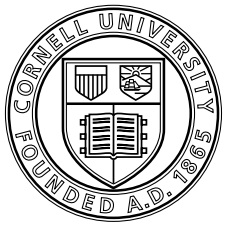

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Animal Science

of the

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Agriculture and Life Sciences

(A Statutory College of the State University of New York)

Cornell University, Ithaca, NY 14853

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2009 Cornell Nutrition Conference for Feed Manufacturers

Conference Program

Tuesday, October 20, 2009

Pre-Conference Symposium by Adisseo

Theme: Impact of Amino Acid Formulation and Nitrogen Efficiency in Dairy Cows

1:00 – 1:10	Welcome and introduction of speakers
1:10 - 1:30	Current dairy industry economics *John Geuss*
1:30 – 2:15	Maximizing milk components and metabolizable protein utilization through amino acid formulation *Dr. Chuck Schwab*
2:15 – 3:00	Challenges of predicting metabolizable lysine content of ingredients *Dr. Sarah Boucher*
3:00 – 3:15	Break
3:15 – 4:00	The role of amino acid nutrition in reproduction of dairy cows *Dr. Jose Santos*
4:00 – 4:45	Formulating for amino acids in CNCPS – present and future *Dr. Mike Van Amburgh*
4:45 – 5:15	Summary and Q & A session *Dr. Brian Sloan*

Wednesday, October 21, 2009

Breakfast sponsored by BFI Innovations

6:30 - 8:00	The triangular relationship: diet, microbial activity and immune function; consequences for the dairy cow's longevity and performance. *Dr. Jan van Eys, North American Ruminant Nutrition Specialist, INVE Technologies/Nutri-Ad*

Morning Session – T. R. Overton presiding

8:30 – 9:00	Recent research with low-starch diets for lactating dairy cows *Dr. Heather Dann*
9:00 – 9:30	Estimating intestinal digestibility of amino acids in the rumen undegraded protein fraction of feedstuffs *Dr. Sarah Boucher*
9:30 - 10:00	Effect of intake on digestibility of NDF in soy hulls *Dr. Mike Thonney*
10:00 – 10:10	Presentation of Maynard Award *Dr. Dale Bauman*
10:10 – 10:40	Break
10:40 – 11:10	Refining nitrogen feeding using current knowledge of recycled urea and microbial nitrogen uptake *Erin Recktenwald*

11:10 – 11:40 aNDF, NDFd, iNDF, ADL and kd : What have we learned?
Emiliano Raffrenato

11:40 – 12:10 Effect of type and length of dietary fiber on growth, efficiency and carcass quality of feedlot cattle
Dr. Mike Baker

12:10 – 1:30 Lunch

Afternoon Session – L. E. Chase presiding

1:30 – 2:00 Negative energy balance and ketosis: consequences and monitoring in transition cows
Dr. Daryl Nydam

2:00 – 2:30 Integrating nutritional and grouping management of transition cows
Dr. Tom Overton

2:30 - 3:00 Gigantism and Sauropod dinosaurs
Dr. Peter Van Soest

3:00 – 3:30 Break

3:30 – 4:00 Phiddling with phenylalanine: influence of dietary amino acids on enzymes of phenylalanine metabolism in the chicken
Dr. Richard Austic

4:00 – 4:30 Perspectives on Cornell contributions to poultry nutrition
Dr. Kirk Klasing

4:30 – 5:30 Panel discussion – interpreting and implementing starch digestibility information in the field
Ralph Ward, Ian Shivas, Paul Sirois, Dr. Charlie Sniffen; moderated by Dr. Mike Van Amburgh

5:30 – 7:00 Cash Bar Reception and Dinner
Sponsored by CHR. Hansen and Cornell University

Thursday, October 22, 2009

Breakfast sponsored by APC, Inc.

6:30 – 7:30 Colostrum feeding follies: trials and tribulations of passive transfer
Dr. Jim Quigley, Vice President and Director of Calf Operations, APC, Inc.

Morning Session – D. E. Bauman presiding

8:30 – 9:15 Food economics and consumer choice
Rob Aukerman

9:15 – 10:00 Demystifying the environmental sustainability of food production
Dr. Jude Capper

10:00 – 10:20 Break

10:20 – 10:50 An evaluation of milk fatty acid composition of whole fluid milk in the United States.
Anne O'Donnell

10:50 – 11:20 Using forages in dairy rations: are we moving forward?
Dr. Debbie Cherney

11:20 – 11:50 Ammonia emissions form dairy farms - What have we learned?
Dr. Mark Powell

11:50 – 12:20 Feeding low crude protein rations to dairy cows – opportunities and challenges
Dr. Larry Chase

12:20 Adjourn – have a safe trip home!

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Erin Recktenwald - Animal Science
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Sarah Boucher - W. H. Miner Research Institute
Jude Capper - Washington State University
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MAXIMIZING MILK COMPONENTS AND METABOLIZABLE PROTEIN UTILIZATION THROUGH AMINO ACID FORMULATION

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University of New Hampshire
and
[2]Central Valley Nutritional Associates, LLC
Tulare County, California

Heger and Frydrych (1989) concluded, largely from research with non-ruminant animals, that when the essential amino acids (EAA) are absorbed in the profile as required by the animal, the requirements for total EAA is reduced and their efficiency of use for protein synthesis is maximized. Research with lactating dairy cows has been shown many times that increasing predicted concentrations of Lys and Met in metabolizable protein (MP) to recommended levels increases efficiency of use of MP for milk protein synthesis. This should not be a surprising observation in feeding situations where Lys and Met are the first two limiting AA. Therefore, it is reasonable to conclude that maximizing milk components and MP utilization in lactating dairy cows requires providing a profile of AA in MP that matches the profile required for the combined functions of maintenance, reproduction, and milk production, and that the MP is provided in amounts that meets but doesn't exceed requirements for optimal health, reproduction and milk production.

Research to date indicates that of the twenty AA that occur in proteins, only the amount and profile of EAA in MP are of concern. Providing a mixture of nonessential AA (NEAA) to post-weaned dairy calves (Schwab et al., 1982), or lactating dairy cows (Oldham et al., 1979; Schwab et al., 1976; Whyte et al., 2006), where one or more EAA were shown to be limiting, were without benefit. It was also observed that infusing the 10 EAA into the abomasum of lactating cows fed protein deficient diets resulted in increases in yields of milk protein that were similar to the yields that were obtained when casein was infused. Collectively, these observations indicate that when AA supplies approach requirements for total absorbable AA, requirements for total NEAA are met before the requirements for the most limiting EAA.

In recognition of these observations, and because Lys and Met had been shown to be the first two limiting EAA for lactating dairy cows fed diets common to North America, NRC (2001) published dose-response plots that related changes in measured percentages and yields of milk protein to model-predicted changes in Lys and Met concentrations in MP. By using a rectilinear model to describe the dose-response relationships, breakpoint estimates for the required concentrations of Lys and Met in MP for maximal content of milk protein were determined to be 7.2 and 2.4%, respectively; corresponding values for maximal protein yield were 7.1 and 2.4%. These were the first estimates ever presented by a Dairy NRC Committee to evaluate a diet for adequacy of AA concentrations in MP, and have proven exceptionally useful for routine users of the NRC (2001) model in their quest to increase milk component yields with similar or lower

predicted flows of MP. Because they can be rather easily achieved, target levels for Lys and Met in MP have typically been 6.6 and 2.2%, respectively. Both values approximate 96% of the concentrations needed, according to NRC (2001), for maximal content and yield of milk protein.

It is recognized that histidine (His) has been identified as the first limiting AA when grass silage and barley and oat diets are fed, with or without feather meal as a sole or primary source of supplemental RUP (Kim et al., 1999, 2000, 2001a, 2001b; Huhtanen et al., 2002; Korhonen et al., 2000; Vanhatalo et al., 1999). Based on NRC (2001) predicted concentrations of Lys, Met, and His in MP for the diets fed in these experiments, coupled with similar evaluations of diets where cows have (or have not) responded to increased levels of Lys and Met in MP, leads us to speculate that His may become the third limiting AA rather quickly in some diets, particularly where barley and wheat products replace significant amounts of corn in the diet.

The purpose of this paper is 3-fold: 1) to share the results of a recent re-evaluation of the Lys and Met dose-response plots using the "final version" of the NRC (2001) model, 2) to share the results of a recent effort to develop the same dose-response plots using CPM-Dairy (v.3.0.10) and AMTS.Cattle (v.2.1.), and 3) to review our approach for maximizing milk components and MP utilization.

RECENT RE-EVALUATION OF THE LYS AND MET DOSE-RESPONSE PLOTS USING THE "FINAL VERSION" OF NRC (2001)

To determine what the "requirements" for Lys and Met in MP are when the NRC (2001) model is used to evaluate diets, the NRC committee used the indirect dose-response approach described by Rulquin et al. (1993). This approach has the "unique benefit" of allowing requirement values to be estimated using the same model as that used to predict concentrations of EAA in MP.

Because the AA submodel [AA equations (see pages 74-81 in NRC 2001)] had to be developed before the final version of the NRC model was available, a beta version of the model was used to predict the concentrations of Lys and Met in MP in the studies used to develop the dose-response plots. Because of this, and concerned that changes may have been made to the rest of the model, as part of model validation, that may have changed predicted concentrations of Lys and Met in MP, Schwab et al. (2009) re-evaluated the Lys and Met dose-response plots using the final version of the model.

All steps, as stated in NRC (2001), were repeated. In brief, generating the dose-response plots involves 5 steps: 1) predicting concentrations of Lys and Met in MP for control and treatment groups in experiments in which postruminal supplies of Lys, Met, or both, were increased and production responses measured, 2) identifying "fixed" concentrations of Lys and Met in MP that are intermediate to the lowest and highest values in the greatest number of Lys and Met experiments, 3) calculating, by linear regression, a "reference production value" for each production parameter in each Lys experiment that corresponds to the "fixed" level of Lys in MP and in each Met

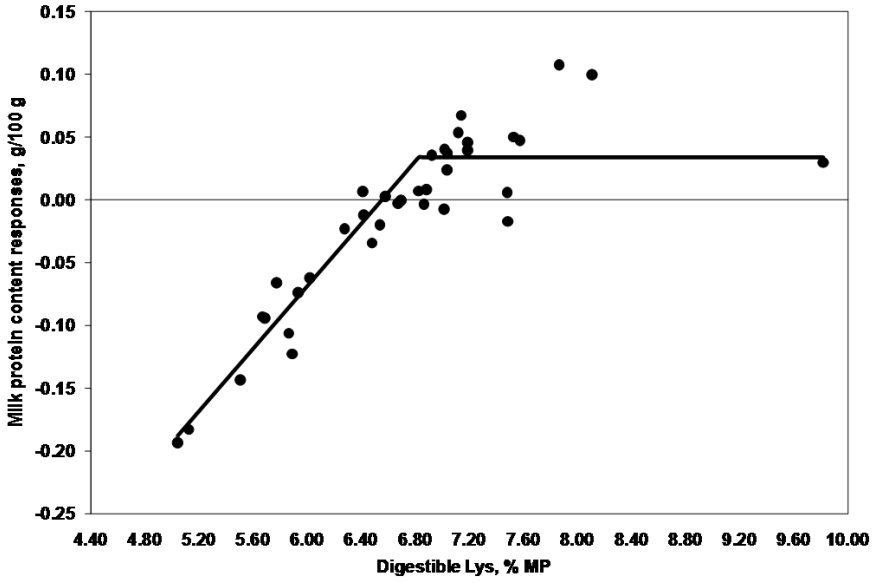
experiment that corresponds to the “fixed” level of Met in MP, 4) calculating “production responses” (plus and minus values) for control and treatment groups relative to the “reference production values”, and 5) regressing the production responses on the predicted concentrations of Lys and Met in MP.

The “revised” dose-response plots that relate changes in milk protein concentrations to changes in predicted concentrations of Lys and Met in MP for the NRC (2001) model are presented in Figure 1. While the plots are strikingly similar to those published in NRC (2001), there are differences between the “published” and “revised” breakpoint estimates for the required concentrations of Lys and Met in MP for maximal content of milk of milk protein. The breakpoint estimates for the required concentrations of Lys and Met in MP for maximal content of milk protein were 6.80 and 2.29%, respectively (see figure legends), slightly lower than the values of 7.24 and 2.38% reported in NRC (2001). The breakpoint estimates for the required concentrations of Lys and Met in MP for maximal yield of milk protein were 7.10 and 2.52%, respectively (plots not shown). These values are also different from the NRC (2001) values of 7.08 and 2.38%. It was concluded by Schwab et al. (2009), from a comparison of the predicted flows of microbial MP and feed MP with the beta and final versions of the two models, along with a re-examination of feed inputs, that the primary reason for the differences in breakpoint estimates was differences in feed inputs for some of the studies. It is suggested that the new values be used as the reference values when using the NRC (2001) model to optimize Lys and Met concentrations in MP for lactating cows.

DEVELOPMENT OF LYS AND MET DOSE-RESPONSE PLOTS FOR CPM-DAIRY (V.3.0.10) AND AMTS.CATTLE (V.2.1.1)

Recognizing that the indirect dose-response approach for identifying optimal concentrations of Lys and Met in MP had not been extended to other models, Whitehouse et al. (2009) repeated the same steps, using the same studies as used for NRC (2001), to generate Lys and Met dose-response plots for CPM-Dairy and AMTS.Cattle. Because ration formulation and diet evaluation programs differ in the approach and assumptions taken to estimate AA supply, it was expected that estimated “requirements” for Lys and Met in MP would be different from those for NRC (2001).

The resulting dose-response plots that relate changes in milk protein concentrations to changes in predicted concentrations of Lys and Met in MP for CPM-Dairy and AMTS.Cattle are presented in Figures 2 and 3, respectively. As noted in Figures 1-3 and Table 1, differences existed among the three models in the breakpoint estimates for the required concentrations of Lys and Met in MP for maximal content and yield of milk protein. This was expected, as models differ in the approach for predicting supplies of AA. These differences lead to differences in predicted supplies of RDP, RUP, MP and MP-AA (Whitehouse et al., 2009). The AA prediction model in NRC (2001) is semi-factorial in nature, where some of the parameters are determined by regression. In contrast, CPM-Dairy and AMTS.Cattle use factorial approaches for predicting AA flows



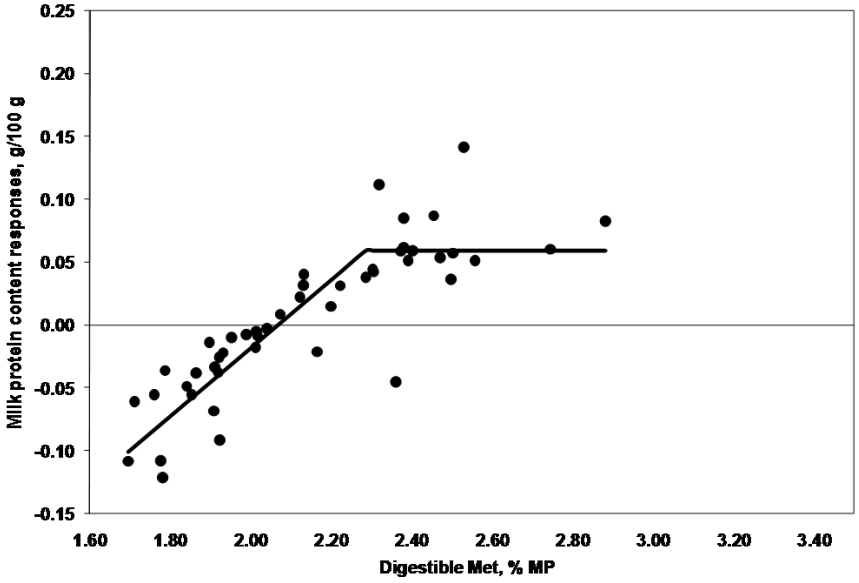
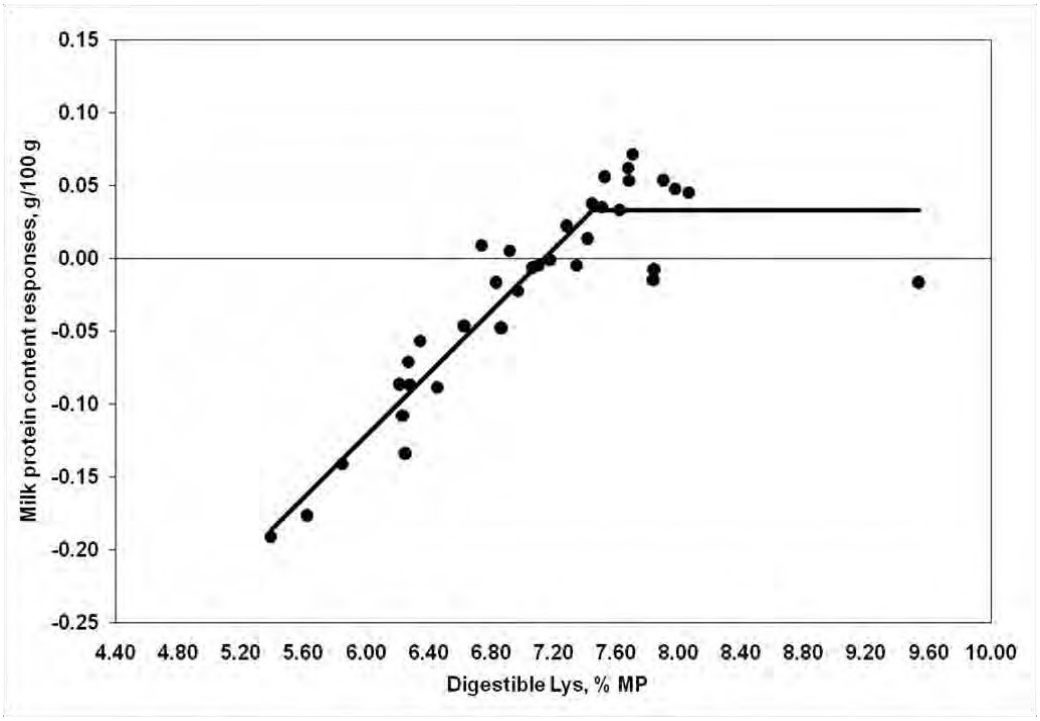


Figure 1. Revised NRC (2001) Lys and Met plots for milk protein concentrations. Regression analysis for Lys was limited to data where Met was 2.07 % or greater of MP. For the linear part of the model y = -0.818 + 0.125x and for the plateau y = -0.818 + 0.125 x **6.80**. Regression analysis for Met was limited to data where Lys was 6.16 % or greater of MP. For the linear part of the model y = -0.560 + 0.271x and for the plateau y = -0.560 + 0.271 x **2.29**.



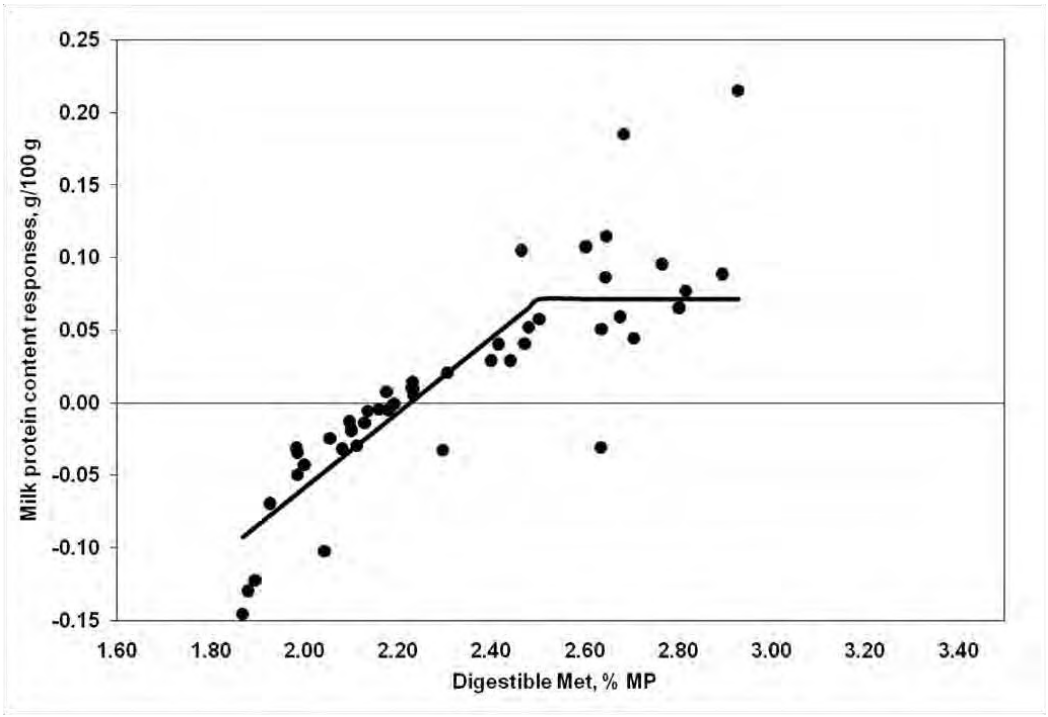
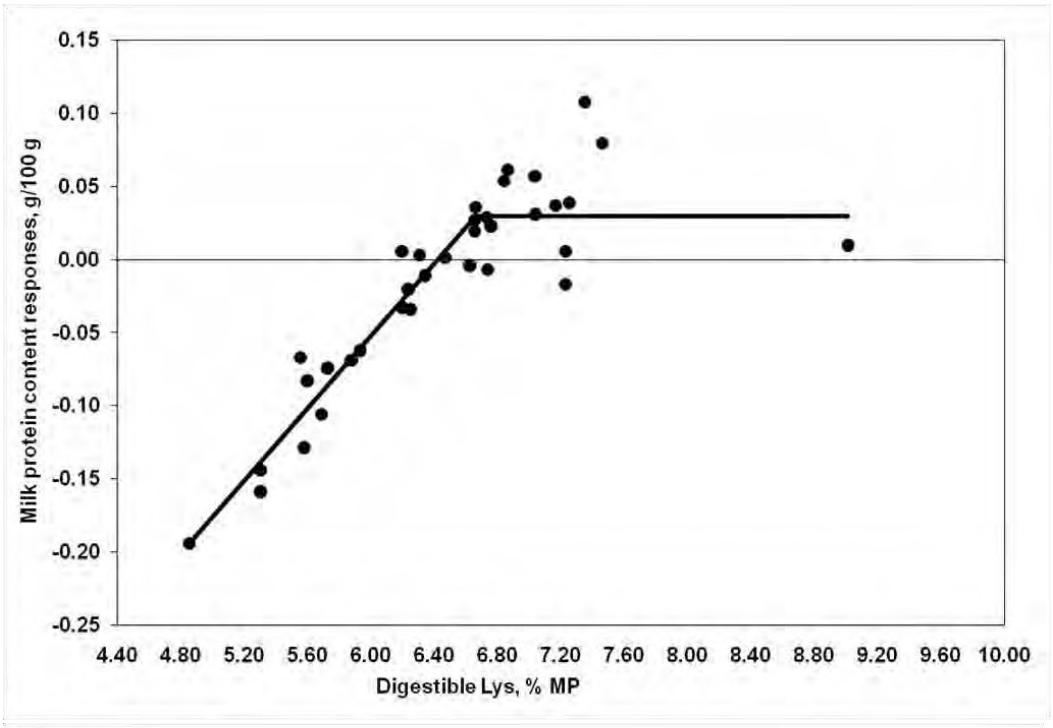


Figure 2. Lys and Met plots for milk protein concentrations for CPM-Dairy. Regression analysis for Lys was limited to data where Met was 2.17 % or greater of MP. For the linear part of the model y = -0.763 + 0.107x and for the plateau y = -0.763 + 0.107 x **7.46**. Regression analysis for Met was limited to data where Lys was 6.65 % or greater of MP. For the linear part of the model y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259 x **2.57**.



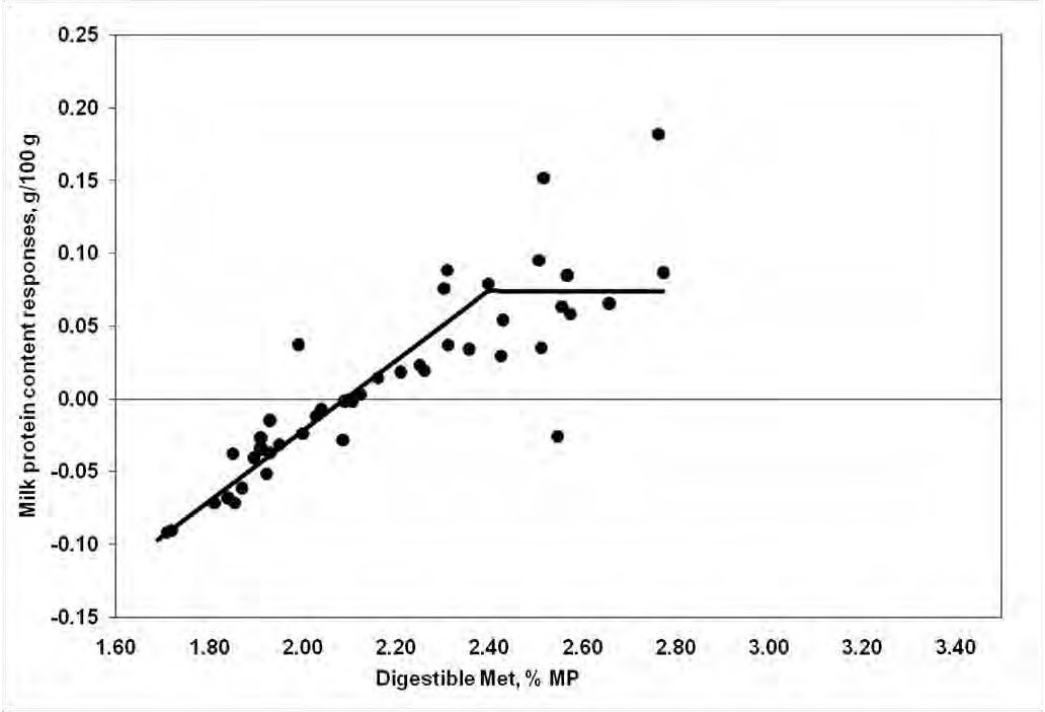


Figure 3. Lys and Met plots for milk protein concentrations for AMTS.Cattle. Regression analysis for Lys was limited to data where Met was 1.94 % or greater of MP. For the linear part of the model y = -0.795 + 0.124x and for the plateau y = -0.795 + 0.124 x **6.68**. Regression analysis for Met was limited to data where Lys was 6.09 % or greater of MP. For the linear part of the model y = -0.506 + 0.242x and for the plateau y = -0.506 + 0.242 x **2.40**.

Table 1. Breakpoint estimates for required concentrations of Lys and Met in MP for maximal content and yield of milk protein for the NRC, CPM, and AMTS models.

Item	Optimal Lys	Optimal Met	Lys r^2	Met r^2	Optimal Lys/Met
	NRC Model				
Content of milk protein	6.80	2.29	.82	.75	2.97
Yield of milk protein	7.10	2.52	.65	.36	2.82
	CPM Model				
Content of milk protein	7.46	2.57	.83	.73	2.90
Yield of milk protein	7.51	2.50	.53	.46	3.00
	AMTS Model				
Content of milk protein	6.68	2.40	.83	.76	2.78
Yield of milk protein	6.74	2.31	.65	.38	2.92

to the small intestine (O'Connor et al., 1993). Prediction models based on the factorial method require the assignment of AA values to model-predicted supplies of ruminally synthesized microbial protein, RUP, and if predicted, endogenous protein. CPM-Dairy (v.3.0.10) uses CNCPSv.5 and AMTS.Cattle (v.2.1.1) uses CNCPSv.6. The latest version of CNCPS has expanded CHO pools, modified CHO A1-B1 degradation rates, the soluble fractions (e.g., sugar, NPN) flow with the liquid phase instead of the solid phase, and the passage rate equations have been updated. The result of these and other changes have led to reductions in ruminal CHO degradation, higher RUP and lower microbial protein flows, and lower predicted flows of Lys and Met to the small intestine, as compared to CPM-Dairy.

MAXIMIZING MILK COMPONENTS AND MP UTILIZATION THROUGH AA FORMULATION

We consider the following 5 steps as being important to maximizing milk components and MP utilization through AA formulation. A brief discussion of each step follows.

Step #1: Feed a blend of high quality forages, processed grains, and byproduct feeds to provide a blend of fermentable carbohydrates and physically effective fiber that maximizes feed intake, milk production, and yield of microbial protein

Microbial protein, based on research to date, has an excellent AA composition for lactating dairy cows. The average reported concentrations of Lys and Met in bacterial true protein approximate 7.9% and 2.6%, respectively; values that exceed the concentrations in nearly all feed proteins (NRC, 2001), and values that exceed the optimal concentrations in MP as estimated by the NRC (2001), CPM-Dairy (v.3.0.10)

and AMTS.Cattle (v.2.1.1) models (Table 1). Realizing maximal benefits of feeding a balanced supply of fermentable carbohydrates on feed intake, milk production, and yields of microbial protein requires use of high quality feeds, adequate intakes of physically effective fiber, well-balanced and consistent diets, unlimited supplies of fresh water, and superior bunk management.

Step #2: Feed adequate but not excessive levels of RDP to meet rumen bacterial requirements for AA and ammonia

Realizing the benefits of feeding a balances supply of fermentable carbohydrates on maximizing yields of microbial protein also requires balancing diets for RDP. Rumen degraded feed protein is the second largest requirement for rumen microorganisms. It supplies the microorganisms with peptides, AA, and ammonia that are needed for microbial protein synthesis. The amount of RDP required in the diet is determined by the amount of fermentable carbohydrates in the diet. Diet evaluation models differ slightly in their estimates of RDP in feeds. The NRC (2001) model typically predicts RDP requirements of 10 to 11% of diet DM. Monitor feed intake, fecal consistency, milk/feed ratios, and MUN to make the final decision. A common target value for MUN is 10-12 mg/dl. Don't short-change the cows on RDP…carbohydrate balancing can be negated with an inadequate supply of RDP. A deficiency of RDP will suppress the ability of the microorganisms to reproduce, but they can continue to ferment carbohydrates. This results in higher feed intake, but milk/feed ratios will be low because of lower than expected synthesis of microbial protein.

Avoid over-feeding feeding RDP to the point that rumen ammonia concentrations markedly exceed bacterial requirements. Not only does it result in wastage of RDP, but research (e.g., Boucher et al., 2007), as well as a summary of N passage studies where rumen ammonia concentrations were also measured (Peter Robinson, personal communication), indicate that rumen ammonia concentrations in excess of bacterial requirements decreases flows of microbial protein to the small intestine.

Step #3: Feed high-Lys protein supplements to achieve a level of Lys in MP that comes as close as possible to meeting the optimal concentration (see Table 1)

If protein supplementation is required, select high quality, high-Lys protein supplements (e.g., soybean and canola meals, blood meal, and fishmeal). Feeding low-Lys feeds such as distiller's grains or corn gluten meal as sources of additional protein is not consistent with balancing for AA. Purposely selecting high-Lys protein supplements has been the only option, at least until the recent release of the first rumen-protected Lys sources on the market, to at least partially compensate for the low content of Lys in the RUP fractions from forages, grains and distiller's grains. Achieving target formulation levels for Lys in MP will become easier, and the value of lower Lys protein supplements extended, if these rumen-protected Lys products can be demonstrated to be cost effective sources of MP-Lys.

Step #4: Feed a “rumen-protected” Met supplement in the amounts needed to achieve the optimal ratio of Lys and Met in MP (see Table 1)

Feeding a rumen-protected Met supplement, in conjunction with one or more of the aforementioned high-Lys protein supplements, is almost always necessary to achieve the correct Lys/Met ratio in MP (see Table 1). We continue to be surprised with first time evaluation of diets how often we see Lys to Met ratios in MP of 3.3 or higher…values as high as 3.5 and 3.6 are not uncommon. “Out of balance” Lys to Met ratios lowers the efficiency of use of MP for protein synthesis and the more “out of balance” the ratios, the less efficient the use.

To achieve the desired predicted ratio of Lys to Met in MP, and to ensure full use of the available MP-Lys for protein synthesis, also requires that a realistic estimate of efficacy be used for the Met product that one elects to feed. Over-estimating the bioavailability of some of the Met supplements has been way too common. Doing so leads to disappointing production outcomes, and the nutritionist and dairy producer believing that balancing for Lys and Met has minimal impacts on animal performance.

Step #5: Don’t overfeed RUP

Three factors determine the cows’ requirement for RUP. These are: 1) supply of microbial protein, 2) RUP digestibility, and 3) AA composition of RUP. Given the rather high probability that a nutritional model will not be very accurate in predicting the cows’ exact requirement for RUP, because of the multitude of factors that affect the requirement for RUP, we suggest that you let your cows tell you how much they need. Routinely used models don’t adjust MP requirements, and thus RUP requirements, for changes in AA balance, so let the let models be initial guides, not the final answer. Don’t be surprised, as a result of balancing for Lys and Met in MP, how little RUP is actually needed in the diet.

Field experience indicates that cows are more responsive to changes in diet RUP content when RUP has a good AA balance vs. when the balance is not good. This makes sense because the nutritional potency of the RUP is greater when it has a good AA balance vs. a poor AA balance.

Balancing for Lys and Met in MP, using the steps as outlined, has led to many important benefits, both in research and on-farm implementation. A summary of published studies and reported observations include: 1) reduced need for supplemental RUP for a given level of milk and milk production, or increased milk and milk protein production with the same intake of RUP, 2) reduced N excretion per unit of milk or milk protein produced, 3) more predictable changes in milk and milk protein production to changes in RUP supply, 4) improved herd health and reproduction, and 5) increased herd profitability. There are many good reviews in the literature summarizing the benefits of enriching rations in metabolizable Lys and Met (e.g., Garthwaite et al., 1999; NRC, 2001; Rulquin and Verite, 1993; Schwab et al., 2007, and Sloan, 1997). More recent experiments highlight the value of increasing concentrations of Lys and Met in

MP on increasing the efficiency of use of MP for milk and milk protein production (e.g., Noftsger and St-Pierre, 2003; Chen et al.,2009).

As expected, the responses that one achieves in balancing diets for Lys and Met in MP depends on ones "starting point". It should also be noted that where it is possible, field nutritionists with experience in balancing for Lys and Met will also lower dietary RDP and/or RUP if the previous diets allow. This has the benefit of often reducing the usual added expense of replacing low Lys protein supplements with high Lys protein supplements and the cost of adding one or more ruminant protected Met sources to the diet. In reducing dietary CP, it is important, for the reasons previously stated, not to cause a deficiency of RDP. When employing these feeding strategies, the results of a 10-herd field study in 2006 indicated a return on investment (ROI) ranging from 1.1 – 5.5; the average ROI was 3.35:1 (Driver, 2007).

We also typically observe ROI of 2.5 or higher. Increases in butterfat content and milk yields are common and contribute to the favorable ROI. Balancing diets for Lys and Met, because of the stated benefits, is an attractive option for increasing dairy herd profitability, even with current low milk prices and high feed costs. It is no longer uncommon to hear reports of increases in milk protein and milk fat concentrations of 0.10 - 0.25 and 0.10 – 0.15 percentage units, respectively, and 2 - 4 lb more milk as a result of balancing for Lys and Met, with little or no effect on feed costs.

The second author of this paper has also had excellent success in balancing diets for Lys and Met. He uses Formulate2, a commercially available ration formulation model that he has developed that uses NRC (2001) as its operating platform. The model not only implements the NRC (2001) model, but fully integrates the model's equations with its optimization processes. Thus, it provides the ability to set and accurately meet MP-AA constraints at the duodenal level while minimizing the cost of doing so.

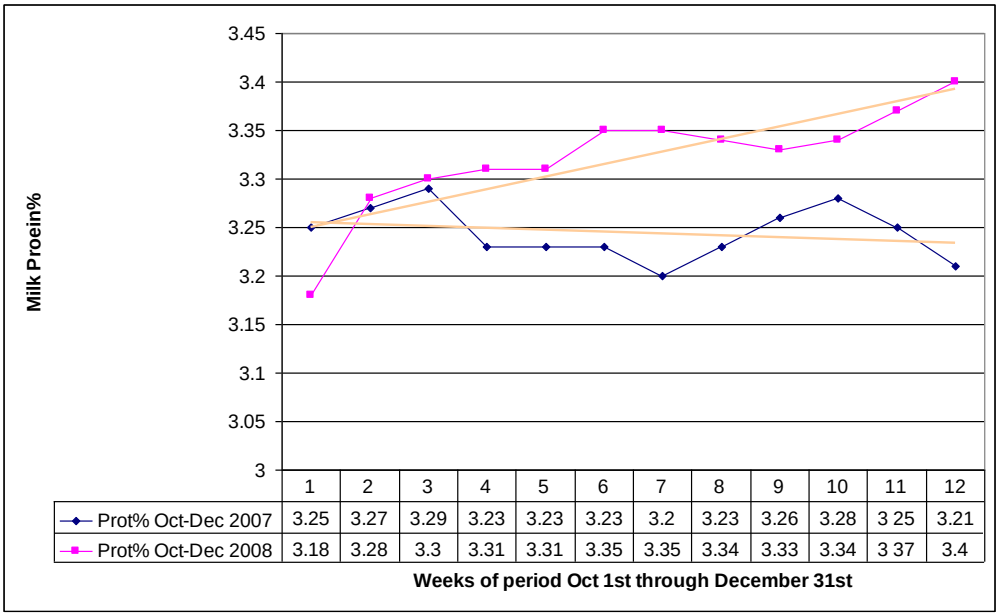
In addition, the work reported by Schwab et al. (2003, 2004) to extend the NRC model to predicting changes in lactation from changes in supplies of MP-Lys and MP-Met has been incorporated into Formulate2 as a pop-up calculator. The calculator permits acquisition of milk flow and milk composition inputs from the background diet record and utilizes the equations developed from this work to generate target values for grams of MP-Lys and MP-Met. The calculator also permits re-calculation of the MP requirement based on a user stipulated concentration of the selected MP-AA (MP-Lys or MP-Met) in MP. As a result, diets are routinely balanced with Formulate2/NRC 2001 for RDP, MP-Lys and MP-Met without regard to CP%.

Using these tools it has been possible to improve concentrations of MP-Lys and MP-Met in the diets of fresh and early lactation animals allowing the reduction of dietary CP levels to a range of approximately 16.0% to 16.5% via reduced feed RUP while maintaining RDP at acceptable levels. As a result, significant improvements in milk protein percent, total milk solids percent and butterfat percentages have been achieved as well as improved yields of these components. Additionally, because of the reduction

of dietary RUP achieved by this approach, a frequent result has been lower total concentrate costs by allowing the inclusion of lower cost feeds that can contribute well to total dietary NFC. This approach has been implemented with herds at all levels of milk production with equal effect.

The primary basis for realizing these responses has been the excellent predictive reliability exhibited by the NRC (2001) model in commercial production environments in terms of NE(l) allowable and MP allowable milk. In all situations where previous diets have been reviewed and revised a significant gap between energy allowable milk and MP allowable milk has been observed. This phenomenon has been evident regardless of which other model was used to formulate the previous diets. In these diets, MP allowable milk has been found to be the fundamental limiting factor in production of milk and milk components and thus the primary factor predicting animal response. Simply closing this gap, even without optimizing MP-Lys and MP-Met in MP, has produced significant results. The additional responses and benefits described previously are obtained when AA balance and reduction of RUP/MP are addressed. It is important to note that what has been described has been achieved by using Formulate2/NRC 2001 "out-of-the-box" without the need to make adjustments to the rates and constants of various model predictive mechanisms.

One example of a farm situation follows. The graphs below illustrate changes in milk protein percentage and milk protein yield when balancing for MP-Lys and MP-Met in a herd of approximately 1700 cows with significant, ongoing management issues and less than average milk production. The two periods shown are the same weeks of the year in different years.

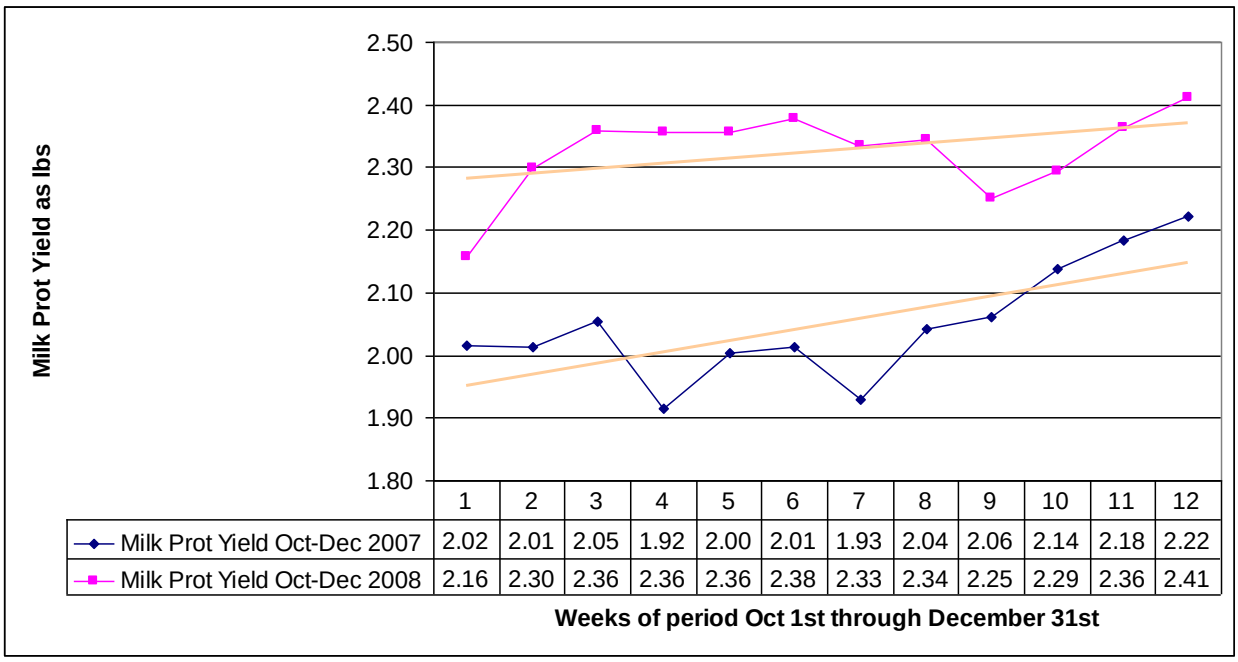


	1	2	3	4	5	6	7	8	9	10	11	12
Prot% Oct-Dec 2007	3.25	3.27	3.29	3.23	3.23	3.23	3.2	3.23	3.26	3.28	3 25	3.21
Prot% Oct-Dec 2008	3.18	3.28	3.3	3.31	3.31	3.35	3.35	3.34	3.33	3.34	3 37	3.4

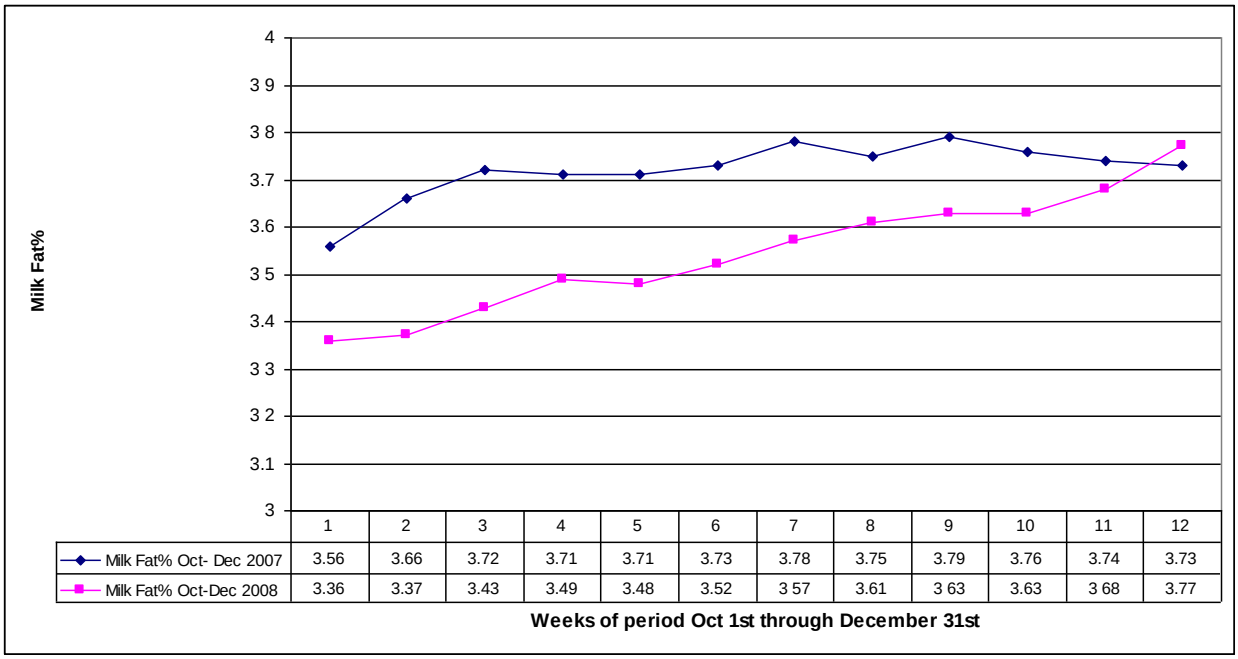
Herd average milk flow was 63.2 lbs for the 2007 period and 70.0 lbs for the 2008 period. The 2007 diets were balanced for MP only with dietary CP at 17.9% which did yield improvement over historical milk protein production. The MP supply targets for the

high cows for both years were very similar; 2846 g in 2007 and 2868 g in 2008 on equal DMI.

