for insulin-like activity, the fact that overall milk yield and dry matter intake were not reduced during treatment are interesting results. The increase in milk protein

yield in the 10-d study reported here support the observations from Dale Bauman's group at Cornell (Griinari et al., 1997a; Mackle et al., 1999; Mcguire et al., 1995) that insulin does indeed influence milk protein production in lactating cows.

IMPLICATIONS AND CONCLUSIONS

Continuing to fine tune dairy cow nutrition management to improve protein efficiency within the cow has large implications for both the environment and the bottom-line of dairy farms. As we continue to improve conversion efficiencies of feed nutrients and nitrogen into milk protein, less nitrogen will be wasted and excreted into the environment. As margins have tightened on farms due to high feed prices and low milk prices, improving nitrogen efficiency is increasingly important to keep farms profitable.

Based on the research reported here, it appears that there is more efficiency to be gained in milk protein production within the cow. With no change in total milk volume, cows treated with insulin glargine had improved use of amino acids and greater milk fat and protein output than control cows. As we continue to learn more about the role of insulin in milk component synthesis, there may be opportunity to fine tune rations to alter circulating insulin concentrations to boost milk protein content without compromising total milk yield or animal health and body condition.

Further research is being carried out to determine the mechanism of insulin glargine action in the mammary gland. The effect of insulin glargine on global protein synthesis within the gland will be examined by looking at protein expression from mammary biopsies collected at the end of the 10-d study reported here. We hypothesize that insulin glargine is acting through mammalian target of rapamycin (mTOR) signaling pathways to improve milk protein synthesis (Menzies et al., 2009; Rius et al., 2010; Toerien et al., 2010).

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A BRIEF REVIEW OF CURRENT AND FUTURE APPLICATIONS OF NUTRIGENOMICS IN THE HORSE

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With the completion of the equine genome sequence state of the art genetic tools are now available for a wide variety of applications in the horse. Similar to many livestock species, maintaining healthy animals through a proper diet is a key concern for most horse owners. Some genetic traits have already been identified that can impact the nutritional management of the horse.

HYPP, or hyperkalemic periodic paralysis, is a serious condition found primarily in the American Quarter Horse and related breeds(Finno et al., 2009). Affected animals are stricken with muscle twitching, weakness and potential collapse soon after work or stress. HYPP attacks can be fatal if the paralysis extends to the respiratory system. Dietary management can reduce the frequency and severity of HYPP. The available genetic test for HYPP conclusively identifies affected individuals the disease and indicates if they are homozygous (and therefore likely to be more severely affected.) This information aids horse owners in making appropriate feeding and management decisions. Dietary prevention includes avoiding high potassium feeds like alfalfa and molasses, feeding several small meals throughout the day, and preventing dehydration. Approximately 56% of halter-bred Quarter horses are affected by HYPP(Tryon et al., 2009). More information, as well as the genetic test, is available from the VGL at UC Davis: http://www.vgl.ucdavis.edu/services/hypp.php.

Polysaccaride storage myopathy (PSSM), aka tying-up or Monday morning disease, is a defect in the ability to store glucose from the diet as muscle glycogen. Signs include limb stiffness and awkward gait which can progress to sweating, reluctance to move and brown-colored urine (Finno et al., 2009). Episodes occur most frequently in horse receiving a high grain diet and are brought on by exercise. Exercise intolerance and a refusal to work can also occur. Episodes may be prevented by a low carbohydrate, high fat diet. Several commercial formulations are already available (Re-Leve by Halloway Feeds, Ultium by Purina, Equi-Jewel from Kentucky Performance Products). Alterations to the training schedule may also be beneficial. PSSM horses should be exercised regularly with only gradual increases in intensity. The genetic cause for the most common type of PSSM, type 1, is known, and a test is available from the Neuromuscular Diagnostic Laboratory at the University of Minnesota: http://www.cvm.umn.edu/umec/lab/home.html (McCue et al., 2008). Type 2 PSSM, which is caused by a yet unknown genetic mutation, can be diagnosed by a muscle biopsy. PSSM1 is most often found in the American Quarter Horse and related breeds as well as draft and warmblood breeds.

New tools created from the equine genome will allow us to attack some of the more complex issues in nutrigenomics. For example, we are currently using the Equine SNP chip, an assay that allows us to examine 50,000 genetic markers at once, to map the genes that contribute to Equine Metabolic syndrome and Cushings Disease. In the future these loci may be used to identify horses at risk for developing metabolic syndrome under normal management conditions and allow for intervention through a preventative diet before they develop EMS and secondary conditions like laminitis. Other research groups throughout the world are examining many diverse nutrition-related genetic conditions in the horse. Developmental Orthopedic Disease (DOD) has been linked to several locations in the genome. Previous work has shown that rapid growth and excessive feeding of weanlings and yearlings contributes to this condition, and that certain breeds are more susceptible. Additional research is needed before an individual's genomic information could be used to tailor a diet to just meet the conditions for optimal growth without developing DOD. Finally, these new genomic tools can be use to understand nutritional processes in the healthy individual, and not just those with disease. How an individual utilizes their diet is heavily influenced by their external environment, as well as internal conditions like the population of gut microflora. The interactions of these environments are extremely complex and difficult to study. By teasing out the genetic component to nutrient utilization the "equitation" for a healthy horse can be simplified, allowing for precise study of these other factors.

While we haven't fully capitalized on the information now available to us from the equine genome the door is open for the pursuit of applications in a variety of venues. Key to our success is the partnership between nutritionists and veterinarians with the experience in the field and geneticists with the knowledge of these novel tools.

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FILLING FEED HOLES: ADVANCES AND CURRENT ISSUES IN FORAGES AND GRAZING MANAGEMENT

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The past few years have seen wild swings in the costs of grain, fuel, and fertilizer, and also market prices for livestock products. These fluctuations have created significant financial challenges for livestock operations, especially those that depend on grain or grain byproducts as their primary source of nutrients. Confinement dairies, feedlots for beef cattle and sheep, and monogastric production systems are particularly vulnerable to these fluctuations (Clark et al, 2010), and the recent series of dairy buyouts is sad evidence of this stress.

Partially in reaction to these risks, many livestock producers around the country have shown a growing interest in altering their operations to incorporate improved forages and intensive grazing as the primary feed source (Kriegl and McNair, 2005). These operations cover a wide array of management systems: they may rely on forages for all or part of their nutrients; they may sell their products through traditional commercial markets or through alternative venues; they may label their products as “grassfed” or “organic”. These operations are not necessarily small or marginal. In response to this growing interest, the USDA recently established market standards for “grassfed” ruminants (Federal Register, 2007) and revised its pasture access rules for organic operations (Federal Register, 2010).

One of the main challenges facing grass-based operations is to overcome low periods of forage growth C to find ways of raising or maintaining forages during the slow months of the year. Winter growth is usually not possible across much of the country, and during the summer, many cool-season grasses experience a Asummer slump@ that greatly reduces yield (Barnes, 1999). Because mechanically-harvested forages are considerably more expensive than grazed forages (Gerrish, 1999), progressive grass-based producers strive to maximize the use of grazing. They utilize a variety of improved forages and grazing techniques, many of which have been developed in the last fifteen years. In this paper, I will describe some of the new forages and grazing techniques currently used by intensive management systems to reduce the monthly variation of forage growth. I will focus on improved pastures, primarily in the northern regions of the country and in the maritime areas of the Pacific Northwest.

NEW FORAGE SPECIES & VARIETIES

Italian Ryegrass

Annual ryegrass (ARG, *Lolium multiflorum*) is a common grass grown throughout the Southeast and is becoming popular in other places of the country due to its rapid

establishment, excellent response to fertility, high yield, relatively inexpensive seed, and high nutritional value (Nelson et al., 1997). Because it is an annual species, its establishment costs can be high relative to its yield. There are, however, at least two main genetic lines of ARG: the *Westerwolds* and the *Italians*. the *Westerwold* cultivars are true annuals. They were developed in the Westerwolde region of the Netherlands (Nelson et al., 1997). Planted in fall or spring, *Westerwold* varieties will set seed in the early summer, and thus provide only a few months of forage. In contrast, the varieties of *Italian Ryegrass* generally require vernalization to stimulate seed development. If a cultivar of Italian Ryegrass is planted in the spring after frosts, it will not set seed during its first year but instead will provide vegetative growth during that first summer and autumn. It will then overwinter, grow vegetatively during the second spring and set seed in its second summer. Italian ryegrass can effectively provide two seasons of vegetative growth for the cost of one planting, including months of high quality forage during the hot summer. A critical management issue with the Italian Ryegrasses is to plant them late enough to avoid vernalization, otherwise they will go to seed during that first year.

Progressive grazers in the Pacific Northwest are using Italian Ryegrass varieties to reduce tillage costs and provide high-quality feed in the summer. These varieties may show promise for the hot summers of the Southeast.

Hybrid Forage Brassicas

The Mustard family (*Brassicaceae*) is a large category of broad-leaved plants that includes turnip (*Brassica rapa*), rape (*B. napus*), the mustards (many genera), cabbage (*B. oleracea*), radish (*Raphanus sativus*), and many common vegetables. Many annuals of the *Brassica* genus are well-known forages, such as bulb turnip, leafy rape, kale (*B. oleracea*), and swedes (rutabaga, *B. napus*). Turnips have traditionally been used for a one-time mob grazing, especially with breeding stock, as these plants can often be grazed in the winter. Turnips can produce high yields of leaf and bulbs, although these are actually two nutritionally-different feeds. There has been a growing interest in new varieties of brassicas that can support multiple grazings and also remain green after killing frosts One of the earliest leafy cultivars was ATyfon,@ a cold-tolerant hybrid of stubble turnip and chinese cabbage which could provide 2-3 grazings in a season. But this cultivar has been supplanted by varieties far more exciting to graziers: the new Hybrid Forage Brassicas, which are much improved hybrids bred for improved leaf yields, quick establishment, multiple harvests, high nutritional quality, and good heat and drought tolerance. These include varieties such as AHunter,@ a cross between turnip and rape, and AWinfred@, a cross between turnip and kale, and others. In addition, many graziers are planting a new hybrid forage radish AGraza@ which is a cross between garden radish and perennial seaside radish 7. *maritimus*) and cabbage. With good soil fertility and sufficient water, these hybrid forage brassicas can be grazed only 50 days after planting and then every 30-35 days thereafter.

Graziers utilize the new forage brassicas in many ways in addition to providing high-quality forage at different times throughout the year. Since these plants can remain productive for a year, producers can plant them in fields with the intention of ultimately

renovating these fields into perennial grass-legume pastures. These brassicas are sometimes planted with sorghum-sudangrass to provide continuous feed through the summer and fall and early winter. Graziers can also use these brassicas to eliminate grass weeds from a field. A field can be sprayed, planted in forage brassicas, and then sprayed multiple times for annual and perennial grasses. The brassicas continue to produce forage, shade out most competition, and thus allow the field to remain in production for that growing season. Brassicas can also help control nematode gastrointestinal parasites. Since brassica fields must be cultivated prior to planting, the annual brassica plants grow relatively free of these nematode larvae. Using brassicas and other annual forages in a parasite control program is a technique that promises to gain importance over time, as there is a growing problem of gastrointestinal parasite resistance to anthelmintics, particularly in small ruminants (Min and Hart, 2003).

Brassicas, however, do have some potential caveats of which producers should be aware. Brassicas are associated with a number of nutritional disorders. Brassicas may contain high levels of glucosinolates that inhibit the uptake of iodine by the thyroid gland and thus cause an iodine deficiency (Cheeke, 1998). Brassicas also contain relatively high levels of sulfur, often greater than 0.4% DM. The combination of high sulfur levels and low fiber levels is associated with the occurrence of polioencephalomalacia (Gould, 1998). Brassicas have also been linked with Acute Bovine Pulmonary Emphysema (Fog Fever, ABPE) due to rumen production of 3-methyl-indole from tryptophan when cattle are moved from dry feed to succulent forage like brassicas. Less commonly, ruminants can also suffer from Brassica Anemia due to the unusual amino acid S-methylcysteine sulfoxide that occurs in this forage (Cheeke, 1998). However, all these problems can usually be managed with proper management and are not compelling reasons for avoiding this forage.

Herbs

Graziers are beginning to use two unusual herb-like plants as mainstream forages: chicory and plantain. Both are found wild in the United States, typically as weeds, but New Zealand geneticists bred improved leafy cultivars of them (Labreveux et al., 2004). These species are broad-leaved perennials with deep taproots and soft, low-fiber, highly palatable leaves. They thrive over a wide range of soil pH, are high-yielding and very responsive to improved soil fertility. Both species are included in pasture mixtures to improve forage variety, ground cover, and seasonal yield.

Of these two species, Chicory (*Cichorium intybus*) is more popular around the U.S, primarily because of its winter-hardiness and good summer growth. The improved commercial variety APuna@ has been available since 1985 (Labreveux et al., 2004), and other varieties has since been released commercially. Producers must manage chicory carefully to reduce its tendency to bolt in the hot summer, although the newer varieties have suppressed this characteristic. Chicory may also have some value in controlling gastrointestinal parasites (see below).

The second herb species, plantain, is not the common plantain found in many gardens.

The plantain genus is quite large. Improved leafy plantain cultivars were bred in the 1990s from Narrowleaf Plantain (Ribgrass, *Plantago lanceolata*) and are marketed commercially under the namesATonic@ and ALancelot@ (Rumball et al., 1997). Plantain offers graziers a different array of traits from other forages. Plantain is winter-active and does not bolt during the summer, which gives it a particularly attractive growth pattern for mild climates such as the Pacific Northwest or areas of the Southeast. Plantain can provide feed during the slow periods of forage growth. It establishes rapidly and is tolerant of a wide range of soil drainage conditions. Plantain is becoming a standard forage in pasture mixtures in the mild areas of the Pacific Northwest.

Although both species are perennials, long-term persistence can be a problem. Their exceptional palatability makes it challenging to maintain them in a sward, particularly under continuous grazing systems (set-stocking) because livestock will preferentially graze them out. Graziers who intensively manage their pastures are usually more successful in maintaining these plants over time.

Novel Endophytes

Tall fescue (TF, *Schedonorus phoenix*, formerly *Festuca arundinacea*) is one of the most widely-grown perennial grasses in the U.S. However, in many areas, particularly in the South and Southeast, the most common TF varieties are heavily infested with a fungal endophyte (*Neotyphodium coenophialum*). This endophyte secretes the ergot alkaloid ergovaline that causes the well-known syndromes of fescue toxicosis. These include fescue foot, summer slump, bovine fat necrosis, reduced gain and milk production, poor reproduction, agalactia in mares, and others (Bouton et al, 2002). Endophyte has a symbiotic relationship with the host grass that gives the infected plant a selection advantage under practical field conditions. Endophyte imparts to the host plant improved drought tolerance and increased resistance to damage by various insects. In fact, turf varieties of TF are sold as Aendophyte enhanced@ because of the agronomic benefits provided by endophyte. But in spite of its endophyte, TF is a primary forage species in many regions particularly in the Southeast, as it is a persistent perennial responsive to good soil fertility, it can provide good yields during spring and summer, and it is also the preferred species for fall stockpiling (Barnes, 1999).

For years, producers have struggled to manage fields of endophyte-infected TF by utilizing a number of traditional management and grazing techniques (Hancock and Andrae, 2009). But recently an exciting new strategy has become available for TF: novel endophytes (sometimes known as animal-friendly endophytes). These are strains of endophyte that do not produce ergovaline but do produce alkaloids that impart drought tolerance and insect resistance to the plant (Hancock and Andrae, 2009). In New Zealand, a novel endophyte strategy has been used for many years to successfully address the problem of ryegrass staggers caused by the perennial ryegrass endophyte *Neotyphodium lolii* (Cheeke, 1999). It wasn=t until the late 1990s, however, that a novel endophyte was successfully introduced into TF (Bouton et al., 2002), which was subsequently released commercially as Max-Q (Hancock and Andrae, 2009). Livestock grazing novel endophyte TF show similar performance as animals grazing endophyte-free TF, but the TF plants

containing novel endophyte show the same persistence and vigor as traditional endophyte-infected TF (Gunter and Beck, 2004). Replanting TF fields with novel endophyte-infected TF cultivars is becoming an attractive option for graziers where TF is a main grass species and hot summers favor it. Renovating old TF fields is not easy because of the large amount of residual seed, but there are a number of strategies that can be followed for a successful renovation (Hancock and Andrae, 2009). As of this writing, alternative novel TF endophytes are beginning to reach the market.

High-Sugar Perennial Ryegrass

Perennial Ryegrass (PRG, *Lolium perenne*) has long been a staple component of productive pastures, particularly in temperate regions with mild winters. PRG consistently provides high-quality forage and responds quite well to high rates of fertilization, although it is generally not as cold-tolerant as other cool-season grasses. A recent development with PRG is attracting the attention of intensive graziers. The Wales Institute of Grassland & Environmental Research (IGER) released a set of PRG varieties that contain elevated levels of water soluble carbohydrates (Downing et al., 2008). These are collectively known as the High-Sugar Rygrasses, and they were originally marketed under the variety names of AberDart, AberAvon, AberEcho, and others. Currently one American company is marketing them under the name of SucraSeed.

The high levels of water soluble carbohydrates may provide some practical advantages. These ryegrasses show improved fermentation characteristics for making silage (Downing et al., 2008) and also a slight increase in vitro digestibility (Lee et al., 2002). They may be more palatable than standard PRG varieties (Jones and Roberts, 1991). Better palatability, however, may be of minimal value in intensive grazing systems where livestock are not given a choice of forages and are removed from a paddock when the target residual pasture mass has been reached. A more important practical aspect of high sugar ryegrasses is that they may improve rumen fermentation under the conditions of good pasture management. PRG is typically planted in fields with good soil fertility. Forages that receive high applications of nitrogen fertilizer often contain crude protein levels greater than 20%, with perhaps one third of this nitrogen as soluble non-protein nitrogen (Van Soest, 1994). The high sugar ryegrasses have been shown to reduce rumen ammonia, probably due to the increase of readily-available energy for the rumen microbes to support microbial protein synthesis (Lee et al., 2002).