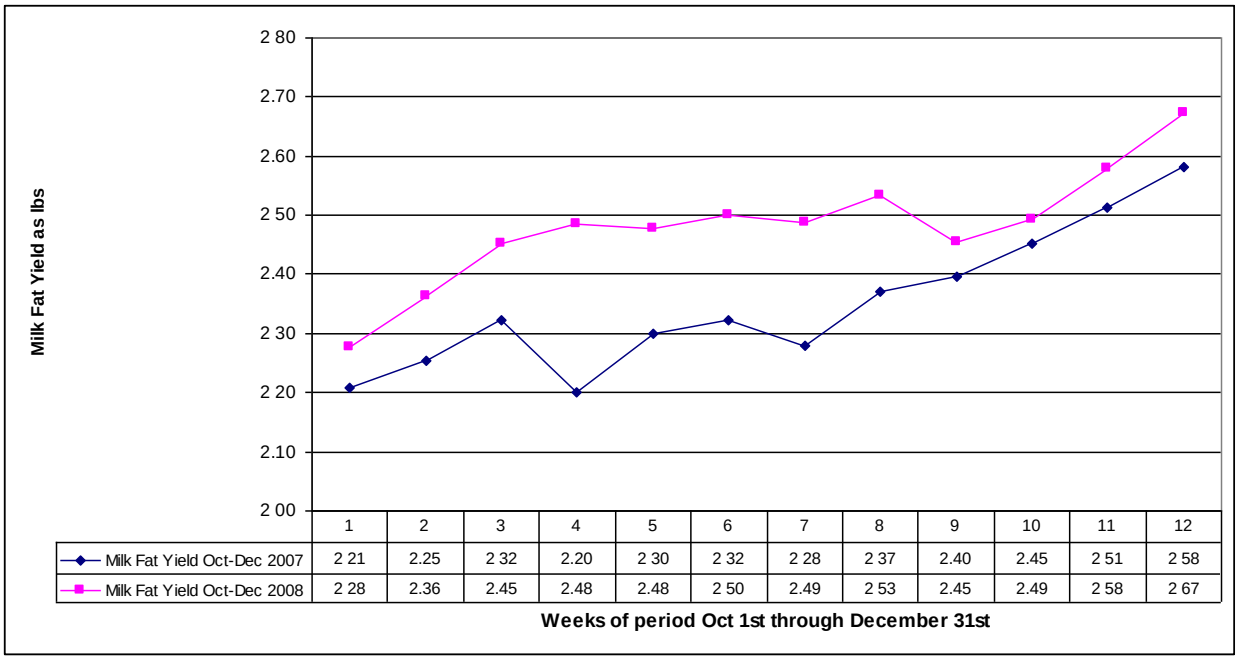
Though diets during the second period were balanced for MP-Lys and MP-Met, no direct attempt was made to minimize RUP/MP. Consequently, CP in the diet of high producing animals was 17.3%. Subsequently, CP in such diets has been reduced to 16.5% with no apparent negative effects.



	1	2	3	4	5	6	7	8	9	10	11	12
Milk Prot Yield Oct-Dec 2007	2.02	2.01	2.05	1.92	2.00	2.01	1.93	2.04	2.06	2.14	2.18	2.22
Milk Prot Yield Oct-Dec 2008	2.16	2.30	2.36	2.36	2.36	2.38	2.33	2.34	2.25	2.29	2.36	2.41



	1	2	3	4	5	6	7	8	9	10	11	12
Milk Fat% Oct- Dec 2007	3.56	3.66	3.72	3.71	3.71	3.73	3.78	3.75	3.79	3.76	3.74	3.73
Milk Fat% Oct-Dec 2008	3.36	3.37	3.43	3.49	3.48	3.52	3 57	3.61	3 63	3.63	3 68	3.77



	1	2	3	4	5	6	7	8	9	10	11	12
Milk Fat Yield Oct-Dec 2007	2 21	2.25	2 32	2.20	2 30	2 32	2 28	2 37	2.40	2.45	2 51	2 58
Milk Fat Yield Oct-Dec 2008	2 28	2.36	2.45	2.48	2.48	2 50	2.49	2 53	2.45	2.49	2 58	2 67

CONCLUSIONS

The adoption of the concept of balancing diets for AA continues to increase. Balancing diets to come as close as possible to meeting model determined optimal concentrations of Lys and Met in MP is the first step to balancing diets for AA. Benefits include: 1) increased yield of milk and milk components, 2) reduced N excretion per unit of milk or milk protein produced, 3) more predictable changes in milk and milk protein production to changes in RUP supply, 4) improved herd health and reproduction, and 5) increased herd profitability. Increases in milk protein and fat concentrations of 0.1-0.25 percentage units for protein and 0.1-0.15 for fat and returns on investment of 2.0 to 3.5 are typical. Increases in milk yield are more common in early lactation cows than late lactation cows, and can be rather significant if balancing for Lys and Met is started before calving. With high feed costs and low milk prices, an important benefit of AA balancing has been the opportunity to increase milk and milk component yields with less RUP supplementation and similar or lower feed costs.

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CHALLENGES OF PREDICTING METABOLIZABLE LYSINE CONTENT OF INGREDIENTS

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SUMMARY

- Of all amino acids in feed protein, lysine is generally the most susceptible to damage during heat processing.

- Identifying in vitro methods to assess damage to lysine that results from heat processing can aid in monitoring quality of feed ingredients.

- Identifying an in vitro method to estimate lysine damage in blood meal is particularly important because blood meal is often specifically fed to increase concentration of lysine in metabolizable protein.

- Several in vitro methods that can be used to estimate availability or digestibility of lysine in blood meal and other feedstuffs are reviewed.

INTRODUCTION

Heat treatment of feedstuffs is utilized to decrease ruminal degradation of feed protein and increase the proportion of RUP (rumen undegraded protein). Heat application for this process needs to be carefully controlled because excess heat can destroy lysine and depress intestinal lysine digestibility (Faldet et al., 1992; Pereira et al., 1998). Monitoring the effect of heat treatment on intestinal digestibility of lysine in RUP (RUP-Lys) is especially important for lactating cows as Lys is often co-limiting with methionine (Met) or second limiting for milk and milk protein production in North America where diets high in corn products are fed (NRC, 2001). When feeds are heated to decrease the proportion of RDP (rumen degradable protein) and increase the proportion of RUP, the greatest benefit will be observed if the RUP is readily digested and RUP-AA (RUP amino acids) are readily absorbed by the animal. Processing methods that increase RUP supply without damaging RUP-Lys should be used.

Monitoring Lys and protein damage during the drying process of wet feeds is also important. The amount of distillers dried grains with solubles (DDGS) fed to ruminants is increasing, and the vast majority of DDGS fed in the U.S. is the resultant by-product of the production of fuel ethanol from corn. The AA profile of corn is not desirable for ruminants, as corn protein has a low content of Lys (2.84% of CP; NRC, 2001). However, standard corn meal is low in protein (9.4% CP; NRC, 2001) and most of that protein is degraded in the rumen and incorporated into microbial protein, which has an improved AA profile. Distillers dried grains with solubles has a higher CP concentration than corn (29.7%; NRC, 2001), and more of the protein in DDGS remains undegraded in the rumen and arrives at the small intestine intact (NRC, 2001). This can negatively

impact the AA profile of metabolizable protein (MP) if other feedstuffs that are high in RUP-Lys are not included in the diet. These effects are further confounded by the fact that RUP digestibility of DDGS is lower than corn meal, and RUP-Lys digestibility for DDGS is lower than the digestibility of the other AA (Boucher et al., 2009a). Therefore, the inferior AA profile of MP that results when feeding DDGS can be further exacerbated by the decrease in digestibility of RUP and RUP-Lys. Decreases in milk protein concentrations have been reported when DDGS replaced corn meal and SBM in the diet of lactating cows (Kleinschmit et al., 2006) and when DDGS replaced wet distillers' grains with solubles (Anderson et al., 2006). Therefore, assessing Lys due to the drying process of distillers grains is critical to the successful feeding of DDGS to lactating dairy cows.

Monitoring Lys damage during the drying of blood meal (BM) is also critical. Blood meal obviously needs to go through a drying process before it is fed to cattle. The Lys concentration of BM is about 9% of CP (NRC, 2001), which makes BM a desirable ingredient to feed to increase Lys concentrations in MP (MP-Lys). However, if the Lys is damaged in the processing of BM, MP-Lys supply will be overestimated by current ruminant nutrition models. Therefore, understanding how heat processing conditions can impact the quality of protein feeds is critical, and developing methods to rapidly and accurately estimate the damage to Lys and protein that results from heat processing will be beneficial. More information regarding digestibility of RUP and RUP-AA, particularly RUP-Lys, is needed.

NUTRITIONAL CONSEQUENCES OF HEAT PROCESSING

Terminology

In order to discuss the nutritional consequences of heat processing feedstuffs, the following terms are defined:

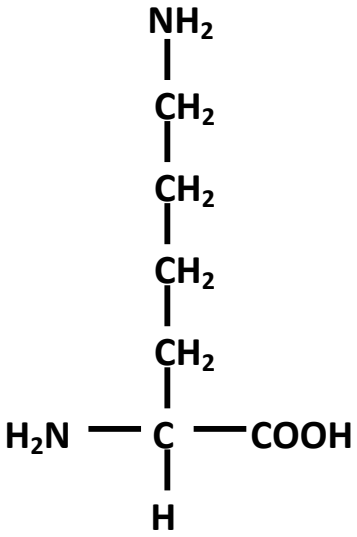
Bioavailability – proportion of ingested dietary AA that is absorbed in a chemical form that renders these AA potentially suitable for metabolism or protein synthesis.

Digestibility – reflects enzymatic hydrolysis and microbial fermentation of ingested proteins and peptides and absorption of AA and peptides from the gastrointestinal lumen.

Blocked lysine – lysine molecules in which the epsilon-amino group is bound to another compound.

Reactive lysine – lysine molecules in which the epsilon-amino group is not bound to another compound.

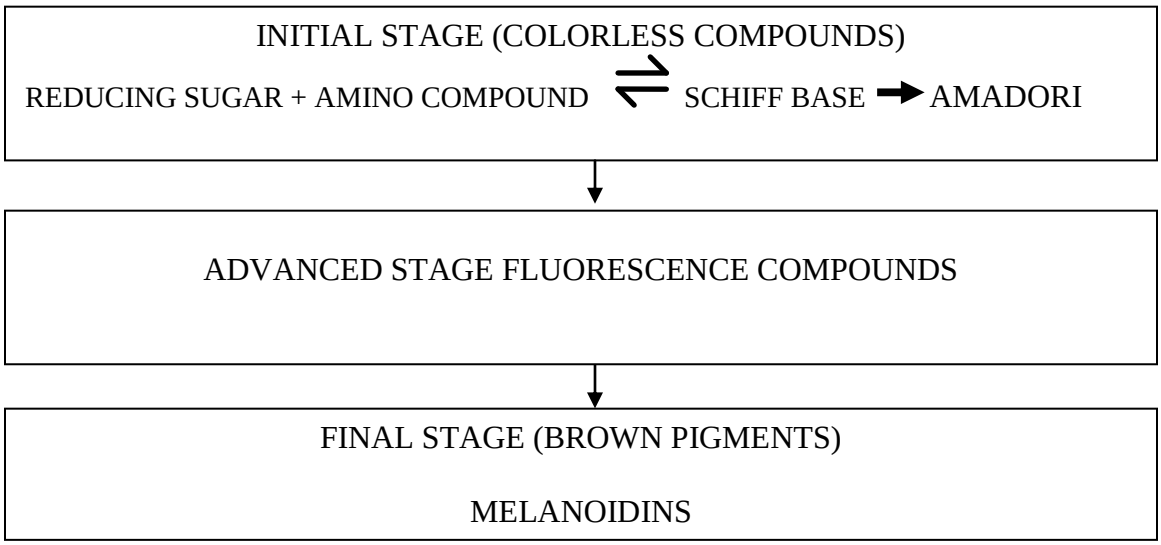
Figure 1. Chemical structure of lysine



Also throughout this paper, digestibility of RUP-AA will refer to intestinal digestibility estimates obtained using the residue after feeds have been ruminally incubated *in situ*, and digestibility of AA will refer to intestinal digestibility estimates obtained using the intact feed.

Heat damage of protein and AA in feedstuffs primarily results from three major reactions: Maillard reaction, AA racemization reactions, and protein cross-linking reactions (Meade et. al., 2005). Of the reactions that occur during heat processing of foods and feeds, the Maillard reaction generally has the greatest impact on nutritional quality (Mauron, 1990). The reaction most commonly occurs in feeds between carbonyl groups of reducing sugars and the free amino group present on the side-chain of Lys (Fig. 1; Meade et al., 2005).

Figure 2. Summary of the Maillard Reaction Adapted from Silván et al. (2006)



Therefore, one of the major nutritional consequences of the Maillard reaction is the destruction or loss of Lys, an essential AA (EAA) that is often limiting for livestock production. The early Maillard reaction is characterized by the destruction of Lys (Figure 2), but as the reaction progresses, cross-links within and between protein molecules form, reducing digestibility of the entire protein molecule or fragments of the molecule.

Estimating Thermal Damage in Feeds: The Furosine Procedure

Furosine analysis has been used for over 40 years as an indicator of thermal damage in foods (Erbersdobler and Somoza, 2007). ε-*N*-deoxyfructosyl-L-lysine is the major Amadori compound formed during the early Maillard reaction (Figure 2). Upon acid hydrolysis, ε-*N*-deoxyfructosyl-L-lysine is released in a constant ratio of 50% lysine, 30% furosine, 20% pyridosine, and 10% other compounds (Mauron, 1990). Therefore, the amount of furosine measured after acid hydrolysis can be used to calculate % blocked lysine according to the following equation (Mauron, 1990):

$$\% \text{ blocked lysine} = \frac{(3.1 \text{ furosine} \times 100)}{[\text{total lysine} + (1.86 * \text{furosine})]}$$

The acid hydrolysis procedure used in furosine analysis is identical to that used in AA analysis of feeds. Therefore, the furosine assay is attractive because furosine and AA content of feeds can all be determined with one procedure. Pahm et al. (2008) used the furosine procedure to estimate blocked Lys content of 33 DDGS samples, and the average blocked Lys content of the samples was 16% with a coefficient of variation of 7%. Pahm et al. (2008) also reported that blocked Lys was correlated with standardized ileal Lys digestibility in swine (R^2 = 0.66). Boucher et al. (2009b) determined the furosine content of 16 rumen undegraded residue (RUR) samples: 3 samples of soybean meal (SBM), 3 samples of expeller SBM (SoyPlus®), 5 samples of DDGS, and 5 samples of fish meal (FM). One of the SBM, SoyPlus, and DDGS samples were heated additionally in the laboratory oven to intentionally depress digestibility of RUP-Lys. Of the 16 samples analyzed, only 9 samples contained furosine, and only the 4 unheated DDGS samples contained appreciable amounts of furosine (Table 1). Blocked RUP-Lys was calculated from the furosine and Lys concentrations of the RUR according to the above equation. The same 16 RUR samples were previously fed to cecectomized roosters to determine digestibility of RUP-Lys *in vivo*. Results of the experiment indicated that blocked RUP-Lys determined via the furosine method was negatively correlated with standardized RUP-Lys digestibility (R^2 = 0.94; Y = 88.43–0.89X; $P < 0.001$, n = 9).

Estimating Thermal Damage in Feeds: The Homoarginine Procedure

Homoarginine is an amino acid that is not found in nature. Homoarginine is formed by the reaction of the epsilon-amino group of Lys (the NH_3 group on the side chain) with *O*-methylisourea. If the epsilon-amino group of Lys is bound to another compound, such as a reducing sugar, then homoarginine does not form. Determining the amount of homoarginine and Lys after the reaction with *O*-methylisourea allows for the determination of the reactive Lys content of a feed, or the amount of Lys in which the epsilon-amino group is not bound to another compound.

Table 1. Blocked RUP-Lys and reactive RUP-Lys content of feeds (Boucher, 2009)

Sample[a]	Blocked RUP-Lys, %[b]	Reactive RUP-Lys, %[c,d]
Heated SoyPlus	-	36.5
SoyPlus 1	-	81.5
SoyPlus 2	0.10	77.4
Heated SBM	-	43.3
SBM 1	0.7	85.2
SBM meal 2	1.0	85.1
Heated DDGS	-	31.5
DDGS 2	26.0	70.3
DDGS 3	7.6	76.8
DDGS 4	15.3	73.7
DDGS 5	21.0	71.3
Anchovy FM	0.4	84.9
Catfish FM	-	71.3
Menhaden FM 1	0.2	79.5
Menhaden FM 2	-	79.1
Pollock FM	-	89.4

[a]SBM = soybean meal; DDGS = distillers dried grains with solubles; FM = fish meal.
[b]Blocked RUP-Lys as a % of total RUP-Lys determined via the furosine method.
[c]Reactive RUP-Lys as a % of total RUP-Lys determined via the homoarginine method.
[d]Blocked RUP-Lys and reactive RUP-Lys do not add up to 100%. Different procedures were used to estimate blocked RUP-Lys and reactive RUP-Lys, and errors and differences in experimental techniques exist.

Boucher et al. (2009b) evaluated the use of the homoarginine method to estimate reactive Lys in the RUP fraction of protein concentrate ingredients commonly fed to dairy cattle (Table 1). The same samples described above in furosine analysis were used in the homoarginine analysis as well. Results of the experiment indicate that reactive RUP-Lys determined via the homoarginine method was positively correlated with standardized RUP-Lys digestibility previously determined in cecectomized roosters (R^2 = 0.90; Y = −12.15 + 1.19X; P < 0.001, n = 16). Unlike the furosine procedure, the homoarginine procedure can be used to determine the reactive lysine content of all feeds evaluated, which makes it a more applicable procedure to the dairy industry. However, the homoarginine procedure requires more than 5 days to complete, which hinders its use for routine monitoring of Lys damage to feeds due to processing conditions.

Estimating Digestibility of RUP and RUP-AA in Blood Meal

In addition to the feedstuffs described above, we have also evaluated the use of several *in vitro* techniques to estimate the digestibility of RUP and the AA in RUP of blood meal (Boucher, 2008). Five samples of BM (2 bovine and 3 porcine) were obtained from various sources in NE, IL, IA, and Canada, and the samples were ruminally incubated *in situ* for 16 h. One of the bovine and porcine BM samples was heated in a laboratory oven to artificially depress digestibility of protein and AA. Due to availability of resources, we were able to analyze a total of 6 BM samples; therefore, the heated bovine BM sample was analyzed in the unheated form as well, but the heated porcine BM sample was not analyzed in the unheated form. The intact BM samples (n =

6) and BM RUR samples after the 16 h ruminal incubation (n = 6) were crop-intubated to cecectomized roosters to obtain *in vivo* AA and RUP-AA digestibility estimates. The average AA and RUP-AA digestibility estimates for the 6 BM samples are presented in Table 2.

Key observations from Table 2 (column labeled rooster):

- Amino acids in all of the in the intact and RUR BM samples were highly digestible in the cecectomized roosters.
- There was little variation in AA and RUP-AA digestibility among the BM samples evaluated.
- Digestibility of AA in the intact feeds was similar to digestibility of RUP-AA in the RUR.

Table 2. Average AA digestibility estimates of BM before & after ruminal incubation (BM RUR) determined in cecectomized roosters and via the original (BM RUR) and modified three-step procedure (TSP; Boucher 2008)

	Method			
	Rooster		Modified TSP[a]	
Digestibility, %[b,c]	BM	BM RUR	BM	BM RUR
Arginine	90.7	91.5	58.9	72.2
Histidine	92.0	92.4	58.2	72.2
Isoleucine	85.5	87.4	49.9	64.9
Leucine	95.9	96.1	57.6	71.3
Lysine	90.5	90.7	62.0	75.4
Methionine	97.5	97.6	65.7	77.7
Phenylalanine	96.1	96.3	59.2	72.4
Threonine	95.5	95.9	60.7	74.4
Tryptophan	96.8	96.5	61.1	70.4
Valine	95.7	96.0	57.4	70.7
TEAA	94.1	94.4	58.7	72.4
NEAA	95.0	94.9	60.7	73.1
Total AA	94.4	94.6	60.4	74.2
CP	-	-	59.5	74.1
CP original TSP[d]	-	-	-	59.7
Avg. of EAA	93.6	94.0	53.2	69.7
Minimum	85.5	87.4	2.3	46.0
Maximum	97.5	97.6	65.7	77.7
St. Dev.	3.8	3.3	18.3	9.0

[a]Modified three-step procedure (TSP) of Gargallo et al. (2006).
[b]TEAA = total essential amino acids; NEAA = nonessential AA.
[c]AA digestibility of the RUR samples in as a % of AA in rumen undegraded protein.
[d]Original TSP of Calsamiglia and Stern (1995).

Modified Three-Step Procedure

The intact and RUR BM samples were also analyzed via the modified three-step procedure (modified TSP) of Gargallo et al. (2006), and the *in vitro* AA and RUP-AA digestibility estimates are presented in Table 2. The AA and RUP-AA digestibility estimates obtained using the modified TSP were lower than estimates from the cecectomized roosters. Estimates of RUP digestibility of the BM samples obtained using the original TSP of Calsamiglia and Stern (1995) are also presented in Table 2. These estimates were lower than RUP-total AA digestibility measured in cecectomized roosters.

Linear regression analysis was used to examine the relationship between AA and RUP-AA digestibility of BM samples determined *in vivo* in cecectomized roosters and *in vitro*. The results indicate that for the intact BM samples there was a trend for a correlation between *in vivo* and *in vitro* digestibility for many of the AA (R^2 for total AA digestibility = 0.73; P = 0.06). For methionine and tryptophan in the BM samples there was a correlation between *in vivo*

and *in vitro* digestibility. However, RUP-AA digestibility estimates of the RUR BM samlpes obtained *in vivo* and *in vitro* were not correlated, except for tryptophan (R^2 for total RUP-AA digestibility = 0.55; *P* = 0.15). Digestibility of total CP in the RUR obtained with the modified TSP was highly correlated to digestibility of total CP in the RUR obtained with the original TSP.

The relationship between *in vitro* digestibility of the intact BM samples and *in vivo* RUP-AA digestibility (AA digestibility estimates obtained by feeding the RUR BM samples to cecectomized roosters) was also examined. There was a trend for a correlation between *in vivo* RUP-AA digestibility and *in vitro* AA digestibility of the intact BM for many AA (R^2 for digestibility of total RUP-AA *in vivo* and digestibility of AA in the intact feeds *in vitro* = 0.73; *P* = 0.06), and a significant relationship was observed for methionine, tryptophan, and alanine.

The modified TSP may be useful in estimating RUP-AA digestibility of BM. However, it is suggested that digestibility of the intact samples be estimated and the ruminal incubation step be eliminated because this will yield more accurate estimates of RUP-AA digestibility. However, the *in vivo* estimates were much higher than *in vitro* estimates; therefore, the database on RUP-AA digestibility of BM using the modified TSP should be expanded because there was such a discrepancy between *in vivo* and *in vitro* estimates. Also, more research is needed with this method to estimate poorly digested BM samples because the samples used in this experiment were all highly digestible *in vivo*.

Homoarginine Method

Results of homoarginine analysis of the BM samples are presented in Table 3. The reactive Lys content of the intact BM samples ranged from 92.6 to 96.7%, and the reactive RUP-Lys content ranged from 45.3 to 93.1%. However, the reactive Lys and RUP-Lys content of the samples was not correlated to Lys and RUP-Lys digestibility determined *in vivo* in cecectomized roosters. For the intact feeds, Lys digestibility *in vivo* ranged from 82.9 to 95.8% and *in vivo* RUP-Lys digestibility ranged from 83.9 to 95.3%. There are several possible explanations for the lack of correlation between *in vivo* Lys digestibility and *in vitro* reactive Lys content. First, there was little variation among *in vivo* Lys digestibility for the BM samples analyzed, which could affect the strength of correlation. However, reactive Lys content theoretically cannot be overestimated using the homoarginine method because the formation of homoarginine is specific to the reaction of O-methylisourea and the epsilon-amino group of Lys; therefore, it is likely that factors other than reactive Lys content affect Lys digestibility in BM. Additionally our sample size was small (n = 6). A larger sample size may have yielded a stronger correlation between reactive Lys determined via the homoarginine method and Lys digestibility *in vivo*.

Table 3. Reactive Lys content of intact and rumen undegraded residue blood meal samples determined using the homoarginine method (Boucher, 2008)

Intact sample	Reactive Lys, %
Bovine BM1	96.66
Bovine BM2	93.05
Heated bovine BM	96.20
Porcine BM1	96.52
Porcine BM2	92.59
Heated porcine BM	94.37
Rumen undegraded residue	
Bovine BM1rr[a]	66.98
Bovine BM2rr	72.56
Heated bovine BMrr	45.30
Porcine BM1rr	71.30
Porcine BM2rr	93.12
Heated porcine BMrr	86.83
RUP-Lys dig. vs. reactive RUP-Lys[b]	$R^2 = 0.05$
Lys dig. vs. reactive Lys[c]	$R^2 = 0.29$

[a]rr = rumen residue.
[b]Digestibility of lysine in rumen undegraded protein (RUP) determined in roosters and reactive lysine in RUP determine via the homoarginine method.
[c]Lysine digestibility determined in roosters and reactive lysine determined via the homoarginine method.

Ohio State Modifications of the Three-step Procedure

In the previously described analysis of *in vitro* AA digestibility of BM samples, it is recognized that the sample size (n = 6) is small. In a more extensive analysis of RUP digestibility, 265 blood meal samples were evaluated using the 3-step *in-situ/in-vitro* method described by Calsamiglia and Stern (1995) with modifications (Table 4; St-Pierre, unpublished observations). Considerable inter-assay and inter-lab variation was observed when the original Calsamiglia and Stern (1995) procedure was applied to BM. Therefore, the following modifications were made at Ohio State: (1) partial standardization of enzymes, (2) use of fuzzy standards (i.e., standards whose analytical values are not precisely known), correction for wash-out, and (3) Bayesian statistics to adjust for inter-assay variation. Using these modifications, the inter-assay variation was reduced five fold compared with the original 3-step procedure. Amino acid digestibilities were corrected such that the sum of all digestible AA was equal to the adjusted digestible protein. While the CP and RUP averages (Table 4) were similar to those reported for ring-dried blood meal in the NRC 2001, the RUP digestibility of 64.6% (Table 4) was lower than the NRC (2001) library value of 80%. Amino acid digestibilities were similar to RUP digestibility with the exception of lysine, which was lower (56.0 $\pm$ 27.1%; data not shown).

While heating of BM can increase its RUP, heating does not consistently alter the digestibility of the RUP once it arrives at the small intestine. This concept is clearly demonstrated in Figure 3, because there is no relationship between the amount of RUP and RUP digestibility as measured in the Ohio State modified 3-step procedure.

Figure 4 clearly demonstrates that Lys digestibility of BM was almost always lower than digestibility of RUP, and as the RUP digestibility of BM decreased RUP-Lys digestibility of BM decreased even more. Therefore, the amount of MP-Lys supplied to the animal when BM is fed can be substantially less than predicted by ruminant nutrition

Table 4. Results from 265 blood meal samples tested by Venture Milling at Ohio State University using a modified Minnesota 3-step procedure (St-Pierre, unpublished observations). Amino acid data is from 238 samples

	Average	S.D.	5th percentile	95th percentile
DM%	89.8	1.65	87.1	92.4
CP, % DM	90.1	3.68	84.4	96.0
RUP, %CP	76.8	14.80	50.4	96.6
RUP digestibility, %	64.6	23.06	19.9	97.6
AA, %CP				
Arginine	4.18	0.31	3.76	4.72
Histidine	6.56	0.81	5.06	7.90
Isoleucine	0.88	0.41	0.45	1.66
Leucine	13.26	0.92	11.50	14.63
Lysine	9.12	0.79	7.44	10.31
Methionine	1.18	0.30	0.75	1.59
Phenylalanine	7.10	0.54	6.30	7.99
Threonine	4.11	0.75	2.96	5.15
Tryptophan	1.36	0.33	0.77	7.80
Valine	8.91	0.61	7.85	9.85

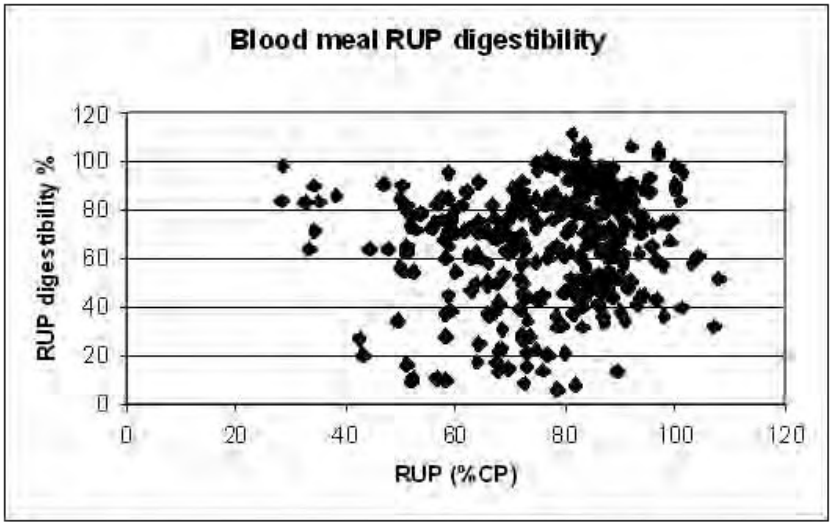


Figure 3. Lack of relationship between RUP (rumen undedgraded protein) and RUP digestibility in blood meal from commercial sources

models, particularly for lower quality BM samples, because the models do not adjust predicted MP-Lys supply based on the digestibility of RUP-Lys nor do they account for the variation in RUP and RUP digestibility. The feed libraries in most ration formulation

software uses the NRC 2001 values for blood meal, which has a high RUP value (77.5% when dry matter intake = 4% of body weight) with high RUP digestibility (80%).

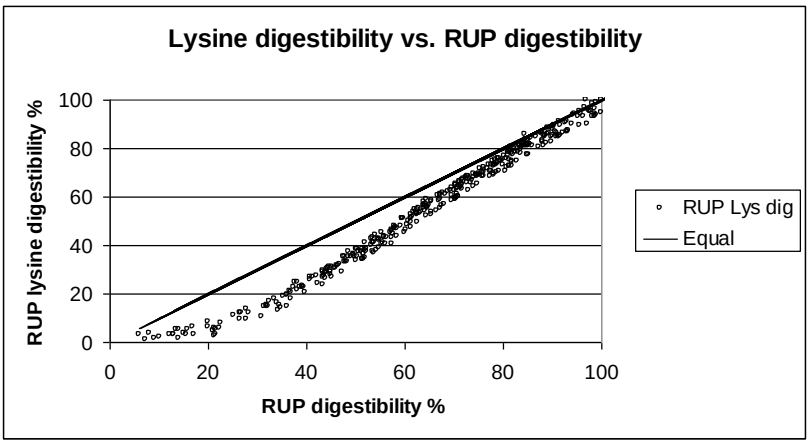


Figure 4. RUP-Lys digestibility versus RUP digestibility as determined by a modified 3-step procedure (St-Pierre, unpublished observations). Measured data is represented by dots; RUP- Lys digestibility equal to RUP digestibility would fall along the line. RUP = rumen undegraded protein

CONCLUSIONS

Heat processing of feeds is commonly used in the production of by-product ingredients and to decrease ruminal degradability of feed protein. If heat processing conditions are too severe, or not carefully controlled, Lys damage can result, which decreases Lys digestibility in the small intestine. Decreased digestibility of total RUP can also result from improper heat processing conditions. For feeds in which the Lys has been damaged due to heating conditions, MP-Lys supplied by those feeds will be over-predicted by current ruminant nutrition models because these models do not account for intestinal digestibility of individual AA in RUP. Therefore, more data is needed to better characterize differences in RUP-AA digestibility within feedstuffs. This data will be particularly important when feeding DDGS and BM because RUP-Lys digestibility can be substantially lower than RUP digestibility for these feeds. Identifying an accurate *in vitro* method that can be used to rapidly and economically analyze more samples for RUP-AA digestibility, particularly RUP-Lys digestibility, may allow ruminant nutrition models to more accurately predict MP-Lys supply from individual feed ingredients. If more accurate predictions of MP-Lys supplied by RUP are realized, herd responses to balancing rations for AA will become more predictable and consistent. Monitoring lysine damage that results from heat processing conditions can also be useful in monitoring quality of protein feeds supplied from various sources.

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THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM: CURRENT AND FUTURE APPROACHES FOR BALANCING OF AMINO ACIDS

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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, Dalex) have been used in the dairy industry to evaluate and formulate rations for more than 10 years. The long-term objective of the CNCPS and CPM modeling effort has been to provide a field usable model the accounts for a large proportion of the variation in ration formulation and animal performance and is based on our current understanding of the biology of both growing and lactating cattle. In recent years, the accumulation of information pertaining to amino acid balancing has outpaced our ability to mathematically characterize and model it appropriately. The purpose of this paper is to characterize where we are with metabolizable protein (MP) and amino acid balancing and what aspects of the mathematical description of the biology might enhance our ability to improve the predictions of amino acid requirements and supply.

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., 1994; 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. An evaluation of most limiting (ME or MP) 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.

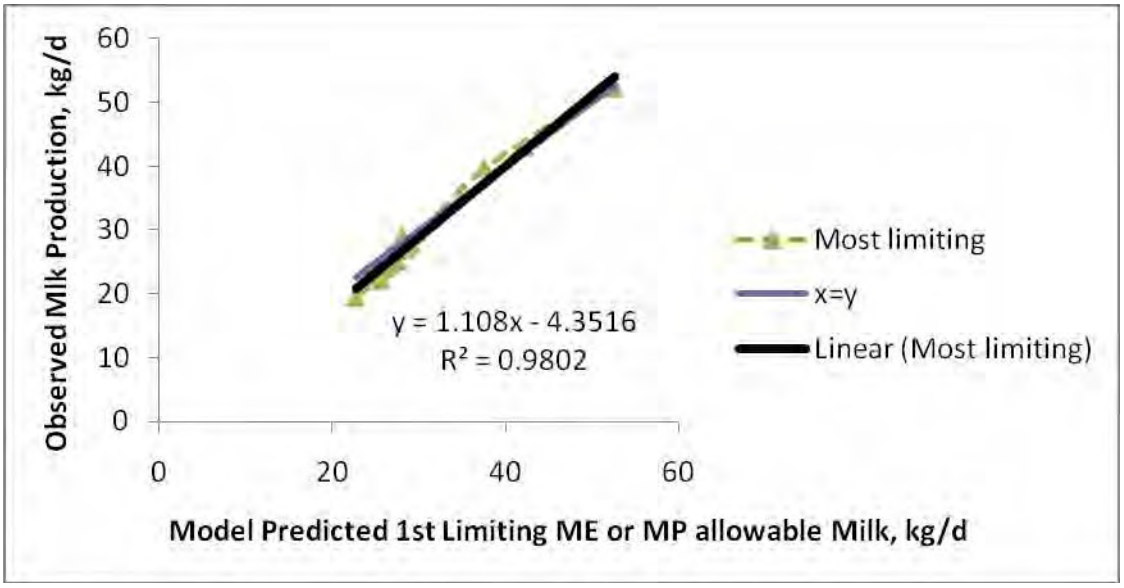

Figure 1. Observed versus predicted most limiting (ME or MP) milk production from the CNCPS. There are 24 comparisons ranging from groups within herds to individual study data with dietary crude protein levels of 12.7 to 17.4%.

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). However, the comparisons described in Figure 1 indicates that when evaluating these data, a static value does reasonably well over a large range in milk production and a dietary CP levels, most likely because the changes in efficiency of use of particular amino acids are within the range covered by the conversion of MP to NP and the individual AA efficiency is hard to detect because the we have little data on AA balancing beyond methionine and lysine. Also, when making comparisons for evaluating AA limitation, the AA in question or MP in general should be at or near limiting through a dose titration to elucidate the optimal efficiency given the ME available for milk and the stage of lactation.

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, Table 7, 100% of the optimum supply). Taking the simple mean of the efficiencies listed in Table 7 of 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).

If this overall value is more appropriate, part of the offset in the CNCPS might be the endogenous protein supply which is not accounted for in the current structure of the model. Given the calculations of Ouellet et al., (2002) and Marini et al., (2008) the amount of the endogenous protein reabsorbed in the cow can be up to 15% of total protein supply. This is currently calculated as a maintenance cost and none of that protein is considered available to the cow. Further, from the data of Ouellet et al., 2002 the rumen bacteria utilized endogenous protein at the same level as the recycled N, which means the microbes utilize protein from endogenous supply. The Dairy NRC (2001) estimated that 5% of the total protein supply could come from endogenous supply but based on the data of both Ouellet et al. (2002) and Marini et al. (2008) this is underestimated by about 50%. To extend this to amino acids supply, we would need to estimate the AA profile of the endogenous protein and then calculate supply and digestibility. There are several data sets available where these estimates have been made and can easily be adopted in the CNCPS (Shabi et al., 2000; Ouellet et al., 2002; and Marini et al. (2008). It will require a reworking of how maintenance requirements are calculated and a change in the number of protein pools that are available to the animal. Implementing a set of calculations to incorporate this would result in predicting the endogenous supply and then allocating it to the microbial pool and the balance to possible re-absorption by the cow.

Further, based on the recent data of Ouellet et al., (2002) the re-absorption of the endogenous protein in the Dairy NRC is appropriate, but proportionately still only accounting for about 50% of the true absorption. More interesting are the endogenous protein contributions to the microbial protein pool. Within their data set, approximately 10% of the microbial protein came from endogenous protein uptake, a value similar to the amount of microbial protein originating from recycled urea (12%). This observation also points out an obvious source of peptides for bacterial growth, which decrease the need for feed peptides currently predicted to enhance bacterial protein yield in the CNCPS.

Amino Acid Composition of Feeds

The CNCPS currently has a feed library that contains the values for AA based on the insoluble residue (Sniffen et al. 1992; O'Connor et al. 1993). With increased knowledge and more robust modeling approaches, this AA profile has been called into question. Does the insoluble residue actually represent the profile of AA that reaches the small intestine? This was the original hypothesis of Sniffen and Van Soest and for some feeds, it is most likely appropriate. A couple studies have been conducted to evaluate the use of the buffer insoluble residues and the conclusions are that for most feeds the intact feed and the insoluble residues are not remarkably different (Tedeschi et al., 2001; Ross, 2004). The most significant differences are in the fermented forages, thus more data is necessary to properly characterize those feeds to provide a better AA profile for use in the model. 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. The soluble proteins and peptides move with the liquid phase from the rumen to the small intestine and supply the cow with AA (Chooi 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. 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.

Amino acid supply from protozoa

The CNCPS does not have a protozoa pool within the rumen submodel, however it is now known that from 5% to at least 20% of the total AA flows from the rumen are from the protozoa (Shabi et al., 2000; Sylvester, et al. 2005; Karnati et al., 2007). The CNCPS currently calculates that the protozoa consume 20% of the estimated bacterial yield, thus the Ymax estimation is reduced from 0.5 g of bacteria per g of carbohydrate per hour to 0.4 g. However, there is really no provision for the ultimate fate of this bacterial growth and results in an estimate of bacterial yield that is static and ignores protozoa metabolism and any AA yield from the protozoa. There are enough data available from the work of Jeff Firkins group and many studies prior to that to engage in a remodeling of the rumen submodel to include the protozoa and develop a true microbial growth model that then drives yield based on the liquid and solid passage rates. This would be a very different rumen submodel than we currently have and would allow for the development of a volatile fatty acid model which is needed if we are to effectively predict the substrates available for milk production that differentiates how the cow utilizes those nutrients. Some of this will be addressed by Recktenwald and Van Amburgh in during this conference.

It is important to recognize that protozoa have a different AA profile, especially with respect to methionine and lysine. The methionine content of protozoa is lower than that of bacteria (24.0 vs 28.4 g/kg of total AA, whereas the lysine content of protozoa is significantly greater than bacteria (121.4 vs 90.3 g/kg total AA) (Shabi et al., 2000). This suggests that under certain formulation conditions, if protozoa were included in the prediction of AA flow, lysine might not be as limiting an AA provided protozoal growth and escape made up a significant portion of the MP supply. Protozoa are lower in BCAA content, thus potentially creating conditions where those AA are more limiting.

Absorbed Amino Acids – Efficiency of Use

Coefficients for the efficiency of individual AA use for pregnancy and lactation have been updated from the original values provided in O'Connor et al. (1993). The revised coefficients for the efficiency of individual AA use for lactation were calculated from summarized data for uptake/output of individual AA by the mammary gland in experiments using dairy cattle (Cant et al., 1993; Clark et al., 1977; Erickson et al., 1992; Guinard and Rulquin, 1995; Hanigan et al., 1992; Lykos and Varga, 1997; Mackle et al., 2000; Metcalf et al., 1996; Spires et al., 1975).

Efficiency factors for use of individual essential AA that are utilized in CNCPS are provided in Table 1. Of note is the substantial standard deviation associated with the

mean values for efficiency of use for individual essential AA for lactation. This represents in part the experimental variation associated with conducting experiments to measure net uptake and output of amino acids across the mammary gland. It is likely, however, that some of this variation is true biological variation in efficiency of use for those AA that are subject to oxidation or transamination to nonessential AA within the mammary gland. Given this variation, it is logical to question what is are acceptable limits to the prediction of amino acid flows and even MP supply. These data suggest that ranges in prediction should be determined and presented so that the user can make a decision about their level of comfort with the formulation.

For methionine, the arithmetic mean yielded an efficiency of use of 100% and this is due to little net use of Met for processes other than milk protein synthesis in the mammary gland, but we must be recognized the role that methionine plays in overall protein synthesis in the body, thus the requirement is most likely several grams greater than we are currently calculating based on our approach – maintenance, pregnancy and lactation with separate efficiencies. It is apparent from many datasets that the ratio of lysine to methionine (3:1) is very well understood and new data are providing absolute quantities that are consistent over years and different approaches (Rulquin et al. 1993; Schwab, 1996; Doepel et al., 2004). The latest version of the model appears to reasonably predict these relationships, for field application.

Lapierre et al. (2007) suggested that it is difficult to separate the efficiency of use of AA for maintenance and lactation as is currently done in CNCPS v6.1. We agree with that assessment and are making provisions to combine the efficiencies of use into a single calculation and utilizing the efficiencies from the Doepel et al. (2004) paper in Table 7 at 100% of optimum utilization as our starting point for evaluating against current data. The efficiency of use of each AA will be difficult to calculate in a factorial approach, as is currently done, thus a more dynamic approach might be needed. However, more experimental data is needed where MP and AA are known to be limiting or at the absolute requirement relative to energy to understand what the interactions are and how far the efficiencies will change without losing milk and milk protein yield. We are working towards studies of this nature and hope to gain some insight over the next few years. We need to move beyond lysine and methionine and begin to understand the interactions with other potentially limiting AA like histidine, the branch-chain AA and others.

Table 1. Efficiency factors for use of amino acids for maintenance and lactation with indices of variation provided for mammary gland efficiencies of use.

AA	Maintenance efficiency of use	Lactation (mammary gland utilization)		
		Efficiency of use	Standard Deviation	Coefficient of Variation
Met	0.85	1.00	0.10	10%
Lys	0.85	0.86	0.13	16%
His	0.85	0.94	0.13	14%
Phe	0.85	0.98	0.08	8%
Trp	0.85	0.85	NA	NA
Thr	0.85	0.79	0.14	18%
Leu	0.66	0.71	0.07	10%
Ile	0.66	0.66	0.07	11%
Val	0.66	0.62	0.09	14%
Arg	0.85	0.34	0.06	17%

Source of Energy and Metabolic Regulation

Data generated over the last 15 years demonstrate that the site of absorption of starch and sugars, and the form of substrate, either glucose in the small intestine or propionate, might impact the animal response to the nutrient and potentially the endocrine signaling in a manner that could affect the efficiency of use of absorbed AA (Knowlton et al., 1998, Rulquin et al., 2004; Raggio et al., 2006, Reynolds, 2006; Lemosquet et al. 2009). These data suggest that as starch is digested in the small intestine, the transfer of glucose into the circulation increases, but the efficiency of transfer of the glucose carbon into lactose production decreases, thus decreasing the efficiency of use of absorbed AA. Reynolds (2006) summarized several studies and concluded that although there is an increase in glucose availability, the tissues drained by the portal vein appear to preferentially utilize the glucose and in addition retain slightly greater body protein. There is an apparent increase in milk volume, but milk fat content decreases so that the energy output of the cow is not increased. With increased post-ruminal starch digestion there is a change in insulin status, but under the conditions study to date, it does not translate into greater milk protein output. Currently in the CNCPS, although the model calculates the amount of starch digested in the rumen and small intestine, there is no provision for changes in how the model treats energy absorbed as glucose in the small intestine versus energy yield from ruminal digestion. To enhance the sensitivity of the model, we need to make adjustments for post-ruminal starch digestion that describes the fate of that energy in a more mechanistic approach – this should allow us to enhance the prediction of efficiency of use of absorbed AA.

Efficiency of use of AA is a combination of the energy allowable production and then the ability to balance the profile and supply of AA to meet the energy driven demand. It is well known that particular hormones such as insulin, IGF-I, and cortisol to name a few have a profound impact on protein synthesis in the mammary gland, thus can alter the

efficiency of use of particular AA (Mepham, 1982; Mackle et al. 2000). It is unlikely within the near future that we will engage in modeling the effect of hormones on milk protein synthesis, but it is likely that the calculation of efficiency will be based more on the predicted ME allowable milk rather than calculating efficiency on level of MP supply per se. We can decrease excess feed protein in the ration and decrease the excretion of urinary N without having a profound impact on the overall efficiency of use – the apparent efficiency increases because we are wasting less protein but the actual efficiency is not improved because more milk protein is not being excreted. So at the moment, in a factorial system, we have a two step process based on N for rumen function and then AA for milk protein output and the challenge is to integrate protein supply with energy supply and associated homeorhetic and homeostatic signaling mechanisms.

SUMMARY

Data now exist to allow us to restructure the CNCPS and improve our ability to predict AA flows and utilization by the lactating cow. This will require substantial re-imagining of the structure and the appropriate data for proper validation.

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RECENT RESEARCH WITH LOW-STARCH DIETS FOR LACTATING DAIRY COWS

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INTRODUCTION

Common recommendations for dietary starch content (dry matter (DM) basis) for lactating cows are 23 to 30% (Grant, 2005), 24 to 26% (Staples, 2007), and greater than 24% (Shaver, 2008). Surveys of dairy herds that produced more than 12,700 kg of milk per cow per year found that dietary starch content ranged between 15 and 30% (Hall and Van Horn, 2001; Johnson et al., 2002; Shaver and Kaiser, 2004; Chase, 2006). The major source of dietary starch for lactating cows is corn according to the Dairy 2007 survey (USDA, 2008). Corn, oats, barley, and wheat were fed to lactating cows in 94, 18, 14, and 7% of herds, respectively. The price for corn grain as a livestock feed has increased substantially during the past two years. Consequently, lower-starch feeding strategies that minimize the amount of corn may be more profitable than higher-starch diets particularly if lactational performance and ruminal fermentation are not compromised.

STRATEGIES TO LOWER THE DIETARY STARCH CONTENT

Recently, strategies for formulating lactating cow diets with high corn prices have been suggested and include using less corn grain and using more high-quality forage and byproduct feeds to provide highly digestible neutral detergent fiber (NDF) and nonfiber carbohydrates (Chase, 2007; Knapp, 2007; Staples, 2007; Shaver, 2008). Summarized in Table 1 are the dietary conditions and dry matter intake (DMI) and milk yield results of studies on replacement of corn starch with nonforage fiber sources (NFFS) and other carbohydrate sources to yield lower-starch ($\leq$ 23%) diets.

Use of NFFS is a practical way to reduce the dietary starch content while maintaining lactational performance. Batajoo and Shaver (1994) replaced shelled corn and soybean meal with wheat middlings (0 to 9%), dried brewers grains (3 to 20%), and soyhulls (SH; 0 to 9%) to provide alfalfa silage-based diets ranging in starch content from 32.9 to 17.6% to lactating cows. Decreasing the dietary starch content linearly decreased DMI, milk protein content, and milk protein yield, linearly increased milk fat content, ruminal pH, ruminal acetate concentration, ruminal acetate:propionate, and total tract digestibility of NDF and starch, and had no effect on milk yield. In another study (Ipharraguerre et al., 2002), SH (0 to 40%) were used to replace corn grain in alfalfa/corn silage-based diets for mid-lactation cows. There tended to be a linear decrease in DMI as SH replaced corn, but the major decrease in DMI occurred at the 30 and 40% inclusion level of SH. Milk yield tended to decrease at the 40% inclusion level. Milk fat content increased linearly with more SH and less starch. Thus, cows can be fed successfully a 19% starch diet containing up to 30% SH.

Table 1. Summary of selected research where corn starch was replaced with nonforage fiber sources or other carbohydrate sources resulting in low-starch (< 23%) diets.

Reference; Treatment information	Dietary content, % of dry matter[1]							
	Treatment	F:C	Forage	NDF	Forage NDF	Starch	DMI, kg/d	Milk, kg/d
Replacement of Corn Starch with Nonforage Fiber Sources								
Batajoo & Shaver, 1994; shelled corn and soybean meal replaced with wheat middlings (0 to 9%), dried brewers grains (3 to 20%), and soybean hulls (0 to 9%)	0, 3, 0	48:52	48 AS	28.2	20.5	32.9	27.6^{L}	40.2
	7, 7, 0	48:52	48 AS	32.9	20.5	28.5	27.2^{L}	39.7
	10, 9, 7	48:52	48 AS	37.4	20.5	24.0	26.7^{L}	39.7
	9, 20, 9	48:52	48 AS	42.9	20.5	17.6	25.8^{L}	39.5
Leiva et al., 2000, study 1; corn hominy (CH) replaced with dried citrus pulp (24%)	CH	33:67	7 AH, 27 CS	35.9	-	26.5	21.4	32.8
	Citrus pulp	33:67	7 AH, 27 CS	36.5	-	15.1	20.9	31.3
Leiva et al., 2000, study 2; corn meal replaced with dried citrus pulp (21%)	Corn meal	46:54	16 AH, 30 CS	33.8	-	19.1	-	31.8^{a}
	Citrus pulp	45:55	16 AH, 29 CS	34.4	-	12.9	-	27.9^{b}
Boddugari et al., 2001, study 1; corn and soybean meal replaced with wet corn gluten feed (0 to 45%)	0	54:46	27 AS, 27 CS	28.2	22.8	30E	24.7^{a}	30.4
	22	54:46	27 AS, 27 CS	35.4	22.8	23E	22.5^{b}	30.5
	34	54:46	27 AS, 27 CS	38.2	22.8	18E	23.1^{ab}	30.8
	45	54:46	27 AS, 27 CS	41.6	22.8	15E	21.8^{b}	29.5
Ipharraguerre et al., 2002; corn replaced with soybean hulls (0 to 40%)	0	46:54	23 AS, 23 CS	29.4	19.0	38E	23.8	29.5
	10	46:54	23 AS, 23 CS	34.4	19.0	31E	24.8	29.3
	20	46:54	23 AS, 23 CS	39.9	19.0	24E	24.4	29.9
	30	46:54	23 AS, 23 CS	44.8	19.0	17E	22.9	29.3
	40	46:54	23 AS, 23 CS	49.4	19.0	9E	22.7	28.3
Broderick et al., 2002, study 1; high moisture ear corn (HMEC) or cracked corn (CC) replaced with dried citrus pulp (19%)	HMEC	60:40	50 AS, 10 GS	27.4	22.0	30.6	20.0	34.5^{A}
	CC	60:40	50 AS, 10 GS	26.2	22.0	31.0	20.9^{a}	33.6^{a}
	Citrus pulp	60:40	50 AS, 10 GS	28.4	22.0	20.0	19.2^{b}	29.9^{Bb}

Table 1 continued.

Reference; Treatment information	Dietary content, % of dry matter[1]							
	Treatment	F:C	Forage	NDF	Forage NDF	Starch	DMI, kg/d	Milk, kg/d
Voelker & Allen, 2003a; high moisture corn replaced with pelleted beet pulp (0 to 24%)	0	40:60	20 AS, 20 CS	24.3	17.1	34.6	24.8^{L}	36.4
	6	40:60	20 AS, 20 CS	26.2	17.1	30.5	25.0^{L}	36.6
	12	40:60	20 AS, 20 CS	28.0	17.1	26.5	25.1^{L}	35.9
	24	40:60	20 AS, 20 CS	31.6	17.1	18.4	22.9^{L}	35.4
Ranathunga et al., 2008; corn replaced with dried distillers grains with solubles (0 to 21%)	0	49:51	-	-	21.0	28.0	25.6^{L}	39.4
	7	49:51	-	-	21.0	24.5	25.0^{L}	37.4
	14	49:51	-	-	21.0	21.0	23.4^{L}	37.7
	21	49:51	-	-	21.0	17.5	22.9^{L}	38.3
Replacement of Corn Starch with Other Carbohydrate Sources								
Valadares Filho et al., 2000; alfalfa silage replaced with high moisture corn (19 to 56%)	19	80:20	80 AS	42.9	37.9	12.3	22.1^{LQ}	31.2^{L}
	31	65:35	65 AS	38.2	31.1	20.7	25.2^{LQ}	36.0^{L}
	44	50:50	50 AS	32.6	23.9	29.5	26.4^{LQ}	39.8^{L}
	56	35:65	35 AS	27.7	16.8	38.3	25.6^{LQ}	43.4^{L}
Oba & Allen, 2003a; ground high moisture corn (HMC) or dry ground corn (DGC) at 32 or 21% starch	HMC, 21	66:34	34 AS, 32 CS	30.1	25.3	21.0	19.7^{b}	33.4^{b}
	DGC, 21	66:34	34 AS, 32 CS	30.5	25.4	21.3	19.6^{b}	34.3^{b}
	HMC, 32	43:57	22 AS, 21 CS	23.1	16.5	31.1	20.8^{a}	38.8^{a}
	DGC, 32	43:57	22 AS, 21 CS	24.2	16.5	32.2	22.5^{a}	38.4^{a}
Broderick & Radloff, 2004, study 1; high moisture corn replaced with dried molasses (0 to 12%)	0	60:40	40 AS, 21 CS	28.2	22.9	31.5	25.3^{Lb}	38.0^{Cab}
	4	60:40	40 AS, 21 CS	29.1	22.9	28.4	25.7^{Lab}	37.5^{Cab}
	8	60:40	40 AS, 21 CS	29.2	22.9	25.2	26.3^{La}	38.9^{Ca}
	12	60:40	40 AS, 21 CS	29.3	22.9	23.2	26.0^{Lab}	36.7^{Cb}

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Table 1 continued.

Reference; Treatment information	Dietary content, % of dry matter[1]							
	Treatment	F:C	Forage	NDF	Forage NDF	Starch	DMI, kg/d	Milk, kg/d
Charbonneau et al., 2006; cracked corn (CC, 47%), ground corn (GC, 47%), GC (35%) + wheat starch (WS, 11%), GC (35%) + dried whey permeate (WP, 11%)	CC	45:55	45 AS	28.9	18.8	26.7	22.7^{c}	34.0^{c}
	GC	45:55	45 AS	27.1	18.8	28.8	24.3^{b}	37.4^{ab}
	GC + WS	45:55	45 AS	25.1	18.8	33.3	24.4^{b}	37.6^{a}
	GC + WP	45:55	45 AS	24.9	18.8	21.6	25.7^{a}	35.8^{b}
Broderick et al., 2008; corn starch replaced with sucrose (0 to 7.5%)	0	60:40	39 AS, 21 CS	30.0	25.6	28.2	24.5^{Lb}	38.8
	2.5	60:40	39 AS, 21 CS	29.2	25.6	27.4	25.4^{Lab}	40.6
	5.0	60:40	39 AS, 21 CS	29.6	25.6	24.5	26.0^{La}	39.4
	7.5	60:40	39 AS, 21 CS	29.6	25.6	21.5	26.0^{La}	39.3
Gozho & Mutsvangwa, 2008; barley (31%), corn (29%), wheat (33%), and oats (31%) as primary starch source	Barley	48:52	18 AH, 30 BS	32.7	-	19.2	26.2	40.2
	Corn	48:52	18 AH, 30 BS	32.9	-	21.8	26.0	38.9
	Wheat	52:48	20 AH, 32 BS	33.0	-	22.4	24.0	36.8
	Oats	48:52	18 AH, 30 BS	35.7	-	15.2	25.2	38.4
Abdelqader et al., 2009; corn and soybean meal replaced with corn germ (0 to 21%)	0	55:45	25 AH, 30 CS	28.3	23.5	24	28.1^{Q}	37.3^{Q}
	7	55:45	25 AH, 30 CS	28.8	23.5	23.3	29.1^{Q}	38.0^{Q}
	14	55:45	25 AH, 30 CS	29.8	23.5	21.6	28.8^{Q}	38.2^{Q}
	21	55:45	25 AH, 30 CS	30.7	23.5	19.3	27.3^{Q}	36.3^{Q}
Arndt et al., 2009; corn (C) replaced with corn bran (CB;19, 38%) in high forage (HF, 64%) and low forage (LF, 38%) diets	HFC	64:36	29 AS, 36 CS	29.7	18.1	25.6	24.8^{bc}	42.0^{b}
	HFCB	64:36	29 AS, 36 CS	42.6	18.1	14.8	24.3^{c}	38.7^{c}
	LFC	38:62	17 AS, 21 CS	23.1	10.9	34.0	26.7^{a}	46.7^{a}
	LFCB	38:62	17 AS, 21 CS	48.4	10.8	12.5	25.9^{ab}	40.5^{bc}

[1] F:C = forage to concentrate ratio, NDF = neutral detergent fiber, DMI = dry matter intake, AS = alfalfa silage, AH = alfalfa hay, CS = corn silage, GS = grass silage, BS = barley silage, E = starch content estimated with CPM-Dairy v.3.
^{L} = linear effect with $P \leq 0.05$, ^{Q} = quadratic effect with $P \leq 0.05$, ^{C} = cubic effect with $P \leq 0.05$, $^{abc\ or\ AB}$ Least squares means within the same column and study without a common superscript differ with $P \leq 0.05$.

Voelker and Allen (2003abc) replaced high moisture corn with 0 to 24% beet pulp to formulate alfalfa/corn silage-based diets with decreasing starch content (34.6 to 18.4%) for lactating cows. Decreasing starch content linearly decreased DMI and microbial nitrogen yield, tended to linearly increase feed efficiency, and had no effect on milk yield and milk composition. Ruminal passage rate of starch, ruminal digestion rate of potentially digestible NDF, and total tract digestibility of organic matter and NDF increased linearly, while ruminal digestion rate of starch decreased linearly with decreasing starch content. Corn hominy was partially replaced with citrus pulp (24%) in corn silage-based diets to provide diets containing 26.5 and 15.1% starch to mid-lactation cows (Leiva et al., 2000). There was no effect of diet on DMI, milk yield, or milk composition. Broderick et al. (2002) used alfalfa silage-based diets and partially replaced high moisture ear corn or cracked corn with citrus pulp (19%). Dry matter intake and milk yield were reduced when the citrus pulp was fed. Boddugari et al. (2001) replaced corn and soybean meal with wet corn gluten feed (CGF; 0 to 40%) in alfalfa/corn silage based diets fed to lactating cows. Dry matter intake tended to be lower for the diets containing the wet CGF. Diet did not affect milk yield or milk composition. Ranathunga et al. (2008) partially replaced corn starch with dried distillers grains with solubles (0 to 21%) in diets for lactating cows. As the starch content decreased from 28.0 to 17.5%, DMI linearly decreased and feed efficiency tended to linearly increase. There was no effect of diet on milk yield or composition.

Replacing corn starch with sugar sources in high forage diets containing alfalfa silage and corn silage is a viable strategy for reducing dietary starch content while maintaining milk yield. Broderick and Radloff (2004) replaced high moisture shelled corn with 0 to 12% dried molasses in diets for mid-lactation cows. Decreasing the starch content from 31.5 to 23.2% linearly increased DMI and total tract digestibility of DM, organic matter, and NDF, but had no effect on milk yield and ruminal fermentation. Broderick et al. (2008) fed lactating cows diets containing 21.5, 24.5, 27.4, and 28.2% starch; corn starch was replaced with 0 to 7.5% sucrose. Decreasing starch content linearly increased DMI, milk fat content, and milk fat yield, linearly decreased feed efficiency, ruminal acetate concentration, and ruminal acetate:propionate, and had no effect on milk yield and ruminal pH.

Another feasible strategy to reduce the dietary starch content is to replace corn with high-quality forage. Valadares Filho et al. (2000) substituted alfalfa silage for high moisture ear corn and soybean meal. As the starch content decreased, there was a linear decrease in DMI and milk yield with the majority of the decrease in DMI occurring at the lowest starch level. There were linear and quadratic responses in milk fat content and yield. Reducing the dietary starch content to less than 20.7% should be avoided when substituting alfalfa silage for corn starch. Oba and Allen (2003ab) fed lactating cows diets containing either ground high moisture corn or dry ground corn at two dietary starch contents (32 and 21%). Dry matter intake, milk yield, and milk protein content were lower for the low-starch diets. Milk fat content, body condition loss, ruminal pH, and acetate:propionate were higher for the low-starch diets. Total tract digestibility (%) of starch was lower for the low-starch diets, but ruminal digestion (%) of starch was not affected by starch content.

RESEARCH AT MINER INSTITUTE WITH LOW-STARCH DIETS

We (Dann et al., 2008) used 12 multiparous, mid-lactation cows in a replicated 3×3 Latin square design study with 21-d periods (7-d collection) to determine the effect of feeding diets containing 18, 21, and 25% starch (Table 2) on lactational performance, ruminal fermentation, and total tract nutrient digestibility (Table 3). Dietary starch was reduced by decreasing the amount of corn grain and increasing the amount of beet pulp, wheat middlings, and distillers grains. Cows were able to maintain high productivity on all diets. Dry matter intake (26.5 kg/d), milk yield (43.5 kg/d), milk fat content (3.54%), milk true protein content (3.14%), and efficiency of milk production (1.65 kg milk/kg DMI) were unaffected by diet. Diet also had no effect on ruminal pH averaged over 24 h (6.06), total volatile fatty acids (150 m*M*), acetate:propionate (2.4), or microbial nitrogen yield (584 g/d). Total tract digestibility of organic matter was higher for the 25% starch diet (69.2%) compared with the 21% (67.3%) and 18% (67.0%) starch diets but was of little biological relevance. Digestibility of NDF and starch was not affected by diet.

Table 2. Ingredient and chemical composition (dry matter basis) of diets containing 18, 21, or 25% starch fed to lactating Holstein cows.

	Diet		
Item	18% starch	21% starch	25% starch
Ingredient composition			
Corn silage, %	30.2	30.2	30.4
Grass silage, %	18.5	18.4	18.6
Alfalfa hay, %	5.0	5.0	5.1
Soybean meal (48%), %	7.1	8.0	8.4
Corn, finely ground, %	3.4	10.1	16.9
Beet pulp, %	6.7	3.4	-
Wheat middlings, %	13.4	10.1	6.8
Distillers grains, %	9.7	8.7	7.8
Other, %	6.0	6.1	6.0
Chemical composition			
Crude protein, %	17.4	17.6	17.2
Acid detergent fiber, %	22.2	20.8	20.0
Neutral detergent fiber, %	38.0	36.5	34.2
Forage neutral detergent fiber, %	24.7	24.7	24.8
Sugar, %	4.8	3.9	3.6
Starch, %	17.7	21.0	24.6
Starch 6-h digestibility, % starch	82.5	77.3	73.6
Rumen fermentable starch, %	14.6	16.2	18.1

In summary, with the sources of corn grain, corn silage, and byproducts fed in this study, we observed no effect on feed intake, milk component production, ruminal metabolism, or microbial protein yield when dietary starch was varied between 18 and 25%. It is important to note that, as dietary starch decreased, ruminal fermentability increased and consequently the range between the 25 and 18% starch diets in rumen

fermentable starch (3.5%-units) was less than the range in starch content (6.9%-units). When predicting the potential impact of starch content of the diet on animal response, we need to consider not only the amount, but the digestibility of the starch.

Table 3. Lactational performance, ruminal fermentation, and total tract digestibility data of lactating Holstein cows fed diets containing 18, 21, or 25% starch.

	Diet				
Item[1]	18% starch	21% starch	25% starch	SEM	*P*-value
DMI, kg/d	26.4	26.9	26.3	0.8	0.51
DMI, % of BW/d	3.68	3.72	3.65	0.10	0.60
Milk, kg/d	42.9	43.4	44.1	1.9	0.60
3.5 % FCM, kg/d	43.1	43.4	43.8	1.8	0.86
Milk fat, %	3.57	3.57	3.48	0.15	0.45
Milk true protein, %	3.09	3.18	3.14	0.07	0.19
Milk/DMI, kg/kg	1.64	1.62	1.68	0.08	0.32
Ruminal pH	6.10	6.01	6.07	0.12	0.76
Total VFA, m*M*	151.8	153.4	145.2	6.0	0.21
Acetate: propionate	2.3	2.3	2.6	0.3	0.70
Microbial nitrogen, g/d	579	590	583	24	0.75
Organic matter TTD, %	67.0[b]	67.3[b]	69.2[a]	0.5	0.009
NDF TTD, %	43.7	43.4	42.3	1.2	0.62
Starch TTD, %	98.2	98.3	98.5	0.1	0.25

[1] DMI = dry matter intake, BW = body weight, FCM = fat-corrected milk, VFA = volatile fatty acids, TTD = total tract digestibility, NDF = neutral detergent fiber.
[ab] Least squares means within a row without a common superscript differ ($P \leq 0.05$).

A low-starch, low-forage dietary strategy may be advantageous when corn starch and forages are either expensive or availability is limited. We (Myers et al., 2009) used 16 mid-lactation Holstein cows in a replicated 4×4 Latin square design study with 21-d periods (9-d collection) to determine the effect of feeding diets containing low-starch (formulated at 19% of DM) and different amounts of forage (52, 47, 43, and 39% of DM; Table 4) on lactational performance, ruminal characteristics, and total tract digestibility. Dry matter intake was lowest (3.47% of body weight) when cows were fed the 52% forage diet and highest (3.67% of body weight) when cows were fed the 39% forage diet ($P = 0.03$). Diet did not affect ($P > 0.10$) milk yield (42.6 kg/d), milk fat content (3.60%), or milk true protein content (3.02%). Because there was an effect of diet on DMI, but not milk yield, efficiency was highest (1.87 lb milk/lb DMI) when cows were fed the 52% forage diet and lowest (1.77) when cows were fed the 39% forage diet ($P = 0.02$). Mean ruminal pH (6.07) and microbial nitrogen yield (450 g/d) were not affected ($P > 0.10$) by diet. As the forage content of the diets decreased from 52 to 39%, the total tract OM digestibility decreased from 65 to 61% ($P < 0.01$) and the total tract NDF digestibility decreased from 39 to 29% ($P < 0.01$). Lower forage diets with low-starch content are a good strategy for feeding high-producing dairy cows under conditions of expensive or limited supplies of corn and forages, but the limit appears to be between 39 and 43% forage with these types of diets when high productivity is expected.

Table 4. Ingredient and chemical composition (dry matter basis) of low-starch diets varying in forage content (52, 47, 43, and 39% forage) fed to lactating Holstein cows.

Item	Diet			
	52% forage	47% forage	43% forage	39% forage
Ingredient composition				
Corn silage, %	37.3	34.0	31.0	27.9
Alfalfa-grass silage, %	14.5	11.1	5.9	0.6
Wheat straw, %	-	2.1	6.2	10.3
Distillers grains, %	11.1	10.3	9.5	8.8
Soybean meal (48%), %	11.0	11.0	11.4	12.2
Wheat middlings, %	7.4	12.5	16.1	19.3
Corn, finely ground, %	5.6	5.4	6.4	7.3
Beet pulp, %	6.2	6.2	6.2	6.2
Other, %	6.9	7.4	7.3	7.4
Chemical composition				
Crude protein, %	17.3	17.7	17.3	18.1
Acid detergent fiber, %	20.5	20.6	19.9	19.1
Neutral detergent fiber (NDF), %	37.4	37.5	37.0	36.0
Forage NDF, %	25.0	23.1	21.7	20.3
Starch, %	20.2	20.8	21.2	21.6
Sugar, %	4.6	4.8	5.1	5.2

WATCH THE COWS WHEN FEEDING LOW-STARCH DIETS

Most of the research conducted with low-starch diets has been short-term (i.e. less than 8 wk) and focused on mid-lactation cows. The long-term effect of feeding low-starch diets to cows in all stages of lactation is unknown. Therefore, when implementing low-starch diets on an entire herd basis, the nutritionist and dairy producer should watch for signs that may indicate that the dietary starch content is too low. Signs include decreased milk production, decreased milk protein content and yield, decreased body condition and weight, increased milk urea nitrogen, and stiffer manure (Staples, 2007). In addition to watching the cows, feed ingredients should be monitored for changes in NDF and starch digestibility. Providing the proper amounts of ruminally fermentable carbohydrates are critical to optimizing ruminal fermentation and generating volatile fatty acids and microbial protein for energy and amino acid use by the cow.

CONCLUSIONS

Corn grain can be replaced with byproduct feeds in lactating cow diets resulting in low-starch (18 to 21%) diets without adverse effects on ruminal fermentation and lactational performance. In particular, diets containing NFFS that provide digestible NDF can support excellent production and feed efficiency with lower than commonly recommended amounts of starch. When a low-starch diet strategy is implemented on a herd, be sure to include feed ingredients that provide highly digestible starch and NDF,

monitor the cow's performance, and analyze feed ingredients for NDF and starch digestibility.

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ESTIMATING INTESTINAL DIGESTIBILITY OF AMINO AICDS IN THE RUMEN UNDEGRADED PROTEIN FRACTION OF FEEDSTUFFS

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SUMMARY

- The current dairy NRC (2001) and CNCPS v.5.1 models recognize differences in intestinal digestibility of rumen undegraded protein (RUP) among feed ingredients.

- These models assume digestibility of individual amino acids (AA) in RUP is the same as digestibility of total RUP, but digestibility of individual AA in the RUP fraction of feedstuffs does vary, particularly lysine digestibility.

- An increased database of digestibility estimates of individual AA in RUP is needed to improve predictions of supply of metabolizable AA by ruminant nutrition models.

- The modified three-step procedure and the IDEA kit assays™ are valid in vitro techniques that can be used to estimate digestibility of individual AA in RUP.

INTRODUCTION

The current poultry and swine NRC models (NRC 1994; NRC, 1998, respectively) allow users to formulate diets based on digestible amino acid (AA) supply. This is possible because the models recognize that small intestinal digestibility of individual AA is not necessarily the same as small intestinal digestibility of total crude protein (CP) in feed ingredients. Therefore, digestibility coefficients are assigned to individual AA in each feed ingredient within the poultry and swine NRC models. In ruminant nutrition models, differences in rumen undegraded protein (RUP) digestibility among feeds are recognized (Sniffen et al., 1992; NRC, 2001), but currently these models assume that the digestibility of individual AA in the RUP fraction of feeds (RUP-AA) is the same as digestibility of total RUP. In order to advance ruminant nutrition models to allow for diet formulation based on digestible RUP-AA supply, it is first important to understand how these models currently predict RUP digestibility of feeds. The RUP digestibility values described in CNCPS v.5.1 (Sniffen et al., 1992) and the dairy NRC (2001) are discussed.

MODEL PREDICTIONS OF RUP DIGESTIBILITY

Cornell Net Carbohydrate and Protein System (CNCPS)

The CNCPS v.5.1 model uses a chemical fractionation method to describe the characteristics of protein in feeds (Sniffen et al., 1992). The model divides feed protein into 5 fractions: A, B1, B2, B3, and C. The amount of protein in each fraction that escapes ruminal degradation is calculated by the model based on relative rates of

degradation and rates of passage. For the protein that escapes ruminal degradation, the model assigns intestinal digestibility coefficients specific to each of the protein fractions. Intestinal digestibility coefficients of 100, 100, 80, and 0% are assigned to the undegraded B1, B2, B3, and C protein fractions, respectively. Therefore, the model does not assign RUP digestibility coefficients to individual feedstuffs, but feed differences in RUP digestibility are indirectly accounted for based on the differences in the proportions of B1, B2, B3, and C in feed protein. For example, fraction C is considered to be completely undegradable in the rumen and completely indigestible in the small intestine; therefore, if feeds contain higher proportions of fraction C, the intestinal absorption of dietary protein will be predicted by the model to be lower than if feeds contain greater proportions of the other fractions.

NRC (2001)

The current dairy NRC (2001) model employs a different approach for estimating RUP digestibility. In NRC (2001), ruminal degradation characteristics of individual feeds were determined based on literature reported estimates of *in situ* determined rates of ruminal protein degradation. The model then calculates the contribution of each feed in the diet to total dietary RDP and RUP based on rates of degradation and rate of passage. Digestibility of the RUP is then calculated using RUP digestibility coefficients assigned to each feed ingredient that contributes to RUP. The RUP digestibility coefficients were determined based on a summary of 54 studies that reported RUP digestibility for individual feed ingredients. The mobile bag technique (MBT) was used in 48 of the studies and the three-step procedure (TSP) of Calsamiglia and Stern (1995) was used in 6 studies. The mean RUP digestibility values reported for each feed were calculated and rounded to the nearest 5 percentage units to emphasize the lack of precision in arriving at mean values. The RUP digestibility coefficients in the NRC (2001) feed library range from 50% for cottonseed hulls and canola seeds to 95% for skim milk powder.

The CNCPS v.5.1 and NRC (2001) models both account for differences in RUP digestibility among feeds, which represents a significant advancement in ruminant nutrition models. However, it is also important to note that RUP digestibility coefficients are not static within a feed type. For example, as stated, the RUP digestibility coefficients in the NRC (2001) model are the average of literature reported values. However, the standard deviation of the mean (SD) for some feedstuffs was quite large when the data was summarized (Schwab, personal communication). For example, the SD for RUP digestibility of grass silage was 22.5, and the SD for the RUP digestibility of canola meal was 10.6. Therefore, if nutritionists rely on model default values for RUP digestibility, metabolizable protein (MP) supply can be over or underestimated by the model.

In addition, the above models do not recognize differences in RUP-AA digestibility within feeds, which is likely due to limited availability of data. However, differences in RUP-AA digestibility within feeds have been reported (Prestløkken and Rise, 2003). Therefore, the development of nutritional models that account for differences in RUP-AA

digestibility within feeds will allow industry professionals to more precisely match AA supply to AA requirements. This will allow for maximal efficiency of use of dietary AA for milk protein synthesis, which can improve herd profitability and decrease N excretion. Adequate data regarding RUP-AA digestibility for a variety of feedstuffs will be needed for the development of such models. Methods that are used to estimate intestinal digestibility of RUP-AA are discussed.

ESTIMATING INTESTINAL DIGESTIBILTY OF RUP AND RUP-AA

Measuring intestinal CP and AA digestibility of individual feed ingredients is difficult in ruminant animals compared with non-ruminant species. This is attributable to microbial degradation of dietary protein in the rumen, ruminal synthesis of microbial protein, and ruminal N recycling. These factors make it difficult to estimate intestinal N and AA disappearance from a single source. Therefore, *in vivo* estimates of RUP digestibility of individual feeds in ruminants are extremely scarce (Hvelplund and Madsen, 1990), and the more simple *in situ* MBT is more commonly used to estimate intestinal digestibility of RUP and RUP-AA (NRC, 2001).

Mobile Bag Technique (MBT)

There are many sources of variation in obtaining intestinal digestibility estimates with the MBT. These sources of variation include: length of ruminal incubation time, fineness of grind of feed, pore-size of the bags, processing of the bags after incubation, sample size, length of pepsin/HCl incubation, and site of bag collection (ileum vs. feces). For the MBT procedure, once the bags have passed through the intestines, the undigested content of the bags is analyzed for AA, and the AA that disappeared from the bags are assumed to be absorbed by the animal. This is a precarious assumption as the small intestine is a live, active organ under physiological and metabolic control. Therefore, RUP and RUP-AA digestibility estimates obtained using the MBT may not be very accurate or precise.

Use of a smaller animal model to obtain RUP and RUP-AA digestibility estimates is easier, less expensive, and likely more accurate and precise compared with the MBT in dairy cattle.

In Vivo Models of Digestibility for Ruminants

Poultry appears to be a viable animal model to estimate RUP and RUP-AA digestibility for ruminants (Titgemeyer et al., 1990). Titgemeyer et al. (1990) evaluated the use of the precision-fed cecectomized rooster assay to estimate intestinal digestibility of AA in cattle. The authors crop-intubated the roosters with freeze-dried duodenal digesta obtained from duodenally and ileally cannulated steers fed 5 different diets. The correlation between the unweighted means for AA digestibility of the duodenal digesta in cattle and in roosters was 0.94 ($P < 0.05$), but the digestibility estimates obtained in the roosters were slightly lower than those obtained in the steers. Although not 100% accurate, AA digestibility estimates in the 2 species were close and

on average did not differ by more than 4 percentage units. Therefore, as the correlation between the AA digestibility estimates obtained in the 2 species was high, the precision-fed cecectomized rooster assay is likely an adequate animal model to estimate intestinal digestibility of RUP-AA in cattle. Experiments using the precision-fed cecectomized rooster assay to estimate intestinal RUP-AA digestibility for protein concentrates commonly fed to dairy cows are described below. Data obtained from these experiments were used in the validation of 2 *in vitro* procedures to estimate intestinal RUP-AA digestibility, which are also described.

Standardized Digestibility of AA and RUP-AA

Recently, Stein et al. (2007) defined several terms in a review article to be used when describing AA digestibility estimates in pig feed ingredients. The authors defined standardized AA digestibility as AA digestibility calculated by subtracting only basal endogenous AA losses (the minimum quantities of AA inevitably lost by the animal) from the outflow of AA. This term will be used in the current paper to maintain consistent terminology in the field.

Boucher et al. (2009a,b) evaluated AA and RUP-AA digestibility of several feed samples using the precision-fed cecectomized rooster assay. Three soybean meal (SBM), 3 SoyPlus® (SP; West Central, Ralston, IA), 5 distillers dried grains with solubles (DDGS), and 5 fish meal (FM) samples were obtained from the Feed Analysis Consortium, Inc. One of the SP, SBM, and DDGS samples were heated to decrease intestinal digestibility of RUP. The samples were fed to cecectomized roosters both in the intact form and after a 16 h ruminal in situ incubation (to obtain RUP-AA digestibility estimates). Standardized AA (feed samples) and RUP-AA (rumen residue samples) digestibility estimates obtained for the different feed types are presented in Table 1.

Key observations from Table 1:

- AA digestibility within feed sample and rumen residue sample categories was similar between SP and SBM

- AA digestibility in the feed samples was similar to RUP-AA digestibility in the rumen residue samples for SBM, SP, and FM

- RUP-AA digestibility in the rumen residue samples was higher than AA digestibility in the intact feed samples for the DDGS samples.

Table 1. Average in vivo AA and RUP-AA digestibility estimated using the precision-fed cecectomized rooster assay for protein concentrates commonly fed to dairy cows[a,b]

	Feed samples				Rumen Residue Samples			
Digestibility, %[c]	**SP**	**SBM**	**DDGS**	**FM**	**SP**	**SBM**	**DDGS**	**FM**
Arginine	93.0	92.5	88.3	90.7	94.7	95.2	91.3	87.3
Histidine	86.3	89.3	80.9	85.2	90.3	88.4	83.9	81.2
Isoleucine	90.1	91.3	85.3	92.9	93.9	94.4	90.0	91.9
Leucine	90.5	90.9	91.2	93.2	94.9	94.2	94.4	92.3
Lysine	84.2	89.5	66.8	87.0	87.2	89.9	72.8	87.6
Methionine	90.2	91.7	88.4	93.2	95.2	95.2	93.4	90.7
Phenylalanine	91.7	92.2	88.7	91.7	95.2	94.9	92.3	89.8
Threonine	86.0	89.3	80.6	92.5	93.3	93.0	87.1	90.4
Tryptophan	93.4	96.1	88.8	97.4	97.5	99.3	91.0	97.9
Valine	87.7	89.6	83.5	91.8	93.9	93.7	90.5	90.6
Essential AA	87.5	90.7	85.4	91.1	93.7	93.9	90.4	89.9
Nonessential AA	88.2	91.3	86.4	89.8	93.2	94.4	92.3	87.7
Total AA	88.7	91.1	86.1	90.4	93.3	94.0	91.6	88.7
NRC RUP dig. estimate	-	-	-	-	93.0	93.0	80.0	90.0
Minimum	84.2	89.3	66.8	85.2	87.2	88.4	72.8	81.2
Maximum	93.4	96.1	91.2	97.4	97.5	99.3	94.4	97.9
Standard deviation	3.1	2.1	7.1	3.4	2.9	3.0	6.4	4.3

[a] Samples heated in the lab were not included in arriving at the average values reported in this table.

[b] n = 2 for SP, n = 2 for SBM, n = 4 for DDGS, n = 5 for FM.

Digestibility of Individual AA vs. Total AA

In order to evaluate the difference between digestibility of individual AA and total AA, the absolute value of the difference between digestibility of individual AA and total AA in intact and ruminally incubated samples was calculated for all feed types (Table 2). The absolute value of the difference between digestibility of individual AA and total AA was greater than zero for all AA and all feed types. The mean difference for the ruminally incubated soy product samples ranged from (mean ± SD) 1.02 ± 0.9 for valine to 11.4 ± 9.5 for lysine. The mean difference between individual and total AA for the ruminally incubated DDGS samples ranged from (mean ± SD) 1.9 ± 1.3 for isoleucine to 25.00 ± 14.00 for lysine. The mean difference between individual and total AA for the ruminally incubated FM samples ranged from (mean ± SD) 0.4 ± 0.3 for isoleucine to 11.0 ± 2.9 for histidine. Because the absolute value of the difference between digestibility of individual RUP-AA and total RUP-AA in the rumen residue samples was greater than zero for all AA, if digestibility coefficients are assigned to individual RUP-AA within these feedstuffs, predictions of metabolizable AA supply may be improved. Also, in the soy product and DDGS samples, RUP-Lys digestibility was the most deviant from digestibility of total RUP-AA. In the FM samples, RUP-His digestibility was the most deviant from digestibility of total RUP-AA. Therefore, accurate estimates of RUP-Lys digestibility will be especially important when Lys is limiting for milk and milk protein

Table 2. Absolute value of mean difference between digestibility of total RUP-AA and individual RUP determined in cecectomized roosters[a]

	Ruminally incubated SP and SBM	Ruminally incubated DDGS	Ruminally incubated FM
	(n = 24)	(n = 20)	(n = 20)
AA	(mean ± SD)	(mean ± SD)	(mean ± SD)
Arginine	4.40 ± 4.59	3.44 ± 5.53	3.87 ± 2.80
Histidine	3.97 ± 1.57	8.02 ± 2.02	10.99 ± 2.92
Isoleucine	1.53 ± 1.44	3.17 ± 3.40	0.41 ± 0.30
Leucine	2.42 ± 2.38	4.17 ± 2.95	0.76 ± 0.50
Lysine	11.35 ± 9.53	25.00 ± 14.00	5.12 ± 3.54
Methionine	3.63 ± 3.28	3.35 ± 3.19	1.01 ± 0.67
Phenylalanine	3.24 ± 2.84	0.84 ± 0.45	1.69 ± 0.64
Threonine	1.09 ± 0.71	5.84 ± 3.12	1.48 ± 1.11
Tryptophan	6.04 ± 2.87	13.88 ± 13.30	8.37 ± 5.28
Valine	1.02 ± 0.86	3.68 ± 5.05	1.28 ± 0.80
Essential AA	0.94 ± 1.02	1.43 ± 1.22	2.11 ± 0.96
Nonessential AA	1.22 ± 1.25	1.18 ± 1.03	4.61 ± 2.10

[a] Heated samples are included in the values reported here.

production and soy products and DDGS are fed, and accurate estimates of RUP-His digestibility will be important when His is limiting for milk and milk protein production and FM is included in the diet.

In Vitro Estimates of RUP and RUP-AA digestibility

Although the use of the precision-fed rooster assay to estimate intestinal RUP and RUP-AA digestibility is a viable alternative to the MBT, an *in vitro* procedure for estimating RUP and RUP-AA digestibility will allow for more routine analysis of feeds for these parameters. The TSP of Calsamiglia and Stern (1995) has been the most common *in vitro* method used to estimate RUP digestibility of feeds. However, the TSP as it was originally developed does not allow for determination of digestibility of individual RUP-AA. Gargallo et al. (2006) modified the TSP to use a Daisy[II] incubator and nylon bags. With these modifications, determination of digestibility of individual RUP-AA is possible.

The same samples described above were also analyzed using the modified TSP of Gargallo et al. (2006) to estimate AA digestibility of the feeds and RUP-AA digestibility of the rumen residues (Boucher et al., 2009c). The feed and rumen residue samples (already completed step 1) were incubated in the pepsin and pancreatin steps (steps 2 & 3) of the modified TSP. Amino acid digestibility estimates (feed samples) and RUP-AA digestibility estimates (rumen residue samples) obtained via the modified TSP are

presented in Table 3. Crude protein digestibility of the samples was also determined using the original TSP of Calsamiglia and Stern (1995).

Table 3. Average *in vitro* AA and RUP-AA digestibility estimated using the modified three-step procedure of protein concentrates commonly fed to dairy cows[a,b]

	Feed samples				Rumen Residue Samples			
Digestibility, %	**SP**	**SBM**	**DDGS**	**FM**	**SP**	**SBM**	**DDGS**	**FM**
Arginine	98.0	98.5	89.2	81.4	93.3	99.2	92.9	86.6
Histidine	96.5	97.2	86.4	81.0	91.3	97.4	92.1	91.3
Isoleucine	95.6	96.8	85.0	75.8	92.1	98.6	93.8	93.4
Leucine	95.3	96.2	86.7	78.3	92.0	98.5	95.5	93.5
Lysine	96.4	97.5	82.9	82.5	93.4	98.3	86.8	94.0
Methionine	96.0	96.5	87.0	76.1	86.5	98.6	94.9	89.6
Phenylalanine	96.1	96.5	86.9	74.0	92.5	98.7	94.6	90.9
Threonine	96.2	96.5	84.5	78.4	90.6	98.2	89.7	91.6
Tryptophan	98.8	100.0	100.0	76.2	94.5	100.0	100.0	97.7
Valine	95.3	96.3	83.6	77.1	91.3	98.2	92.5	91.9
Essential AA	96.3	97.1	86.1	78.7	92.1	98.5	93.6	91.7
Nonessential AA	96.7	97.4	87.3	78.4	91.9	98.5	94.6	88.6
Total AA	96.5	97.2	86.8	78.6	89.9	98.1	93.2	86.9
CP modified TSP	95.6	96.2	84.2	78.0	91.8	97.8	91.8	88.9
CP original TSP	-	-	-	-	78.6	73.0	76.7	73.0
Minimum	95.3	96.2	82.9	74.0	86.5	97.4	86.8	86.6
Maximum	98.8	100.0	100.0	82.5	94.5	100.0	100.0	97.7
Standard deviation	1.2	1.2	4.9	2.8	2.2	0.7	3.5	2.9

[a] Samples heated in the lab were not included in arriving at the average values reported in this table.

[b] n = 2 for SP, n = 2 for SBM, n = 4 for DDGS, n = 5 for FM.

In vitro AA and RUP-AA digestibility estimates obtained with the modified TSP generally agree well with *in vivo* estimates for both the intact feeds and rumen residues reported in Table 1. *In vitro* digestibility estimates (X) for all AA and RUP-AA were highly correlated to *in vivo* estimates (Y; R^2 = 0.93 for total RUP-AA; Y = 31.8 + 0.7X; R^2 = 0.75 for total AA in feed; Y = 45.2 + 0.5X; $P < 0.05$).

In addition, the relationship between *in vitro* AA digestibility of the feed samples and *in vivo* RUP-AA digestibility (using the rumen residue samples) was examined. *In vitro* AA digestibility was highly correlated to *in vivo* RUP-AA digestibility for all AA (R^2 for total AA = 0.76; Y = 44.0 + 0.5X; $P < 0.05$). The strength of correlation was equal for the correlation between *in vivo* RUP-AA digestibility and *in vitro* AA digestibility of the intact feeds and *in vivo* RUP-AA digestibility and *in vitro* RUP-AA digestibility (data not shown). Therefore, further investigation into estimating RUP-AA digestibility from *in vitro* digestibility of AA in the intact feedstuff is warranted because it could potentially eliminate the need to use live animals for any step in determining RUP-AA digestibility.

RUP-Lysine Digestibility Estimated via the Modified TSP

The relationship between *in vitro* (modified TSP; x-axis) and *in vivo* (cecectomized roosters; y-axis) RUP-Lys digestibility estimates is illustrated in Figure 1. *In vitro* RUP-Lys digestibility estimates were higher than *in vivo* estimates for all samples. Although there was a high correlation between *in vivo* and *in vitro* RUP-Lys digestibility estimates (Figure 1), the modified TSP over-estimated RUP-Lys digestibility of all samples. This discrepancy between *in vitro* and *in vivo* RUP-Lys digestibility estimates is likely due to the fact that if the Maillard reaction has occurred, Lys can be present in feeds in forms that are not readily available for absorption in the small intestine (Mauron, 1990). Such Lys compounds could pass through the pores of the bags used in the modified TSP resulting in inflated Lys digestibility estimates. Therefore, to determine if a mean or linear bias was present in the regression model, the residuals (observed – predicted) were plotted against centered predicted RUP-Lys digestibility values (data not shown). Based on the results of this analysis, it was concluded that the modified TSP procedure is an accurate procedure to estimate digestibility of RUP-Lys in SBM, SP, DDGS, and FM. Based on visual assessment of the regression plots, RUP-Lys digestibility determined via the modified TSP will not precisely predict RUP-Lys digestibility for every sample, but on average the modified TSP will yield an accurate estimate of RUP-Lys digestibility.

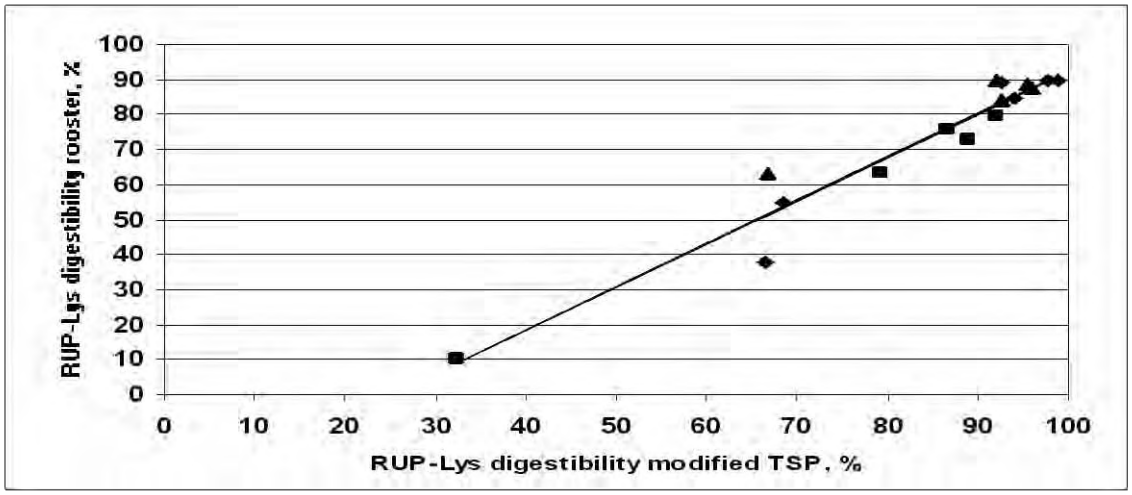

Figure 1. Regression plot of RUP-Lys digestibility of soy product (♦; n = 6), distillers dried grains with solubles (■; n = 5), and fish meal (▲; n = 5) rumen undegraded residue samples determined via the modified three-step procedure and in cecectomized roosters [Y = -31.37 (± 7.30) + 1.24 (± 0.09) X; RMSE = 5.81; R^2 = 0.94, P < 0.0001, n = 16].

Immobilized Digestive Enzyme Assay

The immobilized digestive enzyme assay (**IDEA**) is another *in vitro* assay that can be used to estimate digestibility of protein and AA in feeds (Church et al., 1984; Schasteen et al., 2007). The assay was developed to determine digestibility of protein in

human foodstuffs, and originally required 2.5 d to complete (Church et al., 1984). Schasteen et al. (2007) developed IDEA™ kits (Novus International Inc., St. Charles, MO) to provide a more rapid prediction of protein and AA digestibility than the original IDEA procedure would allow. IDEA kits have been developed for a variety of protein supplements. The kits are currently marketed to predict AA digestibility for poultry feeds.

Boucher et al. (2009c) evaluated the use of the IDEA kits to estimate RUP-AA digestibility. In order evaluate the kits for use in ruminant nutrition, the IDEA values of the rumen residues were regressed on *in vivo* RUP-AA digestibility. The IDEA values of the rumen residues were highly correlated with *in vivo* RUP-AA digestibility of the soy-product and DDGS samples (R^2 for total RUP-AA = 0.83, Y = 24.3 + 152.2X for DDGS; R^2 for total RUP-AA = 0.95, Y = 62.8 + 38.8X for soy products; $P < 0.05$), but for the FM samples, the IDEA values were not highly correlated with RUP-AA digestibility (R^2 for total RUP-AA = 0.47; Y = 72.2 + 132.0X). However, because the IDEA values are used to predict, not measure, AA digestibility, the relationship between the IDEA value of the intact feed and RUP-AA digestibility measured *in vivo* was examined to determine if the IDEA value of the feed could be used to predict RUP-AA digestibility. Based on this analysis, the IDEA value of the intact feed can be used to predict RUP-AA digestibility of rumen residue samples (R^2 = 0.90 for all feed types; Y = 36.0 + 54.0X for DDGS; Y = 54.6 + 41.2X for soy products; Y = 54.3 + 122.7X for FM; $P < 0.05$). Therefore, for a more efficient, cost-effective analysis, it is recommended that for future IDEA analysis, the IDEA values of the intact samples be determined, and that the IDEA value of the intact feed be compared to *in vivo* RUP-AA digestibility to develop accurate prediction equations.