Some progressive grazers are already incorporating the high-sugar ryegrasses into pastures, particularly on dairies and in finishing pastures for beef cattle and sheep. More research is needed to identify the appropriate and most efficient use of these varieties.

Forages Containing Condensed Tannins

One of the primary drawbacks of grazing systems is the problem of gastrointestinal parasites, particularly nematodes. For more than forty years, producers have relied on commercial anthelmintics to reduce parasite loads. Unfortunately, these drugs are becoming less efficacious as the nematodes are becoming increasingly resistant to them,

particularly with small ruminants. An encouraging recent development has been the observation that condensed tannins in some forages seem to have a suppressing effect on nematode gastrointestinal parasites, although the precise mechanism of this mitigation is not well-understood (Min and Hart, 2003).

Condensed tannins are found in a number of forage species, including birdsfoot trefoil (*Lotus corniculatus*), big trefoil (*L. pedunculatus*), chicory (*Cichorium intybus*), sulla (*Hedysarum coronarium*), sainfoin (*Onobrychis viciifolia*), and sericea lespedeza (*Lespedeza cuneata*). Chicory also contains sesquiterpene lactones which may have similar anti-parasite effects (Foster et al, 2009). Much current parasite research in the U.S., however, has focused on sericea lespedeza, which has consistently shown positive results in reducing nematode parasite load in sheep and goats, either as grazed forage or when fed as hay (Moore et al, 2008). Sericea lespedeza is a drought-tolerant, warm season perennial legume that has been widely planted on reclaimed eroded land or fields of low fertility. It grows well on acidic pH soils and does not cause bloat, but is slow to establish and has a high percentage of hard seed (Barnes, 1999). It also has a reputation for low forage quality, which may be in part due to its high level of condensed tannins which discourage livestock from grazing it until it becomes more mature.

The practical management of tannin-rich plants in a grazing system may be quite complex. From a nutritional perspective, condensed tannins have variable effects on animal performance. Tannins are astringent, and high tannin levels can depress intake, but low to moderate levels can improve protein nutrition by increasing the proportion of bypass protein (Cheeke, 1999). Additionally, condensed tannins are secondary plant compounds, and their levels in forage plants can vary greatly with season and genetics (Gebrehiwot et al, 2002). Although tannin-containing forages have long been used in mixed pastures, the use of these plants to control parasites is still a new concept. More research is needed to identify effective forage species and develop practical and reliable forage-management systems that maximize the effects of these forages on parasites.

NEW TECHNIQUES

Management-Intensive Grazing

Rotational grazing has been a recommended practice for decades, but one of the most exciting and practical grazing strategies developed in the past twenty years has been Management-intensive Grazing (MiG, Gerrish, 1999). This is not simply a system of rotating animals through small paddocks. Rather, MiG is a grazing strategy that utilizes animals as harvesting units, maintains the forage in a high-quality vegetative state, and moves animals according to the needs of the different paddocks. Forage is allocated to livestock by fencing an appropriate area to provide feed for a limited number of days, usually a period short enough to minimize the opportunity for grazing regrowth. MiG balances forage quality with efficient yield, supports persistence of perennial forages, and reduces weed infestation. MiG is a system of intensive management, not intensive grazing, since management decisions must be made intelligently in response to changing conditions of forage growth, weather, and economics, and animals nearly always graze

intensively.

Grazers who practice MiG are acutely aware of pasture mass and rates of forage growth. They monitor their paddocks quite regularly, sometimes with weekly measurements of pasture mass using visual estimations, rising plate meters, or equivalent equipment. They generally rely extensively on electric fencing for dividing paddocks. They adjust animal movements and grazing cell size in response to forage growth in each paddock, and these adjustments may change radically over the course of the growing season, depending on the type of forage in each paddock, soil fertility and other soil characteristics, costs, and marketing opportunities. Each year presents a different set of circumstances, and the specifics of animal movement will change in response to these circumstances.

MiG requires significant management input. The traditional concepts of rotational grazing have serious flaws in relation to forage growth, but MiG is designed to be responsive to the vagaries of weather and variability of forage growth. If followed properly, MiG can improve pasture health and soil fertility, support persistence of high-yielding perennial forages, and provide a consistent diet of high-quality feed to the livestock (Martz et al, 1999).

K-Line Irrigation

A recent change in irrigation technology has been the development of the K-Line Irrigation System® by Rx Plastics Ltd. in New Zealand (Rx Plastics, 2010). This system, which is designed expressly for pastures, consists of plastic pods containing sprinkler nozzles positioned every 40-50 feet on an above-ground, flexible, low-density plastic hose. The hose is attached to a primary water source and is moved every 12 or 24 hours by pulling it with an all-terrain vehicle (ATV) in a crisscross pattern across the field. Unlike most other irrigation systems, the sprinkler line can be moved without shutting off the water. Moving a line takes only a few minutes. The K-line system is proprietary, but alternative pod-irrigation systems are becoming available commercially.

Many grazers on the West Coast are installing K-Line systems in pastures, either by retrofitting it onto an existing irrigation system or by designing a new system for each paddock. Some of the advantages of the K-Line system are that it is labor efficient, it supplies a steady gentle stream of water that is less affected by wind shear, it operates at relatively low water pressures, and it can be customized for pastures of all sizes including irregularly-shaped fields. It can be used for any type of improved pasture and also for fields of annuals such as corn, sorghum-sudangrass, and brassicas when the plants are short. On a practical level, the labor-saving feature is critical because it is becoming more difficult to hire temporary workers to attend to irrigation chores. But attracting workers to use an ATV to move K-Line systems is far easier than hiring people to work with traditional irrigation systems.

Irrigation of pastures and hay fields is a common strategy in the western U.S. but is relatively rare in the Midwest or East. The K-Line irrigation system, however, is a radical change from traditional systems, and the economics of using it for supplemental irrigation

in non-traditional settings should be analyzed carefully. Two recurrent problems that face Midwestern and Eastern grazers are the predictable summer slump of forage growth and the unpredictability of rain. The development of the K-Line irrigation system may alter the economics of supplemental irrigation and change the structure of risk management and options for growing summer forages.

FINAL NOTES

These are only a few of the many forages and techniques that progressive grazers are currently utilizing to reduce feed costs and provide a more consistent supply of high-quality forage throughout the year. Others not discussed here include forage species such as gala grazing brome, Persian clover, and the improved varieties of crabgrass, and techniques such as the grazers' wedge and the use of stocking density instead of stocking rate. Although many of the forages and techniques discussed in this paper are relatively new, they are quickly gaining wide acceptance in the field. Additional research is necessary to characterize more aspects of them and identify ways in which they can be used more efficiently and profitably.

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Updates to the Cornell Net Carbohydrate and Protein System v6.1 and implications for ration formulation

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INTRODUCTION

The Cornell Net Carbohydrate and Protein System (CNCPS) has been in development for nearly 30 years, and various versions of the CNCPS or implementations of the program (CPM Dairy, AMTS.Cattle, NDS, DinaMilk) have been used in the dairy industry to evaluate and formulate rations for more than 10 years. The long-term objective of the CNCPS modeling effort has been to provide a field usable model that accounts for a large proportion of the variation in ration formulation and animal performance and is based on a functional mathematical description of the biology of both growing and lactating cattle and their diet and management. Models such as the CNCPS are evolutionary in that as research progresses, model improvements and enhancements occur, provided adequate resources are available for programming and evaluation. This process is similar to the process that occurs when a new Nutrient Requirements of Dairy Cattle publication is produced. Unlike the NRC publications, historically published every 10 years, the CNCPS has been updated on a somewhat continuous basis. Each update has allowed us to predict performance with increased accuracy. However, these updates have at times, caused confusion in the field. This confusion is a combination of changing guidelines and a lack of awareness as to what the changes were and why/how they impact predictions. The objective of this paper is to describe recent updates and explain what impact they have on predictions.

The changes that resulted in the development of CNCPSv6 were described by Tylutki et al. (2008). This paper will focus primarily on the changes that have occurred since the publication by Tylutki et al. (2008) with references to v6.1 but more substantive changes will be highlighted here. The changes will be listed in order of calculation such as maintenance, then growth or lactation or submodel impacting such calculations.

MAINTENANCE

The first change primarily affects growing cattle and the update was to remove the link between the current body condition score (BCS) and maintenance energy requirements. Data from France that was used in the INRA system for lactating beef cattle on pasture made an association between previous level of nutrient intake and BCS and maintenance requirements. As cattle achieved greater BCS theoretically they consumed more energy and thus had larger organ mass and with larger organ mass,

more energy was partitioned to maintenance and away from growth. Thus, prior to v6.1, as the BCS input was increased in growing cattle, the greater the maintenance requirement and the less energy available for growth. The outcome was a difference of almost 0.4 kg/d in ME allowable growth as the score ranged from 1 to 5. This resulted in the potential to overfeed energy to heifers since the model would predict less ME allowable gain than was truly available at an average BCS. This is also true for CPM Dairy.

Another update that impacts the maintenance requirement of all cattle is the calculation of surface area. The equation to calculate surface area used in the CNCPS up to v6.0 was from Mitchell (1928) and that equation ($0.09 \times W^{0.67}$) was derived from sheep weighing from 14, 24 to 38 kg (Berman, 1998). Another equation by Brody (1945) was developed on 50 Holstein cattle from 41 to 617 kg ($0.14 \times W^{0.57}$) and this equation was validated using body measurement data of Holsteins from Heinrichs et al., 1992 by Berman (1998) and adopted for use in the model. The Mitchell equation will under-estimate surface area by 7-10% at 30 to 50 kg (65-110 lb) BW and thus heat loss, and will over-estimate surface area by 23% at 650 kg (1,450 lb) BW at maturity, thus decreasing the effect of evaporative heat loss due to smaller surface area (Berman, 1998)

FEED FRACTIONS AND POOL ASSIGNMENTS, PASSAGE RATES AND RATES OF DIGESTION

Multiple changes were made to correct errors and prepare the model for future development, especially consideration for a VFA submodel. The first step was to expand the CHO pools to four A fractions (VFAs, Lactic, other organic acids, e.g. malate, sugar) as well adjusted CHO kd values downward based on gas production data from Dr. Pell's group. Previous versions utilized a 200-300% per hour kd for sugar. A 300% per hour kd implies rumen retention time of 0.2 hours (12 minutes); a value greater than the mean growth rate of rumen bacteria. The original value for sugar rates came from *in vitro* fermentation studies from Jim Russell's lab using pure cultures of *s. bovis* grown on glucose. To update this, Dr. Pell's graduate students measured mixed sugar fermentation by mixed rumen bacteria using the gas production technique to vary between 40 and 60% per hour (rumen retention time of 100 to 150 min) (Molina, 2002). Updates to the changes in degradation rates of the various fractions are found in Table 1.

Based on the changes in rates of degradation and passage there is a significant impact on soluble pool movement out of the rumen. As an example, the data in Table 2 demonstrates a 16% reduction in sugar (CHO A4) degradability. If a lactating dairy diet fed at 24 kg contains 5% sugar, this results in 192 g less sugar degraded. The 192 grams would equate to approximately 15 g lower MP flow, or approximately 1 liter lower MP allowable milk.

Further, it was assumed PRO A utilization was instantaneous with a kd of 10,000%/hr implying a rumen retention time of 0.6 min. This would imply that any addition of urea would be dissolved and captured by rumen bacteria in 36 seconds, an unrealistic expectation. This value was generated to represent the rate of solubilization and not necessarily microbial uptake. With these changes rates for pools like PRO A kd were reduced to 200%/hr. There were many other updates to the version including: new

Table 1. Feed degradation rates (%/hr) used for CHO and PRO pools in CNCPSv6 and prior to version 6.1

Component	Prior to v6	V6.1
CHO A1 (VFA)	Not modeled	0%
CHO A2 (lactic acid)	Not modeled	7%
CHO A3 (other organic acids)	Not modeled	5%
CHO A4 (sugar)	300-500%	40-60%
CHO B1 (starch)	20-40%	20-40%
CHO B2 (soluble fiber)	20-40%	20-40%
CHO B3 (available NDF)	4-9%	4-9%
CHO C (unavailable NDF)	0%	0%
Pro A (NPN)	10,000%	200%
Pro B1 (soluble true protein)	130-300%	10-40%
Pro B2 (moderately degraded protein)	3-20%	3-20%
Pro B3 (slowly degraded protein, bound in NDF)	0.05-2.0%	For forages, same as the CHO B3
Pro C (unavailable protein)	0%	0%

Table 2. Calculated rumen degradability of several pools using previous and current kd and kp phases.

	Prior to v6			V6.1		
Pool	Kd, %/hr	Kp, %/hr	% degraded	Kd, %/hr	Kp, %/hr	% degraded
CHO A4	500	4	99	60	12	83
CHO B1	20	4	83	20	6	77
Pro A	10,000	4	100	200	12	94

passage rate equations, maintenance requirements for heifers were updated, and error corrections to more appropriately account for microbial ash accumulation, rumen ammonia flow, and updating DMI equations. These changes reduced predicted microbial protein flow approximately 5-7% compared with previous versions.
Historically, NFC was calculated as:

$$NFC = 100 - (CP + Fat + Ash + (NDF - NDIP))$$

This assumed that the protein within NDF remained during the NDF extraction. While true when the NDF assay does not include sodium sulfite, Mertens (2002) AOAC approved NDF assay includes this reagent and we support the use of it as we move

forward. Given that the majority of commercial laboratories routinely use sodium sulfite and amylase to improve filteration, we adopted the AOAC NDF method for use within CNCPS. Thus, NFC is now calculated as:

$$NFC = 100 - (CP + Fat + Ash + NDF)$$

The non-fiber-carbohydrate (NFC) concentration has been decreased (e.g. from 40 to 38.4% DM). This represents another change within the calculations.

The AOAC NDF assay also suggests that NDF should be reported on an organic matter basis (vs. DM basis). This is being further investigated but is expected to be implemented in the near future. There will be an exception list of feeds that will be analyzed either without sodium sulfite (commercial soybean products and animal protein products are the primary feeds affected by this) or we will use another measure to generate an NDF value and associated protein. Since animal proteins do not contain fiber, the use of NDF is not appropriate to begin with and this is under consideration as we move forward. Overall, the net result of these changes are dietary NFC values will be reduced 2-4 units if this change is implemented.

In CNCPS v6.1 the soluble pools, carbohydrate (CHO A) and protein (A and B1), have been re-assigned to the liquid passage rate equation to more appropriately reflect the biology of the cow. Both the solid and liquid passage rate equations were updated and account for a greater amount of variation in liquid turnover than the equation found in v5.0 (Seo et al. 2006). Prior to v6.1 the soluble pools were predicted to flow out of the rumen with the solids passage rate, thus with the high digestion rates and the slow passage rates, all of the soluble fractions were degraded in the rumen. This change in passage rate assignment increases the predicted outflow of soluble components, thus reducing microbial yield and estimated ammonia production and rumen N balance. These changes improve the sensitivity of the model to changes in feeds high in soluble carbohydrates and protein and reduce, but don't eliminate, the under-prediction bias observed in a previous evaluation of the model (Tylutki et al. 2008).

METABOLIZABLE ENERGY

Overall, the model predicts ME allowable milk with reasonable accuracy. An evaluation by Huhtanen using a research dataset indicated an $R^2 = 0.99$ for predicted vs observed ME allowable when evaluated with diets ranging from 12 to 18% CP and milk yields from 15 to 40 kg/cow/d. Our own internal data sets provided similar predicted versus observed relationships when evaluated on a per cow basis among data sets (Tylutki et al., 2008). However, an update that can have a significant change in ME available for milk and tissue is the implementation of the digestibility of fatty acids on an individual fatty acid basis. Previously, the CNCPS used a global intestinal fat digestion coefficient, 95%, for all ether extract appearing at the small intestine. With the work that has been conducted to better estimate fatty acid digestibility, along with the development of the fatty acid submodel in CPM Dairy, we determined the model was more accurate in predicting ME allowable milk if the digestibility of individual fatty acids

were used in place of the global coefficient. The digestibility values used are found in Table 3 and are based on data and reviews from Lock et al. (2006) and Moate et al. (2004).

Table 3. Post-ruminal fatty acid digestibility used in the CNCPS v6.1.

Fatty acid	Post-ruminal digestibility, %
C12	95.4
C14	75.1
C16:0	75.0
C16:1	64.0
C18:0	72.0
C18:1	90.0
C18:2	78.0
C18:3	77.0
Other	58.7

PROTEIN FRACTIONS AND METABOLIZABLE PROTEIN

The first step in this process is to ensure that the model is capable of predicting the MP allowable and the most limiting nutrient MP or ME allowable milk with good accuracy and precision. The current CNCPS/CPM Dairy balances for amino acids using a factorial approach based on the amino acid content of the predicted metabolizable protein (MP) supply and the amino acid profile of the tissue and milk. The approach is identical to that described by O'Connor et al. (1993) with many upgrades and modifications to the prediction of MP supply (Fox et al., 2004; Seo et al., 2006; Lanzas et al., 2007a,b; Tylutki et al., 2008). In order to have confidence in the ability of the model to predict AA accurately, the model needs to be able to account for the MP allowable milk with reasonable accuracy and precision. During the development of CNCPS v6.1 (Tylutki et al., 2008; Van Amburgh et al. 2007), we have refined the model to be more sensitive to MP supply and thus more robust in evaluating the most limiting nutrient under field conditions. This has allowed current users to balance diets at crude protein levels below 16% and maintain milk yield to increase overall efficiency of use and in many cases enhance milk protein output.

Proteins, peptides and free amino acids in the soluble pool can be rapidly degraded, but because they are in the soluble pool, they move with the liquid phase from the rumen to the small intestine and supply the cow with AA. There are now several data sets that demonstrate that the soluble pool of feeds contributes between 5 and 15% of the total amino acid flow to the duodenum of the cow (Hristov et al. 2001; Volden et al., 2002; Choi et al. 2002a,b; Reynal et al. 2007). The pool sizes of the NPN and soluble

true protein have been updated to reflect the presence of small peptides in what was previously considered the NPN fraction (Table 4) (Ross and Van Amburgh, unpublished).