The advantage of the IDEA kits compared with the modified TSP is that the IDEA analysis takes only 1 day to complete, and AA analysis is not needed on the final undigested product as is the case for the modified TSP. This saves time and money when obtaining AA digestibility estimates. However, because the kits are specific to a particular feed type, the use of the kits is limited. In addition, in the experiment described here, a small sample size was used for the evaluation of each kit; therefore, more analysis of the IDEA kits to develop more accurate prediction equations for estimating RUP-AA digestibility of feed ingredients is needed.

The advantage of the modified TSP is that it can be performed by any lab equipped with a Daisy^{II} incubator, and the modified TSP can be used to estimate RUP-AA digestibility of any feed ingredient.

CONCLUSIONS

In the advancement of ruminant nutrition models to better predict MP-AA supply, accurate digestibility estimates of individual AA in the RUP fraction of feedstuffs are needed. This is particularly important for MP-Lys supply because Lys is often limiting for milk and milk protein production and the digestibility of Lys can be quite different from the digestibility of total RUP. Improving predictions of MP and MP-Lys supply will aid in balancing dairy rations for AA and should result in improved predictions of animal

response to an improved profile of AA in MP. The modified three-step procedure and the IDEA kit assays are adequate in vitro procedures to estimate digestibility of individual AA in RUP. Use of such techniques can lead to an improved database of intestinal RUP-AA digestibility.

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EFFECT OF LEVEL OF INTAKE ON DIGESTIBILITY OF NDF IN SOY HULLS

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SUMMARY

Including soy hulls in diets increases feed intake and production in ruminants. This is likely due to the high fraction of fermentable neutral detergent fiber (FNDF) found in soy hulls, which may optimize VFA production for rumen health. High levels of intake of a soy hull diet, however, can decrease digestibility due to increased rate of passage. In this project, the effect of feeding soy hull-based diets at intake levels of 2, 3, or 4% of body weight was quantified in weaned ram lambs and in mature, non-lactating ewes. The apparent dry matter digestibility (DMD) and digestibility of NDF were quantified using chromic oxide as a marker. In ram lambs, apparent DMD decreased by 8.1 ± 1.16 percentage units and digestibility of NDF decreased by 12.1 ± 1.57 percentage units for each 1 percentage unit increase in DMI as a percentage of BW ($P < 0.001$). In mature ewes, digestibility values at low intakes were not as high as in lambs and the depression in digestibility was less pronounced, with DMD decreasing by 2.9 ± 1.28 percentage units and digestibility of NDF decreasing by 4.5 ± 2.00 percentage units for each 1 percentage unit increase in DMI as a percentage of BW ($P = 0.034$). These experiments demonstrated a linear decrease in digestibility of NDF with increased intake.

INTRODUCTION

The high concentration of fermentable fiber in soy hulls has been shown to increase intake and production in dairy cows and sheep (Araujo et al., 2008a; Araujo et al., 2008b; Ipharraguerre and Clark, 2003; Thonney and Hogue, 1999). The improvement in production may be due to optimization of volatile fatty acid (VFA) production in the rumen by rumen microbes. Ruminal fermentation of the non-structural carbohydrates found in grains causes a high proportion of lactic acid production, which can lead to ruminal problems such as acidosis. However, the highly-fermentable fiber found in soy hulls results in optimal ruminal VFA production and does not have the adverse effect of high lactic acid production (Elliott et al., 1995).

The effect of high intakes of soy hull-based diets on digestibility affects the value of soy hulls as a feed ingredient. Higher intake increases rate of passage of feed through the digestive tract, decreasing the time for digestion with a consequent decrease in digestibility (Wagner and Loosli, 1967). A recent experiment on the effect of a soy hull-based diet on production, intake, and digestibility in lactating ewes consuming up to 5.3% of DM as a percentage of BW reported NDF digestibility as low as 32% (Schotthofer, 2007). Thus, the depression in digestibility with increasing intake of diets with high concentrations of highly digestible NDF should be measured to allow for the best use of high NDF ingredients in ruminant diets. The purpose of the experiments in

the present study was to quantify the effect of feeding increasing amounts of a soy hull-based diet on digestibility of NDF in weaned ram lambs and in mature, non-lactating ewes.

MATERIALS AND METHODS

Ram Lamb Experiment

Forty-eight weaned, 3 mo-old, 18 kg ram lambs born in August and September of 2007 at the Cornell University Sheep Farm were housed in pairs in 1.2 x 2.4 m expanded metal floor pens. Each pen was randomly assigned to feed intake corresponding to 2, 3, or 4% of the average starting body weight (2BW, 3BW, and 4BW), with 8 pens of 2 lambs at each intake level. The diet contained 70% soy hulls, 15% corn, 7.9% soybean meal, 4.5% molasses, 1% vitamin-mineral premix[1], 0.75% ammonium chloride, 0.5% chromic oxide, 0.25% vitamin E premix, and 0.025% Deccox.

The diets were fed once daily for a ten-day adaptation period, after which feces were collected under each pen on sheets of plastic for two days. The small amount of feed not consumed was weighed at the end of the experiment. The fecal samples and two feed samples were dried and ground for determination of NDF, dry matter, and chromic oxide concentrations.

Mature Ewe Experiment

Twenty-four non-lactating, mature ewes were housed individually in 1.2 x 2.4 m expanded metal floor pens and fed at 2, 3, or 4% of body weight (2BW, 3BW, and 4BW) with 8 ewes fed at each intake level. Diets were randomly assigned to ewes in pens set up similarly to the ram lamb experiment. The diet contained 72% soy hulls, 20% corn, 2% soybean meal, 4.5% molasses, 1% mineral-vitamin premix[1], and 0.5% chromic oxide. After a ten-day adaptation period, feces were collected for two days on porous netting for easy separation of feces from urine. Uneaten feed was recorded to determine actual feed intake. The feces samples and two feed samples were dried and ground for determination of NDF, dry matter, and chromic oxide concentrations.

Chromium Measurement

Feces were dried in a 60°C oven over a period of 10 days until the dry weights were constant. Feed and dried fecal samples were ground through a 1mm screen in a Wiley Mill. Then 0.2 g of each sample was weighed into an Erlenmeyer flask in duplicate for fecal samples and quadruplicate for feed samples. Then 4 mL of concentrated nitric acid (HNO_3) were added to the flasks and the flasks were heated at 110°C for one hour. The samples were allowed to cool and 10 mL of 70% perchloric acid was added. Then

[1] Premix contained 50% Salt, 5% Deccox, 6% concentrate, 0.5% mineral oil, 2,500 ppm manganese, 30 ppm selenium, 2,000 ppm zinc, 80 ppm iodine, 20 ppm cobalt, 264,552 IU/kg vitamin A, 33,069 IU/kg vitamin D, 2,205 IU/kg vitamin E.

the samples were heated at 220°C for thirty minutes, or until all chromium became oxidized, signified by a color change of the solution to orange.

The solution in the flasks was allowed to cool and transferred to 100 mL volumetric flasks. Distilled water was added to make up 100 mL of solution. Duplicate samples from the volumetric flasks were sent for analysis to the Nutrient Analysis Laboratory at Cornell University for measurement of chromium concentration. The apparent dry matter digestibility (DMD) was found using the equation: DMD = 1 – (Cr as a % of DM in the feed / Cr as a % of DM in the feces).

NDF Measurement

Neutral detergent fiber concentration was determined by weighing 0.5 g of sample into a 600 mL beaker in duplicate for fecal samples and in triplicate for feed samples. To each beaker was added 0.5 g sodium sulfite, 100 mL NDF buffer, and 0.2 mL of heat-stable amylase. The sodium sulfite served to break disulfide bonds in the sample protein matrix, while the buffer solution and amylase digested and solubilized other non-NDF components. The samples were then refluxed for one hour. The hot solution was filtered through Whatman 934-AH 1 µm filter paper in a Gooch crucible, using boiling water to rinse twice and acetone as the final rinse. The crucibles were then dried in an oven at 106° C overnight and hot-weighed. Finally, the sample residues were ashed in a muffle furnace at 505°C overnight, cooled to 106°C, and hot-weighed. Ash determination was used to calculate ash-free NDF. Digestibility of NDF was calculated using the equation: dNDF = 1 – (Cr as a % of NDF in the feed / Cr as a % of NDF in the feces).

Dry Matter and Ash Measurement

The dry matter concentration of each sample was determined by weighing duplicate 1 g samples into 25 mL beakers and drying in an oven at 106°C overnight before recording hot-weights. Finally, the samples were ashed in a muffle furnace at 505°C overnight and hot-weighed to determine ash concentrations.

Data Analysis

The effect of level of intake was analyzed by one-way analysis of variance. A quadratic regression equation was then fitted to the data to quantify the effect of level of intake on apparent dry matter or NDF digestibility. In each case, the quadratic effect was nonsignificant so the simple linear regression equations are reported.

RESULTS AND DISCUSSION

Dry Matter Digestibility

Weight and intake data for ram lambs and mature ewes are shown in Tables 1 and 2, respectively. The initial weights at each intake level were similar within ram and ewe

experiments. However, the final weights of the lambs were affected by intake level (P = 0.004), especially between 2BW and the two higher levels (Table 1). The lambs fed at 2BW lost weight, indicating that this level of intake was insufficient to meet their nutritional requirements. The lambs fed at 3BW also lost weight, but the loss was less than that of the lambs fed at the 2BW level. There was no effect of feeding level on initial or final weights of ewes (Table 2).

As expected, DM intake (DMI) increased as experimental level of intake increased (P < 0.001). The lambs fed at 4BW consumed up to an average of 3.7% of their BW (Table 1). This high intake level was previously observed in lamb feeding experiments from which it was concluded that lambs fed a diet high in dNDF will increase their intake to meet nutritional requirements (Hogue, 1987). The weight loss for the 2BW and 3BW lambs indicates that lambs fed high dNDF diets at ≤ 2.7 percentage units of BW could not consume sufficient nutrients to support growth.

Although the ewes also consumed significantly more feed at each increased experimental intake level, the actual values at 2BW, 3BW, and 4BW were 2.0, 2.6, and 3.1% of BW, respectively. Thus, the ewes at 3BW and 4BW often did not consume the feed offered to them. However, the fact that body weight was maintained at 2BW, 3BW, and only slightly increased at 4BW, suggests that the value of the feed diminished as intake increased.

Table 1. Effect of level of dry matter intake on DM and NDF digestibility by lambs.

	DM intake, % BW				
Item	2	3	4	SE	P-value
Number of pens (2 lambs each)	8	8	8		
Initial weight, kg	17.9	19.0	17.7	0.69	0.348
Final weight, kg	15.6	17.9	18.1	0.51	0.004
Average weight, kg	16.8	18.5	17.9	0.59	0.140
Daily dry matter intake, g	330	495	656	2.4	<0.001
DM intake, % BW	2.0	2.7	3.7	0.09	<0.001
Apparent DM digestibility, %	78.3	75.0	64.4	1.55	<0.001
Daily digestible DM intake, g	258	371	423	8.8	<0.001
Digestible NDF, % NDF	82.0	76.1	60.5	1.96	<0.001

The apparent DM digestibility (DMD) decreased (P < 0.001 and P = 0.002 for lambs and ewes, respectively) with increasing intake. This effect was shown first by Wagner and Loosli (1967) and summarized by Tyrrell and Moe (1975) in dairy cows. More recent authors (Anderson et al., 1988; Araujo et al., 2008b; Schotthofer, 2007) reported similar results in sheep when large quantities of a soy hull-based diet were fed. The rate of passage through the digestive tract of soy hulls is fast when compared to high-forage diets, possibly due to high specific gravity and small particle size (Titgemeyer, 2000). This high rate of passage is increased further by increased intake. While digestibility of soy hulls can be as high as 90% (Anderson et al., 1988), feeding a large quantity of this small-particulate feed can dramatically reduce digestibility.

Table 2. Effect of level of dry matter intake on DM and NDF digestibility by mature ewes.

	DM intake, % BW				
Item	2	3	4	SE	P-value
Number of ewes	8	8	8		
Initial weight, kg	67.1	68.3	61.6	2.28	0.113
Final weight, kg	67.1	68.2	63.2	2.54	0.361
Average weight, kg	67.1	68.2	62.4	2.39	0.213
Daily dry matter intake, g	1321	1786	1945	93.3	<0.001
DM intake, % BW	2.0	2.6	3.1	0.14	<0.001
Apparent DM digestibility, %	68.3	67.0	62.1	1.50	0.002
Daily digestible DM intake, g	901	1198	1204	60.8	0.002
Digestible NDF, % NDF	67.8	64.8	58.8	1.88	0.009

The relationship between apparent DMD and actual DM intake as a percentage of BW was best represented by simple negative linear equations ($P < 0.001$ and $P = 0.034$ for lambs and ewes, respectively) as shown in Figure 1. With 70% and 72% soy hulls in the lamb and ewe diets, respectively, the diets had very similar composition, yet level of intake had a much greater effect DMD for lambs than for ewes. The slopes of the equations show that a one-percentage unit increase in DMI as a percentage of BW decreased DMD by 8.1 ± 1.16 and 2.9 ± 1.28 percentage units for lambs and ewes, respectively. On average, the lambs consumed up to about 3.7% of DM as a percentage of BW (Table 1), while the ewes only consumed up to about 3.1% of feed as a percentage of BW (Table 2). Therefore, most of the ewe data covered only a span for intake of about 1 percent of BW with intake at or near maintenance. The data from the lamb experiment covered a greater span with the higher intake levels much higher than maintenance and lower intake levels below maintenance based upon the weight changes. This may have contributed to the much greater decline in digestibility with increased intake in lambs compared with ewes, but DMD was lower for ewes at the same relative intake compared to lambs, especially at lower intake levels.

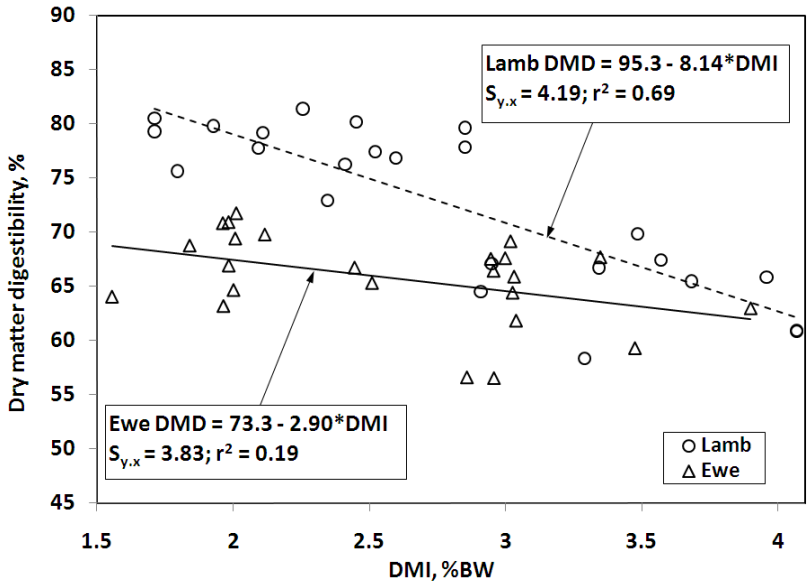

Figure 1. Effect of level of intake on dry matter digestibility by lambs and ewes.

This is surprising in view of the complete review of the early literature by Schneider and Flatt (1975), who concluded that animal age has little effect on digestibility.

NDF digestibility

The digestibility of NDF decreased ($P < 0.001$ and $P = 0.009$ for lambs and ewes, respectively) as intake level increased (Tables 1 and 2). This observation can also be explained by increased rate of passage, which decreased the amount of time for fermentation.

Like the regression between DMD and individual intake levels, there was a simple negative linear relationship ($P < 0.001$ and $P = 0.034$ for lambs and ewes, respectively) between digestibility of NDF and intake as a percentage of BW (Figure 2). The slopes of

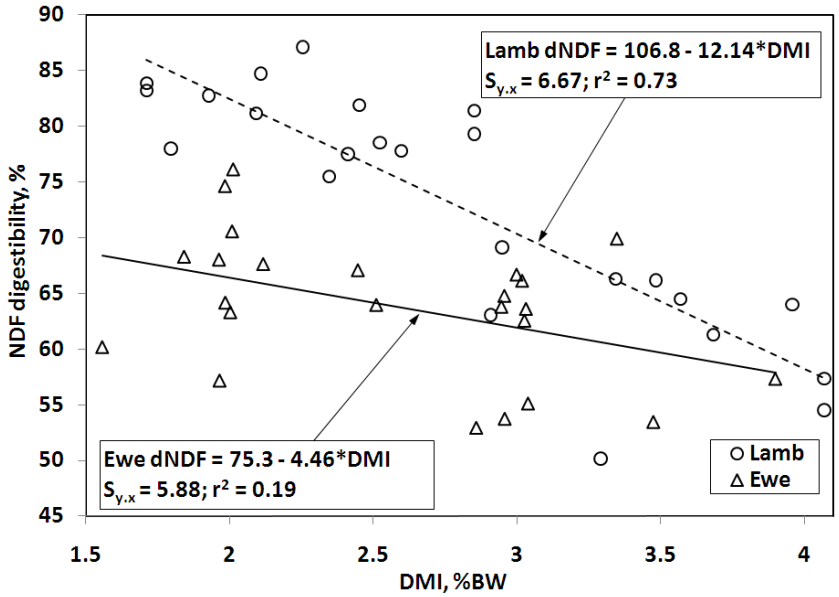

Figure 2. Effect of level of intake on digestibility of NDF by lambs and ewes.

the equations show that a one-percentage unit increase in DMI as a percentage of BW decreased digestible NDF by 12.1 ± 1.57 and 4.5 ± 2.00 percentage units for lambs and ewes, respectively. These were much steeper than the declines in DMD, suggesting that the decrease in DMD was mostly due to the decrease in digestibility (fermentability) of the NDF. In support of this observation, Van Soest et al. (1992) stated: “The depression in digestibility is largely accounted for by the loss of potentially digestible NDF . . .” when describing discounts for net energy and protein.

CONCLUSIONS

In agreement with recent reports (Araujo et al., 2008a; Araujo et al., 2008b; Schotthofer, 2007), increasing feed intake reduced NDF digestibility. Diets with sufficient fermentable NDF often maximize milk production and growth in sheep by increasing intake without sacrificing rumen health. The linear declines for digestibility with increasing intake as quantified in this experiment may be useful to optimize the use of fermentable NDF in feed formulation.

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REFINING NITROGEN FEEDING USING CURRENT KNOWLEDGE OF RECYCLED UREA AND MICROBIAL NITROGEN UPTAKE

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Nitrogen efficiency has been a popular topic in the dairy industry in the last few years due to a desire for reduced excretions. Beside groundwater contamination, NH_3 and nitrous oxides are able to volatilize and form precipitates, acidify precipitation, and decrease ozone, which in turn is detrimental to human and environmental health. As responsible stewards, many dairy producers and nutritionists have taken strides toward eliminating excess N inputs. Work at Cornell is focused on aiding in this process, and much work is being done to understand enhancements in N efficiency in an effort to reduce N excretion and promote greater milk protein exported from the farm. One step is to better understand, and quantitatively describe, urea-N recycling into the gastrointestinal tract (GIT) and its utilization by microbial populations. This process allows seemingly excess N absorbed from the GIT and converted to urea to re-enter the GIT for the purpose of incorporation into microbial protein. Current research has shown that approximately 15-40% of N intake may follow this process, so it is important to describe this accurately for nutritional estimates (Lapierre et al. 2004; Ouellet et al. 2004; Valkeners et al. 2007). Further, enhancements of field usable models like the CNCPS (Tylutki et al. 2008) and CPM Dairy (Boston et al., 2000) require quantitative data to enhance the predictions from the rumen submodel. If we are going to refine the models, then data supporting more of the dynamics of N utilization, including the incorporation of the protozoa are needed to better predict energy and amino acid yields from organic matter intake.

The conversion of N intake into urea ranged from 27-117% among several lactating dairy cow experiments, (Baker et al., 2007; Lapierre et al., 2004; Ouellet et al., 2004; Valkeners et al., 2007). However, excluding a couple potential outliers, based on more typical MP balances, this value is more consistently in the range of 50-70%. The proportion N intake that reenters the GIT as urea-N in dairy cows has been reported at 25-78%, but is most commonly 30-45%. In a typical high producing cow at 600 g N intake, this means that approximately 360 g N is formed into urea and 225 g N reenters the GIT as urea-N (Gozho et al., 2008; Valkeners et al., 2007).

A variety of factors appear to affect N reentry into the GIT. High rumen NH3-N concentrations have been reported to be negatively correlated to urea-N transfer across the rumen wall, while PUN concentration is positively correlated (Kennedy and Milligan, 1980). It is possible that a combination of diffusion and urea transporter activity adjust accordingly to change rumen plasma clearance rates to allow for urea-N movement to a favorable environment, one where N is either low or in demand by the microbial population. The exact mechanisms behind this transfer are currently un-described, but previous work has given insight into patterns of behavior. The kidney is playing a major

role in the movement of urea back into circulation, where facilitated transporters exist all along the gastrointestinal tract and provide the mechanism for transfer into the GIT, but how all of this redirection is controlled is still not fully appreciated (Marini and Van Amburgh, 2003; Stewart et al., 2005).

In addition to N concentrations across the rumen wall, readily available carbohydrates have demonstrably increased urea-N transfer into the rumen. In sheep supplemented with sucrose and/or urea, increases in urea-N recycling were observed for increasing amounts of sucrose supplementation and decreasing concentrations of ruminal NH_3-N (Figure 1, (Kennedy et al., 1981) There may also be influences on recycling via VFA production, carbon dioxide solubility, and pH, but the impact of these factors are currently less well defined in the context of a high producing dairy cow (Remond et al., 1993; Thorlacius et al., 1971).

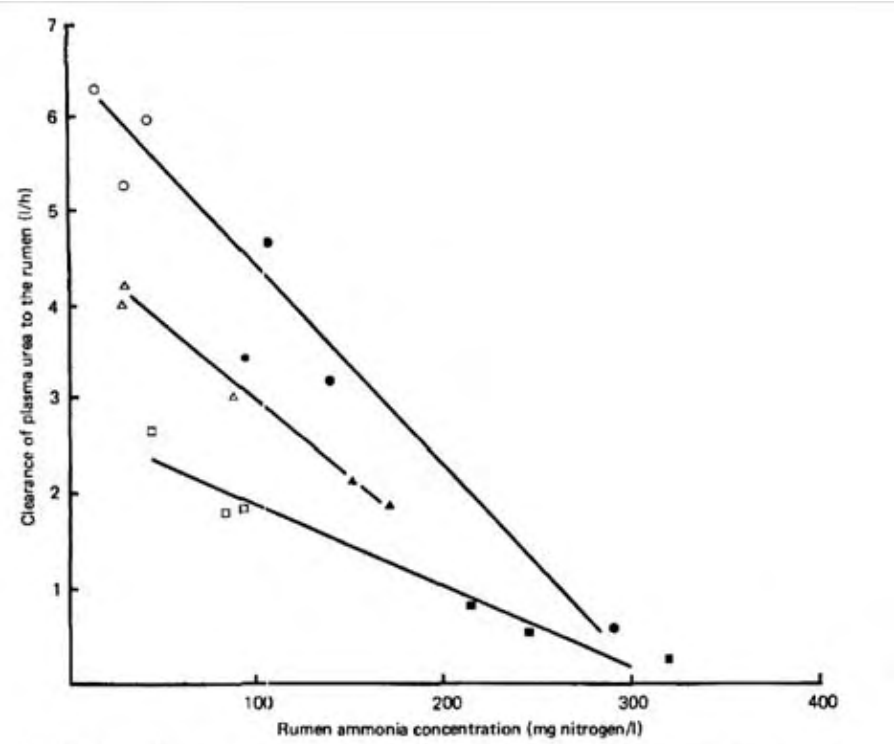

Fig. 1. Relationships between clearance of plasma urea to the rumen with rumen ammonia concentration in sheep given pelleted brome grass (*Bromus inermis*) alone or with sucrose and urea; no urea, (□, △, ○); urea supplemented, (■, ▲, ●). (□, ■) Sheep with no sucrose ($y = -0{\cdot}0082x + 2{\cdot}67$, r 0·97); (△, ▲) sheep with 150 g sucrose/d ($y = -0{\cdot}0159x + 4{\cdot}51$, r 0·99); (○, ●) sheep with 300 g sucrose/d ($y = -0{\cdot}0205x + 6{\cdot}41$, r 0·98).

The amount of urea-N that enters the rumen is one factor, but possibly not the most important factor to consider. A more important value is the amount of recycled urea-N that is incorporated by the microbes and formed into microbial protein. This is the fraction that is able to be utilized by the animal. High concentrations of ruminal NH_3-N from the diet will decrease the capture of recycled N due to the dilution effect of the dietary NH3-N supply. For instance, in lactating cows fed high concentrate diets,

recycled urea-N contributed 37.5% of the duodenal bacterial N, while this was only 12.7% for those fed high forage diets fed similar amounts of N (Al-Dehneh et al., 1997). And in Holstein heifers fed isocaloric diets, increasing N intake from 1.45 to 3.4% of DM decreased the proportion of bacterial N from recycled urea-N from 18.7% on the low N diet to only 4.3% on the high N diet (Marini and Van Amburgh, 2003). If producers are to reduce N waste, the most beneficial improvements will be made in capture of this recycled N in the microbial populations and that is really the balancing procedure the industry has been working towards since the inception of protein solubility and rumen degradable protein concepts.

Experimental work at Cornell

To enhance the rumen submodel of the CNCPS, we need to generate data that provides pool sizes for bacteria and protozoa and allows us to characterize the energy and N requirements of both microbial populations. Work being conducted by Jeff Firkins and colleagues at the Ohio State University is yielding data on protozoa growth and yield and will allow us to further enhance the rumen submodel. Based on our previous experience, we hypothesized that the double labeled urea procedure developed by Lobley et al., (2000) could be used and extended to provide us with a procedure that would allow us to generate data on N utilization by the microbial pools and allow us to quantify the protozoa and the protozoal predation of bacteria. Protozoal predation has been set in the CNCPS and CPM Dairy at 20% and is frequently modified in CPM Dairy assuming that certain feed ingredients modify the efficiency of bacteria. This is an incorrect adjustment because the modification assumes a change in protozoal predation and there is really no data to support modifying that value until now.

Recently our lab has done work attempting to quantify urea-N recycling and its capture in the bacterial and protozoal populations. Twelve multi-parous, lactating Holstein cows were fed diets deficient in either ruminal N or in MP or balanced for both ruminal N and MP. These cows were a subset of a larger study involving 88 cows fed the same diets and monitored for milk yield and components, DMI, BCS, BW over the course of 100 days (Recktenwald, 2007). Three diets were fed consisting of approximately 47% corn silage, 2% wheat straw, 51% a concentrate mix specific to diet objectives. Rations were formulated using CPM Dairy v3.0 for a 625 kg cow producing 36.3 kg milk/d at 3.7% fat and 2.95% protein – typical measurements for animals at Cornell T&R. For this report, diets will be labeled lowRumN, lowMP, and Control. The lowRumN diet contained 14.1% CP, with adequate MP supply (predicted via CPM Dairy v3.0) but deficient in ruminal N (-39 g N/d or 79% of requirement). The lowMP diet contained 14.1% CP, with adequate ruminal N balance but deficient in MP (-145 g MP/d). The Control diet contained 16.3% CP and was predicted to be sufficient in both ruminal N balance and MP supply. Cows were fed 1x/d and 300 mg Rumensin ®/cow/d was added to the TMR. Animals were also given bST per label (Posilac, Monsanto Co.).

The 12 animal subset of fistulated cows were given jugular vein infusions of double labeled $^{15}N^{15}N$-urea for 72 hours (0.0208 g urea/h). Fecal, urine, plasma, milk, rumen fluid, and total rumen contents were collected before infusion for background ^{15}N enrichment measurements and after 72 hours of infusion. Urine and rumen fluid samples were acidified to pH < 2. A representative sample of the total rumen contents was used to isolate 3 microbial fractions. Protozoa were isolated from strained rumen fluid by flocculation, addition of 1% formalin, then centrifugation at 500xg for 5 min. Fluid associated bacteria were isolated from the supernatant by centrifugation at 27000xg for 30 min. Solid associated bacteria were isolated from the solid contents by methycellulose incubation, blending in a Waring blender, incubation in Tween 80 and 1% methanol at pH 2, then centrifugation at 27000xg for 20 min. (Sylvester et al., 2005). Total rumen contents were determined by evacuating the rumen approximately 8 h after feeding, then 1 h before feeding 2 days later. Microbial and rumen content samples were freeze dried for analysis. Enrichment of ^{15}N was determined for microbial, ruminal NH3-N, fecal and milk samples by a NC2500 Carlo Erba elemental analyzer interfaced to a ThermoFinnigan Delta Plus IRMS.

Urine samples were collected via an external funnel system, however, we observed significant leakage of urine through the system which confounded our measurements. Once the cows reached plateau (72 hr of infusion of labeled urea) urine spot samples were taken via vulva agitation and acidified to pH < 2. Fecal grab samples were taken prior to infusion and once the cows reached plateau. Urea was isolated via AG 50W-X8 cation exchange resin, diluted to 6 mmol/L, and reacted with lithium hypobromite under vacuum for analysis by a PDZ Europa Geo 20/20 IRMS (Marini and Attene-Ramos, 2006). Due to technical difficulties collecting total urine and feces, total urine and fecal N were predicted in CNCPS v6.1 using the new equations of Higgs et al. 2009. Higgs developed new equations to predict urinary N (UN) excretion to correct a partitioning problem between urinary and fecal N (FN) excretion in the CNCPS (Higgs et al., 2009). The model was predicting total N excretion with good accuracy, but the partitioning was biased. Incorporating a more accurate fecal N prediction into the current CNCPS framework and correcting a 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 have been incorporated into the latest version of the CNCPS (v6.1). The variation in total collection studies ranges from 10 to 30% (Firkins and Reynolds, 2005; Reynolds and Kristensen, 2008), thus, the predictions from this and other models with measured intakes are most likely more accurate than measuring total excretion and serve as an adequate basis for providing inputs for the N recycling calculations.

Dry matter Intake, milk yield and components, and nitrogen efficiency results for the 12 animals during the infusion period and for all the animals over 100 days are shown in Table 1. The cows given infusions had much lower milk yield than the rest of the animals used most likely because of placement into metabolism stalls for the infusion and total collection period. Animals chosen for infusions were blocked by milk yield over the first 50 d of lactation for treatment assignments. No significant differences were

observed for DMI nor milk measurements in these animals, but milk yield and intake were numerically lower for those fed lowRumN. As formulated, lowMP and lowRumN cows consumed less N (80 and 140 g N/d, respectively). For the full study, milk yield was highest for Control, intermediate for lowRumN, and lowest for lowMP (Table 1). As milk component percentages were similar, milk fat and protein yields generically followed total yields, being highest for Control and slightly less for the low protein diets, but not always reaching significance.

Table 1. Intake, milk yield and components, and nitrogen efficiency of early to mid-lactation cows consuming lowMP, lowRumN, and Control diets over a 100 day period. Infused cows (n=12) a subset of the full study.

	LoMP	LoRumN	Control	SEM	Diet effect
DMI, kg/d	25.2[a]	22.0[b]	24.3[a]	2.79	0.70
Milk yield, kg/d	33.0	27.3	32.5	3.26	0.28
Milk fat %	3.17	2.71	3.16	0.24	0.33
Milk protein %	2.87	2.87	3.04	0.17	0.72
Milk fat yield, kg/d	1.03	0.75	1	0.09	0.10
Milk protein yield, kg/d	0.88	0.88	0.93	0.05	0.71
N intake, g N/d	582	521	659	66.0	0.35
N efficiency, milk N/intake N	0.26	0.25	0.25	0.03	0.96
Full lactation study results (88 cows, 100 d)					
DMI, kg/d	25.5	24.2	25.7	0.40	0.02
Milk yield, kg/d	42.6[b]	43.3[ab]	45.0[a]	0.75	0.06
Milk fat %	2.67	2.54	2.68	0.08	0.37
Milk protein %	2.92	2.9	2.93	0.04	0.85
Milk fat yield, kg/d	1.12[ab]	1.09[b]	1.2[a]	0.03	0.09
Milk protein yield, kg/d	1.23[b]	1.24[b]	1.32[a]	0.02	0.01
N intake, gN/d	584.0	550.4	668.8	9.6	<0.001
N efficiency, milk N/intake N	0.33	0.36	0.31	0.004	<0.001

[a,b]Different subscripts indicate significant differences within a row, $P < 0.05$

For this study, we did not attempt to control for DMI, a common practice for an isotopic infusion study since fluxes in DMI will create more variation in microbial yield and N pool flux, making the statistical analyses less robust. Thus, some of the differences in measurements are not statistically significant, but from an application and modeling perspective the values are appropriate for the feeding conditions and allow for enhanced model parameter development.

Urea-N synthesis was greatest for animals fed Control, intermediate for lowRumN, and lowest for lowMP. Cows fed lowRumN had the highest amount of urea-N entry into the GIT, with slightly less for Control and much lower amounts for lowMP animals (Table 2). As urea-N synthesis is related to N intake, the high urea-N entry rate (UER)

was expected for the Control fed cattle. Even though the N intake was higher for lowMP than lowRumN, more urea-N was produced in lowRumN cows due to higher GIT reentry and less urea-N partitioned to urine (Table 2). Comparing the diets sufficient in ruminal N, lowMP and Control, the proportion of urea-N synthesized that entered the GIT was similar, at 63%, therefore 37% of the liver urea production entered the urine for excretion. However, lowRumN had only 25% of the urea-N enter urine and 75% entered the GIT. This was most likely due to the low ruminal N balance (ruminal NH3-N was only 6.6 mg/dl vs 8.3 and 7.4 for lowMP and Control, respectively) acting as a gradient to effectively pull urea-N from plasma to meet the requirements within the GIT for microbial activity.

Of the recycled urea-N, approximately 48% returned to the urea cycle in lowMP and Control cattle, while this number was only 39% for cattle fed the lowRumN diet (Table 2). Anabolic use was highest for lowRumN, with 60% of the recycled N used for anabolism, and this was only 51% for the other two diets. As these animals were in steady state and NH_3-N and AA transfer reactions in the animal can be considered negligible, this N was effectively used for microbial N synthesis. So not only did the low ruminal N balance diet recycle more urea-N, this N was used more efficiently for synthesis of microbial protein, which enhances the efficiency of use by the cow for milk protein synthesis and other functions.

Table 2. Urea-N kinetic measurements for 12 lactating cows given $^{15}N^{15}N$-urea infusions for 72 h.

	lowMP	lowRumN	Control	SEM	Diet effect
UER	221.6	253.8	293.3	44.0	0.49
UUE	84.26	48.92	108.75	19.59	0.22
GER	137.39	204.84	184.55	45.60	0.57
ROC	63.24	70.85	86.09	14.18	0.49
UFE	0.796	0.416	1.187	0.32	0.35
UUA	73.35	133.58	97.27	37.93	0.58
UER to urine	0.375	0.254	0.374	0.088	0.62
UER to GIT	0.625	0.746	0.626	0.088	0.62
GER to ROC	0.488	0.393	0.485	0.101	0.80
GER to UFE	0.006	0.003	0.007	0.002	0.53
GER to UUA	0.506	0.604	0.508	0.104	0.79

UER = urea-N entry rate; GER = gastrointestinal (GIT) urea-N entry rate; ROC = urea-N re-entering ornithine cycle; UFE = urea-N to fecal excretion; UUA = urea-N utilized for anabolism

With the assumption that all anabolic N use was for microbial N, CNCPS microbial yield estimates, ^{15}N enrichment values, %N of microbes, and microbial pool size measurements were used to determine N fluxes among the three microbial pools. Previous work by (Sylvester et al., 2005) demonstrated that protozoa isolated with the

procedure described here contains 8% liquid associated bacteria. Therefore, all calculations were made to account for this contamination. Bacterial turnover rate was calculated based on CNCPS estimated bacterial N yield divided by g of N contained in the bacterial pools. Protozoal turnover rates are difficult to determine, as little experimental work has been done to elucidate them in vivo. In this preliminary report, it is assumed to be 50-100% of the value for the bacteria and represents both flow out of the rumen and turnover within the rumen.

Enrichment of ^{15}N in rumen NH3-N was significantly highest for the cattle on the lowRumN diets, with similar values for the other two diets (Table 3). It was also numerically highest for the liquid associated bacteria and protozoa pools, with particle associated bacteria having slightly higher enrichments for cattle fed the lowMP diet. Since urea-N recycling was highest for the lowRumN fed cattle, it is no surprise that rumen ^{15}NH3-N enrichment was high. Accordingly, the more enriched rumen ^{15}NH3-N was correspondingly taken up by the microbes in greater proportion on diet lowRumN than in the other diets, leading to more efficient use of this recycled urea-N (as observed by higher UUA values as well). Fecal and milk protein enrichments were also highest for lowRumN, most likely due to more microbial ^{15}N from high recycled N incorporation ending up either as sloughed cells and microbial cell wall in the feces or absorbed and utilized for milk protein synthesis.

Between 16 and 27% of the microbial N were present as protozoal N. This corresponds with previous work by (Sylvester et al., 2005), who reported this value to be 5-13% when measured as flow. The estimates by this method will be for the entire protozoal pool of the rumen and not just the flow of protozoa. The protozoal population may have been higher in these diets due to addition of sugar to the diet (0.8, 2.6, and 3.1% of diet DM for lowMP, Control, and lowRumN diets, respectively). High sugar and starch contents are known to stimulate protozoal growth (Hristov and Jouany, 2005). Based on CNCPS estimates made from MP supply from bacteria, bacterial and protozoal, turnover rates varied from 6.4 to 9.0 times/d, with the highest turnover rates with lowRumN. In a rumen with high protozoal concentrations, the entire bacterial population could be turned over each hour due to their ability to individually consume up to 10,000 bacteria per hour (Jouany, 1996).

Table 3. Enrichment of ^{15}N in fluid and particle associated bacteria, protozoa, rumen NH_3-N, and milk protein in 12 cows given $^{15}N^{15}N$-urea infusions. Values are in atom percent excess (APE).

	lowMP	lowRumN	Control	SEM	Diet effect
Liquid bacteria enrichment	6.85	9.54	6.35	1.88	0.24
Particle bacteria enrichment	5.79	4.73	4.83	1.12	0.59
Protozoa enrichment	5.56	7.80	5.50	1.21	0.15
Fecal enrichment	5.08	6.29	4.59	1.07	0.32
Rumen NH3 enrichment	5.43[b]	11.44[a]	6.09[b]	2.28	0.05
Milk protein enrichment	5.98	6.82	5.54	0.78	0.30

[a,b]Different subscripts indicate significant differences within a row, $P < 0.05$

If protozoal turnover rates were equivalent to bacterial turnover rates, protozoal predation of bacteria was 18.8% of the total bacterial yield for the lowMP diet and approximately 40% for the other two diets (Table 4). If protozoal turnover rates were only 50% as fast as those estimated for bacteria, these values decrease to 9.4 and 20%, respectively. Due to difficulties involved in quantifying predation, previous estimates had approximated predation at 20%. The value from the lowMP diet is consistent with the current CNCPS/CPM Dairy default of 20%, however the other two diets represent a doubling of protozoal predation of bacteria compared to the current estimates in the CNCPS. These results demonstrate that there are most likely wide ranges in predation depending on diet, but it may be higher than the prior estimate. Future work is needed to more accurately quantify these transactions with focus on total protozoal yield and turnover in order to better estimate predation and N transactions within the rumen microbial pools in general.

Table 4. Protozoal predation of bacteria, microbial N production from recycled urea-N, and proportion of intake N used by microbes.

	lowMP	lowRumN	Control	SEM	Diet effect
% of microbial N pool as protozoal N	0.158	0.262	0.268	0.047	0.23
protozoal turnover rate (d^{-1})	7.31	8.99	6.36	1.47	0.47
protozoal predation of bacteria, 100%	0.188	0.401	0.394	0.087	0.20
75% of bacterial turnover rate	0.141	0.301	0.295	0.066	0.20
50% of bacterial turnover rate	0.0941	0.200	0.197	0.0437	0.20
% bacterial N production from recycled N (includes predated bacteria)	0.235	0.574	0.363	0.20	0.54
% protozoal N prod from recycled N, 100%	0.275	0.624	0.379	0.22	0.57
75% of bacterial turnover rate	0.366	0.833	0.506	0.29	0.57
50% of bacterial turnover rate	0.549	1.249	0.759	0.43	0.57
% of intake N as GER	0.246	0.500	0.285	0.11	0.35
% of intake N used by bacteria prod	0.481[a]	0.467[a]	0.362[b]	0.026	0.02
% of intake N used for protozoa prod, 100%	0.091	0.184	0.143	0.045	0.39
75% of bacterial turnover rate	0.069	0.138	0.107	0.034	0.39
50% of bacterial turnover rate	0.046	0.092	0.071	0.023	0.39
% of intake N recycled and used by bacteria (predated included)	0.139	0.326	0.153	0.09	0.39

[a,b]Different subscripts indicate significant differences within a row, $P < 0.05$

Including predated bacteria, 57% of the bacterial N yield originated from recycled urea-N in animals fed lowRumN. Lower amounts, 24 and 36%, were observed for the lowMP and Control diets, respectively. These values were comparable for the protozoal population, 28-62%, with similar trends among diets, and increased to 55-125% if protozoal turnover was only 50% of the bacterial turnover rate. As these percentages coincide with the proportion of N intake that recycled (GER-N), they seem valid, and the bacteria did not appear to preferentially utilize recycled vs. dietary N to a large extent.

Overall, 32.6% of the N intake was recycled as urea-N and utilized by the microbes in the lowRumN diet. This was numerically much higher than for either lowMP (13.9%) or Control (15.3%). It appears that a ruminal N deficiency is able to stimulate urea-N recycling, and in this instance, is also able to allow for greater microbial incorporation of this recycled N if the ration is suitable for high microbial growth – high fermentable carbohydrate availability. This allowed for less urea-N produced in the liver to be eliminated in the urine, decreasing the urinary excretion amount by 55% over the Control diet. Further work will involve refining urea-N recycling equations and defining microbial N transactions to better understand microbial requirements and N handling – especially for the protozoa pool.

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ANDF, NDFD, INDF, ADL AND KD: WHAT HAVE WE LEARNED?

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INTRODUCTION

Animal performance is the product of, nutrient and energy concentration, intake, digestibility, and metabolism. Of the variation in digestible dry matter or digestible energy intake among animals and feeds, 60 to 90% is related to differences in intake, whereas 10 to 40% is related to differences in digestibility (Mertens, 1994). Accurate estimation of forage digestibility is a prerequisite for diet formulation, economic evaluation of forages and prediction of animal responses, besides optimal decision making.

Digestion and passage in ruminants can be empirically and mechanistically described by models of varying complexity. Illius and Allen (1994) made a detailed comparison of the structure and assumptions of the models, which differed principally in the fractioning of feed and in the description of the digestion and passage kinetics. However, accurate and precise predictions of the intrinsic digestion kinetic parameters are critical to the accurate prediction of NDF digestibility and intake. In order to be useful in rumen models, the kinetic parameters should only be limited by the attributes of substrates, i.e. intrinsic characteristics of cell walls. Physical and chemical attributes of the digestion environment should not be limiting factors in the determination of the potential rate and extent of NDF digestion.

Several reviews of digestion kinetics of cell wall carbohydrates (Mertens, 1993a, 1993b; Ellis et al., 1994, 1999) have addressed the problems associated with the estimation of kinetic parameters. The importance of the rate and extent of NDF digestion on OM and NDF digestibility can be demonstrated by simulation with the CNCPS model (Tylutki et al., 2008). Simulation results clearly demonstrate profound effects of these parameters on digestibility and therefore on the supply of energy and microbial protein.

At this conference, Van Amburgh et al. (2003) described a mathematical approach for determining rates of digestion and the application of this approach to in vitro corn silage NDF digestion data. A further objective was to develop a system that allowed for the determination of rates with a minimum number of time points. Since then many commercial laboratories have used the mathematical approach routinely and the kd's have been implemented in models like CPM and CNCPS. A further objective has been to challenge this approach and to refine it for possible improvements. This paper will further review work in the area of NDF digestion and the estimation of rates of digestion. We will summarize our current approach to conducing NDFd and provide information indicating where changes will most likely occur in the near future when estimating rates

of digestion. This involves refinements in methodology and a more complete understanding of the extent of digestion.

Sample Analyses

Conventional and BMR corn silages, perennial ryegrasses, wheat straw, and alfalfa hays were analyzed for fiber fractions using the procedure of Van Soest et al. (1991). Sulfuric lignin was also used as the standard lignin procedure (Van Soest and Robertson, 1980). A small preliminary study indicated that fermentations in-situ for 16 days may not be enough to reach the maximum extent of digestion and this is still being investigated. Extended fermentations were also carried using the ANKOM in-vitro apparatus (DAISY^{II}; ANKOM Technology Corp., Fairport, NY), because of the ease of re-inoculating the samples. This latter system allowed a better estimation of the intrinsic iNDF of the forage after 16 days. Fermentations were then carried for 16 days using the DAISY system and bags of polyester polyethylene terephthalate with 15 µm porosity and 8.5% open area (ANKOM Technology Corp., Fairport, NY). Jars were re-inoculated with medium every 4 days. Fermentations in-vitro were carried out for 6, 12, 24, 30, 36, 48, 72, 96, 120, 144, 168, 192 and 216 hours, in 125 ml Erlenmeyer flasks in a 39 °C water bath under constant CO_2 in Goering and Van Soest buffer (1970), and with renewed medium after 96 hours. In-vitro flasks were inoculated with rumen fluid from the same cows used for the DAISY fermentations, fed hay and grain. Blank samples were run for both fermentations and used to correct for any contamination at each fermentation time. All samples at the end of the fermentations were analyzed for NDF.

Further Evaluations of Rate Estimations for NDF Digestion – Comparison to a Non-Linear Model for a First Order Approach

The earliest attempts to describe the kinetics of digestion have been reviewed by Mertens (1993a, 1993b). The term “rate of digestion” appeared in the 1950s, but the assessments were mainly based on the visual interpretation of digestion curves. Waldo (1970) suggested that if the indigestible residue was subtracted, digestion of potentially digestible cell walls might follow first order kinetics. Two primary methods are used for fitting data to the first order kinetic models: linear regression on logarithmic transformations of undigested residues (ln-linear) and nonlinear estimation of parameters using multiple time points. The regression between the natural logarithm of the potentially digestible NDF (pdNDF) against time results in supporting the hypothesis that pdNDF follows the first order digestion kinetics. In this case, it is critical the estimation or measurement of the indigestible fraction (iNDF) is accurate in order to accurately describe the digestion kinetics.

A further problem in describing NDF digestion and kinetics is that bias can also be associated with early measurement of digestion. Usually, digestion of the pdNDF fraction does not appear to start instantaneously at time zero. Instead there is a lag period during which digestion occurs slowly or not at all (Mertens, 1993a, 1993b) while the fermentation stabilizes and bacterial attachment and enzyme synthesis has occurred. As a result the use of early fermentation points with the logarithmic

transformation and linear regression may result in a biased estimate that is lower than the true rate. Problems associated with the logarithmic transformation-linear regression can be overcome by estimating kinetic parameters using non-linear least squares regression procedures (Mertens and Loften, 1980; Van Milgen et al., 1991; Ellis et al., 2005) but this would require a laboratory to run multiple timepoints. Nonlinear models assume an equal error at each fermentation time, whereas the ln-linear models assume that error is proportional to the size of residue at each time point. And since random errors are typically the largest for early and medium (8-48 hours) incubation times, neither of the approaches seems satisfactory since there is error associated with both. Therefore the only apparent discrepancy with the ln-linear method is during lag when fluxes and variation are low, but residue weights are high. Thus, it does not seem that the multiplicative error distribution associated with logarithmic transformation is a significant problem during parameter estimation.

To evaluate the associated errors between the two approaches, values from in-vitro fermentations were used to obtain simultaneous estimations of rates of digestion (kd_v), lag times (L_v), pdNDF ($pdNDF_v$) and iNDF ($iNDF_v$), through a non-linear first order decay model using PROC NLIN of SAS and the Marquardt algorithm. Initial values for the non-linear iterations were obtained using a linear transformation of the mentioned model (Mertens and Loften, 1980; Moore and Cherney, 1986). The model was:

(1) $$NDF_t = pdNDF_v \, e^{-kd\,(t - L)} + iNDF_v$$

where

NDF_t = concentration of residual NDF after t hours of fermentation when t > L and NDF_t

= pdNDF + iNDF when t < L;

pdNDF = concentration of potentially digestible NDF;

kd = fractional rate of pdNDF digestion;

L = discrete lag time;

iNDF = concentration of indigestible NDF.

Estimations of rates of digestion with the approach by Van Amburgh et al. (2003) were then possible after calculating the pdNDF as NDF – iNDF. Estimates of the iNDF fraction were then found subtracting the 72% sulfuric acid lignin x 2.4 from NDF, according to Chandler (1980) ($iNDF_{2.4}$), or using the residue after 216 hours of in-vitro fermentations ($iNDF_{216}$) or using the residue of the DAISY fermentations ($iNDF_D$). This resulted in respectively $kd_{2.4}$, kd_{216} and kd_D. Estimations were therefore done either calculating a lag or using an arbitrary fixed lag. The calculation of the lag was accomplished using 6 and 24 hours as the two time points needed, since a preliminary analysis showed the highest correlation with the true lag. The discrete arbitrary lag of

three hours was instead used, as an arbitrary proxy of an average commercial laboratory lag. This was done to analyze the option of using only one time point to estimate the rate. The rates of digestion obtained with our approach were then compared for prediction with the rates obtained from the non-linear estimation (kd). The single point rates of digestion were obtained using the 12, 24, 30 and 36 hour time points. Kd's estimated by semi-logarithmic transformation and using the same iNDF as mentioned above were also included for comparison. The non linear estimation used data up to 216 hours, therefore, the log-linear transformation estimation was obtained using different ranges of time points. Prediction accuracy was tested and compared using correlations and the mean square prediction error (MSPE) analysis of Theil (1966) and Bibby and Toutenburg (1977). The non-linear estimation allowed us to obtain an approximated 95% confidence interval for the rates that was used as further comparison tool.

All calculation procedures resulted in equations that described the data very well. Predicted and observed values were highly correlated (*P<0.01*) for both the non linear and linear procedures, also indicated by the similarity of the residual errors of the models. However even if the overall equations tended to describe the data equally well, our objective is the refinement of the mathematical approach for commercial use and therefore the analysis of the bias in the actual values of the parameters obtained due to calculation method. 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. The log-linear transformation gave low correlations mainly when using data further than 48 hours and therefore those rates are not shown here. This is most likely related to the presence of two first order pools as shown by Van Soest et al. (2005). The single time point rate estimations presented the highest correlation for the 24 and 30 hours values. Fermentation times less than 24 and greater than 30 hr are therefore not shown here. In general the lowest MSPE's were obtained when using 24 hours as single time point fermentation with the variable lag and using the last fermentation point (216 hours) resulting from the Daisy system. Higher MSPE resulted when comparing the actual kd, from the non linear model, with the kd obtained using the residue $iNDF_{2.4}$. The higher MSPE values were mainly due to mean bias, however regression slope was not different than unity. The use of Chandler ratio of 2.4 (Chandler, 1980) to obtain the iNDF resulted in general in higher MSPE, showing that the factor is not consistent among forage families and the need for refinement (Huhtanen et al., 2006; Nousianen et al., 2004; Traxler et al., 1997).

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. Even if lag requires two time points, our results show that an average lag of 3 hours can be used with a single time point, however a lab-specific value should be used to obtain the best rate estimations. Results also show that the net effect of using a single time point estimation and the approach presented here cannot be considered biologically different from predicting a kd using a non linear model and different fermentation points. The variation associated with using a single time point was primarily related to the estimation of the iNDF.

ADL AND iNDF

According to the Lucas principle, the iNDF is an ideal fraction since by definition it is digested at a predictable rate of zero. The estimation of the indigestible fraction is not a mathematical or modeling contrivance, but is a critical biological principle upon which the concept of digestion kinetics and rates are based (Mertens, 1994). Often digestion rates are calculated without subtracting the indigestible residue or by subtracting one that is determined at too short a fermentation time. The purpose of long digestion times in kinetic analysis is to obtain a value that is used to estimate an indispensable kinetic parameter. 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 potentially digestible NDF (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. The importance of the iNDF estimation on OM and NDF digestibility, rumen NDF pool and microbial N flow is also demonstrated by the Nordic model of dairy cow metabolism “Karoline” (Danfær et al., 2005). Simulation results clearly demonstrate profound effects of these parameters on OM digestibility and consequently on the supply of energy and microbial protein.

Mertens has shown (1973, 1977) the effect of the fermentation time chosen to represent the iNDF on digestion rate, determined using ln-linear. Subtraction of large iNDF (early time points) results in prediction of greater than true digestion rates. Conversely, subtraction of small or no iNDF results in less than true rates of digestion, because high value of residues at later fermentation times cause a counter-clockwise rotation of the semi-logarithmic regression line. Therefore any error in estimating indigestibility can bias the estimates of fractional rate and lag time as they are sequentially estimated using ln-linear regression (Mertens and Loften, 1980; Moore and Cherney, 1986). Observations of long in-vitro fermentations (up to 216 hours) have shown how digestion was not completed at 96 hr (Van Soest et al., 2005). However, Huhtanen et al. (2006) have suggested that the ultimate extent of NDF digestion may not be reached with in-vitro batch systems and the in-situ approach might be biased due to crucial drawbacks of the traditional nylon bag procedure as discussed by Nousiainen et al. (2004). The close relationship between in-vivo digestibility and the potential extent of digestion (Nousianen et al., 2003) suggests that using prolonged incubations and bags with a small pore size may allow the extent of NDF digestion (and iNDF) to be accurately measured. Nousiainen et al. (2004) determined iNDF by 12 days in situ incubations using nylon bags of small pore size.

Table 1: Comparison between the non-linear reference method and the 24 hours single point approach with both fixed and variable lag, and using alternative iNDF estimations. The values highlighted are the ones falling inside the 95% confidence interval.

	Nonlin	95% c.i.		24 hr with fixed lag			6-24 hr with estimated lag time		
Forage	kd	lower	upper	kd2.4	kd216	kdD	kd2.4	kd216	kdD
FULLTIME	**7.64**	**6.81**	**8.47**	6.60	6.66	6.75	7.01	7.08	7.69
MYCOGEN	**6.81**	**5.59**	**8.04**	5.23	5.36	6.17	5.39	5.54	6.43
HARDIES BMR	**8.18**	**7.38**	**8.98**	6.59	7.43	7.68	6.85	7.79	8.06
HARDIES NORMAL	**6.15**	**5.20**	**7.09**	4.82	5.53	5.76	4.87	5.64	5.89
CORNELL T&R	**6.14**	**5.16**	**7.11**	5.27	6.09	6.21	5.47	6.36	6.49
CPM 238	**5.05**	**4.20**	**5.90**	4.57	5.07	5.35	4.49	5.01	5.31
CPM 249	**5.95**	**4.55**	**7.35**	4.78	5.49	6.68	4.84	5.61	6.91
CPM 250	**5.54**	**4.65**	**6.42**	4.58	4.88	5.22	4.68	5.00	5.37
CPM 251	**6.79**	**6.19**	**7.39**	6.16	6.43	6.83	6.45	6.76	7.20
CPM 252	**5.75**	**4.82**	**6.68**	4.56	4.86	6.00	4.57	4.88	6.11
CPM 259	**5.29**	**4.10**	**6.47**	4.71	5.41	5.66	4.69	5.43	5.70
CPM 260	**4.89**	**4.14**	**5.63**	4.42	5.32	5.43	4.45	5.40	5.75
CPM 261	**5.45**	**4.17**	**6.73**	4.36	5.46	5.89	4.51	5.70	6.18
ALFALFA 83	**6.35**	**5.18**	**7.52**	5.43	7.22	6.67	5.45	7.39	6.79
ALFALFA 93	**8.18**	**6.48**	**9.87**	7.69	8.72	9.46	7.18	8.28	9.08
TIMOTHY 68	**3.89**	**3.00**	**4.79**	2.96	3.55	3.87	2.81	3.41	3.74
TIMOTHY 93	**7.01**	**5.69**	**8.32**	6.27	6.73	7.12	6.22	6.73	7.15
COCKSFOOT 76	**4.40**	**3.73**	**5.08**	3.20	4.09	4.37	3.28	4.24	4.54
WHEAT STRAW 68	**2.39**	**1.88**	**2.91**	1.87	2.20	2.42	1.72	2.04	2.25
WHEAT STRAW 92	**3.41**	**3.01**	**3.81**	2.61	3.04	3.09	2.72	3.18	3.23
slope				1.02	0.92	0.90	1.01	0.91	0.89
Correlation				0.97	0.94	0.95	0.98	0.95	0.97
mean bias				0.86	0.08	0.00	0.77	0.04	0.05
regression bias				0.00	0.01	0.02	0.00	0.02	0.03
unexplained variation				0.12	0.28	0.23	0.09	0.23	0.14
MSPE (%/hr)squared				**0.98**	**0.38**	**0.26**	**0.87**	**0.29**	**0.22**
RMSE				**4.15**	**1.27**	**0.31**	**3.93**	**0.84**	**1.04**

Lignin is generally accepted as the primary entity responsible for limiting the digestion of forages (Besle et al., 1994; Van Soest, 1994). Assuming that any estimation based on long fermentations and made at any time other than infinity is an overestimate of the true asymptotic indigestible residue, several attempts to predict iNDF from lignin concentration have been made (Mertens, 1973; Chandler, 1980; Conrad et al., 1984; Weiss et al., 1992; Traxler et al., 1998). As previously mentioned Chandler et al. (1980) estimated the indigestible fraction as lignin times 2.4, after fermentation between 90 and 120 days in methane digesters. The Cornell Net Carbohydrate and Protein System uses the 2.4 value as ratio between ADL and NDF to estimate iNDF in forages. Despite the small size and origin of the database, the Chandler equation performed well in prediction of iNDF in our observations (Van Soest et al., 2005), resulting in satisfactory regression between predicted and observed (R^2 = 0.94).

Data from Huhtanen et al. (2006) shows a general relationship between permanganate lignin and iNDF measured by 12 day in-situ fermentation, with an overall slope of 2.4, but that relationship does not hold among all the values from Huhtanen et al. (2006). This was also observed by Nousianen et al. (2004) who could not develop an acceptable prediction equation (R^2 < 0.40) for iNDF based on lignin content on different grass silage types. The previous findings from Nousianen et al. (2004) and Huhtanen et al. (2006) refer to cold climate grasses that might result in different relationship between lignin and iNDF due to environmental and agronomic interactions.

Our next objective was therefore to evaluate the consistency of the 2.4 ratio among various forage families. Previous tests for nutritional uniformity indicated an average recovery of 86% for ADL and sintered glass filters with a 40 µm aperture might not achieve complete recovery of fine particles (Robertson, unpublished results; Udén, 2006). Thus, we evaluated ADL and iNDF recovery to assess the ratio between ADL and NDF after improved recovery in order to estimate iNDF. Thirty forage samples of various species and ADL content were analyzed for ADL content in Gooch crucibles of porosity of 40 µm, with or without glass microfiber filters (1.5 µm; Whatman, 934-AH). The same samples were also fermented in situ for 16 d using bags of PPT monofilament fabric with porosity of 15 µm and an open area of 8.5% (Ankom Technology, Fairport, NY). The bags were also inserted in the rumen of two fistulated non-lactating cows. The same samples were also fermented for 16 d in the same bags in a Daisy Ankom System where the medium and rumen fluid were renewed every 4 d. All bags were analyzed for NDF after 16 d. Ratios between ADL and NDF, for estimation of iNDF, were back calculated with the iNDF obtained after the fermentations. Recovery of ADL varied among samples, but was generally higher using the filter paper (Table 2). Improved recoveries for iNDF followed the ADL recoveries, with higher recoveries for lower iNDF forages, when using the filter papers from the in-vitros rather than the bags from the in-situ or from the Daisy. Long fermentations were consistent within specie for both in-situ and in-vitro procedures. The combined improved recoveries of ADL and iNDF resulted in the back-calculated ratio not being constant and different than the 2.4 used so far. The observed ratios were in general always larger than 2.4. Forages lower in ADL/NDF had larger ratios and vice-versa for forages with larger ADL/NDF values. The data suggest the lignin procedure needs to be revised to

improve recovery of lignin particles and the development of forage family and specie-specific equations for the estimation of the iNDF.

The original AOAC lignin procedure for crucibles relied on the use of asbestos as a filtering agent, but the asbestos was rendered a health hazard and removed and another filtering agent was never instituted, thus the variation in the lignin assay is partially a function of the filtering step. Data in Figure 1 shows examples of the lignin/NDF relationships for conventional and bmr corn, grasses and alfalfas. The two types of corn silages and the grasses present differing ratios, while the alfalfas are characterized by a less variable ratio (avg=2.8), with later (4^{th} and 5^{th}) cuts having a different behavior and larger values. More data are needed to allow specific equations to be used for groups of forages. The improved recoveries alter the previous lignin to NDF relationships primarily because of the increased recoveries of both lignin and fermented NDF and this does not invalidate the previous ratio of 2.4 but enhances our understanding to be more forage specific. More accurate and precise iNDF values will allow for better estimations of rates of NDF digestibility to be used in CNCPS.

Table 2. Average increases in recoveries of ADL for different classes of forages using small pore size (1.5 µ) filter papers in Gooch crucibles.

Class of forage	% Increase in recovery
Conv. corn silage	25
Bmr corn silage	36
Alfalfa	3
Grasses	9
Early cut grasses	28

NDFd, kd and Chemical Structure of Lignin Bonds

Finally, when comparing different plants, the negative correlations observed between the extent of digestion and lignin content are relatively good for the same variety among stages of maturity; however, they are weaker within a family and poor when data from plant families are pooled (ie grasses and legumes). The action of lignins seems to depend not only on their amount but also on other factors like cross linking and because of the chemical nature of this heterogeneous compound, it is nearly impossible to extract lignin in any pure form – especially once it polymerizes into ADL.

Lignin action on digestibility depends therefore not only on its content but its qualitative structural aspects. In general lignin is associated with carbohydrate (in particular with hemicellulose) through covalent bonds, hydroxycinnamic acids are attached to lignin and hemicellulose through ester and ether bonds as bridges between them forming lignin/phenolic-carbohydrate complexes. It is generally accepted that the association of phenolic components with carbohydrates presents the greatest barrier to their utilization. It is well established that hydroxycinnamic acids attach to arabinoxylans through ester bonds. A simple dimerization of these esters, even if there is no further attachment to lignin, may be enough to substantially reduce the biodegradability of digestible cell walls. Any further association (ether bonds) between this

hydroxycinnamic moiety and the lignin polymer that forms a bridge between a carbohydrate and lignin would cause a severe reduction in carbohydrates availability. These acids are mainly p-coumaric and ferulic acids. Because of their dual function (hydroxyl and carboxyl) these acids may form bridges between lignins and hemicelluloses.

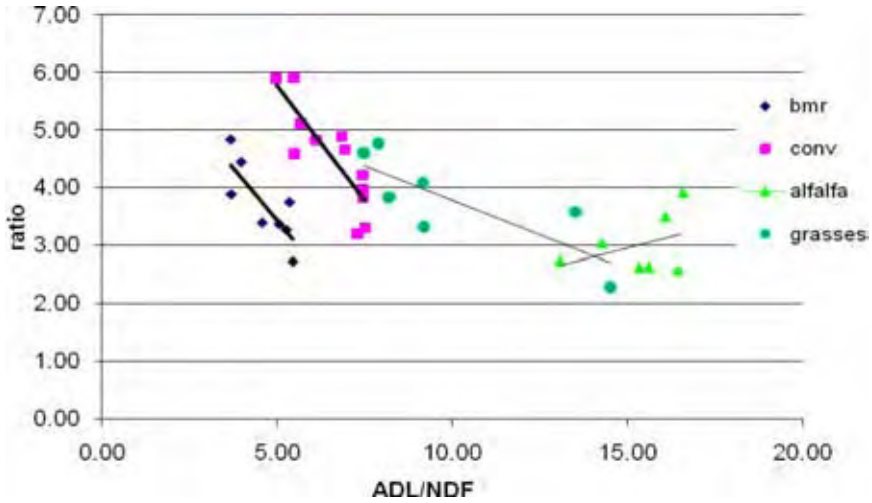

Figure 1. The ratio of lignin as a function of NDF (lignin * y)/NDF to describe the forage specific iNDF pool size (back-calculated from the residues of the NDF fermentations) regressed on the value of ADL/NDF for conventional and bmr corn silages, grasses and alfalfas.

We are aware of the large genetic variability among cell types and within and especially among forage species and families because of the different speed of cell wall change and reproductive maturity. However our objective was to try to integrate recent findings relative to phenolic acids and nutritive value, focusing only on phenotypic correlations. Also, the possible correlations among cell wall components may prevent any type of cause and effect to be determined from these analysis but might lead only to a better prediction of fiber digestibility. We wanted to determine if the presence of measurable ester and ether linkages impact rates as well as extents of NDF digestibility and if these relationships were similar among forages.

Thirty forages including conventional and bmr corn silages, alfalfas, immature and mature grasses were incubated in-vitro for 24hr and 96hr NDFD. Digested residues were analyzed for NDF, ADF and ADL and ester and ether linked para-coumaric acid (PCA) and ferulic acid (FA) were determined in those fractions. Three of the corn silages were chosen based on variable digestibility and fed to 6 fistulated cows for 3 wks in three iso-NDF diets. Diet, rumen and feces samples were taken every 3 hr for three days, 10d from the start of the study. Intact samples, NDF and ADF residues were analyzed for ester and ether linked PCA and FA. Extraction of phenolic acids was by 2N and 4N NaOH treatment, followed by HPLC equipped with a diode array detector using

a reverse-phase column. Ether-linked PCA and FA were calculated as the difference between total and ester-linked phenolic acids. Phenolic acids and respective amounts of their different linkages and lignin types were used in a multiple regression to select those factors explaining most of the variation in kd, 24hr and 96hr NDFD.

Since most esterified PCA on lignin are not covalently attached to other cell wall polymers, they should not directly influence cell wall degradability. However some cell wall models show how they can interfere with ferulate-lignin cross linking and in some cases reduce the proportion of lignin bound to cell wall. Figure 2 and 3 show the relationship between 24hr NDFD and PCA content in NDF and ADF residues respectively. Forage groups demonstrated different relationships for digestibility from positive to negative in NDF residues. ADF residues were instead characterized by a consistent negative relationship among all forage groups and similar results were obtained for 96 hr NDFD.

Relationship between 24hr and 96hr NDFD with esterified FA content in NDF residues are shown in Figures 4 and 5, respectively. Ester-linked FA had generally a negative relationship except in bmr corn for 24hr and positive for 96hr NDFD. Ferulates primarily form as esters of arabinoxylans and later they cross-link through ether linkages with lignin. So esters of FA should not necessarily limit NDF degradation. This has probably more to do with the degree of arabinoxylans substitution.

Ether linkage between FA and lignin has been used a measure of cross-linking between lignin and arabinoxylans and defined as the most important factor limiting energy utilization (Casler, 2001). However in our case we were not able to obtain consistent negative relationship with NDFD. Figure 6 and 7 show the relationship for 24 and 96 hours respectively with divergent relationship for 24 hr and consistent positive ones for 96 hr NDFD. Negative effect of etherified FA on NDFD has been found in elongating internodes in maize but not in internodes that had stopped the elongation process and confirms the hypothesis that secondary cell wall development may mask the negative impact of etherified FA on NDFD. Interestingly also, bmr shows higher content of etherified FA compared to conventional corn in NDF residues, demonstrating that etherified FA is not always a good indicator of cross-linking between lignin and arabinoxylans and this was also shown recently by Marita et al. (2003). However this relationship changes when ADF residues were analyzed for ether linked FA, showing how the solubulization or branching of the lignin structure has in this case more importance than linkages. Acid detergent solution in this case might dissolve those FAs that only etherified (instead of having and ester-ether linkage).

A multiple regression with stepwise selection was run within each forage group using the independent variables lignin type (ADL or Klason lignin) and their difference on an NDF basis and phenolic acids with their specific linkages also on an NDF basis. We wanted to determine how much variation in 24hr and 96hr NDFD and kd was explained by the above mentioned variables. The procedure was not able to find any significant variable for the alfalfas ($P < 0.10$), showing how NDF digestibility cannot be easily explained in legumes by many of these factors. All the other forage groups reached

significance and with R^2 that varied between 0.56 and 0.99. Results confirmed the lack of consistency among the forage types and the properties of the chemical structures and digestibility and rates of digestion. This clearly demonstrates that that factors affecting rates and extents of digestion of NDF vary by forage type and most likely maturity.

Table 3: Independent variables selected and their solution for each forage group and dependent variable.

Dependent	Forage group							
Variable	Grasses		Imm. grasses		Conv. corn		Bmr corn	
kd	ADL:	-0.72	EthFA:	0.87	ADL:	-0.74	KL:	-0.45
	EthFA:	-0.68			EstFA:	-1.07	EstPCA:	1.04
R^2		0.97		0.75		0.79		0.84
24h ivNDFd	ADL:	-0.84	ADL:	-1.12	EthPCA:	-0.75	KL:	-0.62
	EthFA:	-0.52	EstPCA:	0.36			EstPCA:	1.09
R^2		0.98		0.97		0.56		0.92
96h ivNDFd	ADL:	-0.90	KL:	0.16			(KL-ADL):	-0.57
	EthFA:	-0.40	ADL:	-0.59			EthFA:	0.70
			EthFA:	0.48			EstPCA:	0.34
R^2		0.97		0.99				0.84

Significance level = 0.10

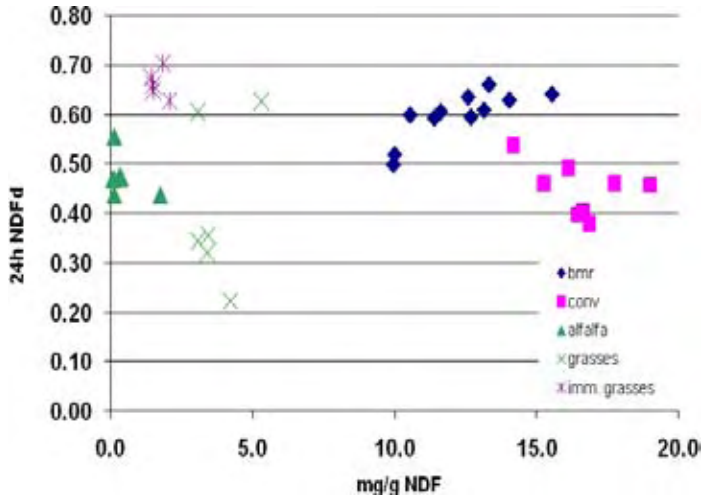

Figure 2: Relationship between 24hr NDFD and ester linked pCA in NDF residues

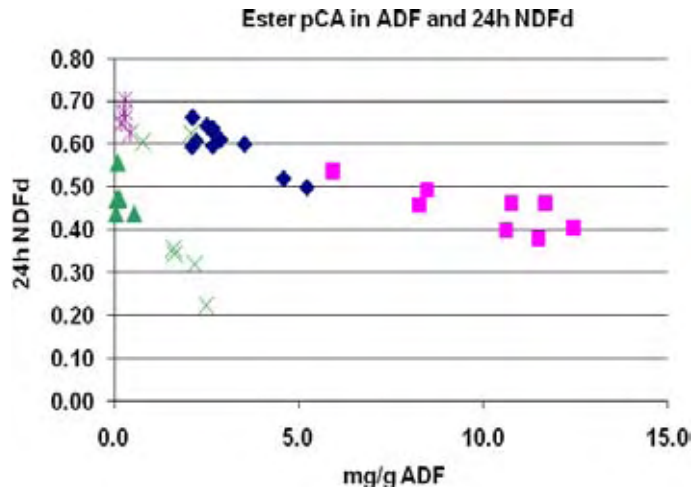

Figure 3: Relationship between 24hr NDFD and ester linked pCA in ADF residues

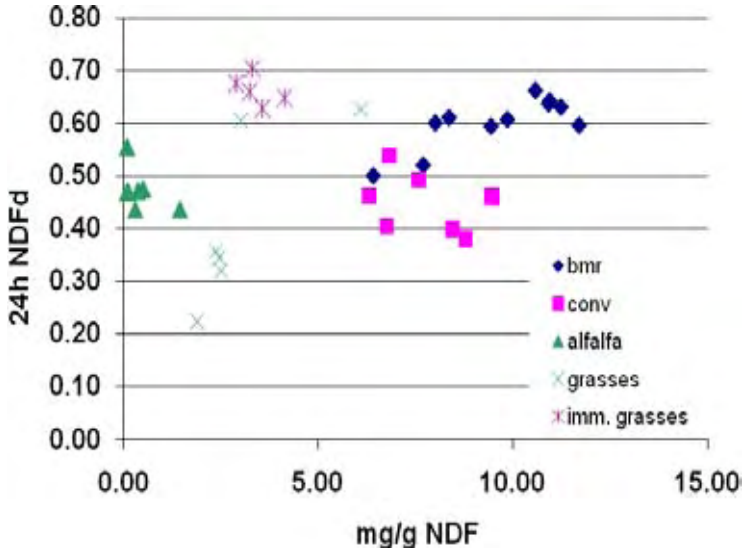

Figure 4: Relationship between 24hr NDFD and ester linked FA in NDF residues

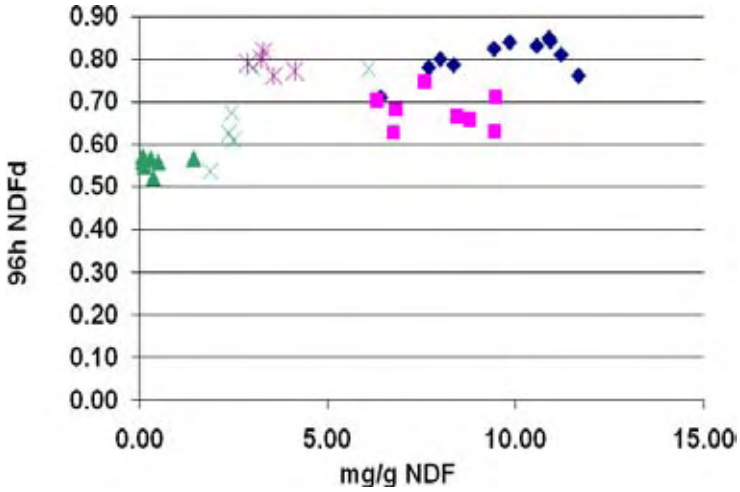

Figure 5: Relationship between 96hr NDFD and ester linked FA in NDF residues

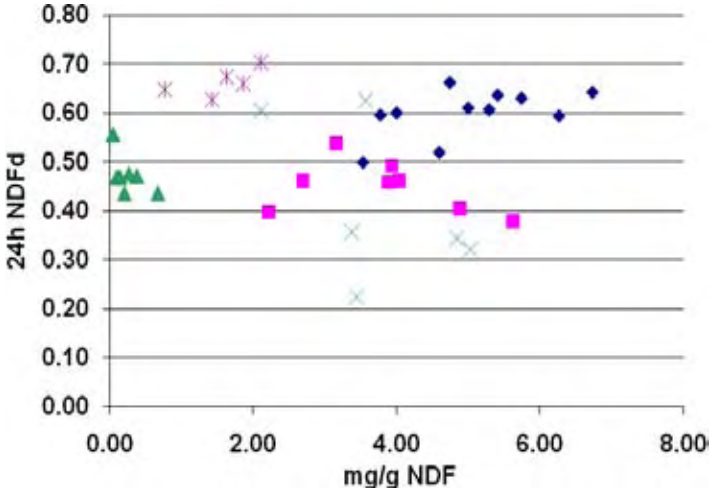

Figure 6: Relationship between 24hr NDFD and ether linked FA in NDF residues

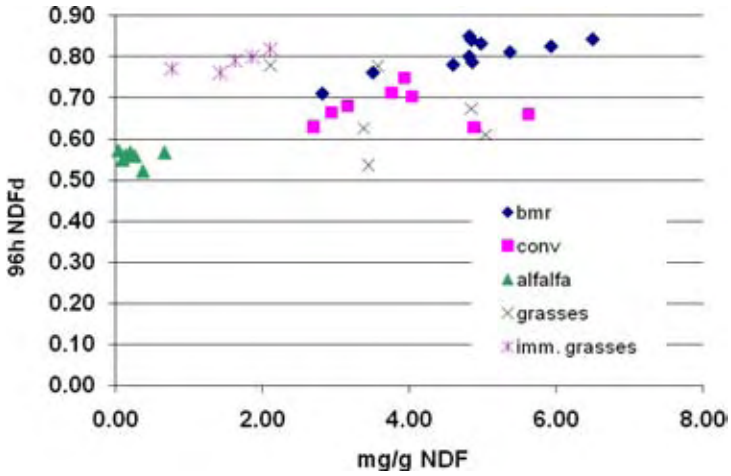

Figure 7: Relationship between 96hr NDFD and ether linked FA in NDF residues

NDFd Procedure

The in-vitro procedure used to obtain a NDFd value is used to calculate a rate of NDF digestion. We therefore want to control of all the factors that would bias the calculation. Besides the estimation of the iNDF, other important factors are the lag and the pool size. It is therefore a must minimizing the lag and avoiding loss of sample during the procedure:

- Fast handling of rumen fluid from the barn to the lab
- Care in keeping the rumen fluid temperature at 39° C and anaerobic
- Filtration of the fluid and not blending
- Total time between barn and inoculation of the samples should not be higher than 20-25 minutes.
- Use of glass microfiber filter 934-AH or GF/C with porosity of 1.5 µ (Whatman)
- Particular care of hot weighing because of filters and moisture variation
- Use of blanks for inoculums/filter loss/gain correction

ADL Procedure

Variation in the ADL procedure has been observed in many laboratories and is especially observed n lower lignin forages, like bmr corn silages, higher digestibility corn silages and immature grasses. Better care should be devoted to this types of forages during the procedure. Use of glass microfiber filter like 934-AH or GF/C is highly recommended along with more attention during the hot weighing. The removal of asbestos as a filtering agent has most likely contributed to increased variation in ADL recoveries. Also, use of blanks is recommended because of possible sulfuric acid degradation of filters. To decrease the chance of filter degradation, the sample can be put in a small beaker (50 to 100 ml) soaked in excess sulfuric acid, stirred appropriately and then filtered in crucibles with filters.

Take Home Messages

- Description of NDF digestion and kinetics have been often biased and limited by laboratory and mathematical errors. A more accurate and precise intrinsic kd estimation is today possible with the tools now available.
- The “anchor points” (lag and iNDF) are confirmed to be the most important factors to prevent bias. Lag can be easily minimized within laboratory. Estimation of true iNDF can be accomplished either by long in-situ or in-vitro digestion using small pore size bags o filters, respectively.
- In the near future calculation of iNDF using forage group-specific equations will be possible, and will need to be validated for environment and climate differences. Rates of digestion and pool sizes will then change accordingly.
- Use of smaller pore size filters is highly recommended for NDF and ADL procedures to avoid loss of fine particles and biased calculations of NDFd, kd’s and iNDF.
- With the exception of legumes, phenolic acids, type of lignin and their different chemical linkages to cell wall carbohydrates explain almost all of the variation in NDF rate and extent of digestion both in-vitro and in-vivo. These factors vary however by forage group and maturity.

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EFFECT OF TYPE AND LENGTH OF DIETARY FIBER ON GROWTH, EFFICIENCY AND CARCASS CHARACTERISTICS OF FEEDLOT CATTLE

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INTRODUCTION

High fiber ingredients often are included in high energy diets to reduce acidosis in feedlot cattle. Historically, fiber length has been thought to help maintain rumen epithelium. However, in a review of his early research (Warner et al., 1956) on factors that affect rumen development, Warner (1991) concluded that the "scratch factor' often thought to be associated with rumen health did not affect papillary development. He further concluded that the volatile fatty acids resulting from fermentation were responsible for the papillary development of the walls of the ruminant forestomach. Yet, the fermentability of the dietary fiber included to maintain rumen function is seldom considered. Because NDF fermentation provides volatile fatty acids useful to maintain rumen function, Thonney and Hogue (2007) proposed that the effective NDF is that portion that is fermentable in the rumen (potentially fermentable NDF, pfNDF). Possibly, we should refer to this as the nutritionally effective fiber.

The purpose of this experiment was to evaluate how level of fermentable fiber and fiber length may affect feed intake, growth, and carcass characteristics of feedlot cattle.

MATERIALS AND METHODS

Crossbred yearling heifers (n = 36, BW = 388 kg) were blocked by weight (light and heavy) and randomly assigned to three diets (Table 1). All cattle were fed diets with 70% whole shelled corn (Table 1). Two diets included early-cut Timothy hay either chopped to 10 cm (Chopped Hay Fiber) or ground to 0.5 cm (Ground Hay Fiber) as 20% of the diet with a premix of protein, minerals, vitamins, and Rumensin in pellet form at 10% of the diet. The third diet included no hay but a different pellet composed of grain byproducts (By-product Fiber) high in pfNDF and a premix similar to that for the hay diets at 30% of the diet. Each diet was formulated to

Table 1. Experimental design (% DM).

	Fiber source		
Item	By-product	Chopped hay	Ground hay
Corn	70	70	70
Timothy hay		20	20
Pellet 1	30	-	-
Pellet 2	-	10	10
Pens	3	3	3
Expected NDF	21	21	21
Expected pfNDF[1]	17	12	12
Heifers/pen (3 pens/diet)	2 light 2 heavy	2 light 2 heavy	2 light 2 heavy

[1]Potentially fermentable NDF.

contain 21% NDF. The By-product Fiber diet was expected to contain 17% pfNDF and the hay diets were expected to contain 12% pfNDF. The formula used for calculating pfNDF was NDF-[100-TDN-Metabolic fecal loss]. The cattle were fed for 82 d in 9 slatted floor pens (4/pen), with 3 pens per diet.

RESULTS AND DISCUSSION

The chemical composition of the diets is shown in Table 2. The calculated pfNDF based on chemical composition was 18, 17, and 17%, for By-product Fiber, Chopped Hay Fiber, and Ground Hay Fiber diets, respectively, instead of the expected 17, 12, and 12%. This was primarily because the Timothy hay had higher NDF and calculated digestibility than expected.

Table 2. Chemical composition of diets (DM basis).

	Fiber source		
Component	By-product fiber	Chopped hay	Ground hay
Dry matter	90.1	89.9	90.0
Crude protein, %	13.7	13.0	13.3
NDF, %	23.1	20.1	21.7
Non-fiber carbohydrates, %	56.6	59.4	58.2
Lignin, %	1.0	0.7	1.0
Starch, %	45.1	50.1	47.6
Fat, %	3.2	3.5	3.3
Ash, %	5.0	5.5	5.1
TDN, %	80	82	80
ME, Mcal/kg	3.1	3.2	3.1

Growth, feed intake, and carcass data are shown in Table 3. There were no significant differences due to fiber source or fiber length on ADG (1.9, 1.8, 1.8 ± 0.04 kg), final weight (550, 548, 542 ± 8.9 kg), DMI (11.8, 12.3, 12.0 ± 0.19 kg), and gain:feed (159, 150, 149 ± 5.8 g/kg).

Rumen epithelia showed no evidence of degradation related to diet. There were no condemned livers. There were no statistical differences due to diet for carcass measurements with the exception of marbling (Table 3). Cattle fed the By-product Fiber diet had higher ($P < 0.05$) marbling scores than cattle fed hay diets (4.2 vs 3.8 ± 0.20) and cattle fed the Chopped Hay Fiber diet had lower ($P < 0.03$) marbling than cattle fed Ground Hay Fiber diet (3.4 vs. 4.1 ± 0.20).

CONCLUSIONS

These results suggest that cattle fed a diet with >15% pfNDF, regardless of fiber source or length, are expected to have equal performance. No advantages were observed for increased Timothy hay fiber length in the diet. While in this small experiment there were no statistical differences among the three diets in growth or feed

intake data, the cattle fed the By-Product diet gained 5% faster and were 6% more efficient than cattle fed the hay diets.

Table 3. Growth, feed intake, and carcass data of heifers (82 days).

		Fiber source				P-value	
Item	n[1]	By-product	Chopped hay	Ground hay	SE	By-product vs hay	Ground vs chopped hay
Initial weight, kg	36	396	397	396	6.6	0.762	0.928
Final weight, kg	36	550	548	542	8.9	0.560	0.610
ADG, kg	36	1.9	1.8	1.8	0.04	0.271	0.542
DMI, kg	9	11.8	12.3	12.0	0.19	0.190	0.255
DMI, % BW	9	2.5	2.6	2.6	0.045	0.170	0.455
DM/Gain	9	6.3	6.7	6.7	0.25	0.235	0.918
g gain/kg DM	9	159	150	149	5.8	0.222	0.863
HCW, kg	36	322	318	318	6.8	0.765	0.978
Dressing percentage	36	58.4	58.1	58.7	0.63	0.734	0.527
Marbling[2]	36	4.2	3.4	4.1	0.20	0.047	0.029
% Choice or higher	9	85	32	74	17.4	0.193	0.146
Back fat, cm	36	1.2	1.3	1.4	0.08	0.336	0.252
Rib eye area, cm^2	36	80	81	82	2.6	0.886	0.669
KPH fat, %	36	1.7	1.6	1.8	0.18	0.381	0.513
Yield grade	36	2.9	2.9	3.0	0.17	0.939	0.733

[1]36 individual heifers and 9 pens (3 pens of 4 for each diet).
[2]Scores: 3 = slight, 4 = small, 5 = modest, 6 = moderate.

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EVALUATION OF THE EFFECT OF NON-ESTERIFIED FATTY ACIDS (NEFA) AND B-HYDROXYBUTYRATE (BHB) CONCENTRATIONS ON HEALTH, REPRODUCTION AND PRODUCTION IN TRANSITION DAIRY CATTLE FROM THE NORTHEAST USA

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INTRODUCTION

Most transition dairy cows visit a state of negative energy balance (NEB) due to increased energy demands after parturition coupled with lagging dry matter intake (Hayirli et al., 2002). The ability to partition available energy for milk production at the expense of reproduction early in lactation (Bauman and Currie, 1980) has made the role of energy balance a key factor in the study of milk production, reproductive performance, and disease occurrence. The metabolites non-esterified fatty acids (NEFA) and/or β-hydroxybutyrate (BHB) are common measures of NEB and/or ketosis in transition animals {290 Duffield,T.F. 2009;}. Although some elevation of these metabolites is normal as these animals balance energy intake and energy demands, excessive elevation can indicate poor adaptation to NEB (Herdt, 2000). Identification of an objective level where NEFA and/or BHB are excessive and cause detrimental effects on health, reproduction and milk production has been difficult due to individual animal variations, normal metabolite elevations during the transition period, and the multiple factors that can affect the outcomes of interest.

The objectives of this study were to: identify critical thresholds above which NEFA and BHB concentrations increase the risk of disease and affect production and reproductive performance; and to investigate the magnitude of these associations in free-stall, TMR-fed herds in the Northeast USA.

MATERIALS AND METHODS

A convenience sample of 104 farms in the Northeast of the USA were selected to participate in a prospective cohort study. All farms consented to participate, and this study was approved by the Cornell University Institutional Animal Care and Use Committee. To be included in the study a herd must have: 1) had greater than 250 milking cows, 2) free-stall housing, 3) fed a total mixed ration (TMR), and 4) participated in DHIA and/or use Dairy Comp 305 (Valley Ag. Software, 2009).

All farms received a standardized consent form, a survey, and case definitions for diseases of interest. The survey collected information on: farm demographics, feeding times in relation to blood collection, voluntary waiting period, and ovulation synchronization protocols. Farm personnel were instructed to document any incident

cases of the diseases of interest: displaced abomasusm (DA), clinical ketosis (CK), and metritis (MET) and/or retained placenta (RP).

Farms were visited once, and during the farm visit, two cohorts of animals were identified: those 14-2 days pre-partum and 3-14 days post-partum. Within each cohort, convenience samples of 15 apparently healthy animals were evaluated. The evaluation included simultaneous blood collection and body condition scoring (BCS) (Ferguson et al., 1994). Guidelines for blood collection and sample handling were based on previous studies (Stokol and Nydam, 2006). Briefly, a plain evacuated red-top tube was used to collect 10 ml of blood from the coccygeal vein or artery. The sera from the pre-partum cohort were analyzed for NEFA and hemolysis. The sera from animals sampled post-partum were analyzed for NEFA, BHB, and hemolysis. For animals sampled, the incidence of the diseases of interest within 30 DIM, time to pregnancy within 70 days post voluntary waiting period and Mature Equivalent 305 (ME 305) milk at 120 DIM were recorded.

Data analysis

Statistical analyses of data were performed using SAS version 9.1 (SAS Institute, Inc., Cary, NC 2004) and ROC curves were obtained using MedCalc (Schoonjans, 2008). Data from the pre- and post-partum cohorts were analyzed separately. Initially data was stratified by parity group (parity =1 or >1), but if the effect of the predictors on the outcome was similar between the two groups they were pooled in the final analyses.

In summary, the analytical approach was done in three stages. The first stage was to identify significant risk factors with a multivariable model where the outcome was the development of any combination of the diseases of interest (DA or CK or MET and/or RP). The second stage, analyzed the continuous significant predictors from the multivariable model with receiver operator characteristic (ROC) curves to identify critical thresholds for prediction of individual diseases (e.g. DA) and any combination of the diseases. Once the range of critical thresholds predictive of disease was identified, the covariates were treated as categorical variables within this range. In the final stage, the magnitudes of the associations between these categorical predictors with disease, reproduction and production were evaluated. For each of these outcomes, three full models were evaluated: pre-partum NEFA and covariates; post-partum NEFA, BHB and covariates; and BHB with covariates.

<u>Evaluation of significant risk factors:</u>

The metabolites, NEFA and/or BHB were the main risk factors and at this level of analysis were treated as continuous predictors. Parity, season, BCS, time of blood collection, and all biologically plausible 2-way interactions were evaluated as covariates in the model. They were modeled with PROC GENMOD using a Poisson distribution, a log link function, p-scale option for over-dispersion, and an exchangeable correlation matrix (Spiegelman and Hertzmark, 2005). This statistical method allows for clustering of cows within herds (i.e. including herd as a random effect) while adjusting for

continuous or categorical covariates. There was no adjustment for varying time spans (offset term) because the length of the time interval at risk was the same for every individual in the sample (Allison, 2007).

ROC curves:

The continuous, significant risk factors identified in the multivariable model were evaluated using ROC curves to determine the critical threshold for predicting disease. The point on the ROC curve that has the highest combined sensitivity and specificity was considered the critical threshold. Interpretation of this critical threshold depends on the area under the curve (AUC), such that if the AUC >0.7 the test is considered accurate (Swets, 1988).

Effect on disease risk, reproduction, and production:

Disease risk

Once the critical thresholds for prediction of disease (DA, CK, MET/RP, or any combination) were identified with ROC analysis, the covariates were dichotomized at the critical threshold. The risk of disease, given these categorical covariates, was evaluated with PROC Genmod, using a poisson distribution, log link function, p-scale option for over-dispersion, and an exchangeable correlation matrix

Effect on reproduction

The effect of elevated NEFA and/or BHB concentrations on reproductive performance was evaluated with time-to-event analysis (PROC Phreg). Cox proportional hazard models (Cox, 1972) were analyzed accounting for clustering of cows within herds. The covariates were: BCS, parity, and ME 305 milk at 120 DIM. ME 305 data was dichotomized based on the median production of the pre- or post-partum group. Animals culled before the end of voluntary waiting period were excluded from the analysis and those not pregnant by the end of the follow-up period were right censored. The proportional hazards assumption was checked statistically by evaluating time dependent covariates and non-informative censoring was evaluated with sensitivity analysis (Allison and SAS Institute, 1995). The categorical metabolite value selected from within the range of critical threshold predictive of disease that resulted in the smallest chance of committing a type I error was kept in the final model.