As the data illustrates, regardless of protein precipitating agent, as filter paper pore size is decreased, the amount of true protein recovered increases. Thus, what historically has been defined as PRO A was severely over-estimating true NPN supply.

Additionally, peptide length does not vary based upon pore size. Based upon these findings, NPN as a percent of soluble protein for forages has been adjusted and this will most likely occur for all of the remaining feeds in the feed library. In earlier versions of the model, the library described the soluble CP fraction of fermented forages as 95% NPN for feeds such as alfalfa silage, 45% has been implemented in the current version. This does not mean that all alfalfa silages fall into this range, but without a functional field applicable assay and given the values we derived, it was a reasonable compromise for this release. Feeds such as soybean meal have been reduced from 25 to 5% NPN % soluble protein. This greatly impacts protein A and B1 pool sizes (Table 5). These shifts in pool sizes, coupled with reduced microbial yield predictions, results in excessive peptide supply for the rumen. Therefore, reductions in dietary RDP requirements (and crude protein) are achievable.

Table 4. Precipitable true protein of trypticase with varying protein precipitating agents and filter paper pore size. The 20 µm pore size represents Whatman 54 filter paper.

PPT Agent	Filter pore, µm	True protein	Filtrate peptide chain length	True Protein, % of largest pore
Tungstic acid	1	34.4	3	1,911%
	6	23.1	4.3	1,283%
	20	1.8	4.2	
Stabilized TA	1	31	3.3	705%
	6	28.5	3.4	648%
	20	4.4	3.6	
TCA	1	2.57	3.4	612%
	6	0.78	4.3	186%
	20	0.42	5	

Table 5. Calculated Protein A and B1 pool sizes using original and updated NPN % soluble protein values using an alfalfa silage as an example.

Component	prior to v6	v6.1
CP % DM	20%	20%
SP % CP	55%	55%
NPN % SP	95%	45%
PRO A + B1 (% DM)	11.00%	11.00%
PRO A (% DM)	10.45%	4.95%
PRO B1 (% DM)	0.55%	6.05%

The soluble proteins and peptides move with the liquid phase from the rumen to the small intestine and supply the cow with AA (Choi et al. 2002; Volden et al., 2002; Hedvquist and Uden, 2006; Reynal et al. 2007), thus, to account for the AA profile of these peptides, we need to provide an AA profile for the soluble pool and as the model moves forward we will be adopting whole feed amino acid values, not the insoluble residue (Sniffen et al. 1992). Thus, the CNCPS was adjusted so that CHO A1-A4 and PRO A-B1 flow with the liquid phase and CHO B1 (starch) always flows with the concentrate solid phase. Table 5 provides an example of integrating the pool phase flow and kd changes. This is currently being done by mathematical manipulation of the pools and rates but a more robust approach is needed to account for more variation in the predicted AA flow.

Further, relative to ruminal N requirements, the previously described peptide requirement was developed from in vitro data from Chen et. al. (1987) and related papers. Data from Broderick and Wallace (1988) reported that peptide uptake by the microbes is a rate limiting step versus peptide formation. This, coupled with PRO B1 being a component of soluble protein, indicates that peptide supply is probably never limiting in the rumen as we have calculated. Also, peptides from endogenous protein flow (Ouellet et al. 2004) are used by the microbes with good efficiency and with ruminal microbial protein turnover there are many sources of peptides not considered when the model was developed. This suggests that feeding to supply peptides for ruminal requirements as has been done for many years causes us to overfeed protein and that the rumen is rarely short on peptides for microbial utilization.

This version of the CNCPS uses an overall efficiency of use of MP to net protein (NP) of 0.67, the same value utilized in the 2001 Dairy NRC (Tylutki et al., 2008; National Research Council, 2001). In addition each amino acid has individual efficiencies for maintenance, growth and lactation and the efficiencies are currently static. Data from recent studies in lactating cattle call into question the use of static efficiencies for either overall MP or specific AA and this makes sense given the possible

roles certain AA have in metabolism (Doepel et al., 2004; Pacheco et al., 2006; Wang et al. 2007; Metcalf et al., 2008).

Metcalf et al. (2008) challenged the use of a static efficiency and observed a range in efficiency of use of 0.77 to 0.50 as MP supply was increased. They further determined using a best fit of data that the optimal efficiency of use of MP to NP was between 0.62 and 0.64 for the average cow. This is quite a bit lower than our current value but is consistent with the data of Doepel et al. (2004). Taking the simple mean of the efficiencies from the Doepel et al. (2004) publication, the average efficiency of use of the essential AA is 62.2%, again lower than the value we are currently using in the model but consistent with the data of Metcalf et al. (2008). Most likely, any change in efficiency of use of MP or amino acids will be associated in a change in ME utilization, thus the absolute differences within one nutrient will be hard to detect or manipulate.

Additional changes have been made to the calculations for metabolic fecal nitrogen. This was a double-accounting error that resulted in under-estimating endogenous protein losses. As this directly impacts maintenance protein requirements, MP maintenance has increased slightly.

PREDICTED N EXCRETION

The CNCPS is designed to be used in the field to predict nutrient excretion as part of a nutrient management decision making process. Through evaluation, the partitioning of urine and fecal N excretion was determined to be inconsistent with total collection studies, thus a study was undertaken to improve this partitioning. In part this was done to help us refine N feeding and excretion in relation to milk. Since urinary urea N is the most volatile form of excreted N and also represents the true excess N, better predictions of urinary N would help nutritionists formulate to decrease this form of N excretion. Data to evaluate model predictions were compiled from published studies (n=32) that reported total collection N balance results. Considerable care was taken to ensure that the treatments included in the data set (n=104) accounted for >90% of the N intake (NI). Unaccounted N for the compiled data set was 1.47% ± 4.60% (mean ± SD). The results showed FN predictions could be improved by using a derivative of an equation proposed by Marini et al. (2008): FN (g/day) = (((NI (g/kg organic matter) × (1 – 0.842)) + 4.3) × organic matter intake (kg/day)) × 1.20, which, when evaluated against the compiled N balance data, had a squared coefficient of determination based on a mean study effect (R^2_{MP}) of 0.73, concurrent correlation coefficient (CCC) of 0.83 and a mean square prediction error (MSPE) of 781. Prior to this, urinary N was being over-predicted by the CNCPS due to inconsistencies in N accounting within the model. Incorporating the more accurate FN prediction into the current CNCPS framework and correcting the endogenous protein calculation error considerably improved UN predictions (MSPE = 970, R^2_{MP} = 0.86, CCC = 0.90). The changes to FN and UN translate into an improved prediction of total manure N (MSPE = 623, R^2_{MP} = 0.96, CCC = 0.97) and were incorporated into the latest version of the CNCPS v6.1.

METHANE PRODUCTION

Due to the pressure being put on the dairy industry to be more environmentally friendly, and due to sporadic requests from groups for predictions, we decided to identify an equation that would provide robust predictions of methane production with inputs currently available in the CNCPS for both beef and dairy cattle. A review of the literature was conducted and several equations were identified. There are many extant equations available for predicting methane and a couple recent evaluations of new prediction equations (Ellis et al., 2007; Mills et al. 2003). We adopted two equations for used in the model, the first equation we adopted was from Mills et al. (2003) (non-linear equation 3, "Mitschelich 3") that included an exponential function describing the increasing effect of ME intake on methane production with an additional ratio for starch/ADF relationships. This equation is specifically for dairy cattle, both lactating and dry, and somewhat complex due to the number of variables requiring quantities of dietary components but easily available within the structure of the CNCPS. The equation is: CH_4 (MJ/d) = 45.98 – ($45.98e^{(-1*(((-0.0011*starch/ADF)+0.0045*MEintake,}$ where starch and ADF are kg of dry matter consumed and ME intake is in megajoules. The equation adopted for beef cattle was from Ellis et al. (2007) and is equation 14b. The equation was chosen because it had the lowest RMSPE (14.4%) and the highest R^2 of the evaluated equations, 0.85. Again, it is a fairly complex equation requiring ME intake, ADF and lignin, but all factors utilized in the CNCPS. The equation is: CH_4 (MJ/d) = 2.94 + 0.0585 * ME intake (MJ/d) + 1.44 * ADF (kg/d) – 4.16 * lignin (kg/d).

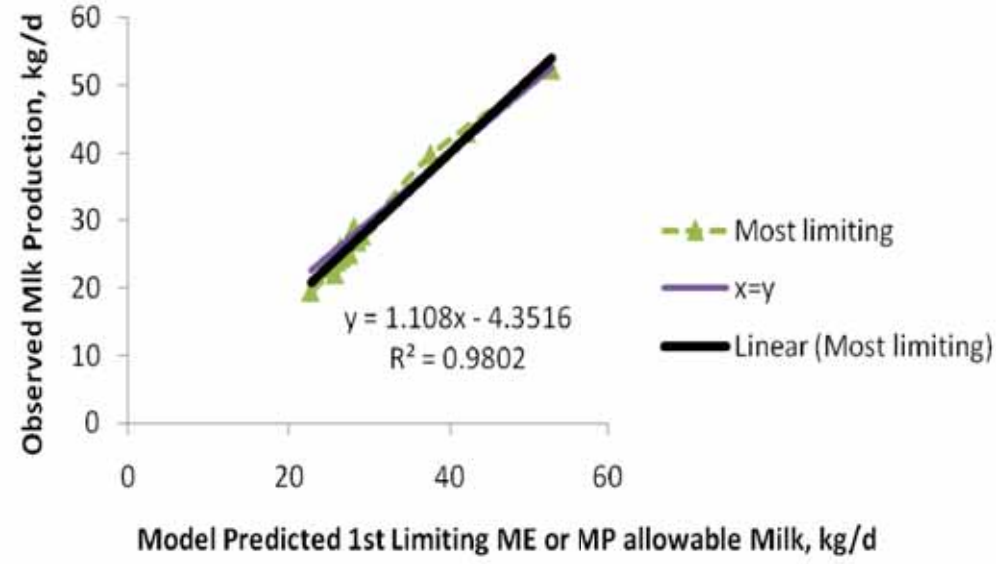
AMMONIA POTENTIAL

The calculation of the ammonia potential of the total N excretion is based on the amount of volatilization of urinary urea N that can occur given the amount of urinary urea excreted daily. This calculation was provided to help guide assessments of the amount of ammonia that could be emitted under typical environmental conditions and requires refining but provides a beginning basis for predicting ammonia volatilization. The calculation assumes 65% of the N in urine has the potential to volatilize under normal circumstances and is sensitive to the amount of N excreted in the urine.

PREDICTION OUTCOME

An evaluation of most limiting (ME or MP) milk is found in Figure 1. Studies and actual farm data are contained in these comparisons and demonstrate that the model is doing a reasonable job in predicting the most limiting nutrient supply, thus this provides us with a reasonable platform from which to start making changes. The evaluation was made from both research and on-farm datasets for lactating dairy cows. The dataset represents cows producing 21 to 52 liters of milk per day fed diets ranging from 12.7 to 17.4% crude protein. Model predicted milk reported is the lower of ME or MP allowable milk. The intercept was not different from zero and the mean prediction bias was less than 1%.

Figure 1. Observed versus predicted milk production as predicted by CNCPSv6.1. Diets range in crude protein from 12.7 to 17.4% DM with milk yields ranging from 21 to 52 liters per day.



As an example, the CPM ver.3 100 lb cow session file was inputted into CNCPSv6.1. Table 6 lists selected output variables from the two programs. In almost all cases, MP allowable production (milk or gain) will be predicted to be higher in CNCPSv6.1 and ME allowable milk reduced. In this case, MP allowable milk is 10.8% greater than in CPMv3 while ME allowable milk is decreased 6.2%. This example in CPMv3 is perfectly balanced for ME and MP while v6.1 suggests opportunity for reformulation. MP from bacterial sources was reduced 6.8% while MP from feed increased 23.8%. This shift changes MP from bacteria from 52% of total MP supply to 44%. As can be expected, these shifts impact amino acid flows and ratios. Microbial protein has a near perfect amino pattern for milk protein production. Thus, reducing microbial yield introduces altered ratios and potentially more variability in ratios as RUP LYS from feed is more variable in composition.

Flows for all amino acids changed as represented by the amino acid balances illustrated in Table 6. LEU and ILE balances changed over 100% while MET and LYS balances increased nearly 50%. These, coupled with the MP balance, suggest reformulation to decrease MP supply, while maintaining AA balance (and ratio) is possible. The LYS ratio (% MP) dropped from 6.9 to 6.6% (a 10% reduction) while the LYS:MET ratio shifts from 3.1 to 3.3:1. In general, we have found that LYS %MP has a larger shift in going from CPMv3 to CNCPSv6.1.

EVALUATING DIETS WITH CNCPSV6.1

Given that the evaluation guidelines nutritionists routinely use when formulating with CPMv3 have changed, the following is an updated list for evaluating diets with CNCPSv6.1:

1. Dry matter intake: Inputted DMI should be within the range of CNCPS and NRC predictions. If it is not, review inputs for bodyweight, environment, and feed amounts.
2. Rumen ammonia should be between 100 and 150%. Diets high in hay silage, or given ingredient availability limitations might be as high as 200%, and although unacceptable from an efficiency perspective, realistic depending on the total forage availability.
3. Peptide balance can be ignored.
4. The considerations given to urea cost can be minimized. However, you can target a urea cost of less than 0.25 Mcal/d.
5. NFC for lactating dairy cow diets can vary between 30 and 42% depending upon sources.
    a. Sugar versus starch versus soluble fiber is user preference in our opinion. Given that cattle require fermentable CHO, sources of fermentable CHO should rely upon local availability and pricing.
6. ME and MP allowable milk should be within 1 kg of each other and should match the observed milk before any ration changes are made. For growing cattle, MP allowable gain should be 0 to 250 grams greater then ME allowable gain.
    a. For replacement heifers, keep lactic acid less than 3% DM. Data from the 1980s suggests a direct link between lactic acid intake and empty body fat composition in growing cattle.
7. peNDF should be greater than 22% DM for lactating dairy cows (8-10% for feedlot cattle).
8. Lysine should be greater than 6.5% MP and Methionine greater than 2.2% MP
9. LYS:MET ratio to maximize milk protein yield should be between 2.80-2.95:1
10. Total unsaturated fatty acid intake should be monitored. Values greater than 500 g/d are a risk factor coupled with quantity and quality of forage NDF (lower quality forages and/or lower quantities of forage NDF fed increase the risk of milk fat depression).
11. Minerals and vitamins. Given that CNCPSv6.1 has implemented the Dairy NRC recommendations for minerals and vitamins (as a dietary supply including bioavailability), we suggest following NRC recommendations.

Table 6. Selected outputs from 100 lb cow session file as predicted by CPM ver. 3.0.10 and CNCPSv6.1.

Component	CPM ver 3	CNCPS v6.1	% Change
Predicted DMI	24.5 kg	24.6 to 27.6 kg	0 to 12%
ME Supply (Mcal)	69.2	64.9	-6.2%
ME Required (Mcal)	66.8	66.3	-0.7%
MP Supply (g)	2,887	3,093	7.1%
MP Required (g)	2,887	2,875	-0.4%
ME allowable milk (kg)	47.6	44.1	-7.4%
MP allowable milk (kg)	45.4	50.3	10.8%
MP Bacteria (g)	1,499	1,374	-8.3%
MP RUP (g)	1,388	1,719	23.8%
MP Bacteria, % Total MP	52%	44%	-14.4%
Ammonia balance (g)	122	100	-18.0%
RDP %DM	11.5	10.0	-13.1%
MP LYS g	199.3	204.1	2.4%
LYS %MP	6.90	6.60	-4.3%
MP MET g	63.5	62.7	-1.3%
MET %MP	2.20	2.03	-7.7%
LYS:MET	3.1	3.3	3.7%
LYS balance g	32.2	48.0	49.1%
MET balance g	10.7	15.6	45.8%
ARG balance g	26.3	25.9	-1.5%
THR balance g	39.7	48.2	21.4%
LEU balance g	2.4	28.1	1,070.8%
ILE balance g	-15.8	3.4	121.5%
VAL balance g	20.4	18.2	-10.8%
HIS balance g	22.2	33.3	50.0%
PHE balance g	52.8	66.3	25.6%
TRP balance g	15.8	14.9	-5.7%
NFC %	40.0	38.4	-4.0%
Diet ME Mcal/kg	2.82	2.65	-6.0%

FUTURE MODELING WORK

The overall ME and MP allowable milk predictions of the CNCPSv6.1 are very good. However; much remains to be done. Efforts are underway to improve the rumen sub-model to include protozoa, nitrogen recycling, a two-pool NDF and two-pool starch fermentation representation, as well as being able to model additives such as monensin. These components are critical in order for the model to then include a more mechanistic lower-tract component to allow predictions of milk components and body composition. Further, excretion predictions will also be improved allowing for more accurate predictions of greenhouse gases.

In 2006, Cornell began offering a licensing program for the integrated model equations. This was done in an effort to allow commercialization of the CNCPS, and to refocus the modeling group towards research activities versus software development and support. Currently, three licenses have been issued to AMTS LLC (NY), RUM&N (Italy), and Fabermatica (Italy). AMTS and RUM&N have licenses for North America. We hope the result is a positive outcome for the end user since it provides them with more professional software and hopefully better software support. This allows researchers to focus on our core strength of research and development of equations and systems and then implement them into the commercially available software.

SUMMARY

Nutritional models are evolutionary. CNCPSv6.1 is the latest evolutionary generation in the CNCPS/CPM path. Between analytical improvements, error corrections, and new research being implemented within the CNCPS framework, model accuracy has been improved. These changes allow the nutrition professional to reduce dietary crude protein levels while maintaining or improving production and profitability. Economics and environmental issues require us to adopt more accurate predictions for the survival of the dairy and beef industries.

Take Home Messages

- Nutrition models are evolutionary should be expected to change with improved understanding of and continue to change as new research is published
- The current version of CNCPS has improved passage rates, feed chemistry and error corrections and will predict greater metabolizable protein supply from feed protein
- Evaluations of herd level nutritional management, when the actual feed chemistry and inputs are used and all other factors are properly characterized, the CNCPS v6.1 is more accurate and precise in estimating ME and MP allowable milk with a lower prediction bias.
- Future model improvements will include the incorporation of protozoa into the rumen submodel, improved predictions of N metabolism on a whole animal basis, the application of a three pool model for NDF digestion and passage, the development

of a VFA submodel and an improved approach for predicting amino acid requirements and supply.

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NDF AND DMI – HAS ANYTHING CHANGED?

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INTRODUCTION

Although the basic biological principles by which fiber affects intake have not changed, our knowledge about the subtle ways in which the characteristics of fiber impact intake regulation and our ability to speculate about the dynamic mechanisms that affect the relationship have changed during the last 15 years. It is well known and accepted that at very high levels of NDF in the ration, intake and animal performance is reduced and at very low levels of NDF, intake also is reduced for very different reasons. This implies that somewhere between these extremes there is an optimum, which maximizes intake. In general, when intake is maximized, animal performance is also maximized. Mertens (1994) provided a broad overview of the basic mechanisms that regulate intake in ruminants. There is no doubt that DMI results from the complex interaction of diet characteristics, animal physiological status, and the feeding environment and management. Allen and Bradford (2009) suggested that the dominant factor regulating intake changes during lactation. In early lactation, hepatic oxidation of fatty acids from body tissue loss regulates intake, during peak lactation, ruminal distension limits intake, and in late lactation, hepatic oxidation of propionate regulates intake when high energy rations are fed to cows with reduced energy demand. Allen et al. (2009) provided an excellent review of the hepatic oxidation theory for physiological regulation of intake.

Although there is ongoing and productive debate about the signals associated with intake regulation mechanisms, this information has not been converted into any type of quantitative system that can be used for ration evaluation or formulation. The physical and physiological mechanisms of intake, first proposed by Conrad et al. (1966) as a unified system, still provide the best framework for developing quantitative systems for predicting intake, or conversely for designing rations that optimize intake for maximum productivity and profitability. Although the NDF-Energy Intake System (Mertens, 1987, 1992) has been discussed at this conference (Mertens, 2002), it will be briefly reviewed to set the framework for discussing the relationship between NDF and DMI. The objectives of this review are to: (1) briefly review the physical and physiological mechanisms of intake regulation from a quantitative perspective, (2) use the NDF-Energy Intake System to define the ration boundary where NDF limits DMI, and (3) use a steady-state model of digestion and passage kinetics to discuss the characteristics of NDF that affect its relationships with DMI.

MECHANISMS OF INTAKE REGULATION

Most nutritionists would accept the concept that animals eat to satisfy some inner drive (we define this as their appetite) associated with their genetic potential or imperative and with their physiological status (maintenance, growth, fattening, pregnancy, and lactation). On of the great difficulties in modeling intake and animal response or in formulating rations is defining the “appetite” of individual or groups of animals. All of our nutrient recommendations or requirements are based on the assumption that we know what the output response (appetite) of the animal will be. Given this assumption, ration formulation becomes a simple “input must equal output” relationship. Nutrients and energy are neither created nor destroyed by the animal, so in the basic sense, input must equal output.

However, animals can shunt nutrients into different products and excrete some nutrients when fed in excess making the prediction of actual intake difficult. One response that is commonly observed is that animals will “over-consume” high energy diets and gain body fat that is unpredicted, and is often considered undesirable by the nutritionists. However, we need to recognize that the animal may have an evolutionary adaptation to accumulate energy whenever it is available as a protective mechanism to enhance survival when dietary energy may not be available in the future. Also, feed availability, social interactions among animals, and interactions with feeding management can modify intake in ways that prevent the animal from attaining its appetite. To simplify the elucidation and discussion of intake concepts, an appetite “target” will be assumed that represents the energy requirement needed to meet the production response we expect of the animal given both homeostatic and homeorrhetic demands for a given physiological state (for example, we would expect cows in early lactation to lose body tissue and we would expect the cows in late lactation will gain tissue reserves).

Physiological Intake Regulation

When we feed low fiber, high energy diets we expect the animal to eat to meet its appetite for a specific physiological state, which we assume is equal to the total energy “requirement” we assign as a target. In simple mathematical terms, physiological intake regulation occurs when the energy intake ($\mathbf{I_e}$) of the animal times the energy density of the diet (**E**) equals the total energy requirement (target) of the animal (**R**):

$$I_e \times E = R.$$

If we solve this equation for I_e, we find that the intake of the animal under physiological intake regulation is a linear function of the animal’s requirement and a reciprocal function of the energy density of the diet:

$$I_e = R / E.$$

Thus, the greater the requirement of the animal, the higher will be its intake, and the greater the energy density of the diet the lower will be its intake. Note that intake is a flux or flow because it has units of time, e.g., joules or Mcals per day. Realize that our definition of energy requirement is a target that may not match that of the animal, which

may gain additional fat (above our “requirement”) when high energy rations are fed. This explains why it is more difficult to predict intake responses to high energy versus high fiber diets, because, depending on physiological status, the animal may accumulate more fat than we want.

Physical Intake Limitation

When we feed high fiber, low energy diets, we typically see that the animal eats until its meets some fill constraint. In simple mathematical terms, physical intake limitation occurs when the filling effect of intake ($\mathbf{I_f}$) of the animal times the filling effect of the diet (**F**) equals the fill constraint of the animal (**C**):

$$I_e \times F = C.$$

If we solve this equation for I_f, we find that the intake of the animal under physical intake limitation is a linear function of the animal’s fill constraint and a reciprocal function of the filling effect of the diet:

$$I_f = C / F.$$

Thus, the greater the fill constraint of the animal, the higher will be its intake, and the greater the filling effect of the diet the lower will be its intake. Note that intake is a flux or flow because it has the unit of time, e.g., filling units per day. Just as the animal can change its “requirement” in response to high energy diets by gaining fat, animals can change their fill constraint in response to high fiber diets. We have all seen the pot-bellied animals that were forced to survive on low energy, high fiber diets. Just as the energy requirement has to be defined as a target rather than the actual appetite of the animal, the fill constraint has to be defined under specific conditions of animal physiological status and nutritional history.

Combining the Mechanisms of Intake Regulation Using a Common Feed Characteristic

There are two problems in making practical use of the simple equations for intake regulation: (1) they represent two different measures of feed and animal characteristics and (2) filling effect must be defined in some measurable way. Fiber, specifically NDF, has unique properties that provide tentative solutions to both problems. Because NDF is related to digestibility of dry matter (**DM**), it can be related to the energy density of diets with reasonable accuracy for predicting intake. The summative equation of Van Soest (Goering and Van Soest, 1970) describes the mathematical relationship of DM digestibility (**DMD**) to NDF, its digestibility (**NDFD**), and the digestibility of its converse , neutral detergent solubles (**NDS** = 100 - NDF):

DMD = NDF*NDFD + 0.98*NDS -12.9, which can be rearranged to show:

DMD = 87.1 – (0.98 – NDFD)*NDF.

Because NDFD is less than 0.98, this equation indicates a negative relationship between DMD and NDF. It also suggests that if we know NDF concentration and its digestibility, we have described most of the variation affecting DMD, which is related to the energy in the feed that is available to the animal. In mixed rations, some of the NDS is starch, which can have a digestibility less than 0.98, but this complication (which can be accounted for by adjusting 0.98 downward depending on the proportion and digestibility of starch in NDS) does not negate the overriding importance of NDF

concentration and its digestibility in determining DMD. Thus, NDF can be used to represent the energy density of the ration and because this relationship is inverse or negative, the equation for predicting intake under physiological regulation (I_e) will be opposite of that for the equation predicting intake under physical limitation of intake.

The filling effect of a feed or diet is more easily related to fiber than is its energy density. By definition and actual effect, fiber for ruminants is insoluble and is indigestible or slowly digesting, thus it takes up space or "fills" the rumen and intestines. The "hotel theory" of Van Soest (1994) postulates that the space occupied by fiber is greater than its mass might indicate because fiber (NDF) is contained in the cell walls of plants and these cell walls encapsulate a volume much greater than that of the walls themselves. This space can often trap fermentation gases, making the apparent volume bigger than the mass would indicate (imagine a balloon that is empty or filled with gas), until the cell wall is disintegrated, allowing the internal space to collapse. When this effect is combined with the effect of fiber particle size on the volume or density of feeds (Mertens, 1980), the combined effect of fiber and its particle size on volume can be immense (simply imagine the volume of chopped straw compared to ground straw of the same mass). Because fiber concentration and particle size is correlated (especially when comparing forages to grains), fiber can represent the "filling effect" of the diet and be used to describe the physical fill limitation of intake. Because NDF is related to most of the properties of "filling effect" it can be an effective proxy for fill it was used to develop the NDF-Energy Intake System (Mertens, 1987, 1992) for formulating diets that determines the forage:concentrate ratio (**F:C**) in a ration that will maximize forage proportion and DMI.

When scaled in terms of ration NDF, these two mechanisms result in curvilinear lines that intersect at points a to d as the milk production target (animal energy requirement) is changed (Figure 1). At the intersection ($I_e = I_f$), the energy requirement and fill constraint are met simultaneously, and this is the point that the ration for a specific target production will have maximum NDF, forage proportion, and DMI. The intersection maximizes fill while allowing the target production to be achieved. Mertens (1987, 1992) developed the NDF-Energy Intake System to define the boundary between rations that cows can feasibly consume and those where fiber or fill limits intake. He observed that cows maximize production of 4% fat-corrected milk (**FCM**), when fed a wide range for forages with corn and soybean meal concentrates, when they consumed 1.25 ± 0.1 percentage of their body weight per day (**% BW/d**) as NDF and used this observation to define C in the calculation of I_f. The NDF-Energy Intake System is based on the unique solution when R/E = C/F. Animal characteristics can be defined as the animal's net energy requirement (**ANER**) and NDF intake constraint (**NDFIC** = 1.25% BW/d):

R = ANER, and
C = NDFIC.

Ration characteristics can be defined in terms of the proportion of forage (**PF**), the net energy concentration in forage (**FNE**) and concentrates (**CNE**) and the NDF concentration of forage (**FNDF**) and concentrates (**CNDF**):

E = PF*FNE + (1 – PF)*CNE, and

F = PF*FNDF + (1 – PF)*CNDF.

After substitution and rearrangement:

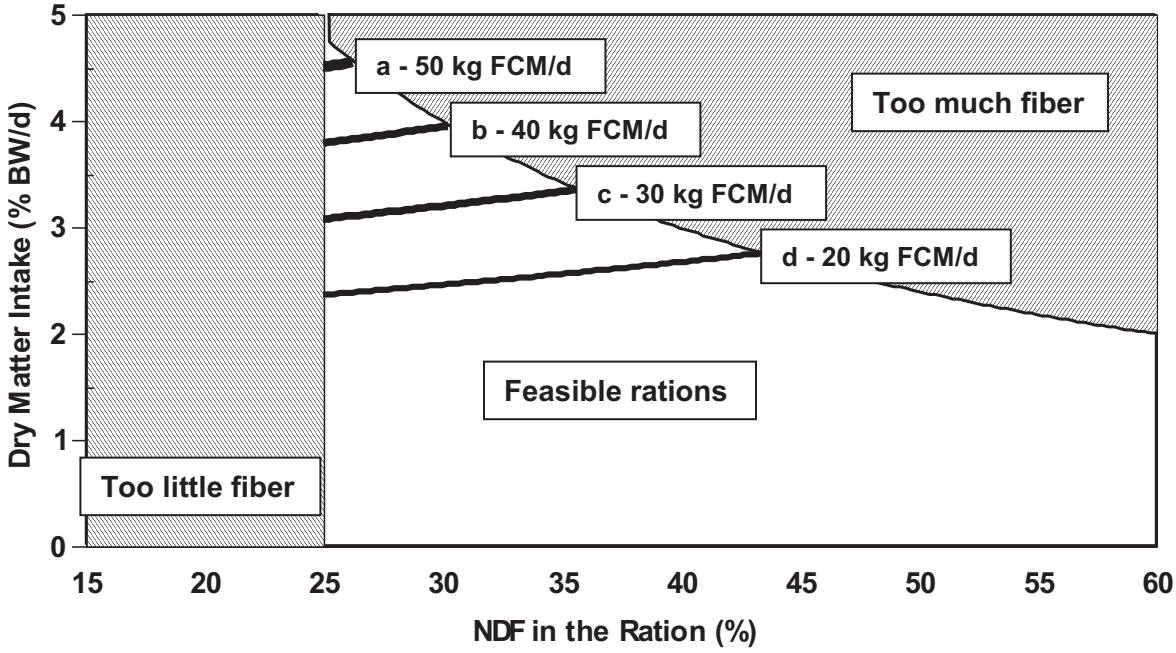
PF = [NDFIC*(CNE) – ANER*(CNDF)] / [NDFIC*(CNE – FNE) + ANER*(FNDF – CNDF)],

Optimum ration NDF = (PF*FNDF + (1 – PF)*CNDF), and

DMI_{max} = NDFIC / Optimum ration NDF.

These last three equations define the maximum forage ration on the upper boundary between feasible rations and those with too much fiber to allow the animal to meet its target production. The lower boundary for ration NDF can be defined by the physically effect NDF (peNDF) minimum requirement (Mertens, 1997), thereby defining the total area of feasible ration solutions.

Figure 1. Identifying the region of feasible rations for dairy cows by combining the NDF-Energy Intake System for determining maximum NDF rations with the peNDF concept for determining minimum NDF rations. Isoclines indicate the dry matter intake for rations corresponding to 20, 30, 40 or 50 kg of 4% fat-corrected milk (FCM) (from Mertens, 2003).



For linear programming solutions for dairy ration formulation, the NDF-Energy Intake System can be described by three equations:

Ration NDF ≤ $\sum$ (b_1*AdjNDF$_1$ +b_2*AdjNDF$_2$ + • • • • • • • • • • b_n*AdjNDF$_n$),

Ration NEL = $\sum$ (b_1*NEL$_1$ +b_2*NEL$_2$ + • • • • • • • • • • b_n*NEL$_n$), and

Ration peNDF ≥ $\sum$ (b1*peNDF1 +b2*peNDF2 + • • • • • • • • • • bn*peNDFn), where b_n is the amount of each feed, AdjNDF is the NDF concentration adjusted for filling effect of small-particle, high-fiber concentrates, and NEL is the net energy of lactation for the feed. While the equation on the previous page describes the upper boundary of feasible solutions, linear programming allows any solution in the feasible area to be selected that maximizes profit or minimizes ration cost.

Figure 1 demonstrates three characteristics of dairy rations that are related to animal and dietary factors affecting DMI. First, as the production of cows increase, the range in available rations becomes narrower and very often DMI will be limited by the NDF processing capacity of the cow. Second, the relationships between DMI and ration NDF are curvilinear for both energy and fill. Third, the relationship between NDF and DMI is complex and cannot be described effectively by linear correlation. Mertens (1994) demonstrated that depending on the production of the animals and the NDF concentration of the ration, the correlation will be positive, negative or zero. At low ration NDF concentrations, DMI will vary greatly among cows depending on their energy demand, but at high ration NDF concentration, the range in DMI among cows will be narrow because all cows are affected by the same fill constraint. The relationship of NDF to DMI is a triangular area and using correlation to test the relationship is nonsensical and prone to disappointment.

The NDF-Energy Intake System has been criticized as too simple (isn't simplicity the key to good models?) and it treats all NDF as if it is alike. The former criticism cannot be taken seriously and the latter fails to recognize that the system is effective because the difference in NDF among sources is much less than the differences between NDF and NDS within the sources for both energy value and filling effect. However, there are differences in NDF that can have an impact on either fill or energy value and the NDF-Energy Intake System can be used to highlight where discrepancies occur and to develop modifications to address them.

One of the main factors affecting the flux of NDF through the animal is particle size. Finely ground NDF will not have the same filling effect or requirement for processing as long forage fiber. Therefore, the NDF of ground, high-fiber byproduct feeds should be adjusted to reflect this difference. It is generally observed that when byproduct feeds with less than 40% NDF are included in rations, intakes are similar to rations containing simple corn-soybean meal mixtures. Thus, it was assumed that the filling effect of these byproduct feeds is similar to a corn-soybean meal mixture and they are given a fill-adjusted NDF (**AdjNDF**) value of 12%. Byproduct feeds with greater than 40% NDF were given a AdjNDF equal to (0.30 X NDF). A table of NDF, AdjNDF and composition of feeds has been compiled by Mertens (1992).