Effect on production

The effect of elevated NEFA and/or BHB concentrations on ME305 milk were evaluated with mixed effects models with herd as a random effect. The covariates were: BCS, season, and when applicable both parity, and the interaction between parity and the metabolite level. In all models, the metabolites, NEFA and BHB were dichotomized and evaluated within the range of values identified as critical thresholds

for prediction of disease. The categorical metabolite value that resulted in the smallest chance of committing a type I error was kept in the final model.

RESULTS

Study population

Of the 104 herds, 4 were excluded from the study due to missing data. 2758 cows from the remaining 100 herds were included in the study and of these cows, 1440 were sampled pre-partum (35 % heifers and 65% cows) and 1318 were sampled post-partum (37% heifers and 63% cows). The number of milking cows per herd averaged 840.

Multivariable analysis

In the three multivariable models, the metabolites were the only significant predictors of any of the diseases of interest: pre-partum NEFA ($p=0.028$), post-partum NEFA ($p=0.0005$) and when BHB was the only main predictor in the model ($p=0.005$) it was also the only significant predictor. No other covariate or interaction term in any of the three multivariable models had a p-value <0.1.

ROC- critical thresholds for prediction of disease

The critical thresholds identified with ROC analysis are summarized in Table 1 with their AUC values. Briefly the NEFA critical thresholds for predicting any of the diseases of interest in the pre- and post-partum cohort were 0.29 and 0.57 mEq/L, respectively and the BHB critical threshold was 10 mg/dL. Figure 1 is a graphical representation of an ROC curve with DA as the outcome and concentrations of post-partum NEFA as the test.

Risk of disease

The risk of disease based on NEFA and BHB concentrations greater than or equal to critical thresholds are also summarized in Table 1. For example, experiencing elevated metabolite levels post-partum increased the risk of developing a DA by up to 10 times, and elevated levels of post-partum NEFA contributed the greatest risk of disease development.

Effect on reproduction

Table 2 summarizes the results of elevated metabolite levels on reproduction with estimates for metabolites and significant covariates reported. Animals with elevated metabolite levels (within the range identified as predictors of disease) took longer to get pregnant; the hazard ratio for pregnancy within 70 days post-voluntary waiting period decreased. Figure 2 is a graphical representation of a Kaplan-Meier curve, where animals with elevated pre-partum NEFA levels took longer to get pregnant.

Effect on production

The results of elevated metabolite concentrations are reported separately for heifers and cows sampled post-partum because the effect of the elevated metabolite thresholds on ME305 milk was different between these two groups. Generally, elevated metabolite levels predicted a decrease of several hundred kilograms of ME 305 milk; however, in heifers sampled post-partum elevated metabolites levels predicted an increase in ME 305 milk. The results of this analysis are summarized in Table 3 with metabolite results and significant covariates reported.

DISCUSSION

This study demonstrated that excessive negative energy balance (as measured by NEFA and BHB concentrations) in the transition period are strong predictors of clinical disease, and negative reproductive and productive performance in cattle from free-stall, TMR-fed Northeastern dairies averaging 840 milking cows. The magnitude of the association between elevated NEFA and/or BHB and diseases of interest, measured by risk ratios, was large (range: 1.75-9.7). The effects of elevated metabolite levels on reproduction decreased the hazard of pregnancy within 70 days post-voluntary waiting on average by 0.2, with parity as the only other significant covariate (cows took longer to get pregnant than heifers). Milk production, showed mixed results, and although further investigation about homeorhesis in heifers is warranted, there was strong evidence of significant ME305 milk loss in cows sampled post-partum and all animals in the pre-partum cohort. Management programs focused on minimizing the risk of these diseases and minimizing negative effects of decreased reproductive and productive performance may consider the following as general guidelines for monitoring NEFA and BHB concentrations in cattle: NEFA concentrations ≥ 0.3 mEq/L for cattle 14-2 d pre-partum; and NEFA concentrations ≥0.6 mEq/L and BHB ≥10 mg/dL for those 3-14 d post-partum.

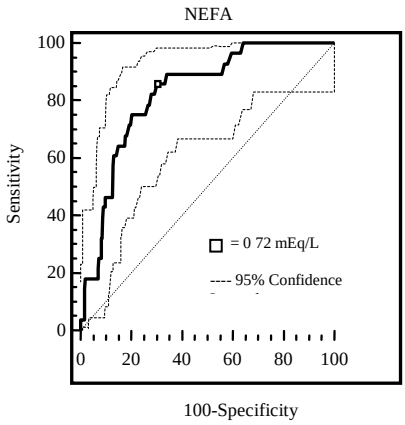

Figure 1. ROC curve determination of critical threshold (upper most left hand corner) for NEFA concentrations predicting DA in animals sampled post-partum.

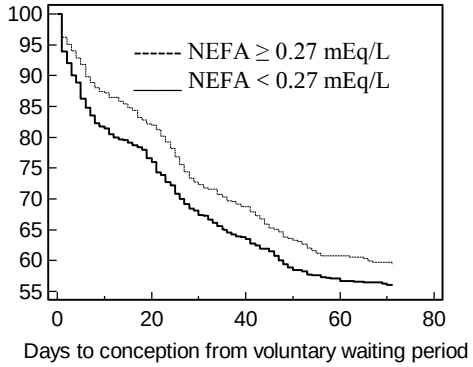

Figure 2. Kaplan-Meier curves of time to pregnancy of cows and heifers with NEFA ≥ 0.27 mEq/L or < 0.27 mEq/L measured in serum 14-2 days pre-partum.

Table 1. Receiver operator characteristic (ROC) curve determination of critical NEFA (mEq/L) and BHB (mg/dL) thresholds as predictors of disease and risk ratios of disease based on these critical thresholds.

Animals sampled pre-partum					
Disease	Critical NEFA threshold[1]	AUC[2]	Risk Ratio	95% RR CI[3]	p-value
DA	0.27	0.6	2.0	1.1 – 3.7	0.03
CK	0.26	0.6	1.8	1.2 – 2.5	0.001
MET and/or RP	0.37	0.6	2.2	1.6 – 3.0	<0.0001
Any 3	0.29	0.6	1.8	1.4 – 2.2	<0.0001
Animals sampled post-partum					
Disease	Critical NEFA threshold[1]	AUC[2]	Risk Ratio	95% RR CI[3]	p-value
DA	0.72	0.8	9.7	4.2 – 22	<0.0001
CK	0.57	0.7	5.0	2.3 – 11	<0.0001
MET	0.36	0.6	16	2.0 – 133	0.008
Any 3	0.57	0.7	4.4	2.6 – 7.3	<0.0001
Disease	Critical BHB threshold[1]	AUC[2]	Risk Ratio	95% RR CI[3]	p-value
DA	10	0.8	6.9	3.7 – 12.9	<0.0001
CK	10	0.7	4.9	3.2 – 7.3	<0.0001
MET	7	0.6	2.3	1.1 – 5.1	0.037
Any 3	10	0.7	4.4	3.1 – 6.3	<0.0001

[1] Highest combined specificity and sensitivity
[2] Area under the curve
[3] Risk ratio confidence interval

Table 2. Cox proportional hazard model of the effect of NEFA (mEq/L), and/or BHB (mg/dL), covariates, and animals clustered within herds on days to conception after voluntary waiting period.

Sampled Population	Variable	Hazard Ratio	P-Value
Pre-partum cohort	NEFA ≥0.27	0.81	0.01
	Parity	0.73	0.0004
Post-partum cohort	NEFA ≥0.72	0.84 0.93	0.05
	BHB ≥10	0.81	0.40
	Parity		0.01
Post-partum cohort	BHB≥10	0.87	0.1
	Parity	0.80	0.01

Table 3. Mixed models for the effect of NEFA (mEq/L), and/or BHB (mg/dL), covariates, and herd as a random effect on milk production measured by 120 DIM ME 305 (kg).

Sampled Population	Variable	Difference in ME milk yield (kg)	P-value
Pre-partum cohort	NEFA ≥0.33	-683	0.001
	Parity	-556	0.01
Post-partum cohort-heifers	NEFA ≥0.57 BHB ≥10	488 -143	0.02 0.5
Post-partum cohort-heifers	BHB≥9	403	0.04
Post-partum cohort-cows	NEFA ≥0.72 BHB 10	-647 -165	0.001 0.40
Post-partum cohort-cows	BHB ≥10	-393	0.04

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INTEGRATING NUTRITIONAL AND GROUPING MANAGEMENT OF TRANSITION COWS

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INTRODUCTION

As described in this proceedings (Nydam et al., 2009), elevated circulating concentrations of nonesterified fatty acids (NEFA) during both the prepartum and postpartum periods and elevated circulating concentrations of B-hydroxybutyrate during the postpartum period are associated with increased risk for metabolic disorders in both heifers and cows, decreased reproductive performance in both heifers and cows, and decreased ME305 projected milk yield in cows but not heifers. Furthermore, Nydam et al. (2009) suggested that more than 15 to 20% of animals with circulating concentrations of these metabolites above threshold values in the target prepartum and postpartum periods would be indicative of a herd-level problem with transition cow management.

One key question relates to prevalence of these elevated concentrations of circulating NEFA and BHB in cows in the 100 freestall dairy farms in the Northeast that were the subject of this study. Of 1472 animals sampled during the prepartum period (2 to 14 d before calving), 45% of heifers (245/540) and 26% of cows (246/932) had NEFA concentrations at or above 0.3 mM. Of 1315 animals sampled during the postpartum period (3 to 14 d after calving), 25% (131/517) of heifers and 33% (267/798) of cows had NEFA concentrations at or above 0.7 mM. Furthermore, 15% (77/517) of heifers and 27% (214/798) of cows had BHB concentrations at or above 10 mg/dL. Herd-level prevalence of elevated NEFA and BHB in this dataset is described in Table 1. This study illuminated that many herds have elevated concentrations of these metabolites during both the prepartum and postpartum periods. Among the most striking findings were the large number of herds (59% of herds) with elevated NEFA in more than 35% of precalving heifers and the large number of herds (30 to 40% of herds) having more than 35% of heifers and cows with elevated NEFA during the postpartum period. Finally, more than 50% of herds had more than 25% of cows with elevated BHB during the postpartum period.

Clearly, there is opportunity for improved transition period management in herds in the Northeast. It is sometimes challenging to pinpoint specific areas within individual farms as it is likely that this substantial variation in overall metabolic health in heifers and cows among farms arises from the net effects of an integrated and dynamic set of facility characteristics, grouping management, and nutritional management factors that are specific to each farm. In the rest of this paper, we will review our current state of knowledge relative to the impact of these factors on aspects of transition cow management and outcomes of importance.

Table 1. Distribution of herds by prevalence of elevated prepartum NEFA or postpartum NEFA or BHB concentrations (Ospina et al., 2009)

	Number of herds by prevalence of elevated metabolite			
Item	< 15 %	15 – 25%	25 – 35%	> 35%
Prepartum NEFA ≥ 0.3 mM				
Heifers	23	3	11	59
Cows	34	16	22	28
Postpartum NEFA ≥ 0.7 mM				
Heifers	43	6	19	30
Cows	24	15	22	38
Postpartum BHB ≥ 10 mg/dL				
Heifers	61	8	17	12
Cows	30	18	26	25

GROUPING AND FACILITY FACTORS AND TRANSITION COW MANAGEMENT

Stocking density

Of all of the grouping/facility factors that have been evaluated in the context of transition cow management, stocking density of groups during the prepartum period has received by far the most attention (Cook and Nordlund, 2004; Nordlund et al., 2007). Unfortunately, most of the current recommendations (e.g., optimal stocking density at 80% of headlocks or 30 in of bunk space per cow; Cook and Nordlund, 2004) are based largely on observational work rather than randomized trials in which the benefits of decreased stocking density were observed in primiparous cows only. Although field experience certainly corroborates the benefits of decreasing stocking density in many situations, these observational studies do not lend themselves to truly determine the optimal stocking density, and the optimal stocking density surely varies across farms based upon other grouping management/facility characteristics.

Recently, Proudfoot et al. (2009) evaluated the effects of competition at the feedbunk on aspects of behavior in transition cows. Cows were assigned to either competitive (2 cows per Insentec feeding bin) or noncompetitive (1 cow per bin) treatments beginning 18 d prior to expected calving and continuing through 18 d postpartum. Competitively fed primiparous and multiparous cows were displaced from the feed bunk 3 and 2 times, respectively, as frequently as noncompetitively fed cows. Other than displacements, competition did not affect most other indices of feeding or meal behavior in primiparous cows. In multiparous cows, competitive feeding resulted more visits but less eating time and a trend for lower DMI in the week before calving, less feeding time in the first week postcalving, and higher feeding rate in the second week postcalving. Interestingly, competitive feeding resulted in significantly more standing time and less resting time in multiparous cows during the prepartum period and during the first week postcalving.

In a study conducted in a similar manner by the same research group, Hosseinkhani et al. (2008) sought to determine whether competitive feeding affected sorting behavior during the prepartum period as assessed by changes in particle size. The TMR averaged 49.5% DM and the Penn State Particle Separator particle size distribution of the original TMR (DM basis) was as follows (18.1% top screen, 32.3% middle screen, 33.4% third screen, and 16.2% bottom pan). Regardless of competitive feeding treatment, cows sorted against longer particles at both 4 and 12 h postfeeding. Although competitive feeding did not affect sorting behavior and did not affect overall daily feeding time or DMI, competitively fed cows had faster feeding rates and lower meal frequency. Consistent with these findings, Krawczel et al. (2009) reported that increasing stocking density at the feed bin (100, 133, 150, and 200%) increased feeding rate during the first 21 d postpartum. In the absence of freestall overcrowding, they determined that stocking density at the feed bin did not alter overall DMI or standing behavior.

In summary, although stocking density certainly appears to be an important factor affecting overall transition period health and performance outcomes, controlled research is largely lacking that gives both the optimal range and the weight of this factor compared to other factors that will be discussed subsequently in this paper. Although it is difficult to determine conclusively, we suspect that use of the Insentec systems as used in the studies referred to above may have moderated the effects of overstocking compared to overstocking applied across a full feed bunk.

Commingling primiparous and multiparous cows

We believe that eliminating the commingling of primiparous and multiparous cows that is common during both the prepartum and postpartum periods remains a major opportunity for freestall dairy farms in the Northeast. Our major basis for this argument is described in Table 1. Although we have not quantified the effects of grouping management factors within this dataset, we would suggest qualitatively that a majority of the herds enrolled in the study would have had a separate first lactation heifer group beyond the fresh group, some of the herds would have had separate fresh groups for first lactation and older animals, and virtually none of the herds would have had separate groups for heifers and cows during the close-up period. We believe that these patterns are reflected in the distribution of the prevalence of elevated NEFA in primiparous animals during both the prepartum and postpartum period. A staggering 70% of herds had more than 25% of their primiparous animals with elevated NEFA during the prepartum period, which clearly indicates that DMI is being compromised in these animals. Furthermore, nearly 50% of herds had more than 25% of their primiparous animals with elevated NEFA during the postpartum period. Although controlled research on commingling is even more lacking than that for stocking density, it is worth noting that the effects of stocking density reported by Nordlund et al. (2007) above were confined to milk yield responses in primiparous cows. Furthermore, primiparous cows had higher responses of cortisol to ACTH challenge than multiparous cows following their introduction to a commingled environment (Gonzalez et al., 2003).

Clearly, this is an area that requires further randomized trials and potentially focus at the field level.

Pen moves

One of the major areas of focus by Cook and Nordlund (2004) was the issue of the number of pen moves made during cows during both the prepartum and postpartum periods. In many freestall transition management systems, it is not uncommon for cows to make five to six moves during the six-week period around calving. They advocated for elimination of group moves 2 to 5 days before calving and shortening of the period spent in a post-fresh monitoring group. Their basis for this was a collection of older studies that suggested that social adaptation to new groups ranged from 48 h to 7 d, with low rank cows more affected by the regrouping. More recently, von Keyserlingk et al. (2008) reported that midlactation cows introduced to a new group had substantially (2.5X) more displacements from the feedbunk on the day of mixing compared to the 3 d prior to regrouping and slowly declined on the two days thereafter. Lying bouts and time also were decreased in a similar manner on the day of mixing with recovery in the days thereafter. Milk yield was decreased by about 3.5 kg/d on the day of mixing only (43.4 vs. 39.7 kg/d). Although controlled evidence specifically focused on pen moves and their timing during the transition period is largely lacking, the overall practice of streamlining grouping changes during this time appears to have yielded dividends on farms in terms of fresh cow health and calving management.

A practice that currently has received some attention in the field, but not controlled research to the authors' knowledge, is the concept of all-in grouping management for close-up cows such that the typical weekly move of cows into a close-up group would occur to a different small pen each week on a rotational basis. Cows would calve in the same group and new cows would not be introduced into this group. Anecdotal evidence suggests that this strategy may have merit, although the requirements for herds to maintain other aspects of grouping management (e.g., stocking density, hygiene) applies in this scenario as well.

Heat stress abatement

Another prepartum management strategy not commonly observed on dairies in the Northeast is evaporative cooling applied to prepartum pens. Israeli workers (Wolfenstein et al., 1988) applied evaporative cooling (sprinklers and fans) to cows during the entire dry period. Temperatures during the study period averaged 75°F at 0700 h and 88°F at 1400 h. Cows subjected to cooling maintained rectal temperatures ~ 0.5°C lower than controls and yielded 3.6 kg/d during the first 150 d postpartum. Recently, Avendano-Reyes et al. (2006) conducted two studies focused on cooling strategies for dry cows. In the first study, they determined that soaking cows without fans to evaporate the water was not effective in cooling dry cows. In the second study, they determined that cows subjected to evaporative cooling during the entire dry period had increased yields of milk and milk fat during the first 8 weeks of lactation compared to noncooled controls. Minimum and maximum temperatures averaged 73°F (THI 66)

and 109°F (THI 94) during the study period, respectively. Although these are high compared to typical Northeast conditions, we believe that heat stress in cows that calve in typical summer conditions contributes to lower milk yield during the fall in these animals; furthermore, this scenario is compounded by overstocking in transition groups during summer months because of poor reproductive performance during the previous summer.

ONE-GROUP AND TWO-GROUP NUTRITIONAL MANAGEMENT OF DRY COWS

Recommendations for nutritional management of cows during the dry period have evolved substantially during the past 5 to 7 years. The industry has largely abandoned the "steam-up" concept with higher energy diets fed to dry cows during the close-up period as advocated by the authors of the 2001 Dairy NRC in favor of controlled energy strategies for dry cows during both the far-off and close-up periods (Drackley, 2007). Furthermore, controlled energy strategies have lent themselves toward more widespread adoption of low energy, one-group dry cow programs.

Drackley (2007) has advocated diets for both far-off and one-group dry cow programs with the following specifications (0.59 to 0.63 Mcal of NEL/lb of DM; 12 to 16% starch, and 40 to 50% forage NDF in the total diet). While we believe that energy and nutrient densities in these ranges are appropriate for cows during the far-off period and cows during the close-up period, they may be too low in many cases if these diets are fed to heifers during the prepartum period, especially in commingled scenarios with varying stocking densities. Goals for NEL intake of both heifers and cows during the far off and close up periods range from 15 to 18 Mcal/day. For most prepartum heifers, this likely means energy densities in the vicinity of 0.66 Mcal/lb, so a reasonable compromise would be 0.64 to 0.66 Mcal/lb if the same diet is fed to both cows and heifers during the prepartum period. Of course, actual energy densities of diets should be based upon actual farm DMI to achieve the energy intake targets specified above.

Limited research has focused on relationships between nutritional management during the far-off and close-up periods with consideration to one-group approaches to dry cow nutritional management. Dann et al. (2006) fed diets during the far off period to achieve 80 (actual 77), 100 (actual 95), or 150 (actual 160)% of NRC-predicted energy requirements followed by a close-up diet fed either above energy requirements (average 135%) or restricted to below energy requirements (80%). Surprisingly, close-up feeding strategy did not affect periparturient metabolism or performance. Cows overfed during the far-off period had lower subsequent DMI and calculated energy balance along with elevated BHB and NEFA during the first 10 d postpartum. Richards et al. (2009) recently compared a controlled-energy (~0.60 Mcal/lb NEL) feeding strategy with a high energy diet (~0.73 Mcal/lb NEL) fed for the entire dry period and a two-group feeding strategy in which cows were fed the controlled energy diet during the far-off period and the high energy diet during the close-up period. As expected, the cows fed the high energy diet during the entire dry period gained more body condition during the dry period and lost more body condition during the postpartum period. Cows fed the controlled energy diet had lower postpartum NEFA, BHB, and liver fat compared

to cows fed the high-energy diet. Metabolic health parameters for cows fed the two-group strategy were more similar to the controlled energy one-group diet than the high energy diet.

Collectively, these data continue to support the concept of moderating energy intake during the entire dry period, regardless of specific dietary grouping strategy. One-group dry period diets can provide additional flexibility for farms to vary their times in close-up groups to manage stocking density or other factors without concern for nutritional support. We advocate that strategies for macromineral formulation of one-group dry cow diets mirror that used for close-up diets as prevention of hypocalcemia and related disorders is a critical part of nutritional management during the prepartum period.

SUMMARY AND CONCLUSIONS

Substantial variation in overall metabolic health in heifers and cows among farms arises from the net effects of an integrated and dynamic set of facility characteristics, grouping management, and nutritional management factors that are specific to each farm. Although much of the focus on grouping management strategies for transition cows has focused on stocking density and pen moves, our field study data suggest that commingling of cows and heifers may be a major issue for our Northeast freestall dairies. Furthermore, the lack of effective heat abatement during the dry period on the vast majority of Northeast dairy farms likely compromises milk yield and other aspects of health and performance in cows that calve during typical summer months. Application of one-group, controlled (lower) energy dry cow nutritional management strategies is conducive to flexibility in grouping strategies for dry cows; however, formulation likely needs to be customized among farms to account for differences in DMI and also whether the same diet will be fed to both cows and heifers during the prepartum period.

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SAUROPOD DINOSAURS AND GIGANTISM: A REPORT OF AN INTERNATIONAL CONFERENCE

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The herbivorous Sauropod dinosaurs of the Jurassic period, about 140 – 210 million years before present (mybp), were the largest land animals that ever lived. This presentation is in part a report of the symposium on Sauropods held at the University of Bonn, Germany in November, 2008. The conference was attended by a group of specialists addressing the issues of size, activity, blood pressure, digestive physiology, and diet of these creatures. Since no modern land animals of such size exist today, extrapolations from the largest and tallest contemporary herbivores, elephants and giraffes, were used to model the Sauropods. Recent discoveries in Patagonia have found even larger Sauropods (Argentinosaurus), exceeding 120 feet head to tail and weighing an estimated 100 metric tons. Footprints of related Sauropods have been found in Chile measuring 2 – 2½ feet in length with an estimated thigh bone size at 10 – 12 feet (Kissel, 2007; Moreno and Benton, 2005).

Dinosaurs first appeared in the Triassic period about 220 million years ago (mybp), and were the dominant land animals for 150 million years, through the Jurassic and Cretaceous periods. Most of them went extinct at the end of the Cretaceous period (65 mybp), except birds which are now recognized as dinosaur relatives. After the extinction that might have been due to a giant meteoric impact, small mammals which had been minor members of the ecosystem, proliferated and became the new dominant species in the ensuing Cenozoic period (Novacek, 2002).

The relationship of the dinosaurs and birds is shown in Figure 1. The herbivorous Sauropods included the giant dinosaurs, the principal subjects of this paper. The carnivorous Theropods, which include Tyrannosaurus rex and its relatives, also includes the birds which eventually branched off. Another branch includes the less related Ornothiscians, which include the tank-like horned dinosaurs (Triceratops), Ankylosaurs (types with plate like projections on the spine), parrot and beak types.

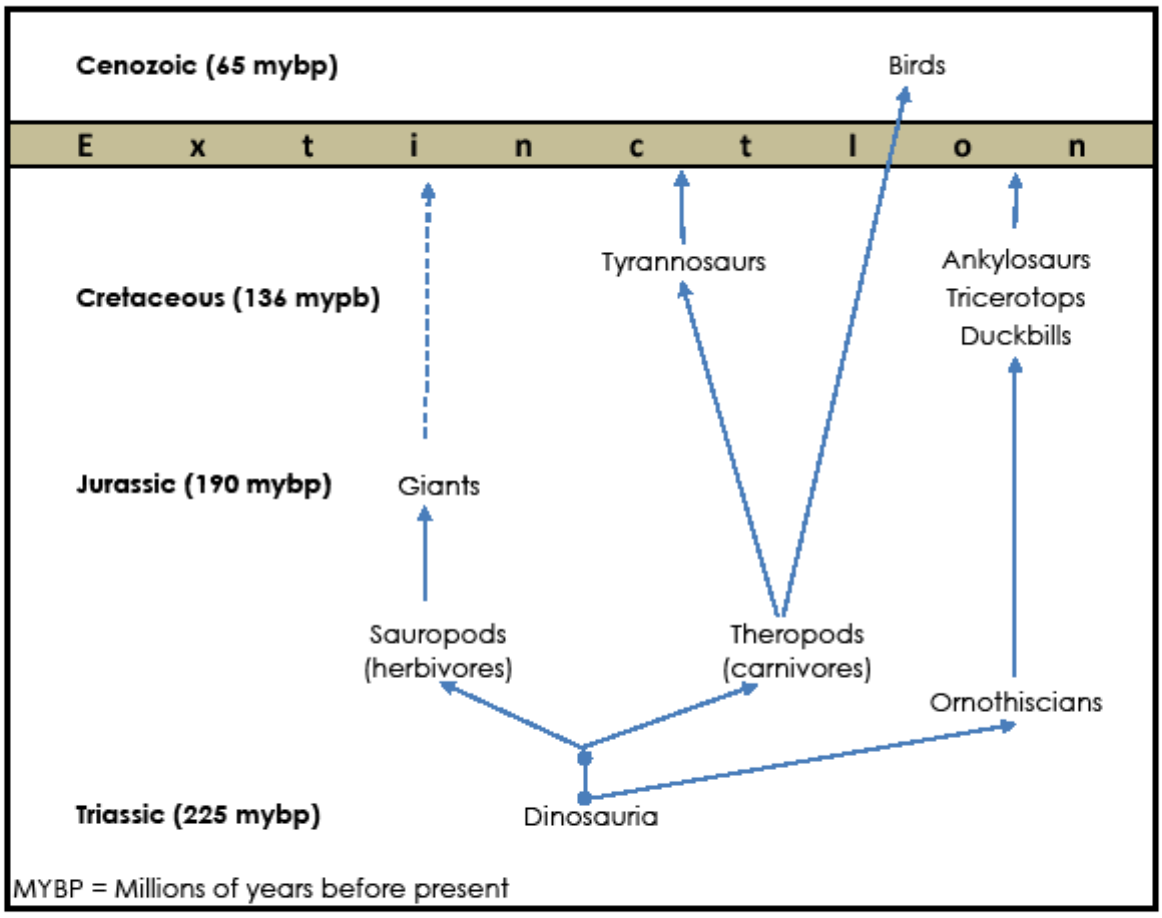

Figure 1: Revised taxonomic relationships among sauropods, tyrannosaurs, birds and ornothiscian dinosaurs. Integrated from Long, etc. (2008) and Novacek (2002).

In the past 20 years there has been a very great revision of dinosaur taxonomy. Birds are now considered dinosaurs (Long et al., 2008) and were the only species to escape the Cenozoic extinction. Modern birds seemed to have survived in parts of Gondwanoland, distant by half the earth from the 110 mile diameter Chicxulub crater in Yucatan (Cracraft, 2001-02). Gondwanoland was the ancient continent when South America, Africa, India, and Antarctica were joined before the plate shift that formed the current continental structure and allowed for the formation of the current seas. The Chicxulub crater is thought to be formed by a large meteor object that forced enough dust and debris into the atmosphere to cause a significant decrease in the radiant heat and sunlight, thus starving the dinosaurs to extinction if not destroying them outright

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Homeothermy vs. Ectothermy

Classical taxonomy considered dinosaurs as reptiles, which are cold blooded. However, since birds are warm blooded, the question can be asked where was the warm bloodedness inherited from and from whom. The problem focuses on when did

the warm bloodedness start and whether other related dinosaurs may have been warm blooded. Opinion remains divided as to whether the Sauropods and the early birds were cold blooded and later, the birds evolved warm bloodedness, or alternatively Sauropods and birds shared a common base of warm bloodedness (Dunham et al., 1989). Factors favoring warm bloodedness in Sauropods and other dinosaurs are shared by modern mammals. Sauropod footprints show that limbs were columnar like elephants, with legs positioned directly under their bodies, rather than splayed to the side as in lizards (Schweitzer, 2005). Another view is that large dinosaurs were passively homeothermic (Dunham et al., 1989; Novacek, 2002). Their great size would have reduced their weight to surface area relationship. Thus the ability to dissipate heat would also be reduced, and might have been warmer by virtue of their tremendous metabolic activity, muscle and organ function. That is, they were so large that getting rid of heat may have been difficult. The consensus at the symposium in Bonn was that the Sauropods may not have been as warm blooded as mammals, but were substantially higher in temperature than living reptiles (Sander and Claus, 2008), primarily because of the body weight to surface area relationship.

Blood pressure

Bakker (1987) shows diagrams of giant Sauropods feeding to the tops of trees (40 – 50 ft.) Physiologists do not believe this because the difference in blood pressure at those heights would cause unconsciousness by lack of blood flow to the brain. The problem is more severe for homeotherms than in a cold blooded animal. This leads to an argument that Sauropods were cold blooded and browsed closer to the ground (Kissel, 2007). However, an Apatosaurus with a hip bone length of 10 feet and a neck of 17 feet could raise its head conservatively 20 feet, according to Kissel. This may be compared to the giraffe, our tallest contemporary browser at 18 feet. In addition, Kissel does not consider tripodal position (tail plus hind limbs) which would allow some additional height, when front limbs were lifted off the ground, and a position likely needed for mating.

Respiratory modifications like increased oxygen and scavenging capacity may also have contributed to the ability to grow to such great size. Modern birds inherited a respiratory modification, increasing oxygen efficiency that may have been a factor in dinosaur evolution. Special airsacks in birds allow highly efficient respiration at high altitude (Berner et al., 2007). Migrating cranes can fly over the Hindu Kush at 24,000 feet, from Central Asia to India, and not suffer from high altitude oxygen deprivation (Ackerman 2004). This adaptation may have made it easier for Sauropods to browse at higher levels in the trees. The Sauropods also evolved lightweight bones that enabled them to be huge. This feature was likely inherited by birds and a factor enabling them to develop flight (Carrano and O'Connor, 2005). Further, birds also inherited feathers from dinosaurs (Long et al., 2008).

Dinosaur diets

The plants eaten by the herbivorous dinosaurs were very different from contemporary forages. Modern grasses and legumes are only about 30 million years old and did not exist in Mesozoic times. Fossils indicate conifers, cycad, ferns, ginkgo, Equisetum (horsetails), and some now extinct taxa existing during the period of the Sauropods (Hummel et al., 2008).

Hummel, et al. (2008) have collected existing species and conducted in vitro digestion (gas production) using inocula from sheep fed grass. These results are shown in Table 1. Ash content and in vitro digestion of NDF were not measured, so the estimated digestibility of fiber is very approximate. However, the gas production which estimates metabolizable energy would be sufficient to support large herbivores. The values in Table 1 may be low from the point that microbes in the rumen of the sheep may not be adapted to these plants, especially when used as inocula without prior exposure.

Table 1: Estimated digestibility of NDF (D.NDF). Data of Hummel et al., 2008, recalculated according to the summative equation table 25.6, p. 412 in Van Soest (1994)

Sample	**Est D**	**NDF**	**D.NDF**
Grasses	70	62.8	46
Forbs	64	37.8	15
Dicot browses	46	43.2	2
Ginkgo	53	27.5	-
Araucaria	58	65.2	36
Podocarp	37	62.3	12
Conifers	57	51.3	21
Cycads	38	65.3	14
Ferns	48	62.8	2
Tree Ferns	40	63.6	11
Equisetum	72	48.4	33

Evolution of gut fermentation

The microorganisms dominant in the rumen and the hindguts of modern non-ruminants are taxonomically diverse, comprising archea (methanogens, acetogens), bacteria (cellulolytics and others), fungi (also cellulolytic), and protozoa. These groups evolved in the Precambrian, > 570 mybp, and existed in bog, soil and inoculated the

guts of evolving herbivores before 300 mybp (Hume and Warner, 1980). No existing mammalian herbivores have enzymes to breakdown cellulose, hemicellulose and related dietary fiber carbohydrates, the digestion of which is dependent on fermentation (Van Soest, 1994). This was also likely true for the herbivorous dinosaurs.

For these reasons much comparative metabolism of contemporary herbivores and their behavior have been extrapolated to the hypothetical herbivorous dinosaurs. Particular problems relate to retention time and extent of fermentations. Most of the available data is from mammalian species. Gut fermentation in reptiles and birds like ostriches is poorly documented and not mentioned in Hume and Warner (1980) or Hume and Sakaguchi (1991) on evolution of gut fermentation. Rumen fermentation is sensitive to temperature (Francis et al., 1978; Westerman, 1996) so that homeothermy would favor efficient gut fermentation. Dunham, et al. (1989) comment that most ectotherms cannot digest food unless their core temperature is above 20°C. Gut fermentations in cold blooded animals are poorly documented (McNab, 2002) and would be thermodynamically unfavorable.

Retention Time

No existing mammalian herbivore has a mean retention time exceeding four days. This is the time limit for secondary methanogenic fermentation which would convert acetate and other VFA to methane (Chandler et al., 1980) which would seriously reduce metabolizable energy. Data showing longer retention time with body size only applies to smaller herbivores below 1000 kg that are limited by food intake and retention time (Figures 2 A,B,C,D and Table 2).

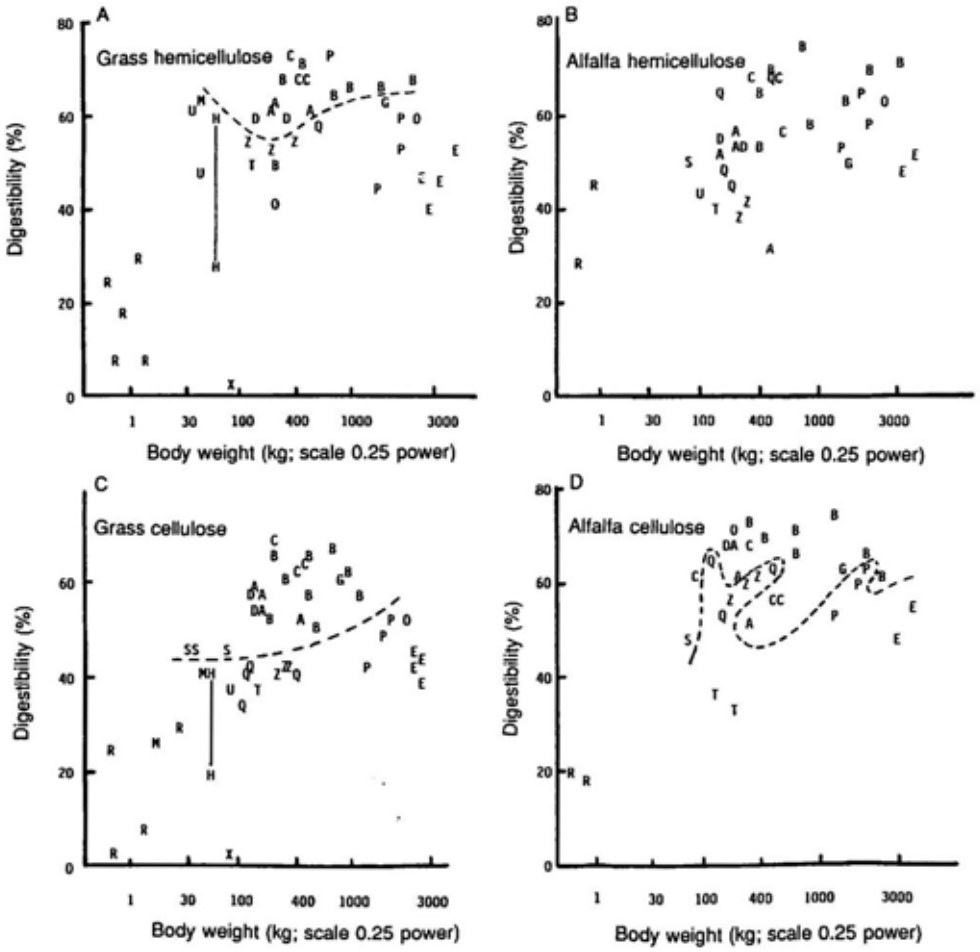

Figure 2 A,B,C,D: The relation between body weight and ability to digest hemicelluloses and cellulose from alfalfa and grasses in diverse species of ruminants and nonruminants. (Van Soest, 1994. See table 2 for identification of species).

Table 2: Key to the identification of animal species in Figures 2 A,B,C,D

Group	Number of Species	Body Weight Range kg	Description	Symbol
Non-Ruminants				
Man	1	66-85	Male Students	H
Monkey	1	40	Baboon	M
Rodents	3	0.05-3	Rat, rabbit, vole[a]	R
Perissodactyls				
Equids				
Horses,	5	132-460		Q
Tapirs	2	147-298	Asian, American	T
Zebras	3	272-354	Grevy, mtn, plains	Z
Rhinocerus	3	1285-2041	Black, white, Indian	P
Artiodactyls				
Hippopotamus[a]	2	181-2268	Pygmy, river	O
Swine	1		Domestic	Y
Other	3	15-123	Beaver, panda, monkey	X
	2	2665-2873	Elephant	E
Ruminants				
Antelope	4	193-454	Eland, gemsbok, milgai,	A
Bovids	7	280-816	Cattle, buffalo, bison	B
Camelids	3	96-544	Camels, guanaco	C
Deer	2	190	Wapiti, barasingha	D
Giraffe	1	904		G
Caprids	2	46	Sheep, goat	S

[a] Pregastric fermenters

Data for balances in elephants and rhinos indicate faster turnover of gut contents than would be expected for their size. Retention time is related to extent of fiber digestion, which differs between ruminants and non-ruminants when fed poor quality grass diets, but disappears when higher quality diets are fed. Figure 3 compares the strategies of the cow and horse of similar size. The strategy of the horse is applied to other equids, rhinos and elephants. This leads to different feeding strategies between ruminants and non-ruminants (Figure 3). Equids and elephants do not need to reduce particle size as much as ruminants, because their digestive tracts do not have the restricting orifices for particle retention. The ecological consequence is that equids and elephants are less restricted in diet quality because they have a faster passage of indigestible lignified material, which is the limitation of the ruminant which has to process indigestible material until it can pass. Thus elephants have lower digestibilities

of fiber than ruminants while equids and elephant have higher intakes. Balances for African and Asian elephants are shown in Table 3.

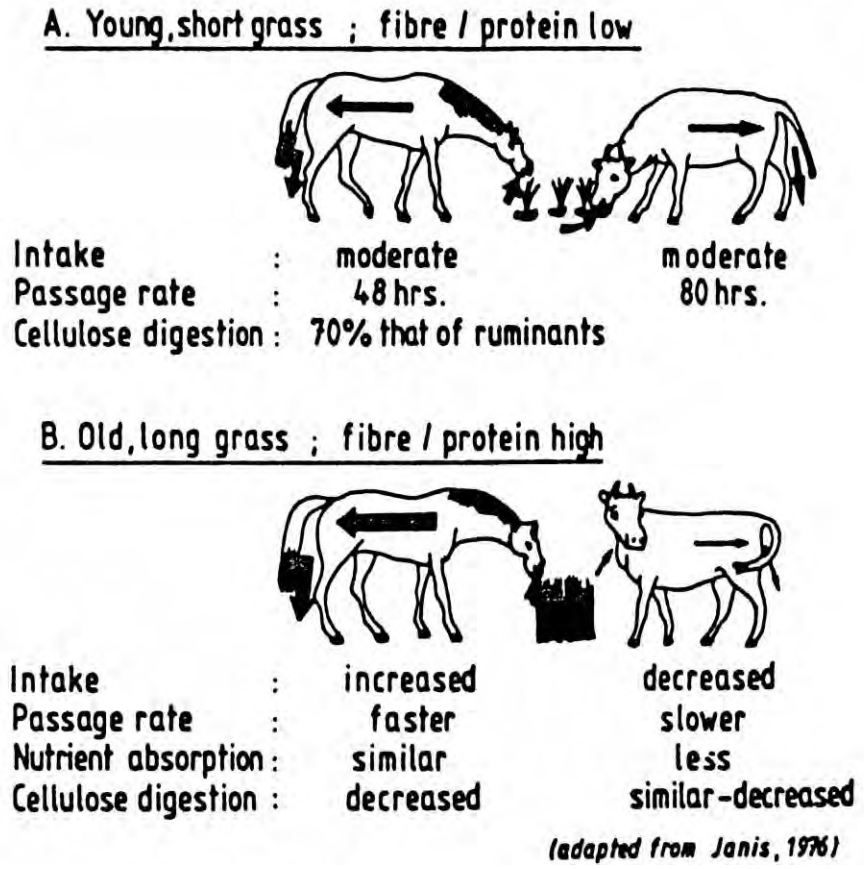

Figure 3: Differences in feeding strategies of horses and cattle. Adapted from Janis (1976) by W. Von Engelhardt; published in Van Soest (1994)

Table 3: Digestion and passage in African and Asian Elephants fed on grass hays. *Source*: Data from Hackenberger, 1987. In Van Soest 1994.

	African	Asian
Number of observations	39	42
Body weight (kg)	2805 ± 506	2623 ± 813
Intake per day (% body weight)	1.7 ± 0.3	1.3 ± 0.3
Mean retention (h)	22 ± 2.0	26 ± 2.5
Digestibility		
Dry matter (%)	38.3 ± 3.0	43.1 ± 3.2
Neutral-detergent fiber (%)	38.1 ± 3.2	42.5 ± 3.0
Cellulose (%)	40.4 ± 3.1	44.3 ± 2.9
Hemicellulose (%)	41.2 ± 1.4	46.0 ± 3.0
Protein (%)	44.8 ± 6.8	54.0 ± 5.7
Fecal nitrogen (FN) (%)	0.89 ± 0.11	0.65 ± 0.08
Net metabolic loss (M_i) (%)	11.1 ± 1.7	13.3 ± 1.7
FN/M_i	0.080	0.049

The problem of retention time and extent of digestion applies to the herbivorous dinosaur which adds the problem of forage quality as shown in Figures 2 A-D. These figures show that there are limiting qualities of cellulose with ruminants versus non-ruminants, whereas the easier fermentable hemicelluloses obscure the differences among species. The animal size factor is only significant below 1000 kg.

The problem of size and feeding behavior is relevant in Sauropods. The giant herbivores grew from 10 kg hatchlings to over 100 tons – five orders of magnitude (Sander and Clauss, 2008). The juvenile size is in the range of limits of small herbivores. The infant dinosaurs must have practiced selective feeding. Quality ceased to be important at sizes greater than 2000 kg. Chewing and mastication were also less important at the larger sizes.

Gastroliths

The ingestion of stones into the gut may have been a substitute for chewing, and has been suggested in Sauropods with limited dental development (Weishampel and Norman, 1989). This is termed the gastric mill in the dinosaur literature. However, Wings and Sander (2007) indicate that the gastric mill was not a feature of the Sauropods. The gastic mill is observed in birds like the ostrich that swallow stones that reside in the gizzard and grind the ingesta. This serves to break the hulls of seeds and reduces need for chewing. The hoatzin is the only bird with a fully documented rumen-like fermentation which is in the crop (Grajal et al., 1989). Stones in the gut of the hoatzin are not reported.

Gastroliths were likely a feature of some dinosaurs and fossils have been associated with gut stones (Wings and Sander, 2007). In the case of ruminants, the evolution of rumination was likely a feature increasing digestive efficiency in smaller animals (Van Soest, 1994). This may well have been the case of some of the herbivorous dinosaurs that might have used the gastric mill as a means of increasing food intake. In the case of the large Sauropods, particle reduction might not have been important in view of digestion and passage.

The ruminant literature indicates that grinding does not increase digestibility measured by digestion balances (Moore, 1964), although it is often claimed. Upon fine grinding, apparent in many in vitro studies, Uden (2005) indicates that the determination of fiber through filters creates a systematic loss of fines, to give a false expression of increased digestibility. Czerkawski and Cheng (1988) indicate particle size is not a factor in continuous fermenters. They quote:

> “The digestibility of hay chopped to about 1 cm length is similar in the Rusitec and in sheep given the same diet. There is no chewing action in Rusitec, and the washed and dried fiber matrix becomes discolored and brittle, but the size and shape of the particles appear to be unchanged. This

> supports the hypothesis that the fibrous feeds are attacked from within and that it is not necessary to break them down physically."

This digestion may be due to fungi that extend their hyphae into stomata and other cracks in the outer cell walls and penetrate inner structures. (Orpin and Joblin, 1988).

Overview

The digestive physiology and metabolism of large contemporary herbivores has been applied to the very much larger Sauropod dinosaurs. The largest Sauropods existed in the Jurassic period and decreased somewhat in the Cretaceous (Dingus et al., 2002). There was a switch from Sauropods to Ornithopods as dominant herbivores in the Cretaceous (Moreno and Benton, 2005). The suggestion that high oxygen levels were a factor influencing size must be discounted, because high levels of oxygen occurred earlier in the Paleocene carboniferous ~300 mybp, and levels in the Jurassic were lower than at present (Berner et al., 2007). There is no satisfactory explanation for the enormous size of the giant Sauropods. The problems of homeothermy and blood pressure remain controversial, although the physiology of the Sauropods was likely different from contemporary cold blooded reptiles.

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PHIDDLING WITH PHENYLALANINE: INFLUENCE OF DIETARY AMINO ACIDS ON ENZYMES OF PHENYLALANINE METABOLISM IN THE CHICK

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Phenylalanine (Phe) is one of the few amino acids that nutritionists take for granted because it is among the least limiting in conventional animal diets. However, it is an indispensable amino acid and the immediate precursor of the semi-indispensable amino acid, tyrosine (Tyr). The major fate of Phe that is not used in protein synthesis is conversion to Tyr (Figure 1) in a reaction catalyzed by phenylalanine hydroxylase (PAH). If the Tyr is in excess of the needs for synthesis of biogenic amines (i.e. dopamine, nor-epinephrine and epinephrine) and melanin pigment, the excess is processed through several metabolic steps to acetoacetate and fumarate. Phenylalanine can be decarboxylated to phenylethylamine, a minor pathway (Edwards and Blau, 1972), or it can transfer its nitrogen atom to α-ketoglutarate via the activity of phenylalanine-α-ketoglutarate aminotransferase or to pyruvate via phenylalanine-pyruvate aminotransferase activity (PAT) (Shih, 1975). The product of transamination can be reduced to phenyllactate, converted by oxidation to o-hydroxy-phenylacetate or by decarboxylation to phenylacetate (Edwards and Blau, 1972). Phenypyruvate, phenyllactate, phenylacetate, o-hydroxy-phenylacetate and have been observed in blood and urine of normal and phenylketonuric humans (Langenbeck et al., 1992; Scriver et al., 2001; Crow et al., 2008). Presumably, Phe can be transaminated in one tissue and further metabolized in a different tissue. This further metabolism could include the re-synthesis of Phe from phenyllactate and phenylpyruvate by the actions of the appropriate dehydrogenases and aminotransferases.