FACTORS AFFECTING THE RELATIONSHIP OF NDF TO THE FILLING EFFECT

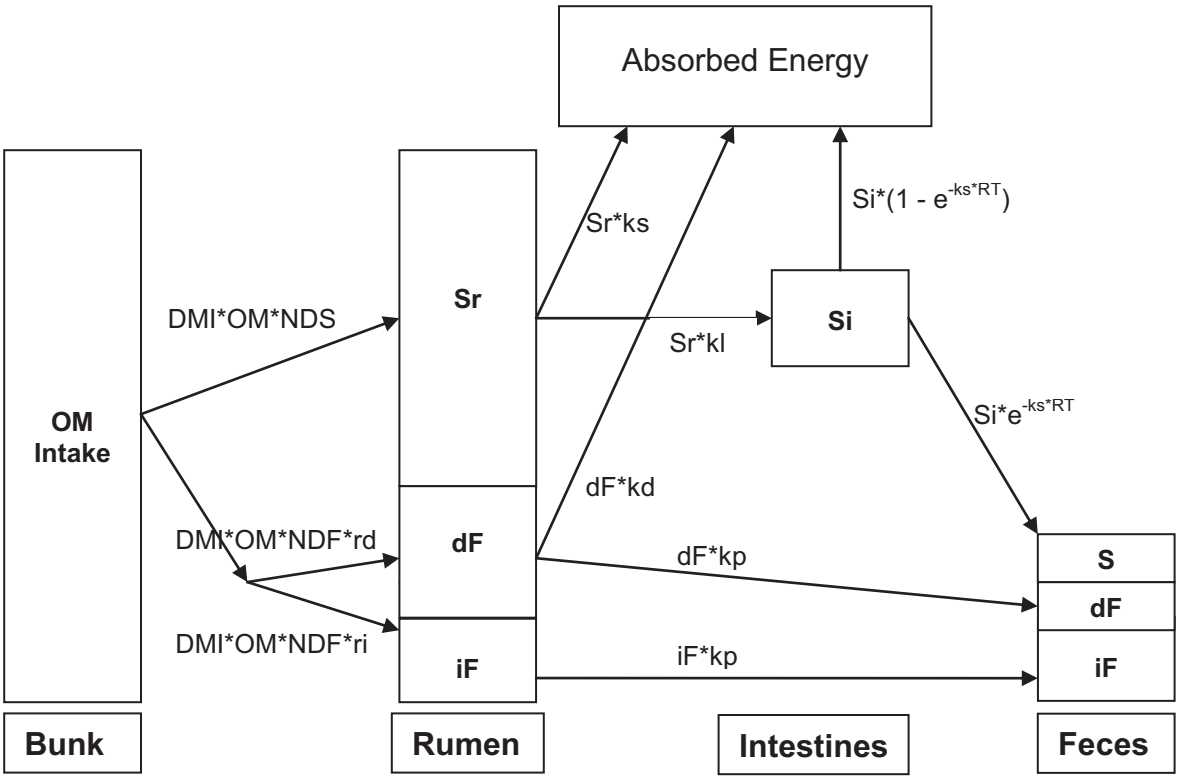
Although the fundamental biological principle that fill can limit intake as we maximize forage use in dairy rations has not changed, our ability to define fill more accurately can and should be changed by investigating and using characteristics of NDF that affect the filling effect of a feed or ration. In addition, our definition of the fill intake constraint in terms of NDF should be reviewed and refined as needed. Finally, we need to evaluate filling effect in the context of the dynamic processes of digestion, particle size reduction, and passage. These topics will be focus of the remaining discussion to highlight what has or should be changed to improve the relationship between NDF and DMI.

One of the most interesting characteristics of the mathematical description of the physiological and physical mechanisms of intake regulation is that the animal constraints (fill and energy) are flows or fluxes (they have units of time). The response (intake) is also a flow or flux. This realization has important ramifications for our understanding of fill and the relationship of NDF to DMI. The NDF intake constraint is actually the amount of NDF that can be processed by the animal each day or hour. Thus, we have to consider any factor that affects the dynamic flow or transformation of NDF in the animal. In addition, to relate fill to the capacity of the rumen requires that the flow of NDF be related in some way to the amount or volume of NDF in the rumen. These consequences of the simple theories of intake regulation indicate that the relationship between NDF and DMI is more than NDF concentration in the ration, but that it should be affected by any factor that affects the flow and volume of NDF. These issues can only be addressed by reframing the concept of fill and intake as a dynamic flow of NDF through the animal. To enhance our understanding and uncover changes that might be needed to describe the relationship of NDF to DMI, the simplest possible model of digestion and passage was developed that mimics the kinetics described in many nutritional models, including CNCPS. The simple model aggregates many of the nutrient flows associated with the soluble part of the diet, but maintains the essential elements associated with NDF that are needed for a discussion of fill and the physical limitation of intake by NDF (Figure 2).

The model shown in figure 2 can be solved analytically at steady-state conditions to provide equations that can calculate pools and absolute rates; and thereby demonstrate the direction and relative impact of the factors affect the relationship between NDF and DMI. Several assumptions were needed to develop the simple steady-state model:

1. NDF will be expressed as amylase-treated NDF organic matter (**aNDFom**) which requires that the NDF intake constraint will be adjusted from 1.25 to 1.20 percent of body weight per day (**% BW/d**),
2. The small particle fraction of the feed and in the rumen will be defined as particles passing through the 1.18-mm sieve using vigorous vertical shaking,
3. The ash content of all rations will be kept constant a 5% of DM to simplify calculations,
4. The aNDFom concentration, proportion of NDF that is indigestible, and the fraction of NDF <1.18 mm for the ration will be calculated using the proportions of forage and concentrate in the ration,
5. DMI is calculated from the ration aNDFom using the NFIIC = 1.20% BW/d to insure that intake is fill-limited,
6. Starch concentration is a separate input and was set at 25% of DM for all comparisons,
7. Fraction of indigestible NDF (**iNDF**) and fractional rate constant of fiber digestion (**kd**) are inputs that will be adjusted to match values commonly observed for each feed type,
8. The fractional rate constant of passage for large NDF particles is an input and fractional rate constant of passage for small NDF particles is assumed to be 4 times the large particle rate, and the fractional rate constant of passage for NDS is assumed to be 4 times the weighted average passage rate of fiber,

Figure 2. Block and arrow diagram of the simplest kinetic model that describes the fill limitation of intake; where OM = organic matter, rd = fraction of NDF that is potentially digestible, ri = fraction of NDF that is indigestible, dF = ruminal potentially digestible fiber, iF = ruminal indigestible fiber, kd = first-order digestion rate constant for digestible NDF, kp = digestible and indigestible fiber passage rate, Sr = ruminal detergent solubles, ks = first-order digestion rate constant for detergent solubles, kl = liquid passage rate, Si = intestinal detergent solubles (assuming a plug flow system with a retention time = RT).



9. The “filling effect” of a ration is assumed to be the volume of fiber particles in the rumen (volume is assumed to affect the stretch receptors that send the feedback signals to the brain) and the estimate of large and small particle volumes are crude relative values that generate rumen content volumes typically reported in the literature – this is the weakest assumption in the model, and
10. All pools in the rumen are assumed to be continuously mixed compartments and the intestines are assumed to be a plug-flow compartment.

This model simulates the effects of changing particle size by adjusting an average passage rate. Multi-compartmental models (Mertens and Ely, 1979, 1982) much more accurately simulate particle size effects and should be the basis for future model development considering the dynamics of NDF digestion and passage. The model calculations are provided in an Excel spreadsheet so that you can do other simulations. The model is copyrighted, so please cite this paper when referencing the use of the model.

The model was designed to demonstrate the relative effects of various factors on the relationship between NDF and DMI, and validation was limited to comparison to the data of Voelker Linton and Allen (2008) who evaluated the ruminal kinetics of cows fed alfalfa (**AS**) or orchardgrass (**OG**) silages. When simulation was done assuming (1) NDF intake was adjusted to match the DMI observed (the intake of the animals apparently was not limited by NDF), (2) the NDF and iNDF concentrations that were reported, and (3) the digestion rates of 0.075 and 0.058 that were observed, the steady-state model predicted NDF kp of 0.030 and 0.024 h^{-1} for AS and OG compared to observed values of 0.029 and 0.024 h-1 for the kp of iNDF. The kp results of the model were predicted by assumed differences in the proportion of small particles in each forage (0.15 for AS and 0.05 for OG). The model-predicted ruminal iNDF pools were 4.35 kg for AS and 2.78 kg for OG compared to observed values of 4.4 and 2.7 kg. Total ruminal aNDFom was predicted to be 5.32 kg for AS and 4.78 kg for OG compared to observed values of 5.6 and 5.1 kg. The bias of model predictions is probably related to the assumption in the model that iNDF and potentially digestible NDF (**pdNDF**) have the same kp. Voelker Linton and Allen (2008) reported slower rates of passage for pdNDF, which are difficult to reconcile with the model assumption that iNDF and pdNDF are components of the same plant tissue. The model predicted total tract NDF digestion of 1.74 kg/d for AS and 2.77 kg/d for OG compared to the observed of 1.8 and 3.0 kg/d. The model predicted a slightly higher fill volume for OG compare to AS, which corresponded with the slightly lower observed DMI of OG compared to AS.

The model was also evaluated by comparing alfalfa and grass as the sole forage sources fed in a ration containing 29% aNDFom. For alfalfa, the assumed parameters were 40% aNDFom, .50 fraction of fiber indigestible, kd = 0.08 h^{-1}, .15 fraction of fiber particles < 1.18 mm, and kp =0.029 h^{-1}. For grass, the assumed parameters were 55% aNDFom, 0.20 fraction of fiber indigestible, kd = 0.06 h^{-1}, .05 fraction of fiber particles < 1.18 mm, and kp =0.023 h^{-1}. Interestingly, the steady-state model predicted a ruminal total NDF pool of 7.09 kg for alfafa and 6.36 kg for grass. To obtain, the same ruminal NDF pool of 6.48 kg, the NDFIC of the alfalfa-based ration would have to be decreased to 1.10% BW/d and of the grass-based ration would have to increased to 1.23% BW/d. Mertens (unpublished) observed that in general legume diets obtain optimal NDF intakes slightly below the average of 1.25% BW/d and grasses generated NDF intakes greater than the average. The steady-state model as develop seems to be able to mimic these subtle response differences.

The steady-state model solutions were used to evaluate forage factors that could change our understanding of the relationship between NDF and DMI (Table 1). The defender model will be the NDF-Energy Intake System, which assumes that the NDFIC flux is a constant 1.2% BW/d. In most cases, the factor in question was changed by 10% in the direction that would reduce ruminal fill. Then, the NDFIC flux coefficient was changed to generate the baseline ruminal NDF pool and determine how much the defender model would be affected by the factor in question. Using a 50:50 mixture of alfalfa and corn silage as the forage source, and assuming an aNDFom intake of 1.2% BW/d, the simple steady-state model predicts ruminal pools of NDF (and fill volume) of 6.48 kg (and 76.7 L) when the forage proportion of ration was 0.70 (recognize that half

of the forage is corn silage containing about 50% grain; therefore the true F:C is about 52:48). This diet and result will be the baseline for comparison of factors affecting the relationship between NDF and DMI for these types of rations.

Table 1. Effects of changing forage or ration NDF characteristics on the steady-state predictions of ruminal NDF and fill volume (item in **bold** font was changed).

Ration or forage characteristics	Baseline	F:C ratio	Forage NDF	Forage kd	Forage indig
Forage proportion in the ration	0.700	**0.630**	0.700	0.700	0.700
Forage aNDFom (% DM)	40	40	**36**	40	40
Forage indigestible NDF (fraction of NDF)	0.35	0.35	0.35	0.35	**0.315**
Forage <1.18 mm (fraction of NDF)	0.150	0.150	0.150	0.150	0.150
kd of pdNDFom (h^{-1})	0.060	0.060	0.060	**0.066**	0.060
kp of large aNDFom particles (h^{-1})	0.0180	0.0180	0.0180	0.0180	0.0180
kp of small aNDFom particles (h^{-1})	0.0720	0.0720	0.0720	0.0720	0.0720
kp of aNDFom (h^{-1})	0.0281	0.0288	0.0283	0.0281	0.0281
Ration aNDFom (% DM)	30.5	28.4	27.7	30.5	30.5
Fixed aNDFom Intake (%BW/d)	1.200	1.200	1.200	1.200	1.200
Ruminal aNDFom (kg)	6.48	6.37	6.45	6.33	6.23
Fill (volume of ruminal aNDFom, L)	76.7	74.2	76.0	74.9	73.7
aNDFom digestibility (% NDF)	0.440	0.435	0.439	0.453	0.462

Changing Ration NDF Concentration By Changing F:C

When the forage proportion of the ration is reduced 10% to 0.63 (true F:C of 47:53), the model predicts that the rumen pools of NDF (and fill volume) would diminish to 6.37 kg (and 74.2 L). It would require an increase in the NDFIC from 1.20 to 1.221 % BW/d to achieve the baseline ruminal total NDF pool, but the fill volume would be reduced (75.5 L vs 76.7 for the defender model). This suggests that if F:C is changed the resulting changes in ration NDF concentration and small particle (<1.18 mm) fraction would change rate of passage and the make fill no longer the limiting constraint for intake. Although interesting, it does not reflect poorly on the defender NDF-Energy Intake System because the system would predict only one F:C ratio that would maximize DMI and NDF in the diet to meet the NDFIC.

Changing Forage Quality

Change aNDFom. The steady-state model indicates that decreasing the NDF of the forages by 10% (40 to 36% of DM) changes the rumen pools of NDF (and fill volume) to 6.45 kg (and 76.0 L). The NDFIC would only have to be increased from 1.20 to 1.206 % BW/d to move the rumen pools to 6.48 kg NDF (76.4 L). The NDF-Energy Intake System indicates that cows would increase DMI (if they had the potential to increase productivity) and the steady-state model indicates about the same increase in DMI while maintaining a similar rumen pool of NDF. This result suggests that the NDF-Energy Intake System can accurately accommodate changes in forage NDF concentration, if other characteristics of the feeds are not changed. If productivity was at the target for the cows (i.e. they would not respond with greater production), the NDF-Energy Intake

System would recommend when the forage NDF decreased 10% to increase forage proportion in the ration to about .80 and the steady-state model predicts (not shown) rumen pools of 6.49 kg NDF (and 76.9L) in this situation.

Change kd. When kd of the aNDFom in the forages is increased by 10% (0.060 to 0.066 h^{-1}) the NDF-Energy Intake System would indicates no change in DMI (because the forage NDF stays constant) but the steady-state model indicates that the rumen pools of NDF (and fill volume) would decrease from 6.48 kg (76.7 L) to 6.33 kg (74.9 L). This suggests that the animal have greater ruminal capacity and NDFIC would have to be raised from 1.20 to 1.228 % BW/d for rumen pools to equal the baseline value. Thus, differences in the digestion rate of NDF are not accounted for by the NDF-Energy Intake System.

Change in the proportion of indigestibility. Reducing the fraction of NDF that is indigestible from 0.35 to 0.315, decreases ruminal pools of NDF (and fill volume) to 6.23 kg (73.7 L). To obtain the baseline ruminal NDF pool of 6.48 kg, the NDFIC would have to be increased to 1.249% BW/d. The current NDF-Energy Intake System, which is based solely on NDF concentration cannot adjust the ration F:C to take advantage of improvements in NDF digestibility due to changes in rate or extent of digestion.

Oba and Allen (1999) compiled data from seven experiments with 13 comparisons and concluded that a 1%-unit increase in NDF digestibility (**NDFD**) measured in situ or in vitro resulted in an increase of 0.17 kg DMI and 0.25 kg FCM. Mertens (2006) added ten additional experiments to the original database of Oba and Allen (1999) and adjusted all in situ and in vitro measures of NDFD to a fermentation time of 48 h (**IVNDFD48h**, %). Mertens (2006) also included the effect of ration NDF (**RNDF**, %) content on dairy cow responses. Allowing an intercept for each trial, the regression coefficients within trial between forage IVNDFD48h or RNDF and cow responses for FCM (kg/d), DMI (kg/d), or NDF intake (**NDFI**, % of BW/d) were:

FCM = Trial + 0.139*(IVNDFD48h) - 0.520*(RNDF); R^2 = 0.977.
DMI = Trial + 0.0970*(IVNDFD48h) - 0.312*(RNDF); R^2 = 0.949, and
NDFI = Trial + 0.00485*(IVNDFD48h) - 0.0237*(RNDF); R^2 = 0.930.

The regression coefficients from Mertens' analysis for IVNDFD48h were smaller than the values determined by Oba and Allen (1999) for DMI (0.097 versus 0.17) and FCM (0.139 versus 0.25). Note in the equations generated by Mertens (2006) that the effect of changing RNDF is 2 to 3 times greater than the effect of changing NDFD. Thus, rations should be formulated first for NDF concentration as the NDF-Energy Intake System proposes, and then fine-tuned for differences in NDFD.

In research trials, the proportion of NDF from the experimental forage is often maximized; however, under most practical feeding situations single forages typically supply only 30 to 50% of the ration NDF. Additional equations were calculated from the dataset in which the IVNDFD48h was weighted by the proportion of NDF in the ration supplied by the experimental forage. These equations determined the regression coefficient assuming all of the NDF was obtained from the experimental forage and can

be used to calculate the effect of IVNDFD48h of the forage for any proportion of NDF obtained from forage:

NDFI = Trial + 0.00585*(wt)*(IVNDFD48h) - 0.0253*(wt)*(RNDF); R^2 = 0.936; where (wt = 1.00) for the regression coefficient obtained.

Increasing the kd from 0.06 to 0.066 would change the IVNDFD48h from 61.4 to 62.3% and with a ration containing 0.7 forage, the regression coefficient indicates that NDFIC should be increased from 1.20 to 1.204% BW/d. Decreasing the fraction of indigestible NDF from .35 to .315 would change IVNDFD48h from 61.4 to 64.7, with a increase in NDFIC from 1.20 to 1.214% BW/d. Although the direction and relative magnitude are similar, the steady-state model predicted much larger changes in NDFIC of 1.228 and 1.249% BW/d for the respective changes in rate and indigestibility to keep the NDF pools in the rumen similar to the baseline ration. It appears that changing NDF digestibility by either increasing factional digestion rate or decreasing indigestible NDF would affect the relationship between NDF and DMI.

Changing Particle Size, Particle Size Reduction, or Rates of Passage

Although the chemical nature of NDF and its digestibility can alter the relationship between NDF and DMI, its physical properties can also have impact on NDF flow through the cow. It is obvious that the relationship between “filling effect” and fiber is more complex than simply measuring the NDF concentration of a feed. Particle size of forages, the rate of particle size reduction, and rates of passage can affect the flow of NDF from the rumen and thereby affect relationship of between NDF and DMI (Table 2).