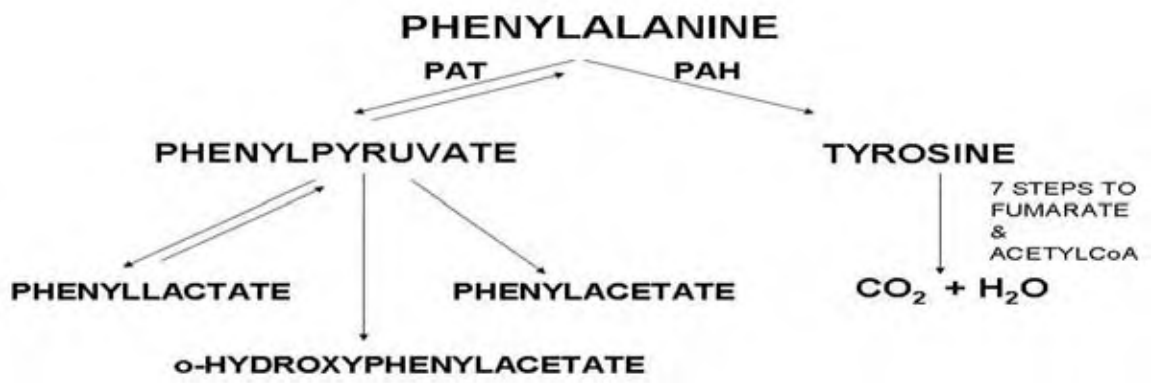

Figure 1. Alternative pathways of phenylalanine metabolism.

Current knowledge of Phe metabolism is based mainly on investigations in humans, rats, and mice. The activity of PAH is restricted to liver cytosol, and to a small extent to kidney cytosol. Phenylalanine is both substrate and an activator of PAH (Shiman et al., 1982). Interestingly, certain other amino acids also can activate the enzyme *in vitro* (Kaufman and Mason, 1982). Although Phe is an activator, excess Phe inhibits enzyme activity (Freedland et al., 1964). The activity of PAH in mammals is regulated by cyclic AMP-mediated phosphorylation of the enzyme (Kaufman, 1986; Tipper and Kaufman, 1992).

Phenylalanine-pyruvate aminotransferase is more widely distributed than PAH. It is reported to be most active (per gram of tissue) in liver, approximately half as active in kidney, and much less active in brain tissue (Sanchez-Urretia and Greengard, 1977). Cell fractionation of rodent liver and kidney revealed that the mitochondrial fraction has the highest activity but activity also has been detected in cytosol and nuclear fraction. PAT has been observed in a variety of species including the rat, mouse, guinea pig, rabbit, pig, dog, human, and chicken (Minatogawa et al., 1977). The kinetic properties of PAH and PAT favor the metabolism of Phe via PAH. Metabolism of Phe via PAT increases, however, when tissue concentrations of Phe are abnormally high as in PKU. PAT and histidine-pyruvate aminotransferase are probably the same enzyme. Other aromatic amino acids (Tyr, Trp, kynurenine, and 5-hydroxytryptophan) can serve as substrates of the purified enzyme (Noguchi and Kido, 1976; Noguchi et al., 1976). Phenylalanine-α-ketoglutarate aminotransferase has received less research attention than PAT and is not addressed in this report. It should be recognized, however, that it catalyzes the formation of the same major product, phenylpyruvate, as PAT.

A genetic disease, phenylketonuria (PKU), is the result of impaired activity of PAH, or enzymes that are involved in the synthesis of the reduced cofactor, tetrahydrobiopterin (Scriver, et al., 2001). The incidence of hyperphenylalaninemia and phenylketonuria is such that newborn infants are routinely screened to detect these problems that, left untreated, lead to mental retardation. Hundreds of mutations of PAH have been detected in humans. Depending on the mutation, these can result in absence of PAH activity or altered kinetic properties (K_m , V_{max}) of PAH (Scriver et al., 2001) It seems reasonable to assume that genetic variants also occur in animal populations. Knowledge of the factors that influence the pathways of Phe metabolism is important in understanding the requirements for Phe and Tyr and the potential consequences of altered metabolism of the two amino acids.

Research on rat and mouse models of PKU revealed that the negative consequences of hyperphenylalaninemia on brain amino acid profile (i.e. elevated Phe concentration in brain) could be alleviated by the administration of a mixture of large neutral amino acids (Binek-Singer and Johnson, 1982). The effect was believed to be due to competition of the neutral amino acids and Phe for carrier-mediated transport of the amino acids into the brain. Research in this laboratory (Austic et al., 1999) indicated that a 5% dietary supplement of large neutral amino acids in the diet of rats in a maternal model of PKU (Brass et al., 1982) resulted in markedly lower Phe concentrations in fetal brain and also in maternal and fetal blood. The lowering of Phe in all three compartments suggested that

competition of large neutral amino acids for entry into brain was not the only effect, and perhaps not even the primary effect, of the large neutral amino acids in lowering brain Phe concentrations. The lowering of blood phenylalanine levels by amino acid mixtures has more recently been observed in humans (see Rocha and Martel, 2009).

Amino acid imbalance was defined historically as the growth depression that occurs when the second limiting amino acid or a mixture of amino acids lacking the first limiting amino acid is added to the diet (Harper, 1956). Studies of amino acid imbalances in chickens revealed that a 5% supplement of an imbalancing mixture of indispensable amino acids (lacking the first limiting amino acid) to the diet resulted in increased activity of the enzyme that catalyzed a rate-limiting step in the catabolism of the first limiting amino acid. More specifically, these studies demonstrated that hepatic threonine dehydrogenase activity in chicks and rats, and branched-chain keto acid dehydrogenase activity in chicks, significantly increased in dietary Thr and Ile imbalances (Davis and Austic, 1994; Park and Austic, 1998), Torres et al. (1999) reported that adding an imbalancing mixture of amino acids lacking histidine to the diet of rats increased liver histidase activity. Keene et al. (2001) observed that threonine dehydrogenase, histidase and phenylalanine hydroxylase activities increased, and plasma Phe concentration decreased, when a 5% supplement of a mixture of amino acids lacking the amino acid in excess was added to diets containing a growth-depressing excess of threonine, histidine or phenylalanine in chicks. The results of the latter study were consistent with the notion that the effects of the mixture of LNAA in the experiments on PKU in rats might be due in part to alterations of PAH activity.

Based on the above-mentioned research, it seemed possible that chicken models of amino acid imbalance and toxicity might shed some light on the nature of the effect of large neutral amino acids on the tissue free amino acid profiles in experimental PKU. Lartey and Austic (2008) demonstrated that a phenylalanine imbalance could be produced in chicks by adding a 10% supplement of indispensable amino acids lacking Phe (IAA-Phe) to a diet that was marginally adequate in Phe. They attempted to determine whether similar changes in hepatic PAH activity might occur in Phe imbalance and excess as for rate-limiting enzymes in the other amino acid imbalances and excesses. The addition of an imbalancing mixture of AA lacking Phe (IAA - Phe) to a diet marginally adequate in Phe did not alter PAH activity as had been observed for the rate-limiting enzymes in the imbalances of other amino acids. It did, however, result in a significant decrease in the concentration of Phe in plasma. Hepatic PAH activity increased in response to excess dietary Phe or a combination of excess Phe and the IAA-Phe supplement (Lartey and Austic, 2008; 2009). Experiments (Lu and Austic, 2009) were done more recently to determine whether the activity of PAT in the alternate pathway of Phe metabolism is influenced by Phe imbalance or Phe excess. Selected experiments from these reports are presented to illustrate the effects of dietary amino acids on PAH and PAT activity.

MATERIALS AND METHODS

Day-old male chicks of the ISA Brown laying strain were reared in thermostatically controlled battery brooder cages with raised wire floors and were provided feed and water *ad libitum*. They were fed a pre-experimental semipurified diet for 7 d followed immediately by the experimental diets for an additional 10 d. The basal diet (Table 1) was similar in composition to the diet of Lartey and Austic (2009). It contained, by calculation, 0.46% Phe and a CP content of 21.8% and met all of the nutrient requirements of the NRC (1994), except Phe, of brown egg-laying pullets to 6 wk of age.

Table 1. Composition of the basal diet.

	g/Kg
Isolated soybean protein (93% CP)	73.0
Amino acids[1]	63.4
L-Phe	2.0
L-Glu	79.6
Cellulose	30.0
Vitamin premix[2]	12.0
Mineral premix[2]	65.9
Glucose H_2O	634.1
Corn oil	40.0
Nutrient composition (% of diet)	
ME kcal/kg of diet	3,240
CP	21.8
Ca	1.21
P (nonphytate)	0.72
K	0.60
Lys	1.10
Met	0.38
Met + Cys	0.81
Phe	0.46
Tyr	0.57
Phe + Tyr	1.03

[1] Supplied the following amino acids (g/kg of diet): Thr, 4.9; Cys, 3.3; Met, 2.9; Val, 5.2; Ile, 5.4; Leu, 7.8; Tyr, 3.7; His (free base), 2.5; Lys·HCl, 8.1; Arg (free base), 7.9; Trp,1.5; Gly, 3.9; Ser, 6.3. All amino acids were L-isomers.

[2] See Lartey and Austic (2009) for composition.

A mixture of indispensable and semi-indispensable L-AA minus Phe (IAA - Phe) was added to the diet at the expense of glucose monohydrate to cause a Phe imbalance and to correct Phe toxicity. The composition of the mixture in g/kg of diet was Thr, 8.6; Tyr, 8.1; His (free base), 5.5; Arg (free base), 17.4; Ile, 12.0; Leu, 17.2; Lys HCl, 17.9; Met, 2.1; Cys, 1.3; Trp, 2.1; Val, 11.5; $NaHCO_3$, 10.3, and glucose monohydrate, 18.5. All AA were in the L-form. The supplement provided 10% amino acids when added to the diet at a level of 13.2%. Phenylalanine was added to the basal diet at the expense of glutamic acid on an equimolar basis.

Three dietary protocols were used in the experiments of Lartey and Austic (2008,2009) and Lu and Austic (2009). Protocol 1 diets were basal (0.46% Phe), imbalance (basal + 10% IAA - Phe), and imbalance corrected (imbalance + 1.12% Phe). Protocol 2 diets were basal (0.46% Phe), Phe excess (basal + 2% Phe), and excess corrected (Phe excess + 10% IAA-Phe). Protocol 3 included 3 dietary levels of Phe (0.46, 1.58, and 2.46%) and 2 levels of IAA - Phe supplement (0 and 10%) which were provided to chicks in a 3 x 2 factorial arrangement of treatments. There were 5 replicates of 2 to 3 chicks per treatment in the experiments involving protocols 1 and 2, and 5 replicates of 2 chicks per treatment experiments involving protocol 3. Chicks were adapted to a semi-purified diet (Lartey and Austic, 2008) for 7 days and then fed the experimental diets for 7 to 10 days. At the end of the experiments they were asphyxiated in CO_2. Fresh livers were used for PAH assay according to Powell et al. (1999). Livers, kidneys, whole brains and samples of *pectoralis major* muscle for PAT assay were frozen immediately in liquid nitrogen, and held at -80°C until analysis of PAT activity according to George et al. (1967). Data were analyzed by two-way ANOVA followed as appropriate by Tukey's pair-wise comparisons of means.

RESULTS

The weight gains and feed intakes and PAH activities of chicks in experiments using Protocol 1 and 2 are shown in Table 2. The imbalancing mixture of amino acids (IAA-Phe) depressed the growth of chicks, and the growth depression was prevented by the addition of Phe to the diet (experiment 1). Feed intake was lower in chicks that received the imbalance and imbalance corrected diets than in chicks that received the basal diet. Hepatic PAH activity did not differ between chicks fed the basal and imbalance diets, but the activity was higher in chicks fed the imbalance corrected diet than in chicks fed the basal diet or imbalance diet. In the experiment 2 the chicks that received excess Phe had lower weight gains than chicks fed the basal or excess corrected diets: the weight gains of the latter groups did not differ. Feed intake was not significantly affected by treatment. Hepatic PAH was significantly higher in chicks fed the excess corrected diet than in chicks fed the other two diets.

Table 2. Effect of Phe imbalance on growth, feed intake and liver PAH activity in chicks.

Diet	Phe %	IAA-Phe %	Weight Gain[1] g/chick/day	Feed Intake[1] g/chick/day	PAH[2] nmoleTyr/ min/g liver
Experiment 1[3]					
Basal	0.46	0	8.7[a]	22.3[a]	21.0[b]
Imbalance	0.46	10	5.6[b]	14.3[b]	19.0[b]
Imbalance corrected	1.58	10	10.3[a]	17.0[b]	34.1[a]
SEM			0.50	1.19	2.32
Experiment 2[3]					
Basal	0.46	0	11.6[a]	28.8	22.0[b]
Excess Phe	2.46	0	8.0[b]	23.7	27.2[b]
Excess corrected	2.46	10	12.3[a]	22.5	38.0[a]
SEM			0.59	1.52	2.84

[a,b] Means within columns and experiment with different letters differ (P≤0.05).
[1] Means of 5 replicates of two chicks per treatment.
[2] Means of 5 replicates of one chick per treatment.
[3] Data from Lartey and Austic (2009).

The results of an experiment to determine the activity of PAT in chicks that were subjected to Phe imbalance are presented in Table 3. Weight gains were lower in chicks fed the imbalance diet than in chicks fed the basal and imbalance corrected diets as observed previously (Table 2, experiment 1). No significant differences in feed intake were detected. The activity of hepatic PAT was higher in chicks that received the imbalance and imbalance corrected diets than in chicks fed the basal diet (P≤0.05).

Protocol 3 was employed in two experiments to determine the responses of PAH and PAT to Phe imbalance, Phe excess and corrected imbalance and corrected Phe excess (Table 4). The growth and feed intake responses were similar in both experiments (4 and 5) and generally reflect the responses reported in Tables 2 and 3

Table 3. Effect of Phe imbalance on hepatic PAT activity (experiment 3).

Diet	Phe %	IAA-Phe %	Weight Gain[1] g/chick/day	Feed Intake[1] g/chick/day	PAT[2], nmol phenylpyruvate/ min/g liver
Basal	0.46	0	9.3[a]	18.2	37.2[b]
Imbalance	0.46	10	5.8[b]	16.3	66.9[a]
Imbalance corrected	1.58	10	9.7[a]	17.1	57.8[a]
SEM			0.44	1.14	3.22

[ab] Means with different superscripts are different (P≤0.05).
[1] Means of 5 replicates of 3 chicks fed the experimental diets 10 days. Data from Lu and Austic (2009).
[2] Means of 5 replicates of 2 chicks sampled at 10 days.

and in other experiments that are not shown. Weight gain and feed intake were depressed by Phe imbalance and excess in both experiments and were corrected by the same treatments as indicated in Tables 2 and 3. The two-way analysis of variance revealed that there was a significant effect of Phe and a significant interaction of Phe and the IAA-Phe mixture on growth rates of chicks in both experiments. Feed intake was significantly depressed by the IAA-Phe mixture in experiment 4. The interaction of Phe and IAA-Phe in experiment 5 reflected the fact that the addition of IAA-Phe to the basal diet depressed feed intake whereas the addition of the same mixture to the diet containing excess Phe tended to increase feed intake. Further, the addition of Phe to the imbalance diet tended to increase feed intake whereas the addition of Phe to the basal diet tended to reduce feed intake.

Hepatic PAH activity (experiment 4) was significantly increased by Phe ($P \leq 0.001$). There was no significant effect of IAA-Phe, nor was there a significant interaction of Phe and IAA-Phe.

Several organs and tissues were assayed for PAT activity in experiment 5. Liver and kidney were similar in activity per g of tissue. The activities in both organs were significantly higher ($P \leq 0.001$) in chicks that received IAA-Phe than in chicks that did not receive the supplement: no effect of dietary Phe level or interaction of Phe and IAA-Phe was detected. The activity of PAT was highest in liver and kidney, lowest in brain, and was more than twice as high in pectoral muscle than in brain. The pooled means of PAT activity were higher for liver and kidney from chicks that received 10% IAA-Phe as compared to chicks that received no IAA-Phe. The dietary treatments did not significantly affect PAT activity in brain or pectoral muscle.

DISCUSSION

Examples of amino acid imbalance in poultry exist for nearly all of the amino acids that are indispensable in diets for chickens. These studies add Phe to the list. We have used a 10% amino acid mixture lacking the first limiting amino acid as a dietary addition to ensure that a strong response to the imbalance would occur. However, in earlier studies of threonine and isoleucine imbalances (Davis and Austic, 1994; Park and Austic, 1998), a 5% mixture was effective in inducing an imbalance.. The strength of the response is undoubtedly determined by the level of the first limiting amino acid in the basal diet and the amount of imbalancing mixture of amino acids added to the diet. In the present studies, growth depressions from Phe imbalance and Phe excess and growth improvements from the corrective additions of Phe and IAA-Phe, respectively, were consistently robust.

The activity of PAH was not affected by imbalance (see Tables 3 and 4) and this result was consistent with several other experiments that are not shown. The response of PAH to Phe excess and Phe excess plus IAA-Phe was not consistent. In experiment 2 (Table 2), for example, PAH was not significantly affected ($P > 0.05$) by excess Phe alone, but it increased in response to the combination of excess Phe and IAA-Phe. This pattern of PAH has been seen in some, but not all, of other experiments that have not been included in this report. The increase in PAH activity in chicks that received excess Phe (experiment

4, Table 4) is an example of a response that has been observed in some other experiments: The conflicting results in experiments 2 and 4 are impossible to explain at this time.

The activity of PAT has been detected in four tissues of the chicken. Two organs, liver and kidney, have similar activities, and PAT in both organs increased when the basal, imbalance, or excess diet was supplemented with the mixture of amino acids. The activity of PAT in liver and kidney, as assayed under optimal conditions *in vitro*, is about the same as that of PAH in liver. We conclude that the activity of PAT, but not PAH increases in chicks that are subjected to Phe imbalance. This is another example of the increased activity of an enzyme capable of catalyzing the degradation of the first limiting amino acid in an amino acid imbalance. Measuring the end products, phenylacetate and o-hydroxyphenylacetate, in future experiments might provide an indication of the biological significance in the increases in PAT activity under conditions of amino acid imbalance.

The marked decrease in the concentration in plasma of the target amino acid (either the first limiting amino acid or an amino acid in excess) when a mixture of indispensable amino acids is added to the diet is a phenomenon that does not have a clear explanation at the present time. Possibly the changes in the activities of enzymes of amino acid catabolism, changes in protein turnover, or competition of amino acids for intestinal absorption or renal reabsorption are responsible in whole or part for the decrease. Determining the molecular mechanisms of this phenomenon would add significantly to our understanding of amino acid metabolism in nutrition and medicine.

Table 4. Growth, feed intakes, PAH and PAT activities of chicks subjected to phenylalanine imbalance and phenylalanine excess using Protocol 3.

			Experiment 4[1]			Experiment 5[2]					
								PAT nmolphenylpyruvate/min/ g of tissue			
Diet	Phe %	IAA-Phe %	Weight Gain g/ chick/day	Feed Intake g/ chick/day	PAH nmol Tyr/ min/g L	Weight Gain g/ chick/day	Feed Intake g/ chick/day	Liver	Kidney	Brain	Muscle
Basal	0.46	0	8.0[a]	17.7	23.4	10.1[a]	18.9[a]	46.0	53.2	7.8	18.9
Intermediate	1.58	0	7.8[ab]	17.5	37.6	9.2[a]	18.5[a]	51.8	49.6	6.5	19.6
Excess	2.46	0	5.9[bc]	16.8	48.3	6.4[b]	14.7[b]	58.1	65.8	8.2	17.6
Imbalance	0.46	10	5.7[c]	14.6	24.6	6.5[b]	15.3[b]	74.7	71.5	7.8	17.8
Imbalance corrected	1.58	10	8.4[a]	14.6	46.0	10.4[a]	17.4[ab]	73.6	75.2	7.2	18.0
Excess corrected	2.46	10	8.1[a]	14.6	44.1	8.8[ab]	16.6[ab]	71.0	72.9	7.5	19.0
SEM			0.46	0.82	1.30	0.36	0.34	3.19	4.52	0.53	1.00
Phenylalanine											
0.46%					24.1[B]			60.4	62.4	7.8	18.4
1.58%					41.4[A]			62.7	62.4	6.8	18.8
2.46%					43.8[A]			64.6	69.4	7.8	18.3
IAA-Phe											
0%					36.4			52.0[B]	56.2[B]	7.5	18.7
10%					38.2			73.1[A]	73.2[A]	7.5	18.3
Source of variation											
Phe			*	NS	***	***	***	NS	NS	NS	NS
IAA-Phe			NS	***	NS	NS	NS	***	***	NS	NS
Phe x IAA-Phe			***	NS	NS	***	***	NS	NS	NS	NS

[abc] Means in a column without a letter in common are different (P≤0.05).
[AB] Pooled means with different superscripts are different (P≤0.05).
[1] Means of 5 replicates of 2 chicks per diet after 7 days of experiment. Data from Lartey and Austic (2009).
[2] Means of 5 replicates of 2 chicks per diet after 10 days of experiment. Data from Lartey and Austic (2009).
[*] (P≤0.05); [***] (P≤0.001); NS, not significant.

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PERSPECTIVES ON CORNELL'S CONTRIBUTIONS TO POULTRY NUTRITION

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Cornell University's faculty and students have lead animal nutrition research since the beginning of the modern era of agricultural science. In poultry nutrition, this tradition started with James E. Rice and continues today. For the last 75 years we have known more about the nutrition of the chicken than that of any other species and much of this amazing bounty was due to fundamental research at Cornell. This review examines the contribution of Cornell's faculty to our understanding of poultry nutrition. Prior to detailing Cornell's contribution I will set the stage with the state of poultry nutrition prior to Cornell's domination of the discipline.

POULTRY NUTRITION BEFORE CORNELL

The 200 plus extant species in the Order Galliformes are omnivores, including the jungle fowl (*Gallus*) that gave rise to modern chickens. Jungle fowl live in tropical and subtropical forests, especially bamboo forests with an understory of tall herbaceous shrubs and abundant termite populations (Beebe, 1990; Collias, 1967; Johnson, 1963). In natural habitats Red Jungle Fowl (*Gallus gallus*) consume fruits and berries from trees and herbaceous shrubs, seeds from a variety of plants and especially bamboo, nuts, young shoots and leaves, petals, tubers, and the eggs, pupae, grubs and adults of insects, earthworms, snails, centipedes and small lizards. The breeding season usually matches the annual cycle of insect availability.

The modern turkey was domesticated from *M. gallopavo* in Southern Mexico, which now appears to be extinct in the wild. We know a lot about the foraging habits of other wild turkey sub-species due to their popularity among hunters. Turkeys live in forested areas and depend heavily on foods produced by trees and of animal origin (Klasing, 2005; Korschgen, 1967; Leopold, 1948). Acorns, beechnuts and other seeds are consumed whole without shelling. Buds, fruits, and young leaves are picked carefully from plants. Scratching is done to locate grasshoppers, crickets, beetles, ants, ticks, fly larvae, spiders, millipedes, snails and small vertebrates. Insects are of critical importance to poults and survival is low in habitats without large insect populations.

Although there is little recorded history of the nutritional management of poultry during the period of domestication, it is likely that they foraged for most of their nourishment and were provided with a minimum amount of human food scraps to keep them returning for shelter each evening. As semi-domesticated chickens spread from Asia into areas with less scavengable foods, provision of additional rations became crucial. When chickens reached the Middle East, they were given daily rations of grains and food scraps to supplement their somewhat limited scavenging resources.

During the late 1700s in Europe, the discovery of chemical composition of foods and the principles of energy and nitrogen balance resulted in a scientific approach to nutrition. This fundamental information was applied to the feeds and feeding of poultry in the mid 1800s. The transfer of European knowledge to the US facilitated the development of a nascent poultry industry in the Northeastern US. Diets in the late 1800s contained grain, meat scraps, greens, spoiled milk, charcoal, and bone or oyster shell. In 1889, the Animal Husbandry Department at Cornell hired James E. Rice to work in the area of poultry husbandry. Rice is credited as the first academic poultryman.

POULTRY NUTRITION AT CORNELL

The Rice Years: 1889-1916

James Rice, the first academic poultry specialist in the US, was a generalist and conducted research on building design, incubation, flock management, marketing, and profitability. He also conducted applied research on genetic selection, nutrition (Rice, 1907), and environmental physiology geared toward adapting poultry production to the New York environment. The challenge that Rice faced in nutrition was that jungle fowl are not granivores and the domestic chicken is stuck with the need for the extra nutrients that insects and other foods provide in their natural diet. Insects are not available during most of the year in Ithaca and the best substitutes - meat, milk and eggs - are expensive. Rice needed to figure out how to take the square peg of a tropical omnivore and force it to thrive in the round hole of temperate granivore. From Rice onward, most of the research at Cornell has characterized the imbalances and deficiencies of amino acids, vitamins, and minerals inherent in grains and other seeds fed to poultry. This research has allowed feed manufactures to correct the grain-based diet fed to modern poultry to make it more reflect an omnivore diet. A nutritionist was eventually hired and Rice could remove nutrition from his expanding portfolio.

The Heuser, Norris and Scott Years, 1916-1979

In 1916, Gustave F. Heuser was hired by Cornell as the first poultry nutritionist. He transformed research on poultry feeding from "nutritional husbandry" into "applied nutritional science" and later, by collaborating with Leo C. Norris, into a “basic science”. During Heuser’s early years he investigated the value of nutritional value of feed ingredients and the appropriate combinations of these ingredients to optimize the phase-feeding of chicks, pullets and hens. In the process, he became interested in rickets and other nutrient deficiency diseases that developed when chicks were fed grain-based diets. He paid close attention to research developments in the medical sciences and applied those developments to poultry. In the 1920s, chickens were becoming a model laboratory species for discovery of vitamins and trace minerals and for elucidating their physiological functions. Heuser collaborated with Norris in cutting edge research designed to hunt for these nutrients, but kept his program anchored in applied poultry nutrition. Heuser’s book “Feeding Poultry” (Heuser, 1946) was the most important book on the subject of its day. It is still in print and readily available. In the first addition of his book, the chemical identity of the vitamins folate and B_{12} and the

essentiality of selenium had yet to be discovered. By the time the last edition was published, all of the nutrients needed for the growth and reproduction of chickens were known. Also of interest - his discussions of high protein feed ingredients focused on meat and milk products. Greens were also discussed thoroughly because of their contribution to vitamins and minerals needs of poultry. Soybeans were barely mentioned in the feedstuffs chapter because "…they are not fed to any extent to poultry". However, meat and milk were in very short supply during World War II and Heuser spent considerable effort investigating vegetable protein sources and micro-nutrient supplements as replacements for meat and milk in poultry diets. Over time, this permitted the amount of meat and milk in poultry rations to be continuously decreased.

Because of his knowledge of biological chemistry, Norris was hired to join Heuser in the nutrition wing of the Poultry Husbandry Department in 1923. Norris carried out a tour de force transformation of "applied poultry science" into "basic science". Norris realized the importance of formulating diets based on purified ingredients of well characterized nutrient composition. By adding or subtracting highly purified ingredients while monitoring chick growth and health, he could narrow in on novel nutrients. He and his very productive students identified and characterized many trace nutrients that were "unidentified growth factors" or prevented rickets, pellagra, curled toe paralysis, perosis, or dermatitis. His work greatly contributed to the discovery of the essentiality of niacin, riboflavin, pantothenic acid, folate, B_{12}, and manganese (Nesheim and Kratzer, 2005). His discoveries permitted the scientific formulation of diets based on known nutrient levels in feedstuffs and known nutrient requirements of poultry. This development catalyzed the rocketing growth of the broiler industry and the use of vitamin and mineral premixes in place of greens and animal products.

Milton L. Scott studied under Norris towards a PhD from 1942-1945 and discovered the essentiality of folate for growing chicks (Nesheim, 2003). He joined the faculty of the poultry husbandry department and continued the tradition of researching trace nutrients that are deficient in grain-based diets of poultry. Milt Scott and his students uncovered the role of selenium and vitamin E in poultry nutrition and contributed greatly to the understanding of vitamins and minerals in poultry nutrition. Scott was also interested in comparative nutrition and included turkeys, ducks, pheasants, pigeons, fish and humans in his research domain. Scott wrote a series of books on animal nutrition. "Nutrition of the Chick", written with Nesheim and Young (Scott et al., 1969), continues to be the standard reference for poultry nutritionists worldwide.

By the end of the 1950s Heuser, Norris, Scott and their students had contributed substantially to the discovery of most of the essential micro-nutrients for poultry and had worked out the practical applications of these discoveries. During this time corn came to dominate grain production and soybeans production had increased from a curiosity crop to the dominant high protein crop. The poultry industry was becoming a way to channel the bounties of corn and soybeans into high-value food products. This trend was accelerated by the idea of a "protein gap" that dominated the politics of human nutrition in the 1960s. Increasing the production of plants and animals that could prevent the eminent protein shortage was a priority. Thus, a refined understanding of the dynamics

of energy and protein nutrition became a priority at Cornell. F. H. Hill, M. C. Nesheim and R. J. Young, the junior faculty in the nutrition wing of the poultry husbandry department, diverted their interests from micro-nutrients to protein and energy nutrition. Nesheim developed an interest in arginine requirements of chickens while collaborating with Cornell's geneticist R. K. Cole. This interest in arginine prompted him to recruit R. E. Austic, a PhD student working on amino acid nutrition at UCDavis, to help him.

The Austic years, 1968 –

Austic's research career focused tenaciously on the metabolic basis for nutritional interactions between amino acids. He leveraged the fast growth rate and genetic malleability of the chicken to uncover the fundamental changes in enzymology, amino acid transport and appetite regulation that underlie amino acid interactions.

Early work. After receiving a BS at Cornell in 1963, Dick Austic began PhD study in Nutrition at University of California at Davis. Fred Hill and Leo Norris had previously left Cornell and were already firmly entrenched at UCDavis at this time. His major professor was C. R. Grau who had spent his career indentifying essential and semi-essential amino acids for growing chicks and had recently set his sights on determining nutrient requirements of developing avian embryos. Austic's dissertation research focused on the aromatic amino acid needs of 3 day old embryos. This was no small task because doing meaningful nutrition work on 3 day old embryos had never been accomplished at that time or since. Austic refined a technique for completely replacing the yolk of an egg with a nutritionally appropriate defined media that supported the growth and development of early embryos in ovo (Austic et al., 1966). Austic manipulated the phenylalanine and tyrosine contents of this media and discovered that tyrosine was an essential amino acid for the development of the central nervous system and the eye (Austic and Grau, 1971; Grau et al., 1965). Defects in eye pigmentation and development of the neural retina and lens was due to the inability of the early embryo to convert phenylalanine to tyrosine (Austic and Grau, 1971). Although impact factors of journals had yet to be invented, Austic published his thesis research in the top journals: Science, Developmental Biology, Journal of Nutrition.

After a hiatus of 30 years, Austic and colleagues investigated the developmental trajectory of phenylalanine hydroxylase (PAH), the enzyme that converts phenylalanine into tyrosine (Powell et al., 1999). They found that liver PAH activity is present at a low level in 11 d chick embryos and increases several fold at hatching and then declines post-hatch. And now, some 40 years later, Austic's most recent papers (Lartey and Austic, 2009, 2008) further examines chick PAH activity in the context of his more recent interest in amino acid imbalances.

Arginine nutrition. After finishing up at UCDavis, Austic began work with Nesheim to investigate the reasons why arginine requirements are highly variable in chickens. They manipulated arginase activity by genetic selection and by the dietary balance of amino acids (Austic and Nesheim, 1970). Lysine, isoleucine, tyrosine, histidine and phenylalanine were found to induce renal arginase, even when dietary arginine levels

were deficient and arginine needed to by tightly conserved. This markedly increased the dietary requirement for arginine. In addition to inducing arginase and causing urinary losses of arginine, Austic found that excess dietary lysine reduces hepatic glycine transamidinase, which reduces creatinine synthesis (Austic and Nesheim, 1972) and has a direct affect on food intake (Austic and Scott, 1975). Further studies on the degradation of arginine via arginase identified the chicken pathway for synthesis of proline (Austic, 1973a; Austic 1973b, Austic 1976; Austic and Nesheim, 1971). Interestingly, Austic found that the capacity of this pathway to produce proline is limited and cannot always supply the complete proline requirement.

After uncovering the complex metabolic interactions of arginine with other amino acids, Austic's interest in arginine shifted towards its role in the immune system. Austic reasoned that the chicken's inability to synthesize arginine via the urea cycle could be used to dissect its role in nitric oxide (NO) production. NO is a key effector molecule that phagocytes use to kill microbes and cancerous cells and is indispensible for innate immunity. Su and Austic (1998) found that chicken macrophages could use L-arginine as well as several arginine dipeptides as substrates for NO synthesis. Furthermore, strains that differ in arginine requirements for growth may differ in their arginine needs for NO production (Kwak et al., 2001). Austic and colleagues also demonstrated that the dietary arginine level effects tumor load induced by Rous sarcoma virus and that the requirement for inhibiting tumors is higher than that for growth (Taylor et al., 1992). This be the result of arginine's influence on the magnitude of cellular immune responses (Lee et al., 2002). These additional needs for arginine during an immune response are exacerbated by the increase catabolism of arginine the occurs during the acute phase response (Klasing and Austic, 1984). Austic and his students capped off work on arginine by teasing apart the broiler chick's dietary requirement for this amino acid (along with lysine) over 2-3 week increments during the growth period (Labadan et al., 2001).

Uric acid and gout. Early in his career at Cornell, Austic became interested in a form of hereditary uricemia that caused articular gout in white leghorns. He worked with geneticist R. K. Cole to select for a hyperuricemic trait that spontaneously occurred in a research population of chickens. Genetic selection produced a hyperuricemic line (HUA) that had a high incidence of gout and a control line with normal uric acid (LUA) and rarely had gout (Austic and Cole, 1972; Cole and Austic, 1980). Austic employed a novel method to continuously collect urine from chickens in order to measure rates of uric acid production and excretion (Austic and Cole, 1972, 1974). Uric acid production from excess dietary nitrogen occurred at a normal rate but excretion was impaired. By infusing different amounts of uric acid i.v. he found that the HUA birds had impaired renal clearance of uric acid. This means that the HUA birds had high levels of blood uric acid because the balance point between uric acid excretion and uric acid production occurred at an abnormally high concentration. Because uric acid has low solubility it precipitates out in the joints and adjacent bursae, especially in the feet. Uric acid is transported by an anion exchange system in the kidney tubules (Kuo and Austic, 1987a) and this is impaired in the HUA line (Kuo and Austic, 1987b). Further studies using kidney slice preparations found that reduced uric acid transport in the HUA line was

related to the involvement of sodium and potassium cations in the transport process (Austic and Cole, 1976). Nutrition studies confirmed that electrolytes influence uric acid metabolism (Austic, 1982). Interestingly, HUA chicks conserve potassium better than LUA chicks when fed a low potassium diet; presumably because they excrete less uric acid via the tubular route, which causes potassium excretion, and compensate by spillage from glomerular filtration, which doesn't cause potassium loss (Austic, 1983).

Amino acid imbalances and antagonisms. Over time, Austic's interest in amino acid interrelationships broadened beyond aromatic amino acids and arginine. Austic and graduate student Trevor Smith explored the metabolic basis for the antagonizing effect of one branched-chain amino acid on the others (Smith and Austic, 1978). They found that the antagonism was strongest when leucine was in excess and that leucine caused an increase in the catabolism of isoleucine and valine. The lopsided ratio of tissue BCAAs modulates protein synthesis and depresses appetite. Austic and post-doc Chris Calvert further dissected the relative importance of appetite (Calvert et al., 1982) and found that 70% of the growth depression could be accounted for by reduced food intake. Isoleucine is susceptible to an imbalance as well as an antagonism. Excess dietary levels of large neutral amino acids, especially histidine, methionine, phenylalanine, tryptophan, and tyrosine, cause an imbalance (Park and Austic, 2000). This is likely because large neutral amino acids share a common transport pathway with isoleucine and disrupt its entry into cells.

Austic next turned his attention to threonine, an amino acid that he had previously observed to decrease arginase activity. It was known from work in rats that threonine metabolism was sensitive to the levels of many other amino acids and it was easily imbalanced. Determining the metabolic basis of this imbalance required an understanding of the enzymology for threonine catabolism in the chick. Graduate student Allen Davis and Austic (Davis and Austic, 1982a) carefully detailed the tissue distribution and activities of the three important catabolic enzymes: threonine aldolase, threonine dehydrogenase, and threonine dehydratase. Yuan and Austic (Yuan and Austic, 2001) characterized the physical, enzymatic and sequence characteristics of chicken threonine dehydrogenase. The enzyme exists as a dimer with a MW of about 70 kd and a Michaelis constant for L-threonine of 5.38. Adam Davis and Austic (Davis and Austic, 1994) found that chicks fed threonine-imbalanced diets have markedly elevated hepatic threonine dehydrogenase. In fact high levels of a cocktail of amino acids excluding threonine induce threonine dehydrogenase much more than high levels of threonine itself (Davis and Austic, 1997) and the induction occurs within a few hours of consumption of the imbalanced diet (Yuan et al., 2000). Thus it appears that excess amino acids induce threonine catabolism, which depletes it from the free amino acid pool. Skewed plasma and brain amino acid ratios result in anorexia. An equalized feeding study indicates that decreased food intake is the major factor causing decreased growth with a threonine imbalance (Davis and Austic, 1982b). The practical implication of this research is that a threonine imbalance results in a greater requirement for dietary threonine. This has important ramifications in broiler production (Rangel-Lugo et al., 1994) because the experimental diets used to titrate the threonine requirement are low in protein but diets used in industry have higher protein. Thus, the

practical threonine requirement is higher than that determined with experimental diets and also that recommended by NRC.

One of the common themes from Austic's research on threonine and isoleucine imbalances is that the rate limiting enzyme responsible for catabolizing the imbalanced amino acid is induced. In a series of experiments designed to examine the robustness of this conclusions, Keene and Austic (Keene and Austic, 2001) found that it is the dietary level of protein, not the dietary level of individual amino acids, that is the primary determinant of the activity of amino acid degrading enzymes in liver. The increased activity of these enzymes may be the mechanism by which dietary protein alleviates the adverse effects of excessive levels of individual amino acids and minimizes toxicities.

Electrolyte balance. Austic's discovery of an interaction between potassium and uric acid piqued his interest in dietary electrolytes in general and he began exploring effects of electrolyte balance on amino acid metabolism, calcification of egg shells, and bone growth. Electrolytes have a wide variety of effects on amino acid metabolism (Austic and Calvert, 1981). Graduate student R. L. Scott (Scott and Austic, 1978) found that high levels of dietary potassium could ameliorate the antagonism of arginine by lysine because potassium increases lysine catabolism via increasing hepatic lysine-α-ketoglutarate reductase activity. Heightened lysine catabolism normalizes the ratio of lysine to arginine in plasma, and presumably the brain, stimulating food intake. High dietary chloride, on the other hand, exacerbates the lysine-arginine antagonism (Calvert and Austic, 1981), perhaps by inducing arginase (Austic and Calvert, 1981) or by increasing lysine absorption (Riley and Austic, 1989). Potassium and chloride make the diet more alkaline or acidic respectively, but these effects are sufficiently buffered to prevent high dietary levels of these electrolytes from affecting the pH of the blood and the gastrointestinal tract, with the exception of the crop (Riley and Austic, 1984). This suggests that the actions of these electrolytes on lysine and arginine metabolism are not mediated by a change in pH.

The coccidiostat monensin acts by catalyzing the exchange between Na^+ and H^+ which alters the Na^+ gradient across membranes (Smith and Austic, 1980). Because electrolyte gradients are important in amino acid transport, Austic and his students suspected that monensin would affect amino acid transport (Riley et al., 1986). Indeed, monensin decreased intestinal absorption of lysine and methionine but increased tryptophan and arginine absorption. The modulation of amino acid transport by monensin might explain the observation that it decreases growth of uninfected chicks. Electrolyte balance also affects egg shell quality with high dietary chloride impairing shell thickness and strength (Austic and Keshavarz, 1988). The adverse effects of high dietary chloride are exacerbated when dietary calcium is low because it increases calcium excretion, which is likely a consequence of acidemia (Keshavarz and Austic, 1990). Diets made acidic by addition of the anions chloride, sulfate, and mono-, di-, and tribasic phosphate increase the severity of tibial dyschondroplasia in broiler chicks (Ruiz-Lopez et al., 1993).

In practical nutrition, it is important to have the correct balance of dietary anions and cations in order to minimize negative impacts on the animals acid-base balance. Lopez and Austic (Ruiz-Lopez et al., 1993) determined that among anions, chloride has a much greater acidogenic effect than sulfate, which has a greater effect than monohydrogen phosphate. Thus, expressing the dietary balance of minerals using simple equations such as [(Na + K + Ca + Mg) – (Cl + P + S)] does not effectively capture the effect of minerals on acid-base balance.

Although it is beyond the scope of this review, dietary cation-anion balance markedly affects amino acid nutrition in swine and fish. Austic and his students played a seminal role in uncovering these interactions (e.g. Chiu et al., 1988; Madubuike and Austic, 1989; Patience et al., 1987a, b).

Future prospects. Cornell's excellence in poultry nutrition catalyzed the formation of the poultry industry in the late 1800s and fueled its explosion during the 1900s. This was done by focusing on basic sciences that underlie the relatively applied field of nutrition. This formula worked from Rice through Austic and the fundamentals have not changed. Chickens were the first agriculturally important animal to have its genome, and phenome, especially nutritional needs, understood thoroughly. Poultry's efficiency at turning the overproduction and refuse of agriculture into nutritionally valuable foods that are highly desired by US consumers continue to boost its growth. The utility of the chicken for conducting basic research married with the wholesome position of poultry products in human health bodes well for poultry nutrition as valued discipline in academia. Animal production is undergoing a transformation from being a detriment to the sustainability of carbon, nitrogen, and mineral cycles in the world towards being a valued solution. Poultry production is just beginning this transformation and Cornell should lead the way.

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INTERPRETING AND IMPLEMENTING STARCH DIGESTIBILITY INFORMATION IN THE FIELD

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In lactating dairy rations in the northeast, the majority of the fermentable NFC is usually provided as starch. Starch rates of disappearance are highly variable. Recognizing the sensitivity of ration formulation models to rates of disappearance, many formulators and feed chemists strive to define the pool sizes of fermentable carbohydrate fractions and their respective rates of disappearance in the rumen of the dairy cow.

CPM-Dairy is a tremendous biology lesson, but one needs to understand the logic behind it to take advantage of its capabilities. Users of this formulation program quickly learn that predictions of microbial protein yield, metabolizable protein, and milk yield are sensitive to the rates of disappearance and the rates of passage used for each of the feedstuffs being fed. These predictions are especially sensitive to the pool size and rates disappearance of the fermentable NFC fractions.

One of the challenges in the field is recognizing and understanding the methods used by labs to quantify and qualify starch characteristics. New tests are available and can improve our ability to model diets. Cumberland Valley Analytical Services (Hagerstown, MD) reports an enzyme available starch analysis to estimate the amount of starch that may disappear in one hour. How can we take advantage of this information? Presently, I use this as the maximum rate (kd) of disappearance for the Starch (CHO:B1) pool. When analyses of consumed feedstuffs, estimates of their rates of disappearance, actual feed intake, environmental definitions and animal production information are accurately entered into CPM-Dairy v3, the model should predict close to the actual animal performance. The correlation between observed milk and model-predicted milk was high (r^2=0.89) when CPM-Dairy was recently evaluated with data from 228 herds, (Tedeschi *et al.*, 2008).

Over past years (1999 – 2003), we had the opportunity to investigate formulation with *in situ* starch analysis for 3 starch disappearance time points: 0hr, 6hr and 24hr. (Karkalas 1985) This allowed us to estimate rates of disappearance for formulation of diets in CPM Dairy v3. In practical terms, it is important to 'know where the ditches are nutritionally' for ration formulation. By defining the amount of fast starch and slow starch in diets, we can potentially prevent too much of the former causing a negative impact on feed intakes (Allen, 2009) and/or negatively impacting on animal health. Conversely, should fermentability rate be too low in the rumen, there may not be enough starch to fuel consistent microbial growth and a risk of aggravating the post-ruminal environment exists.

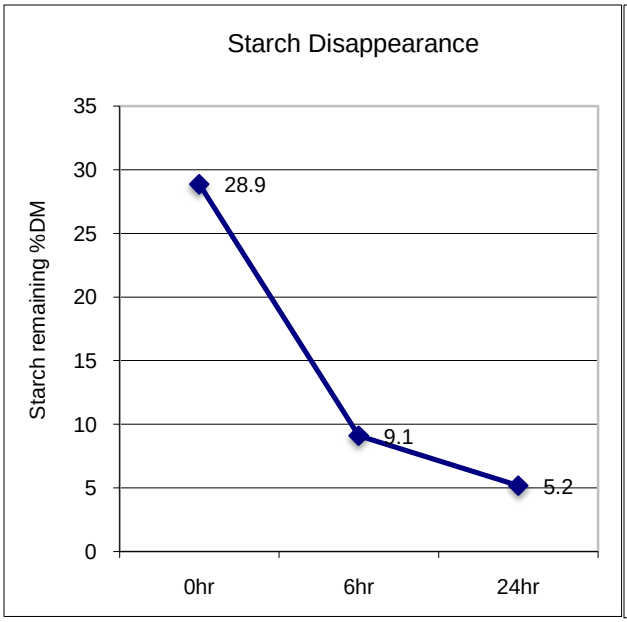

Figure 1: Starch disappearance over time (RFS Technologies)

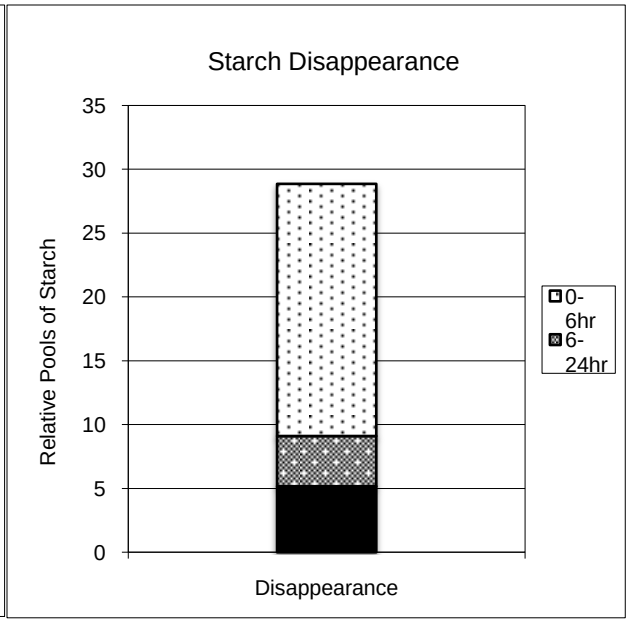

Figure 2: Starch pool size by time of disappearance

Given these considerations, the additional information offered by 3 starch time points can be a valuable tool for diet formulation and ration management purposes.