Changing the proportion of large (>1.18 mm) particles. If we decrease the proportion of particles retained on a 1.18 mm sieve with vigorous vertical shaking by 10 % from 0.85 to 0.765 by fine-chopping we increase the proportion of small particles from 0.15 to 0.235. Not only does this change the particle size distribution in the rumen, but it also will change the absolute flows of NDF out of the rumen predicted by the steady-state model because it changes the average rate of passage of NDF (the average kp of aNDFom is increased from 0.0281 to 0.0323 h^{-1}). The steady-state model predicts that changing forage particle size has a dramatic effect on reducing the ruminal NDF pool (5.84 kg), which is greater than any change predicted by changing the chemical or digestion kinetics of fiber. The NDFIC used in the NDF-Energy Intake System was obtained from cows fed coarsely chopped hays and silages. It appears from the steady-state model that the effects of forage particle size should be incorporated into the system when forages are finely chopped. The steady-state model indicates that the NDFIC would be increased to 1.332% BW/d to obtain the baseline ruminal NDF pool size.

Table 2. Effects of changing forage or ration physical characteristics or passage rates on the steady-state predictions of ruminal NDF and fill volume (item in **bold** font was changed).

Ration or forage characteristics	Baseline	Forage PS[a]	Both PS[a] kp	Large kp	Small kp
Forage proportion in the ration	0.700	0.700	0.700	0.700	0.700
Forage aNDFom (% DM)	40	40	40	40	40
Forage indigestible NDF (fraction of NDF)	0.35	0.35	0.35	0.35	0.35
Forage <1.18 mm (fraction of NDF)	0.150	**0.235**	0.150	0.150	0.150
kd of pdNDFom (h^{-1})	0.060	0.060	0.060	0.060	0.060
kp of large aNDFom particles (h^{-1})	0.0180	0.0180	**0.0198**	**0.0198**	0.0180
kp of small aNDFom particles (h^{-1})	0.0720	0.0720	**0.0792**	0.0720	**0.0792**
kp of aNDFom (h^{-1})	0.0281	0.0323	0.0309	0.0296	0.0294
Ration aNDFom (% DM)	30.5	30.5	30.5	30.5	30.5
Fixed aNDFom Intake (%BW/d)	1.200	1.200	1.200	1.200	1.200
Ruminal aNDFom (kg)	6.48	5.84	6.03	6.24	6.26
Fill (volume of ruminal aNDFom, L)	76.7	63.0	65.5	67.7	74.1
aNDFom digestibility (% NDF)	0.440	0.420	0.426	0.433	0.433

[a]Particle size

Changing rates of passage. Numerous factors could potentially change rates of fiber passage from the rumen that are independent of particle size (discussed in the previous paragraph). Buoyancy and functional specific gravity can affect the floating characteristics of particles, leading to their entrapment in the large particle mat and reduction in rates of passage. Particles can be more fragile or friable making it easier and more rapid to reduce the size of large particles. Mat formation and entrapment or release of small particles could affect their rates of passage. In addition, simple density of fiber particles (Mertens, 1980) could play a role in positioning them in the zone of escape in the reticulum and increasing their rate of passage (Allen and Mertens, 1998).

The steady-state model suggests that changing rates of passage of both large and small particles greatly reduces the ruminal pool of NDF to 6.03 kg. Changing either the large particle passage rate (which in reality is mainly a particle reduction rate) or the small particle passage rate has similar effects on ruminal NDF pools. The results of the steady-state simulations clearly demonstrate that if fiber can be changed to modify its passage rate and if there is a method for measuring these changes in friablility, density, etc. then these effects must be incorporated into the NDF-Energy Intake System for it to correctly adjust the NDFIC for ration formulation.

Adjusting Concentrate NDF For Filling Effect

Mertens (1992) attempted to make a simple modification of NDF values for fibrous by-product feeds due to their typically small particle size. The steady-state model confirms that the NDF in concentrates have much different impact on ruminal NDF pool size. When a forage with 40% aNDFom is the sole diet, the model predicts a ruminal NDF pool of 7.23 kg and a fill volume of 94.0 L. When a concentrate with 40% aNDFom is the sole diet, the model predicts a ruminal NDF pool of 3.14 kg and a fill volume of

19.9 L. The ruminal NDF pool of the concentrate is only 0.434 of the forage pool. The fill volume of the concentrate is only 0.211 of the forage fill volume. Mertens (1992) proposed an adjustment factor of 0.30 for concentrates with more than 40% NDF and a constant fill-adjusted NDF of 12% for concentrates under 40% NDF. It appears that this estimate is in the ballpark, but much more should be done to derive adjustments for individual feeds to improve the effectiveness of the NDF-Energy Intake System when used with fibrous byproduct feeds.

CONCLUSIONS

What has changed in the relationship between NDF and DMI? Probably nothing in the basic biological principles involved or in our qualitative understanding about the roles of the factors involved. Does NDF content of the diet always regulate intake? No, but there are always specific situations were it is the controlling factor, and due to the nutrient demands of high levels of milk production, it is more often the controlling factor for the intake of lactating cows than in the past. What has changed is our quantitative understanding of the factors affecting NDF intake and the mathematical tools available to gain additional insight into the magnitude and direction in which the chemical, physical and bioavailable attributes of NDF can influence intake.

It is clear that the chemical concentration of NDF in feeds and in the ration is the single most important factor defining the effect of NDF on DMI. The NDF-Energy Intake System of Mertens (1987, 1992) uses this relationship to effectively adjust the F:C of rations to define the upper boundary for intake at a given target of milk production and to describe the ration that maximizes the forage content of the ration for a target level of milk production. Next in importance is particle size of the NDF because it not only affects the fill volume in the rumen, but it also has a dramatic affects on the passage of NDF through the animal. The simple mathematical descriptions of the physical and physiological mechanisms of intake regulation make it clear that it is the flows of nutrients or residues that define the intake constraint.

Finally, characteristics of the fiber that influence its digestibility and physical breakdown will have lesser impact that NDF concentration and particle size on the relationship between NDF and DMI, but these effects can be significant and should not be ignored. Increasing NDFD by first decreasing the indigestible fraction and second by increasing the rate of digestion will increase the intake of NDF or reduce its impact on DMI. Feeds with NDF that disintegrates more rapidly due to digestion or chewing activity will not only occupy less space in the rumen but also pass more quickly leaving space for additional intake.

To use the knowledge we gain about the flow of NDF through the cow and its impact on DMI we need quantitative feed formulation systems that builds on the simple framework described by the NDF-Energy Intake System. Dynamic and steady-state models can provide additional information about the mechanisms that alter the basic relationship between NDF and DMI.

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THE CHANGING ROLES OF INSULIN DURING THE TRANSITION PERIOD

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The overall profitability and health of dairy cattle benefit from successful management during the transition period (Drackley, 1999). The metabolic adaptations that support the onset of lactation include increased mobilization of fatty acids from adipose tissue and increased hepatic gluconeogenesis (Bell, 1995). Orchestration of nutrient partitioning is necessary to meet new metabolic demands corresponding with a simultaneous decrease in dry matter intake around calving, requiring mobilization of body fat reserves (Bauman and Currie, 1980). These responses increase circulating non-esterified fatty acids (NEFA), which when taken up by the liver in excess induce triglyceride accumulation and increase risk for fatty liver and other disorders such as ketosis, milk fever, retained placenta, and mastitis (Goff and Horst, 1997). Many of these relationships relate to the changing roles of insulin. A more thorough understanding of these roles may enable us to continue to improve the management of transition dairy cattle and further reduce the incidence of metabolic disorders.

Insulin is a protein hormone secreted by the β-cells of the pancreas which stimulates translocation of glucose transporters such as GLUT4, resulting in glucose uptake by tissues. In ruminants, volatile fatty acids from the gastrointestinal tract are the major energy source rather than direct sources of glucose. Thus, insulin plays a slightly different role in ruminants vs. nonruminants, though many aspects of insulin metabolism are the same. Over the past two decades, the concept of insulin resistance has garnered much research attention with the increasing prevalence of obesity, Type II Diabetes, and metabolic syndrome in humans. Insulin resistance involves changes in sensitivity (the amount of hormone required to illicit a response) or responsiveness (the maximum response to a hormone) (Kahn, 1978). In ruminants as in humans, insulin resistance can involve changes in sensitivity, responsiveness, or both. For the purpose of this paper, the term insulin resistance will be used with the implication that the relative importance of sensitivity or responsiveness is not known in most cases. In addition, insulin resistance is an over-arching term – in the transition cow insulin resistance occurs in different tissues during different metabolic states. We believe that the relationships of insulin, insulin resistance, and adipose tissue function is particularly important in the transition cow.

The major effects of insulin on adipose tissue are increased lipogenesis and inhibition of lipolysis. Accordingly, if adipose tissue is resistant to insulin, the net effect will be mobilization of body reserves and increased plasma NEFA. This is a normal response that occurs in early lactation in high producing dairy cattle. However, it is beneficial to avoid extreme loss of body condition score in early lactation. As will be discussed, it appears as though cows that gain more body condition during the dry

period have more dramatic increases in NEFA around the time of calving. It may be analogous to why obese people are more likely to be Type II Diabetic. In ruminants, there are also concerns related to maximizing dry matter intake (DMI). High plasma NEFA will serve to further drive down DMI at a time when it is already low, sending the animal into more severe negative energy balance. It is this vicious cycle that dairy producers would like to avoid. What roles might changes in insulin metabolism play in this cycle, especially for cows that are over-conditioned during the dry period, and how does stage of production alter management of these signals?

FAR-OFF DRY

Cows during the early far-off dry period are experiencing relatively low metabolic demands as fetal growth is just beginning to accelerate and cows are no longer lactating. Fetal and uterine tissues are insulin-independent and so as energy demands increase with days of pregnancy, maternal peripheral tissues will become more insulin resistant in order to support fetal growth (Bell, 1995). These changes in the role of insulin have effects on energy metabolism in dry cows.

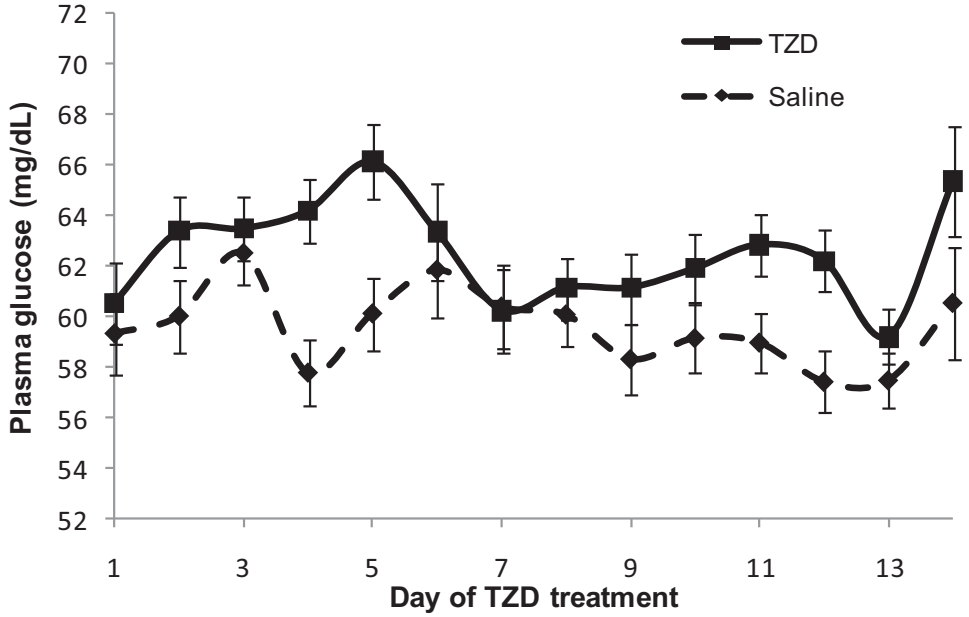
Much of the work in far-off dry cattle has investigated the effect of dry cow diets on the transition period. Researchers have sought to answer why cows that are over-fed during the dry period have more severe decreases in dry matter intake and higher incidences of metabolic disorders during the transition period. It is our hypothesis that increased body condition, or at least increased dietary energy level, makes cows more insulin resistant, leading to abnormal metabolic regulation. Cows fed 178% of calculated energy requirements during the entire dry period, had greater insulin responses to glucose challenge, indicating insulin resistance (Holtenius et al., 2003). Likewise, ewes that are over-conditioned have decreased insulin sensitivity as measured by the hyperinsulinemic-euglycemic clamp technique (Bergman et al., 1989). It is also likely that increased adipose deposition in ruminants results in changes in regulation of adipose tissue hormones such as leptin, TNF-α, adiponectin, and others. For instance, in the study mentioned above, over-fed cows also had higher circulating leptin levels, which is an energy metabolism and intake regulator (Holtenius et al., 2003). These changes will further disrupt insulin signaling.

Dann et al. (2006) found that cows fed 150% of NRC requirement in the far-off dry period had higher NEFA and BHBA and lost more body weight post-calving despite having higher insulin levels. This would further suggest insulin resistance effects on adipose tissue metabolism. Overfeeding during the dry period results in increased esterification rates in adipose tissue prepartum (concurrent with higher circulating insulin) and greater lipolytic rates (and thus higher NEFA) post-partum (Rukkwamsuk et al., 1999b). The practical result proves that cows overfed during the dry period loose more body condition postpartum. Additional post-partum effects will be discussed in later sections.

Recently, a study was completed in order to determine the effects of the insulin-sensitizing agent 2,4-thiazolidinedione (TZD) and dietary energy level on responses to

glucose and insulin challenges during the dry period (Schoenberg et al., unpublished data). Multiparous Holstein cows (n = 32) approximately 50 days prior to expected calving date were dried-off and assigned to one of two dietary energy levels for three weeks (High (H) 1.58, or Low (L) 1.46, Mcal/kg NEL) and treated daily the final 2 weeks with 4.0 mg TZD/kg BW (T) or saline (S) in a completely randomized design. Cows fed the L diet had lower DMI (12.8 vs. 16.1 kg/d; $P < 0.001$) and higher plasma NEFA (103.3 vs. 82.4μEq/L; $P < 0.001$) than cows fed H. Figure 1 shows that TZD treatment increased plasma glucose ($P = 0.03$).

Figure 1. Concentration of plasma glucose for cows administered TZD or saline for two weeks. Values are least squares means with SEM; n = 16 for TZD and Saline; $P = 0.03$.



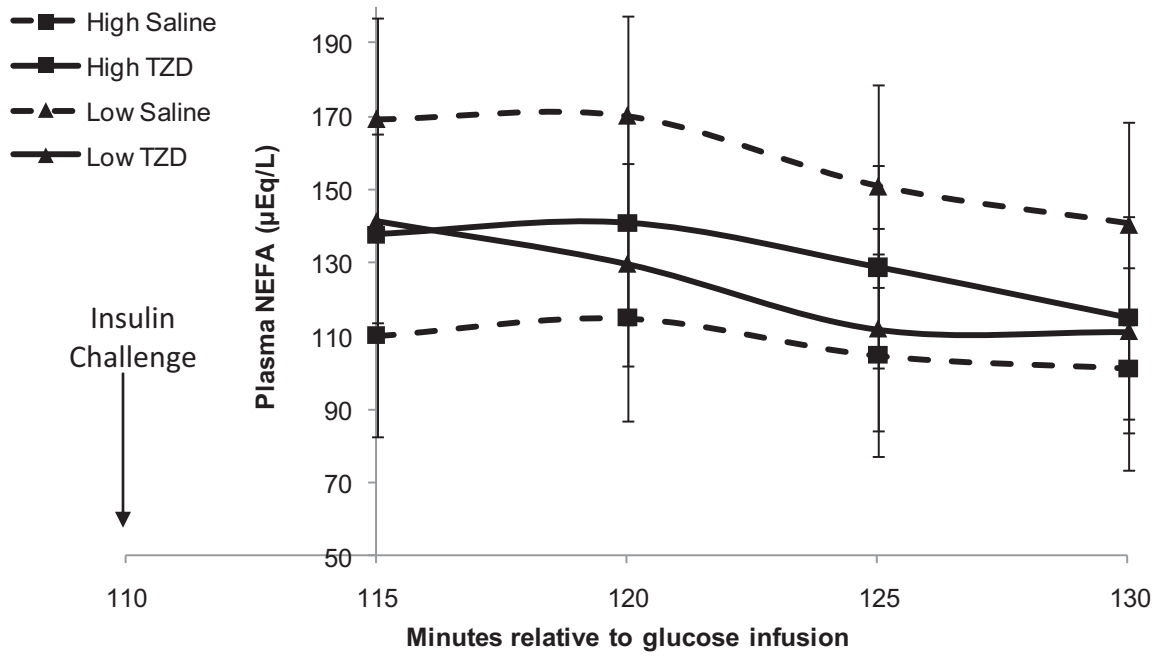
After two weeks of TZD treatment, all cows were subjected to an intravenous glucose tolerance test (GTT; 0.25 g dextrose/kg BW) followed 110 minutes later by an insulin challenge (IC; 1.0 μg/kg BW). Table 1 shows plasma glucose responses to GTT while Table 2 shows plasma NEFA responses. Response measures to glucose and insulin challenges often involve area under the curve (AUC) calculations to estimate response over time after the challenge. There was a trend for cows fed the H diet to have lower 90 min area AUC for plasma glucose during GTT. Since the glucose AUC was smaller, this suggests greater glucose clearance and thus less insulin resistance. However, L-fed cows had more negative (given we are measuring decreases in NEFA) NEFA AUC and greater NEFA clearance during GTT, suggesting differential responses to dietary energy level in tissue glucose and fatty acid metabolism. TZD is a peroxisome proliferator activated receptor-gamma ligand, meaning its effects will be specific to adipose tissue. Thus, affecting lipid metabolism more dramatically than whole-body glucose metabolism might be expected.

During IC, TZD-treated cows had a tendency for a more negative glucose AUC during the first 15 minutes, suggesting that TZD-treated cows may be less insulin

resistant. Figure 2 shows plasma NEFA response to insulin challenge. Again, effects on lipid (vs. glucose) metabolism were more noteworthy during IC. Body condition score had significant influence on many response variables measured including glucose AUC and clearance rate during GTT, and NEFA AUC during IC. Interaction of diet and TZD

was only significant for NEFA responses to IC. These results indicate that energy level and insulin-sensitizing agents during the dry period can have differing effects on glucose and lipid metabolism. Further work is necessary to fully determine potential interaction and the lasting effects on metabolism in the transition period.

Figure 2. Plasma NEFA response to insulin challenge after 3 wks of two different dietary energy levels (Low or High) and 2 wks of TZD or Saline administration. N = 8 for each treatment combination. Values shown are least squares means and error bars represent SEM. NEFA AUC was calculated for the 15 min post-insulin challenge and was lowest for the Low TZD group (*P* = 0.04; Diet*TZD, *P* = 0.04).



CLOSE-UP

Towards the end of pregnancy, nutrient demands increase by 75% (Bauman and Currie, 1980). Additionally, the timing of greatest energy demand for fetal growth corresponds with mammary development. In order to meet the increasing energy demands, peripheral tissues become insulin resistant and glucose and insulin levels decrease as calving approaches (Bell and Bauman, 1997). Uterine tissues, which are insulin-independent, take up 46% of the maternal supply of glucose during this time (Bell, 1995). This “glucose sparing” effect preferentially partitions nutrients to pregnancy.

Table 1. Effects of diet and TZD-treatment on glucose response to i.v. glucose tolerance test

	Diet				TZD				TZD*Diet Interaction					
Measure[a]	High	Low	SEM	*P*	Saline	TZD	SEM	*P*	High Saline	High TZD	Low Saline	Low TZD	SEM	*P*
Basal glucose (mg/dL)	67.8	61.2	1.6	**0.01**	65.0	64.0	1.6	0.64	70.0	65.6	60.1	62.4	2.2	**0.14**
Minimum glucose (mg/dL)	57.3	54.7	2.0	0.38	55.6	56.4	2.0	0.79	57.9	56.7	53.4	56.0	2.8	0.50
Maximum glucose (mg/dL)	182.2	180.6	4.4	0.81	182.2	180.6	4.3	0.79	187.6	176.7	176.8	184.4	6.2	**0.14**
CR_{30} (%/min)	2.61	2.5	0.13	0.53	2.51	2.59	0.13	0.67	2.66	2.56	2.37	2.62	0.18	0.35
$T_{1/2}$ (min)	27.4	29.6	1.5	0.32	29.1	27.9	1.5	0.57	27.2	27.6	31.0	28.2	2.1	0.45
T_{basal} (min)	49.3	55.1	2.8	0.16	53.8	50.6	2.8	0.43	49.9	48.8	57.8	52.4	3.9	0.59
AUC_{30}	1826	1979	97	0.28	1998	1808	97	0.18	1946	1706	2049	1911	137	0.71
AUC_{60}	2029	2418	178	**0.13**	2367	2080	178	0.26	2164	1893	2571	2266	252	0.95
AUC_{90}	1895	2410	231	**0.13**	2289	2017	231	0.41	2021	1771	2558	2263	327	0.95

[a] Basal glucose = mean glucose concentration at -15 and -5 min prior to GTT; Minimum glucose = minimum glucose during GTT; Maximum glucose = maximum glucose during GTT; CR_{30} = clearance rate during the first 30 min of GTT; $T_{1/2}$ = Time to reach half maximal glucose concentration; T_{basal} = time to reach basal glucose concentration; AUC_{30} = area under the curve during the first 30 min of GTT [mg/dL x 30 min]; AUC_{60} = area under the curve during the first 60 min of GTT [mg/dL x 60 min]; AUC_{90} = area under the curve during the first 90 min of GTT [mg/dL x 90 min]

Table 2. Effects of diet and TZD-treatment on plasma NEFA response to i.v. glucose tolerance test

	Diet				TZD				TZD*Diet Interaction					
Measure[a]	High	Low	SEM	*P*	Saline	TZD	SEM	*P*	High Saline	High TZD	Low Saline	Low TZD	SEM	*P*
Basal NEFA (μEq/L)	92.2	142.4	14.8	**0.03**	119.2	115.4	14.7	0.86	87.0	97.5	151.4	133.3	21.0	0.50
Minimum NEFA (μEq/L)	44.0	54.0	4.2	**0.11**	50.6	47.4	4.2	0.59	43.7	44.2	57.4	50.5	5.9	0.54
CR_{60} (%/min)	0.63	1.35	0.18	**0.01**	0.98	1.0	0.18	0.94	0.60	0.66	1.36	1.34	0.26	0.86
$T_{1/2}$ (min)	136.3	92.2	31.4	0.33	145.6	83.0	31.4	0.17	203.4	69.1	87.7	96.8	44.5	**0.12**
GSRN	49.6	40.6	3.1	**0.05**	48.1	42.2	3.1	0.19	51.7	47.5	44.5	36.9	4.4	0.71
AUC_{30}	-641	-1086	246	0.21	-819	-906	246	0.81	-501	-780	-1138	-1032	348	0.58
AUC_{60}	-1827	-3247	586	**0.09**	-2432	-2642	586	0.80	-1534	-2120	-3331	-3165	829	0.95
AUC_{90}	-2137	-4838	880	**0.04**	-3413	-3562	880	0.91	-1774	-2599	-5349	-4326	1244	0.49

[a] Basal NEFA = mean NEFA concentration at -15 and -5 minutes prior to IVGTT; Minimum NEFA = minimum NEFA during IVGTT; $CR6_0$ = clearance rate during the first 60 min of IVGTT; GSRN = glucose-stimulated reduction of NEFA (% of basal); $T_{1/2}$ = Time to reach half maximal NEFA concentration; AUC_{30} = area under the curve during the first 30 min of IVGTT [μEq/L x 30 min]; AUC_{60} = area under the curve during the first 60 min of IVGTT [μEq/L x 60 min]; AUC_{90} = area under the curve during the first 90 min of IVGTT [μEq/L x 90 min]

Interestingly, if cows are overfed during the far-off dry period as discussed previously, they still lose more body weight and have lower DMI during the transition period independent of the close-up diet (Dann et al., 2006). During late pregnancy, metabolic demands shift to fetal growth and the tissues of the dam become increasingly

insulin resistant in order to supply substrates for the fetus while maternal tissues increase reliance on NEFA and ketones (Bell, 1995). These changes in metabolic priorities might help to explain why far-off diets seem to have a more lasting effect on energy balance and metabolic health in early lactation.

EARLY LACTATION

As a cow transitions into early lactation, the mammary gland requires up to 80% of total body glucose turnover (Bauman and Currie, 1980). Keep in mind that this demand is independent of insulin signaling as the mammary gland response is insulin-independent. Circulating levels of NEFA increase as a cow approaches calving and intake begins to decrease (Bell, 1995). Artificial hyperlipidemia was used in dairy cattle to mimic the increases in NEFA during late pregnancy, and successfully illustrate the negative effects that rising NEFA have on insulin signaling (Pires et al., 2007). Given decreased glucose clearance during a GTT and IC, the authors concluded that excessive elevation of NEFA during the dry period and early lactation will lead adipose tissue to be further insulin resistant, further drive down dry matter intake, and perpetuate a cycle of metabolic disorders in these cows (Pires et al., 2007).

When cows have higher BCS at calving (induced by feeding increasing levels of DMI throughout the dry period as discussed), they experience prolonged negative energy balance in lactation as compared to cows that gain less (Agenas et al., 2003). On the other hand, cows are able to cope metabolically post-partum with under-nutrition occurring during the dry period vs. over-nutrition. Cows that have moderate to low energy balance during the dry period and are offered high-quality nutrition during early lactation loose less weight and produce the same amount of milk (Agenas et al., 2003).

In the transition from late pregnancy to early lactation, not only do plasma insulin levels fall, but adipocytes also become insulin resistant (Bell and Bauman, 1997). The end results are increased transcription of lipolytic enzymes (lipoprotein lipase, acetyl CoA carboxylase) and stimulation of glucose transport which is ultimately observed as onset of milk production concurrent with “dumping” of body reserves and a rise in NEFA (Vernon and Pond, 1997). The overall effects of these changes are that glucose is spared for the mammary gland in order to support milk production. In this way, cows are able to lose body condition at the expense of milk production and they are able to coordinate the metabolic changes associated. Figure 3 summarizes the metabolic changes that occur in early lactation.

One of the major changes occurring during early lactation is decreased lipogenic activity in adipose tissue. While the overall effect of decreasing lipogenic activity of adipose tissue is to supply milk fat precursors to the mammary gland, there are additional negative consequences on tissues throughout the body. Primarily, the liver is affected if uptake of fatty acids exceeds its ability to oxidize them or export them in the

form of very low density lipoproteins (Goff and Horst, 1997). Cows given free access to feed during the dry period have as much as two-fold higher postpartum hepatic triglycerides (Van den Top et al., 1996). The resulting accumulation of triglycerides in the liver, known as fatty liver, decreases the gluconeogenic capacity (Goff and Horst, 1997). If the liver has decreased ability to make glucose to support milk production, this further extends the amount of lipolysis which must occur in order to support lactation

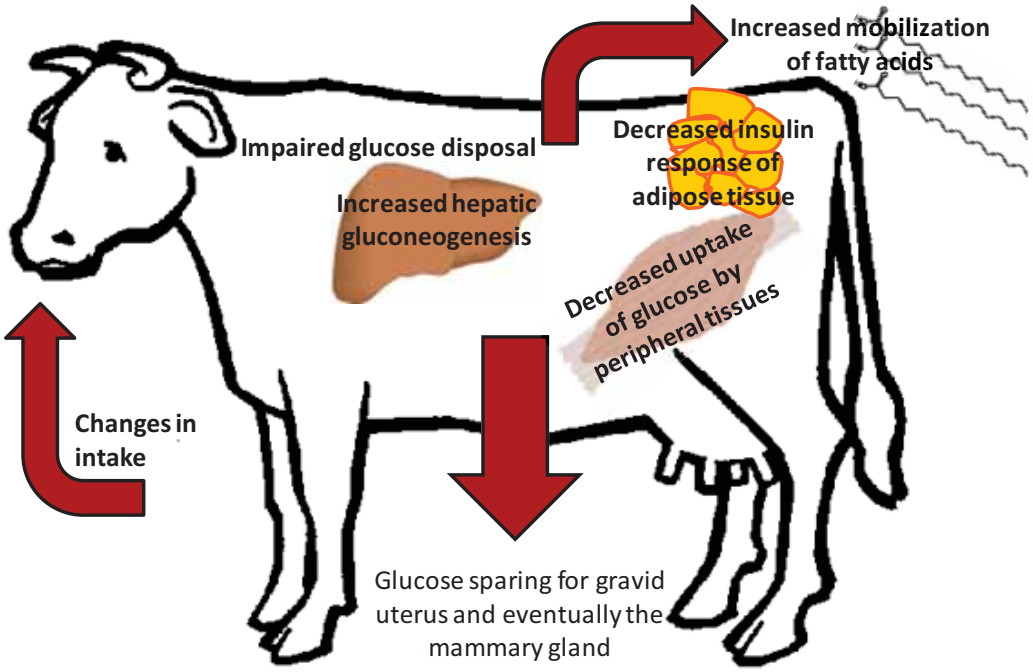


Figure 3. Metabolic adaptation during the transition period.

(Rukkwamsuk et al., 1999a). As already mentioned, NEFA themselves drive down DMI and thus the cow in early lactation finds herself in a vicious cycle driving her further into negative energy balance.

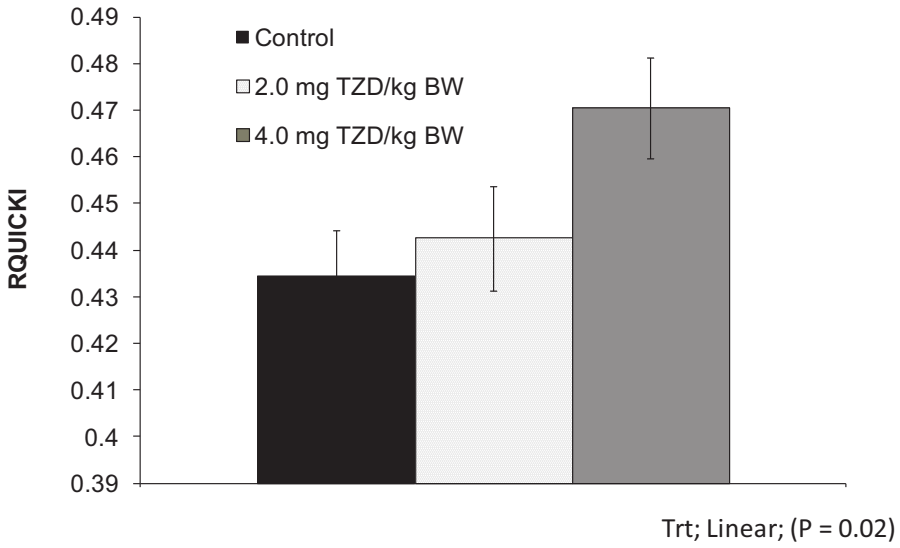
Therefore, there are several control points, as described by Drackley (1999), that perturb the system and result in fatty liver: delivery of NEFA to the liver, uptake of NEFA into mitochondria via CPT-1, regulation of mitochondrial ketogenesis, regulation esterification steps, portioning of newly synthesized triglycerides between secretion and storage, and export of very low density lipoproteins. One way to improve the metabolic health of transition dairy cattle, and especially of those over-conditioned during the dry period, would be to decrease the delivery of NEFA to the liver while still maintaining milk production.

The potential for the use of an insulin-sensitizing agent in transition dairy cattle has recently been explored to do just that. Smith et al. treated dry cows three weeks pre-partum with TZD and saw increased DMI peri- and postpartum intakes (2007), as well as decreased NEFA and liver trigylcerides postpartum (2009). RQUICKI has been used as a relative measure of insulin sensitivity in ruminants (Holtenius and Holtenius, 2007).

A greater RQUICKI, calculated via plasma insulin, glucose, and NEFA values, suggests greater insulin sensitivity. Figure 4 shows the linear response of RQUICKI postpartum.

There may be future potential to use TZD or other insulin-sensitizing agents to improve the metabolic health of transition dairy cattle. Cows that are over-conditioned during the dry period are likely to benefit most from this intervention. At the very least, there is opportunity to affect insulin signaling in these animals in order to further characterize the mechanism by which these cows have metabolic perturbations.

Figure 4. The effects of prepartum TZD treatment on a postpartum insulin sensitivity measure, RQUICKI. A greater RQUICKI suggests greater insulin sensitivity as determined by the following calculation: (1/[log(glucose) + log(insulin) + log(NEFA)])). N = 40. Values represent least squares means and error bars SEM, *P* = 0.02.

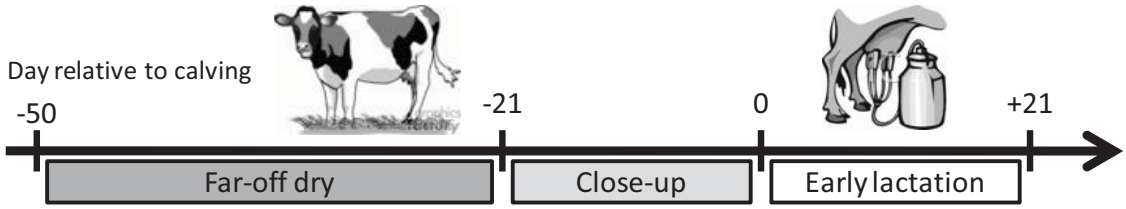


CONCLUSIONS

Tissue responses, circulating levels, and the very roles of insulin change throughout the dry period and into lactation as shown in Figure 5. We are beginning to understand those changing roles and their effects on the metabolic health of dairy cattle. The relationships shown in Figure 5 are related to ideal management conditions where cows are not overfed nor gain excessive body condition during the dry period. However, as discussed, feeding high dietary energy levels during the dry period can make cows more severely insulin resistant. This increased resistance will lead to more dramatic negative energy balance and increased incidences of metabolic disorders during the transition period. This is not unlike the insulin resistance phenomenon we see in overweight Type II Diabetic humans. Therefore, it is beneficial to maintain appropriate relationships between insulin, glucose, and fatty acids to support milk production in early lactation under normal metabolic regulation. This knowledge will help dairy producers make better management decisions, especially during the dry period. The

reason why cows overfed during the dry period eat less, make less milk, and have more metabolic disorders during transition is likely related to alterations in insulin resistance. Therefore, managing metabolic responses during this period will allow for identification of or prevention for insulin resistant animals. These management decisions will be related to the changing roles of insulin at these time points.

Figure 4. Relative changes in insulin, glucose, and NEFA metabolism during the transition from late pregnancy to early lactation.



Relative levels; (under appropriate management)

Plasma insulin	High	Moderate	Low
Plasma glucose	High	Moderate	Low
Plasma NEFA	Low	Moderate	High
Insulin resistance	Low	High	Moderate

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HOW MUCH GAS DO COWS PRODUCE?

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The livestock sector has been reported to be responsible for 18% of the total anthropogenic greenhouse gas (GHG) emissions on a worldwide basis (FAO, 2006). The GHG's considered in this publication are carbon dioxide, methane and nitrous oxide. It is important to remember that this is a global estimate and does not differentiate between animal types, production systems or animal productivity. A recent report from the same organization used a life cycle assessment approach to estimate GHG emissions from the dairy sector (FAO, 2010). In this report, the global dairy sector contributed 2.7 to 4% of the total anthropogenic GHG emissions. The lower figure includes emissions associated with milk production, processing and the transportation of milk and milk products. The higher figure adds the emissions related to meat production from culled or fattened animals. This report also provides information on regional emissions. Regional emissions range from 1.3 to 7.5 kg CO_2 equivalent per kg of fat and protein corrected milk (FPCM). North American and Europe had the lowest GHG emissions per kg FPCM. The highest value was in Sub-Saharan Africa. In this report, the U.S. provided about 16% of the total world milk production but about 8% of the total GHG emissions associated with the dairy sector.

Total GHG emissions in the U.S. increased by 13.5% from 1990 to 2008 (EPA, 2010). However, there was a 2.9% decrease between 2007 and 2008 due primarily to changes in fuel used in the transportation sector. The agricultural sector accounted for 6% of the total U.S. GHG emissions in 2008. During this same time period, methane emissions decreased by 7.5% and nitrous oxide emissions declined by 1.3%. Enteric fermentation from ruminants accounted for 25% of the total methane emissions in 2008. This is a 6.4% increase since 1990. Dairy cattle were responsible for about 23.5% of enteric methane emissions while beef cattle accounted for 71.5% of these emissions.

Even though it is not a GHG, EPA also has an inventory of ammonia emissions from animal operations (EPA, 2004). In this report, they estimated ammonia emissions from 2002 through 2030. Total ammonia emissions from animal operations were reported as 2.42 million tons in 2002. This was projected to increase to 2.67 million tons in 2030. Dairy and beef cattle accounted for 23 and 27% of these total emissions in 2002. The total N lost as NH3 from dairy operations was estimated to be 38%.

NITROUS OXIDE

Nitrous oxide (N_2O) is a concern since it has a global warming potential (GWP) of 310 times that of carbon dioxide (EPA, 2010). The majority of the N_2O emitted on dairy farms is from soil and manure. The quantity of nitrous oxide emitted directly from dairy cattle is very small. A recent study from Japan estimated that the daily emission of N_2O-N from cattle was 5.2 (range 2 to 9.9) mg/day (Kurihara et. al., 2010). A paper from California reported a N_2O emission rate of about 0.02g/cow/hour for cows housed in

environmental chambers (Mitloehner et.al, 2009). A simulation model approach has also been used to examine total N_2O emissions from a dairy farm (Chianese et.al, 2009d). This model used a 100 cow dairy herd housed in a free-stall barn with milk production of 19,800 lbs/cow/year. Manure was stored as slurry and spread on the cropland twice per year. The base run had a total yearly N_2O emission of 681 kg. This was divided into 485 kg from crop production and 197 kg from manure storage. The total yearly N_2O emissions could be reduced to 421 kg/year by making more efficient use of the nitrogen fertility program and using a cover crop on the corn land.

CARBON DIOXIDE

Agriculture is not identified as a major source of carbon dioxide (CO_2) emissions (EPA, 2010). However, CO_2 emissions do occur on farms due mainly to animal respiration and decomposition of soil organic matter (Chianese et al., 2009b). These same authors conclude that CO_2 emissions from animal respiration accounts for about 90% of the total carbon dioxide emissions on a dairy farm. The average daily CO_2 emission for dairy cows producing 63 lbs of milk per day was 6,137 liters (Kinsman et, al., 1995). The range was 5,042 to 7,427 liters/day over a 6-month monitoring period. A recent paper reported the CO_2 emissions using data summarized from the USDA Energy Metabolism Unit (Casper and Mertens, 2010). This is a large dataset obtained from animals using the indirect respiration chambers. This includes >1.200 individual lactating cow trials with an average daily milk production of 51 pounds per day. The range in milk production was from 11 to 125 pounds per cow per day. Key points from this paper are:

- The average daily CO_2 emission was 5,309 liters/day (range = 2,035 to 8,682).
- Daily CO_2 emission was highly related to milk production and dry matter intake. Higher producing cows had higher CO_2 emissions.
- The average CO_2 emission was 0.14 g/kg of milk per day. The range reported was 0.96 to 0.54 g/kg milk.
- Higher producing cows had lower emission factor per unit of milk produced.

Total farm CO_2 emissions were determined on the model farm described above using a simulation model (Chianese et. al., 2009b). The net yearly CO_2 emission was 150,479 kg. This increased by 22% if the farm increased alfalfa acres and decreased corn acres. However, if corn replaced all of the non-permanent grassland, yearly CO_2 emissions were lowered to 35,198 kg.

AMMONIA

Ammonia emissions from agriculture are receiving attention due to air quality concerns. More importantly, ammonia emissions represent losses of N from the farm and usually indicate a lower efficiency of N use. The primary means of ammonia emissions by ruminants is as a result of conversion of urea-N in urine to ammonia. The following points summarize this process:

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

A key factor in reducing ammonia losses on a dairy farm is to balance rations decrease N excretion in manure. Figure 1 contains an example of the relationships between N intake and manure N excretion. Note that manure N excretion increases with the higher CP rations.

Figure 1. Nitrogen intake and manure N excretion in dairy cows (Olmos Colmenero and Broderick, 2006)

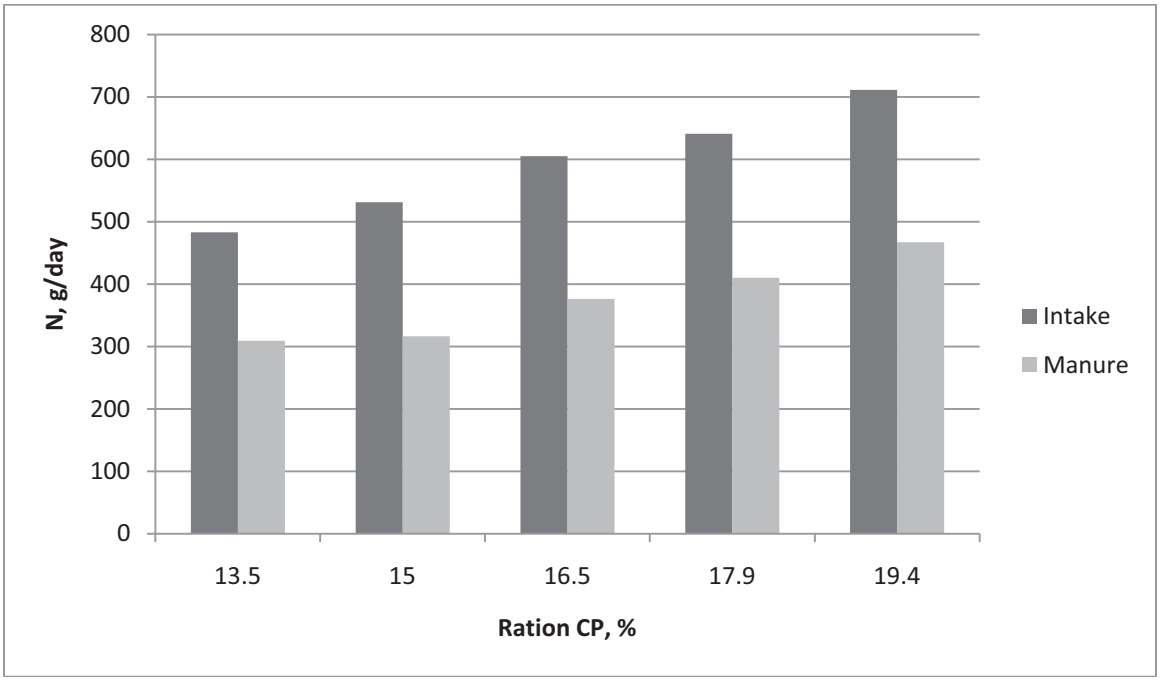
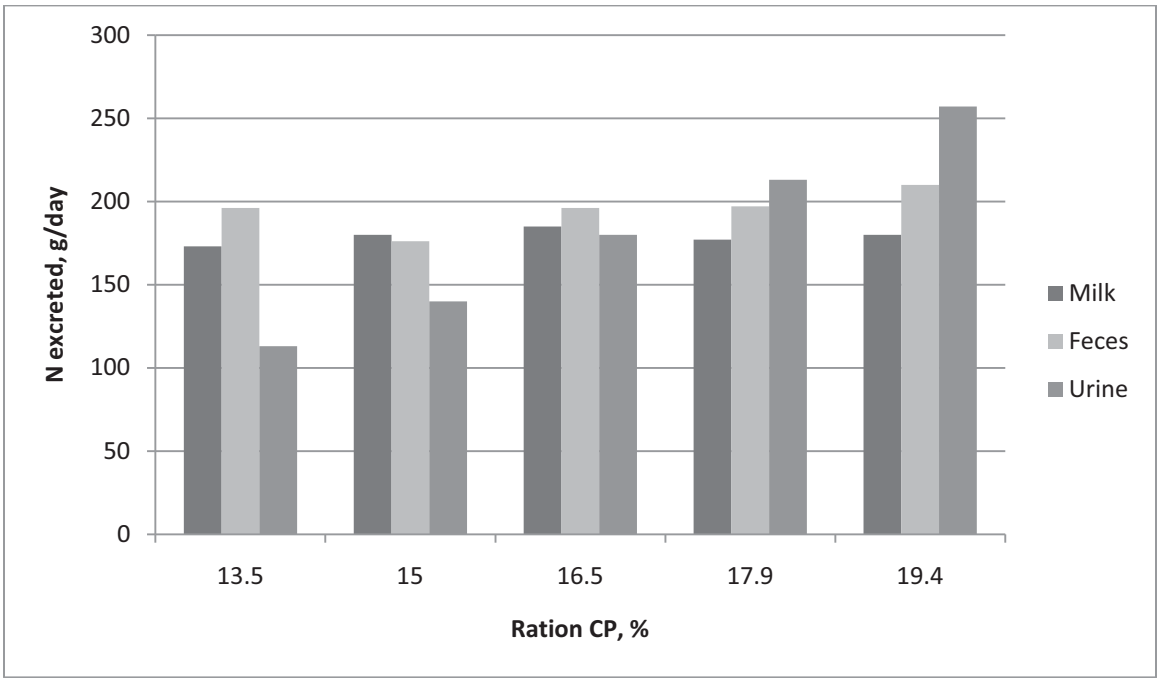


Figure 2 contains the N excreted in the milk, urine and feces from this same trial. It is interesting to note that daily N excretion via milk and feces is relatively constant across this range of ration CP levels. Milk production of the cows in this short term trial was about 80 lbs/day and was not significantly different between ration CP levels. The main route of excreting N as ration CP increased was via the urine. The urea-N proportion of the total urinary N increased from 55.4 to 81.8% as ration CP increased. This would indicate a higher potential for ammonia volatilization with higher ration CP levels.

Figure 2. Daily N Excretion in Milk, Urine and Feces (Olmos Colmenero and Broderick, 2006)



How do these higher urinary urea-N (UUN) levels correspond to ammonia emissions? Growing dairy heifers were fed total mixed rations with 9.6 or 11% CP (James et. al., 1999). Heifers fed the lower CP ration consumed 14% less N/day and had a 28.1% decrease in ammonia emissions. There was a linear relationship between N intake (g/day) and UUN excretion (g/day). The relationship between daily N intake and NH3 emissions was also linear. Both UUN excretion (g/day) and ammonia emissions (gN/cow/day) increased as ration CP levels for lactating dairy cows increased from 15 to 21% CP (Burgos et, al., 2007).

The relationship between milk urea nitrogen (MUN) and ammonia emissions have also been reported. A number of papers have reported positive relationships between MUN and ammonia emissions (van Duinkerken et. al., 2005; Powell et al., 2008 and Burgos et. al., 2010). The results from these papers indicate that equations to predict ammonia emissions could be included in models using either UUN or MUN as the predictive function.

A major nutritional variable that influences N use efficiency and potential ammonia emissions is the rumen balance of RDP relative to requirements. One paper examined this question using diets with 12.9, 13.4 and 15.4% CP for dairy cows producing about 66 lbs. of milk per day (Agle et. al., 2010). Urinary N excretion and cumulative manure NH3 emissions were reduced on the lower CP diets. Another paper used a large number of diets varying in alfalfa (as % of the forage fed), starch and MP levels to evaluate N metabolism in dairy cows (Weiss et. al., 2009). Alfalfa comprised between 25 and 75% of the total forage fed. Diet starch levels ranged from 22 to 30%. These

diets contained 10.7% RDP but MP ranged between 8.8 to 12% of diet DM. Ammonia produced per gram of manure increased as diet MP increased. As higher levels of alfalfa were fed, the ammonia produced per gram of manure decreased. Ammonia produced per unit of manure was lowest and milk protein yields were highest for a diet containing 75% of the total forage as alfalfa, 11% MP and 30% starch.

The carbohydrate balance of the ration can interact with ration N to alter ammonia emissions. One trial reported that replacing ground corn with steam flaked corn lowered manure ammonia emissions (Burkholder et al., 2004). A trial was done using high CP diets (22%) for late lactation cows that contained different carbohydrate sources (Hristov et.al, 2005). They found that proving a rapidly fermentable carbohydrate source lowered rumen ammonia concentration and shifted some of the excreted N from the urine to the feces.

A number of papers have reported estimated annual ammonia emissions for dairy cattle. A yearly emission factor of 84 lbs of NH3/dairy cow/year was reported by EPA (2004). An annual emission of 88 lbs NH3/dairy cow/year was reported from on-farm research on a 185 cow dairy in Washington (Rumburg et.al, 2008). Three dairy herds in Wisconsin were monitored and annual NH3 emissions of 41.8 to 44 lbs/cow were determined (Flesch et. al., 2009). A paper from Idaho reported an annual emission estimate of 125 lbs/.cow (Bjorneberg et. al., 2009). An annual NH3 emission factor of 20.7 lbs. has been reported for an open-lot dairy in Texas (Mukhtar et.al, 2008). These papers indicate some variation in these emission estimates. These differences are probably related to a number of factors include measurement technique, ration fed and environmental conditions. When EPA moves ahead with ammonia emission regulations for dairy and livestock farms, it will be critical to have a uniform and accepted method of estimating ammonia emission factors since on-farm monitoring is probably not realistic. A process based model will most likely be needed to determine ammonia emission factors.

METHANE

Methane is the GHG that has recently been receiving the most attention in popular press articles. Enteric methane emissions are produced by ruminants as a result of microbial breakdown of carbohydrates in the rumen. It is important to point out the changes that have taken place in methane emissions by dairy cattle over time. Table 1 contains data on methane emissions from dairy cattle in the U.S. in 1944 and 2007. The data in this table is **only** for milking cows and does not include replacement heifers and dry cows. Methane production was calculated using the CNCPS 6.1 model (Tylutki et. al., 2008). Capper and Bauman (2009) reported a 43% reduction in methane emissions when comparing dairy production systems of 1944 with 2007. In this paper, they included both dry cows and heifers in addition to milking cows. They also made adjustments for breeds and forage feeding systems.

Table 1. U.S. Dairy Cow Numbers and Methane Emissions

Item	1944	2007	2007, % of 1944
Milk cows, millions	25.6	9.1	35.5
Milk, lbs/cow/year	4,572	20,267	443
Total U.S. milk production, million lbs	117,023	185,602	159
Milk, lbs/cow/day	15	66	440
Methane, Mcal/day/cow	3.05	5.3	174
Methane, liters/cow/day	332	580	175
Total methane, liters/day, millions	8,499	5,278	62
Methane, liters/lb. milk	22	8.8	40

The main sources of methane emissions on dairy farms are enteric emissions and manure. Enteric methane accounts for about 75% of the total on-farm methane emissions (EPA, 2010). Mean daily methane production was reported as 587 liters/day for cows averaging 63 lbs/day (Kinsman et. al., 1995). Other studies have reported daily methane emissions ranging from 420 to 763 liters/cow/day (Chase, 2006). A second approach is to express methane production as a percent of the gross energy intake. Beauchemin et. al. (2008) indicated that 6 – 10% of the total energy intake was emitted as methane. The average methane production for lactating dairy cows was 5.49% of gross energy (GE) intake using the USDA Energy Metabolism Unit database (Wilkerson et. al., 1005). The range was 2.53 to 7.82 % of GE. These same workers reported an average methane production of 7.89% of GE for dry cows with a range of 3.47 to 10%. It is not clear how low this value could be and still maintain rumen function and milk production.

A simulation approach was used to examine methane emissions from dairy farms (Chianese et. al., 2009c). The same model dairy farm as described in the nitrous oxide portion of this paper was used in this simulation. This model indicated that the annual methane emission factors for the milking cows, dry cows and replacement heifers were 233, 127.6 and 169.4 lbs/animal unit. The weighted average value for the whole herd was 312 lbs/cow/year. Total yearly whole farm methane emission was 46,624 lbs with 68% of this from the animals and 32% from manure storage. These base runs used a ration that was 50% forage. Annual methane emissions increased by 15.6% if a ration containing 60% forage was fed. The annual methane emission was reduced by 8% if a high forage diet and seasonal grazing was used. The enteric methane emissions were increased by 2.3% when the high forage diet plus grazing was used. However, there was a 71% reduction in methane emissions from manure when this management change was implemented.

There are a number of strategies that can be used to alter methane emissions on dairy farms (Beauchemin et. al., 2008; Chase, 2008). These include using higher quality forages, feeding higher grain diets, using ionophores and the addition of various fats or

oilseeds to rations. However, there has been some variation in the amount of methane reduction associated with these in reported research studies. These diet alterations are already being used in many herds. In addition, there are a large number of proposed additives that could be added to diets to lower methane emissions. These include yeasts, tannin extracts, essential oils, fiber degrading enzymes, saponins and compounds such as garlic or oregano. While all of these may have potential to reduce methane emissions, additional research is needed before they will be routinely added to dairy rations.

SUMMARY

The dairy cow does emit considerable quantities of carbon dioxide, ammonia and methane. However, minimal quantities of nitrous oxide are emitted by the animal. There will be continuing pressure on the industry to further reduce ammonia and GHG emissions. There are a number of basic nutritional principles that can be used to approach this situation. The use of simulation models will also be important as part of this evaluation process to “estimate” the potential shifts in emissions due to alterations in variables such as level of milk production, forage quality, level of forage in the ration, rumen nitrogen and carbohydrate balance and feeding system (TMR, grazing, etc.). It will be important to evaluate the whole farm response in addition to the animal component (Chianese et. al., 2009a). The whole farm approach permits an evaluation of changes in emissions due to shifts in cropping programs, manure storage and manure application procedures. A key consideration in this process must be farm profitability and sustainability. It will also be critical to estimate the number of dairy cows and milk production levels in future years. Many of the larger scale emission reductions are looking ahead 10 – 30 years. Thus, any future reductions will be a combination of changes in emissions per animal and the total number of animals in the population.

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