Recognition of the inherent flaws in set time point digestibility determinations has led to a greater interest in gas fermentation applications. RFS Technologies Ottawa,Canada, began to move to gas *in vitro* techniques to predict the extent of substrate digestibility and rates of disappearance, noting the benefits of multiple time point analysis. The gas techniques allowed a multifaceted approach, enabling the measure of lag time, gas yield and ultimately VFA and methane. Gas *in vitro* technology has been used since 1960 to help investigate rumen fermentation balance (Wolin 1960). Using an integrative approach, RFS Technologies analyzed multiple (112) TMRs for their *in vitro* gas profiles to determine the desired profiles needed to bench mark gas yield results. Production data was collected for herds or for groups within herds consuming the respective TMRs. Herds included in this data set were using neither BST nor an ionophore and they had to employ a third party recording system to measure milk production. TMR samples and associated herd data were collected from Ont., Que., NY, PA and MD. The *in vitro* gas profiles were processed using two pool logistic equations. The *in vitro* gas profiles were matched with their herd's respective DHI test results. Thirteen characteristics including gas production data, pool size, asymptotes, wet chemistry and *in situ* results were analyzed through stepwise linear regression to develop a predictive equation for milk production. While integrative in its nature, the equation has successfully been used to manipulate diets.

Gas *in vitro* technology can be used to improve the descriptions of ration ingredients. Chai *et al.,* 2004, observed a useable relationship between gas production data and measured starch degradation (r^2=0.80) for all samples tested. For feed ingredients high

in starch content and corn silages, the relationship was higher (r^2=0.96). While the pools are not homogeneous, the researchers concluded that for individual samples, starch degradation could be accurately estimated by gas production and starch content. The system offers an inexpensive alternative of investigating rumen fermentation kinetics in starchy feedstuffs. “Whilst the degree of dietary starch degradation occurring in the rumen is a major influence on total utilization of starch, the rate of degradation may be more important in terms of feed management” (Chai, *et al.*, 2004). One can use the predicted rates of starch disappearance generated from these equations for improved ration formulation in CPM Dairy.

Gas *in vitro* technology can also be used as a diagnostic tool for TMRs by comparing gas yields against benchmark profiles. Using this technology we strive to formulate diets that are rumen friendly and optimize rumen microbial protein and VFA yields.

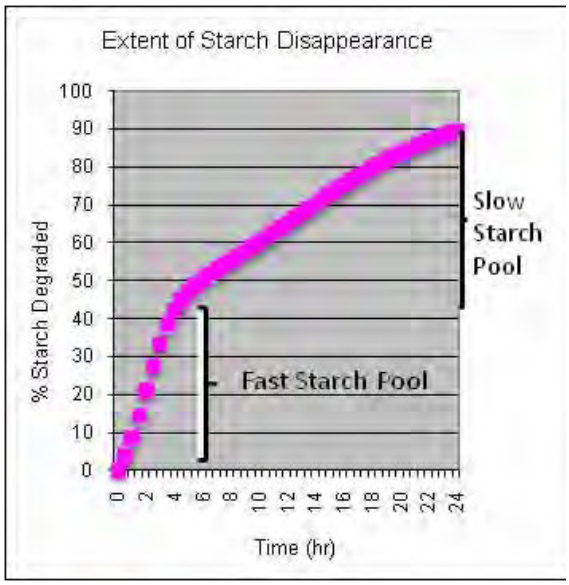

Figure 3: Extent of starch disappearance predicted by using Chai et al., 2004 equations for gas yield of corn silage

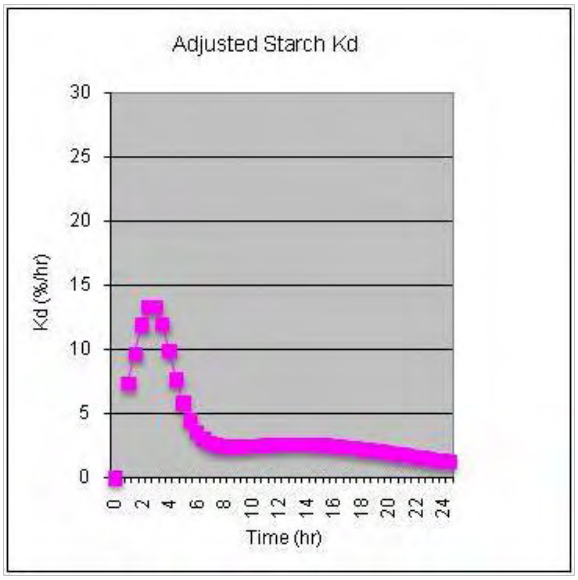

Figure 4: Rate of Disappearance predicted by using Chai et al., 2004 equations for gas yield for corn silage

The rates of starch disappearance in the rumen vary greatly depending on corn genetics, moisture, particle size, vitreousness, etc. (Hoffman 2009) Summaries from Cumberland Valley Analytical Services (Ward, 2008) illustrate how rates of disappearance of starch change over time as the corn silage steeps in the silage acids. It is a challenge for the nutritionist to know what these rates are and how much they are changing.

One can use rates of disappearance determined from available lab analysis to help meet formulation recommendations published in CPM-Dairy Help files. This resource suggests that 20 to 22% of the starch on a DM basis should be fermentable in

the rumen. If a formulator does not have the opportunity to employ *in vitro* or *in situ* laboratory results, what can one do to adjust the rates of disappearance of the starch pools? Certainly, take into account moisture, particle size and color. Recognize the impact of vitreousness of corn grain on the rates of starch disappearance in the rumen (Allen, 2009). Adjust the CHO:B1 Kds to reflect these characteristics. Many studies have observed a strong negative relationship between endosperm vitreousness and *in situ* starch or DM degradability (Hoffman, 2008). Allen *et al.,* fed eight ruminally and duodenally cannulated lactating dairy cows corn with 25 or 66% vitreous endosperm. Feeding cows 66% vitreous endosperm corn reduced ruminal and total tract starch digestion by 19.1 and 7.1 percent respectively. Once you have adjusted the CHO:B1 Kd compare cow data with formulation predictions.

Summary

Starch is a major component of the fermentable NFC in dairy rations in the northeastern US. CPM-Dairy predictions are sensitive to rates of starch disappearance. Understanding starch rates of disappearance is important in formulating diets to meet and not exceed the needs of the rumen bacteria. Gas *in vitro* technology can be a useful tool in defining the kinetics of feedstuffs. Compare the production responses predicted by the formulation program to the actual production data collected on the farm.

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INTERPRETING AND IMPLEMENTING STARCH DIGESTIBILITY INFORMATION IN THE FIELD

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INTRODUCTION

As corn prices increase and dairy farm margins are tightened, it is absolutely essential that starch digestibility be maximized while at the same time controlling rumen acidosis. If dietary starch is lowered, the rumen microbes and the cow must use the remaining starch.

Grinding increases the amount of surface area that the rumen microbes can attach to. Thus, grinding increases starch digestibility. Cornmeal may be ground either coarsely or finely. It has been recommended that 67% of cornmeal should pass through a kitchen flour sifter (~ 1.18 mm). This equates to an average particle size of 1100 microns (Hutjens, 2008). High-moisture corn should be rolled prior to feeding if it is 28-32% moisture but it should be ground to a smaller particle size if it has less than 25% moisture.

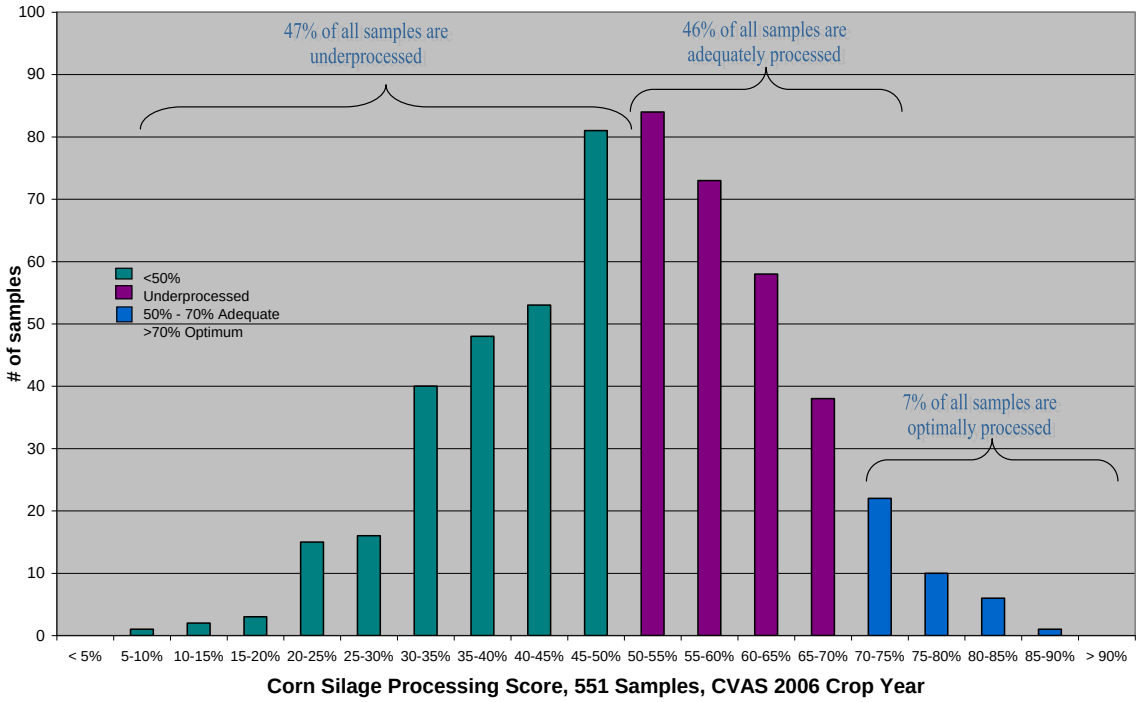
Heat and pressure make starches more rapidly fermentable (Huntingdon, 1997). Gelatinization is defined as the irreversible destruction of the crystalline order in a starch granule so that the surface of every molecule is made accessible to solvents or reactants, including the rumen microbes. Gelatinization in feed is brought about by a combination of moisture, heat, mechanical energy, and pressure. It increases the speed at which enzymes and microbes can break down the linkages of starch to yield energy and microbial protein. Steam flaking, extrusion, and pelleting all cause starch to gelatinize. However, the degree of “cook” is highly dependent on the amount of moisture, pressure, and heat actually obtained during each of these processes. There are guidelines on bushel weights for steam flake corn as well as gelatinization scores for that corn.

Corn starch type also effects digestibility. Taylor and Allen (2005) increased rumen starch digestibility of corn grain in lactating dairy cows from 35 to 57% when corn grain with a floury endosperm was fed rather than vitreous corn grain.

With higher corn grain prices, many dairy producers are increasing the percentage of corn silage in diets. Maximum utilization of corn silage is essential. It is also important to recognize that the starch content of corn silage varies. As with all forages, routine nutrient analysis is needed for optimum production efficiency. Particle size analysis may also be helpful.

Proper corn silage processing at harvest is essential. It is generally recommended that corn silage be cut at a 0.75 inch (1.9 cm) Theoretical Length of Cut and that a 2-3 mm roller clearance be maintained so that all corn kernels are crushed. Silage can be analyzed at laboratories using a Ro-Tap Shaker with nine sieves to obtain a "Corn Silage Processing Score" (CSPS). CSPS is the percentage of corn silage starch that passes through a 4.75 mm screen. Experts have suggested that >70% CSPS is optimal, 50-70% is average, and <50% is inadequate (or too coarse) (Grant, 2008). However, with >70% CSPS, rations may need to be adjusted for increased starch availability and potential sub-clinical acidosis by reducing supplemental starch and paying close attention to effective fiber levels and evidence of TMR sorting.

Data generated from Cumberland Valley Analytical Services (CVAS) of the CSPS analysis (Ward, 2006) of corn silage data from the 2006 crop year is presented below. The mean score is 51% on 551 corn silage samples evaluated. The wide distribution of CSPS implies that this may be a valuable tool in defining an aspect of starch degradability.



Time after ensiling of corn silage may be important to consider for diet formulation. Newbold et al. (2006) found that 3-hour *in situ* starch digestibility increased with storage time from 53.2% at 2 months to 69% at 10 months after ensiling. If starch levels are not reduced when feeding more fully ensiled corn silage, cows may experience more sub-clinical acidosis and the opportunity to save money on supplemental starch will be wasted.

All of the guidelines for starch digestion have been general with minimal quantitative information that could be placed in a nutrition model for ration formulation purposes. We were all willing to go more or less “shooting in the dark” on this with inexpensive corn. However, this all changed in 2008 with $5.00/bu corn. Now the feed industry and the producers they serve have become very interested in the utilization of the starch from the corn and other starch sources. Our challenge is to develop laboratory methods that will allow us to measure the digestibility in the cow.

THE PROBLEM

The challenge for us is that we have three sites of digestion of starch in the cow: The rumen, the small intestine, and the large intestine (Huntington, 1998, Owens and Zinn, 2005). Our focus has recently been on the digestion in the rumen. The research from the Netherlands (Hindle et al, 2005) has provided some understanding of digestion along the tract. More recently, the work from Michigan State (Oba and Allen, 2003, Taylor and Allen, 2005, Allen et al., 2008) has provided not only an understanding of digestion over the whole tract but also an understanding of the effects of starch type and processing.

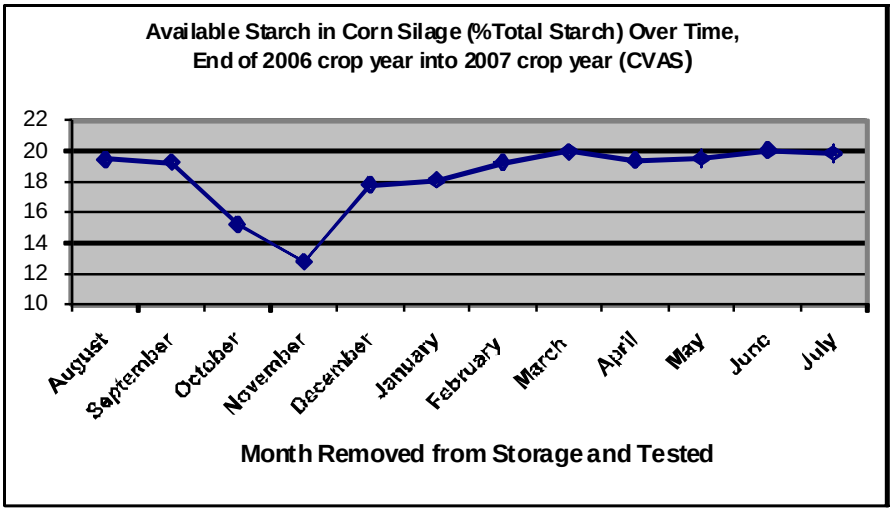
The problem in predicting starch digestion in the cow is magnified by differences in starch sources, particle size and passage rate through the different segments of the GI tract. We can measure fecal starch to estimate whole tract digestion, but this does not give us digestion in the various segments of the tract. There have been many studies over the years on duodenal appearance of starch, providing an estimate of the apparent digestion in the rumen. In many of these experiments, they did not correct for the microbial starch content in order to obtain true ruminal starch degradability. Unfortunately, many of these studies were conducted with low producing cows. There have been many studies done with beef cattle as well. However, it is difficult to apply much of this information to dairy cattle because of diet type and intake level.

The challenge is to incorporate into our nutrition models the means to predict the digestibility of the starch that comes from different dietary sources. This will rely on analytical methods for particle size and degradability that must then be combined in some type of an index or analyte that can be used for modeling. These methods must be commercially robust: provide analytical range, be rapid to run, be repeatable, and economical. As well, they must correlate to animal models. To date, while there have been some attempts to address this need, but no major effort has been undertaken.

VARIATION IN STARCH AVAILABILITY

We have long known that there is variation in starch availability to the cow among feedstuffs. The obvious is that corn and sorghum starch is, on average less available than wheat starch. This concept has been presented in seminars for many years. Then, we began to become more sophisticated by indicating that particle size, moisture, steam flaking and many other factors can affect the availability of starch. There have been many cow studies showing how the starch has variable availability because of

processing, maturity, etc (Theurer, 1998). Shaver and his colleagues proposed the new concept of vitreousness among different corn hybrids (Correa et al, 2003). He related this to the starch availability to the cow. Laboratories historically in the steam flake industry have been using a gelatinization score system to provide the industry with a means of calibrating the steam flaking process and the quality control of the final product. Weld Laboratories (www.weldlabs.com) is an example of this type of lab.

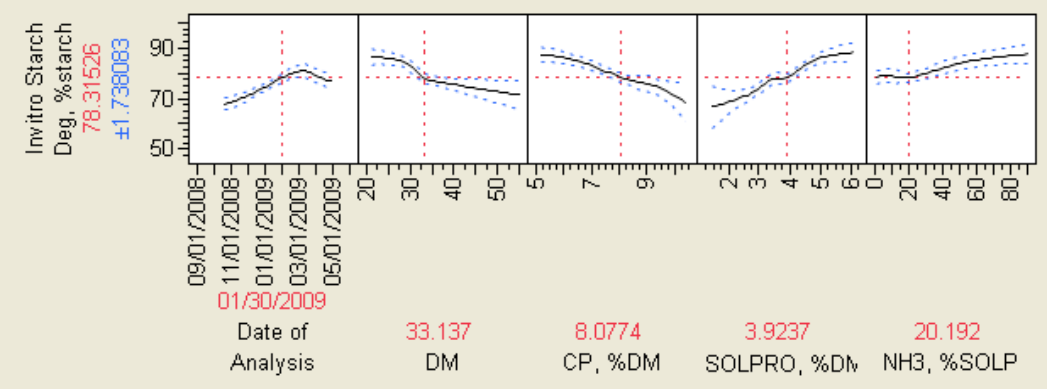


Cumberland Valley developed an enzymatic test for measuring starch availability with which they were able to develop NIR equations. Above is a figure representing over 15,000 corn silage samples from New York. Samples from August and September represent 2006 crop corn silage that at that point had been ensiled for more than 10 months. Moving into October and November, available starch drops, presumably as the percentage of unfermented corn silage samples increases coming from the 2007 crop year. This is particularly interesting, in that this suggests that there is variation over the year in corn silage starch ruminal availability and it might take a few months for starch to become more available to the cow after ensiling the corn. Available starch data were shown to be correlated (Ward and de Ondarza, 2008) to the time course of fermentation data as well as ammonia and soluble protein.

Below is a subset of data, again from CVAS, using the 7h invitro starch digestion measurement. This shows ranges in the availability within corn type and then in corn silage the impact of DM at ensiling on the ruminal availability of starch, demonstrating that as the corn becomes more mature the ruminal starch availability, on average, is reduced.

Feedstuff	*N*	*DM range*	*IVSD 7*	*SD*
Corn grain	123	87.5	60.9	8.1
High Moisture Corn	103	72.9	64.1	8.9
Snaplage	20	58.0	73.9	8.5
Corn silage	107	<28.0	80.1	7.5
	204	28 to 32	79.7	8.7
	224	32 to 36	77.5	9.5
	102	36 to 40	73.3	10.2

Below is an expansion of the corn silage dataset (697 analyses of normal and BMR corn) that shows some of the relationships that occur over a range of nutrient changes. This is a non-linear analysis of the data set. The vertical red line represents the mean shown in red below. It is possible, in modeling, to move this vertical line to the right or left to examine the interactions or change in the shape of the other curves that are displayed. As shown with the 2-hr enzymatic data, the starch degradability improves with time. This is a reflection of the amount of time that the corn silage is in the silo. Of equal interest is that as the DM increases there is a decline in starch fermentability. The protein concentration at a fixed solubility or the range in soluble protein also affects the degradability of the starch of the corn.



The increase in protein could be correlated with the maturity of the corn and it also could be a reflection of the deposition of the prolamin proteins and increases vitreousness. The protein solubilization relates to the ensiling time and breakdown of the prolamin proteins. The NH_3 is related to the soluble protein, but does enhance the model prediction.

MODELS

NRC 2001 recognized that there were differences among starch sources in the digestibility. A processing adjustment factor (PAF) was incorporated based on the literature for the NFC fraction. This was for whole tract digestion. There was recognition that there were differences in starch digestion among feedstuffs. They assigned ground corn a factor of 1.0 and then steam flake and high moisture corn had

factors in excess of 1.0 and corn not ground well or corn silage a factor less than 1.0. They pointed out that at maintenance, the predictions might not be correct but at about 3 times maintenance (for the high-producing cow) the predictions would be close. There is the additional factor that this does not take into account the variable amounts of sugars, fermentation acids, and soluble fiber in the NFC.

In CPM Dairy 3.08, the carbohydrate submodel was expanded beyond the model used in CNCPS 5.0 by separating the sugars and the fermentation acids and the starch and the soluble fiber. This improved the model by separating the NFC components into more unique pools most of which could be estimated directly by routine analyses (total sugars, fermentation acids and starch). The soluble fiber was not routinely being analyzed and was calculated by difference. In reality, this fraction is the residual, by difference from the NFC. Additionally this fraction had not only pectic substances but also fructans from grasses and plant organic acids. It should be added that this pool is the sum of the accumulated analytical errors of the other analytes in the feedstuff. In that CNCPS and CPM each use rates of digestion and rates of passage to estimate starch digestion, attempts were made to estimate the rates of digestion of starch in the rumen. This was based on the limited literature at the time of the model development. Given the estimates were limited to very few feedstuffs with minimum information on the processing, the estimates were guesses at best but were radiated through various vectors such as DM, particle size and processing method. Given this, the platform user could adjust these rates if there was improved information available. There was recognition that the starch escaping fermentation should have a variable digestibility in the intestine. Unfortunately, the model does not differentiate the small and large intestinal segments. However, the user can assign a variable estimate of digestion to the remainder of the GIT. The default numbers were again based on a limited database with an expansion of this database to other feedstuffs through various vectors such as DM, fineness of grind and processing method such as steam flaking. Recently (Tylutki et al, 2008) there has been a recognition in CNCPS 6.1 for the need for an expanded carbohydrate model beyond CPM. Additionally, due to additional literature that the rates of fermentations for the sugars and starches of many of the feedstuffs were found to be in error and there was a need for correction.

RAPIDLY AND SLOWLY DEGRADABLE STARCHES

It is generally recommended that high production dairy rations contain 21-27% starch. At the same level of total dietary starch, however, one ration containing a large amount of fast fermenting starches such as barley, high-moisture corn, or bakery product may result in acidosis, whereas a ration containing a more slowly degradable starch like cornmeal may not. A high extent of starch availability is desired but a combination of rapidly and slowly available starches will help with acidosis control.

For efficient growth of the rumen microbes to occur, the availability of carbohydrate and protein to the microbes must be synchronized on an hourly basis. Work with continuous cultures of rumen microbes showed that microbial yield decreased curvilinearly from 34.2 to 10.3 g bacterial nitrogen per kg DM digested as the

nonstructural carbohydrate / rumen degradable crude protein ratio widened from 1.9 to 8.9 (Hoover, 1987, Stokes et al., 1991). Aldrich et al. (1993) showed that microbial protein yield was highest (262 g/d) when a rapidly digestible protein source as fed with a rapidly digestible starch source and lowest when a slowly digestible protein source was fed with a rapidly digestible starch source (214 g/d).

As part of de Ondarza's Ph.D. work at Michigan State (Roe, 1994), a two-pool model for starch digestion was developed with rapidly available starch plus glucose (S_1) and slowly available starch (S_2). The measurements of the starch plus glucose fractions were based on 2 h and 8 h in situ starch disappearance. The amount of S_2 was calculated as the natural antilogarithm of:

[(ln(starch remaining at 2 h (%DM)) + [2 * [(ln(starch remaining at 2 h (%DM)) – ln (starch remaining at 8 h (%DM)))/6]].

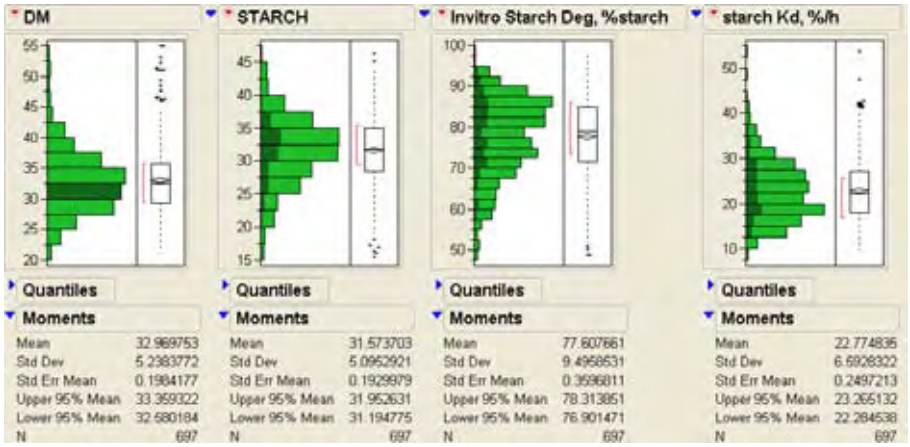
The amount of S_1 was calculated as the difference between the total amount of starch in the feed and S_2. S1 was assumed to have a Kd of 90%/h. Rates of S_2 degradation for different feeds were determined according to the amount of starch disappearance from 2 h to 8 h of incubation in situ. The rates of degradation of S_2 were determined using the following equation:

[[(ln (starch remaining at 2 h (%DM) – ln (starch remaining at 8 h (%DM)))/6 *100].

It is felt that as we progress we will increase our nutrition model sensitivity with a two-pool model. Unfortunately, we are constrained, now to a one-pool model. The equation for this is below:

Kd-7 = (((LN(Starch, %DM))-(LN(((100-7H IV starch Deg, %starch)/100)*Starch, %DM)))/7)*100

Below are the distributions for 697 analyses done by chemistry by CVAS. The highlighted bar for DM is close to the mean DM. The highlighted distributions that result from this shows that there is a range in starch digestibility and the Kd's For those using the expanded carbohydrate model from CPM or from CNCPS 6.1, note the range of Kd's. The default Kd's in CPM have, for the most part been in the 30's.



This brings up the question of how real these are. Work by Oba and Allen, 2003, shows measured ruminal starch degradability's in rations that were corn silage/alfalfa silage based. The results are below. The contrasts were high moisture corn and ground dry corn. Unfortunately, they did not show 7-h starch degradability's. The ruminal starch degradability for the ration was 71% for the high moisture corn high starch ration and only 58% for the low starch ration. In contrast the dried corn ration (the same hybrid was used to produce the high moisture and dry corn grain) was 47% and 46% starch digestion for the high and low starch rations.

Table 4. Effects of corn grain conservation method at two dietary starch concentrations on digestibility of starch.

	High starch		Low starch			*P*-value		
	HM[1]	DG[2]	HM	DG	SE	Starch[3]	Corn[4]	INT[5]
Starch								
Intake (kg/d)	6.2[b]	7.0[a]	3.9[c]	4.1[c]	0.1	<0.001	<0.001	<0.01[6]
Digested in the rumen								
(kg/d)	4.3	3.3	2.4	1.9	0.2	<0.001	<0.001	0.14
(%)	71.1	46.9	58.5	45.9	3.5	0.08	<0.001	0.13
Passage to duodenum (kg/d)	2.2[b]	4.2[a]	1.9[b]	2.4[b]	0.2	<0.001	<0.001	<0.01
Digested in the intestines								
(kg/d)	1.9[b]	3.8[a]	1.6[b]	2.2[b]	0.2	<0.001	<0.001	0.02
(% of intake)	30.5	53.6	41.1	53.4	3.7	0.21	<0.001	0.19
(% of duodenal passage)	86.2	89.6	83.8	86.9	1.7	0.13	0.06	0.99
Digested in total tract								
(kg/d)	5.9[b]	6.6[a]	3.7[c]	3.8[c]	0.1	<0.001	<0.01	0.02
(%)	95.8	94.2	93.3	93.0	0.6	<0.01	0.16	0.26

[1]High-moisture corn.
[2]Dry ground corn.

First, the overall starch degradability's are lower than what we, on average, see in the rations that we feed. Is the experiment wrong? Looking closer at the protocol, the corn used was a hybrid that is known for its vitreousness. When they went to low starch then the main source of starch was the corn silage. If this hybrid was the same as the corn grain and it was being fed in the fall of the year, then it is possible that this explains what we are seeing in this experiment.

Note that the whole tract digestibility shows a lower digestibility for high and low starch. This could be as a result of the impact of the starch from the corn silage. It is obvious that the cow will digest a significant amount of the starch in the rest of the GIT, with a lot of compensation for the differentials that might be seen in the rumen. The question is where is the digestion taking place? Is it in the small intestine or in the hindgut? We would like most of the starch escaping ruminal fermentation to be digested in the small intestine. This does not necessarily happen. Unfortunately, we do not have a hind gut model nor do we have a significant amount of data in lactating cows to help us build a model. We have far to go on this. We are able to change the digestibility for the rest of the GIT

The bottom line question that we need to answer: Is the 7-h invitro degradability a good first step until we can develop better assays, and will the use of two pools improve our sensitivity in predicting cow performance?

ANALYTICAL METHODS

For good estimates of starch digestibility we need good estimates first of the starch in the feedstuffs. There are still some issues with the analytical methodology that do need to be resolved. However, progress and refinement of these methods are being made.

There are two approaches that can be used to improve the estimates of starch digestibility. The first is the whole tract digestion. The Wisconsin group (Blasel et al, 2006) has proposed an improved estimate of whole tract digestibility. This will allow a more quantitative estimate of the PAF factor in NRC 2001. To date there are a couple of labs offering this assay methodology. This group recognized several years ago that there was a wide range in the vitreousness of the starch in various corn sources. This was exciting because the work provided insight into some of the variances in the response to the feeding of corn that were not being captured in our nutrition models. Hoffmann, (Larson and Hoffmann, 2008) also at the University of Wisconsin, hopefully might have a refinement of this approach in the recognition that the prolamin proteins were highly positively correlated with the vitreousness in the corn. There may be an opportunity to use this laboratory method (currently being refined) to provide better estimates of ruminal and intestinal starch availability.

The second approach is to estimate the digestion of the starch in the rumen and in the rest of the GIT. As discussed above, the CNCPS model tries to address this. However, up to this point there have not been good methods to make the types of measurements from which rates could be estimated. One would think the *in situ* procedure intuitively would be a good means to estimate the starch availability. Of historical interest, but highly relevant to this discussion, Agway, formerly of Syracuse, NY developed what they called the RAP/RAC system (Nocek, 1996). This was accomplished through extensive *in situ* measurements of ruminal degradability of protein and carbohydrates of many feedstuffs using highly standardized procedures for conducting insitu assays. The issue with the insitu procedure is the time cost, the need to have access to cows with ruminal cannula, the potential variation due to bag pore size and its impact on particle loss and microbial access as well as the particle size of the material that is placed in the bag. However, the approach proposed in this patent was ahead of its time. Pell and her colleagues suggested that a gas method might offer a means to address this issue. This is currently being used in one commercial lab in North America.

The use of an amylase enzyme is an attractive option due to simplicity and elimination of the need for access to cannulated cows. This is routinely used by several laboratories to provide gelatinization scores for the steam flake industry and is, to a limited degree, an assay provided by a few forage labs to provide a relative available starch value. This approach is not, at this point, been standardized so that it could be used for ration formulation purposes. Unfortunately, this approach also needs to have a “gold standard” for calibration.

In the last several years Allen's team (Allen et al, 2008, Oba and Allen, 2003) has conducted several studies with high producing cows with ruminal and duodenal cannulae to measure the changes in starch digestion. He has related his measurements to standardized 7-hour invitro starch degradability measurements and then a measurement of fecal starch to calculate the digestibility of the starch in the rest of the GIT.

The challenge is to develop equations that will use an estimate or estimates of ruminal starch disappearance over time to provide either a single rate or preferably at least two rates for two pools.

This is a complex challenge in that starch degradation characteristics are impacted by dry matter, particle size, and chemical degradability. Any approach will rely on analytical methods for particle size and degradability that must then be combined in some type of an index that can be used for modeling. Approaches will be different for corn silage versus corn grain and starch containing ingredients.

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TECHNOLOGY'S ROLE IN THE 21ST CENTURY: FOOD ECONOMICS AND CONSUMER CHOICE WHY AGRICULTURE NEEDS TECHNOLOGY TO HELP MEET A GROWING DEMAND FOR SAFE, NUTRITIOUS AND AFFORDABLE FOOD

Jeff Simmons
Elanco Animal Health

INTRODUCTION

Today there are nearly 1 billion hungry people around the globe. Yet in only 50 years, our growing global population will require an estimated 100 percent more food than we produce today. Unfortunately, we will certainly not have 100 percent more high-quality land available to grow twice the amount of grain or two times more livestock. The U.N. Food and Agriculture Organization (FAO) reports that added farmland will help produce only 20 percent of the additional food our planet will need in 2050, and 10 percent will come from increased cropping intensity. Accordingly, the FAO concludes that 70 percent of the world's additional food needs can be produced only with new and existing agricultural technologies.

The consequences of failing to use these science-based technologies and innovations will be disastrous. Food producers in industrialized and developing nations alike require technology to ensure a sustainable supply of safe, nutritious and affordable grains and animal protein to satisfy a rapidly growing demand. For this reason, and many others, we all share in the responsibility to ensure that new agricultural technologies—as well as those proven safe and effective over decades—continue to be available.

EXECUTIVE SUMMARY

- The U.N. projects world population will reach 9+ billion by mid-century and has called for a 100 percent increase in world food production by 2050. According to the U.N., this doubled food requirement must come from virtually the same land area as today.
- The U.N. Food and Agriculture Organization (FAO) further states that 70 percent of this additional food supply must come from the use of efficiency-enhancing technologies.
- Driven by food production efficiency, agriculture can achieve the *"ultimate win"* for consumers worldwide—affordability, supply, food safety, sustainability and ample supplies of grain for biofuels. Three key concepts—*collaboration, choice* and *technology*—emerge as the pathway to this success

Key Data

In **50** years, the world **population** will require ▶ **100%** more **food**, and ▶ **70%** of this food must come from efficiency-improving **technology**

WILL GLOBAL POPULATION GROWTH OUTPACE OUR ABILITY TO MEET THE DEMAND FOR FOOD?

Some argue it already has. In December 2008, an estimated 963 million people around the world didn't get enough to eat. About 42 percent of these chronically hungry people live in two of the world's most populous developing nations: India and China. Because of malnutrition, one in four children in second- and third-world nations (W2 and W3) is underweight for his or her age.

This is an unacceptable situation today and will require a new approach to food production to avert an even worse scenario in the coming decades. That's because world food demand is expected to increase 100 percent by 2050. Consequently, the U.N. FAO projects that global production of meat and dairy protein will almost double by 2050.

FEEDING OUR 3 "WORLDS"

Economists classify our world into three socioeconomic groups:

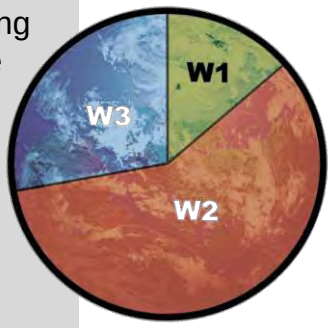
First World (W1): Affluent, industrialized nations and regions including the United States, Western Europe, Japan, South Korea and Australia. *Total estimated population, 2008: < 1 billion.*

Second World (W2): Nations where the key challenge is balancing resources and needs; these include China, India, Eastern Europe and Latin America. *Total estimated population, 2008: 3-4 billion.*

Third World (W3): Nations that are consistently in dire straits, such as Bangladesh, Haiti and most of Africa. *Total estimated population, 2008: 1-2 billion.*

Population estimates used for this graphic:

W1 = 0.9B, W2 = 3.8B and W3 = 1.8B



This increased global demand will be driven by a steady increase in population growth from today's 6.7 billion to 9+ billion at the midpoint of the 21st century.

This rise in population will be characterized by a growth in affluence, primarily in W2 nations, that will create the largest increase in global meat and milk consumption in history. Much of this increase parallels a rise in living standards in developing nations where more people can afford to replace low-cost grains in their daily diet with higher-cost sources of protein. China is a prime example of this trend. Compared to other W2 nations such as India, China has made more progress in reducing hunger among its growing population. In 1985, meat consumption in China was roughly 44 pounds per person per year. By 2000, this had increased to 90 pounds per person annually, a figure that's projected to more than double again by 2030.

A GROWING CONSENSUS:

The growing challenge of feeding the world

What a few experts have to say:

"Science and technology must spearhead agricultural production in the next 30 years at a pace faster than the Green Revolution did during the past three decades."

– Dr. Jacques Diouf, *Director-General, Food and Agricultural Organization of the United Nations*

"Policy responses to protect the poor from food price rises are urgent and need to be designed in a way that is conducive to stimulating greater agricultural production in the long run."

– Dan Leipziger, *World Bank Group Vice President for Poverty Reduction and Economic Management*

"Backyard vegetable gardens are fine. So are organics… But solutions to the global food crisis will come from big business, genetically engineered crops and large-scale farms."

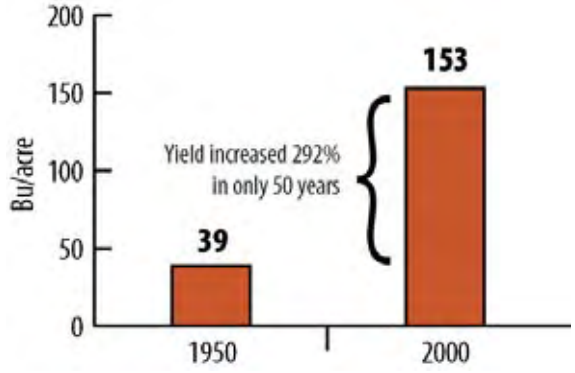
– Jason Clay, *World Wildlife Fund*

LAND: THE ONE RESOURCE WE CAN NEVER PRODUCE MORE OF

Coinciding with increases in worldwide demand for animal protein is the reality of growing constraints on natural resources, with land a key limiting factor. Based on U.N. FAO projections, 13 percent more land in developing countries will be converted to agricultural use over the next 30 years. On a *global* basis, this represents a net increase in available cropland of only 1 percent—from the 39 percent of global land area used in 2008 to a total of 40 percent. This land expansion will account for only 20 percent of future increases in food production. According to the U.N., 70 percent of the rest must come from increased use of new and current yield-enhancing technologies. About 10 percent will come from increased cropping intensity (harvesting more crops per year from every acre).

With respect to increasing output, there is good news. During the last half of the 20th century, agricultural productivity in many W1 nations expanded at a phenomenal rate. For instance, the average yield of corn in the U.S. rose from 39 to 153 bushels per acre (Figure 1).

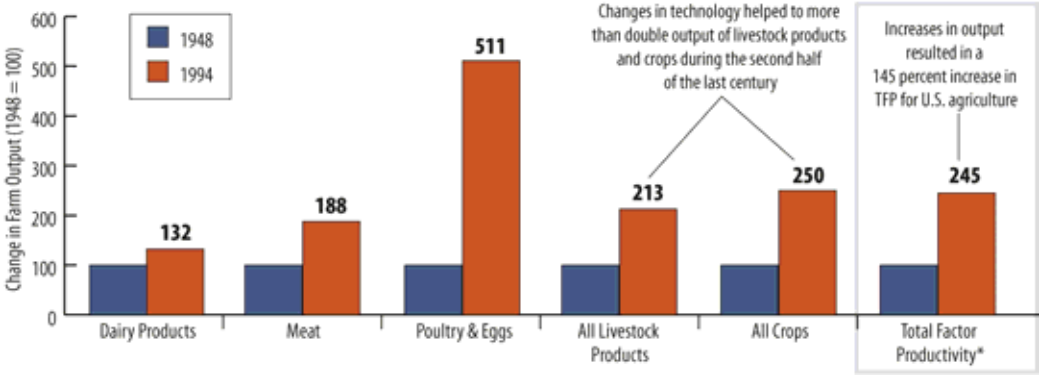
Figure 1. U.S. Corn Yield per Acre: 1950-2000 (USDA Economic Research Service Data)



The USDA calls new technologies a "primary factor" in improvements in agricultural productivity, such as a 292 percent increase in U.S. corn yields from 1950 to 2000.

In addition, a comparison of U.S. farm output for 1948-1994 showed substantial productivity increases for all livestock and grain products, including an 88 percent increase in meat production and a 411 percent increase in the output of eggs and poultry. Combined, these improvements resulted in a 145 percent increase in total factor productivity (TFP)[1] for the U.S. agriculture industry (Figure 2).

Figure 2. U.S. Farm Output & Productivity: 1948-1994 (USDA Economic Research Service Data)



With 1994 farm output for livestock and grain products more than doubling the baseline output of 1948, total factor productivity (TFP) for U.S. agriculture during the last half of the 20th century improved by nearly 150 percent. According to the U.S. Department of Agriculture (USDA), this difference in TFP resulted from factors including changes in technology, efficiency and scale of production.

[1] Overall rate of productivity is most commonly expressed as total factor productivity (TFP), a ratio of outputs to inputs (both measured as an index). TFP captures the growth in outputs not accounted for by the growth in production inputs.

This should give us ample reason to believe we can meet the world's growing need for food. Why? Because according to the USDA Economic Research Service, the development of new technologies—including advances in genetics, nutrition, disease and pest control and livestock management—was an important factor in these 20th-century productivity improvements. Refining these technologies, and discovering new ones, will be critical to our success in expanding on productivity improvements in this century.

With respect to *optimizing* land use for agriculture in the coming decades, however, the news is not so encouraging. The reasons for this are many and complex, but two of them are of paramount importance. First is the growing need to balance the use of agricultural land with the need to minimize the impact of agriculture on the global environment—particularly with regard to greenhouse gas emissions, soil degradation and the protection of already dwindling water supplies. Few would argue against the imperative to employ only those agricultural technologies that have a neutral or positive impact on our environment. To do otherwise is to sacrifice our long-term survival in favor of short-term gains.

The second reason involves the conflicting pressure to reallocate the use of current cropland from growing food to producing grains for biofuels (see sidebar).

Successfully responding to both these additional challenges—protecting the environment and balancing the world's need for energy and food—will require a complex and multifaceted approach. For now, regardless of how we respond to these challenges, both will inevitably affect the cost of food in W1, W2 and W3 nations alike.

GRAIN FOR FOOD OR GRAIN FOR FUEL:

Can we have both?

The USDA projects that about one-third of the 2009 U.S. corn crop will be converted into ethanol. Still, this new technology for revolutionizing energy production has also produced worldwide debate about the trade-offs in using cropland to produce fuel rather than food.

Consider: when U.S. ethanol production began ramping up in 2005, corn was less than $2/bushel. Within two years, this had doubled to $4 and a year later peaked at nearly $8/bushel, resulting in significant pressure on the food industry.

Can we raise enough food to feed the world while helping the U.S. and other nations achieve a higher level of energy independence? If history is any guide, the answer is yes, but only as long as we continue to invest in the technology necessary to make ethanol production, grain production and food production even more efficient.

THE CONSUMER PERSPECTIVE

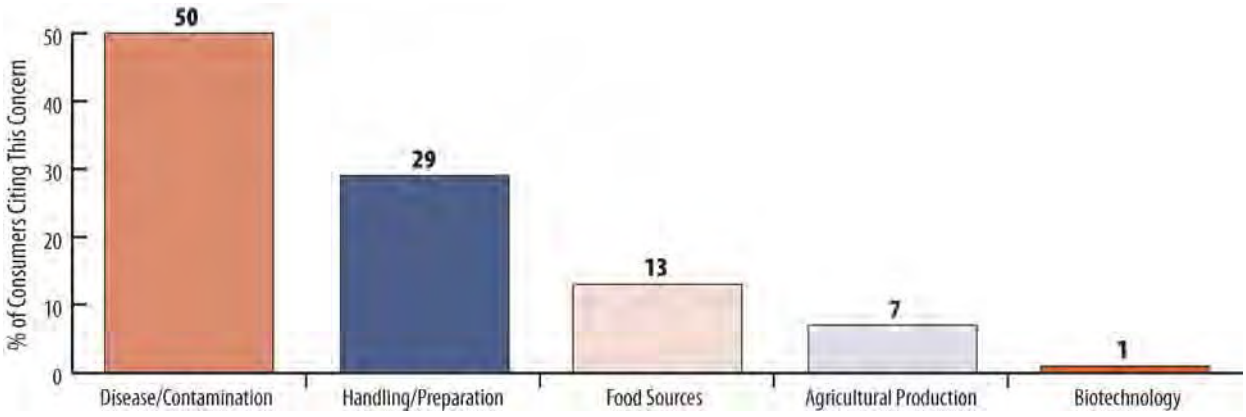
When it comes to the global food supply, what does the average person think about? Does he or she worry daily about food safety and agricultural technologies and methods? Experts continue to debate the answer to this question.

On the one hand, food contamination scares—such as those involving milk from China, peppers from Mexico, beef from some U.S. meat processors and peanut products from Georgia—have created understandable consumer concern about the safety of the world's food supply.

On the other hand, a 2008 survey by the International Food Information Council revealed that when consumers are asked about specific food concerns, half indeed cited "disease and contamination" at the top of the list.

Yet only 7 percent reported that they worry about agricultural production methods, and 1 percent cited biotechnology as a top-of-mind concern (Figure 3).

Figure 3. Consumer Concerns Regarding Food Safety

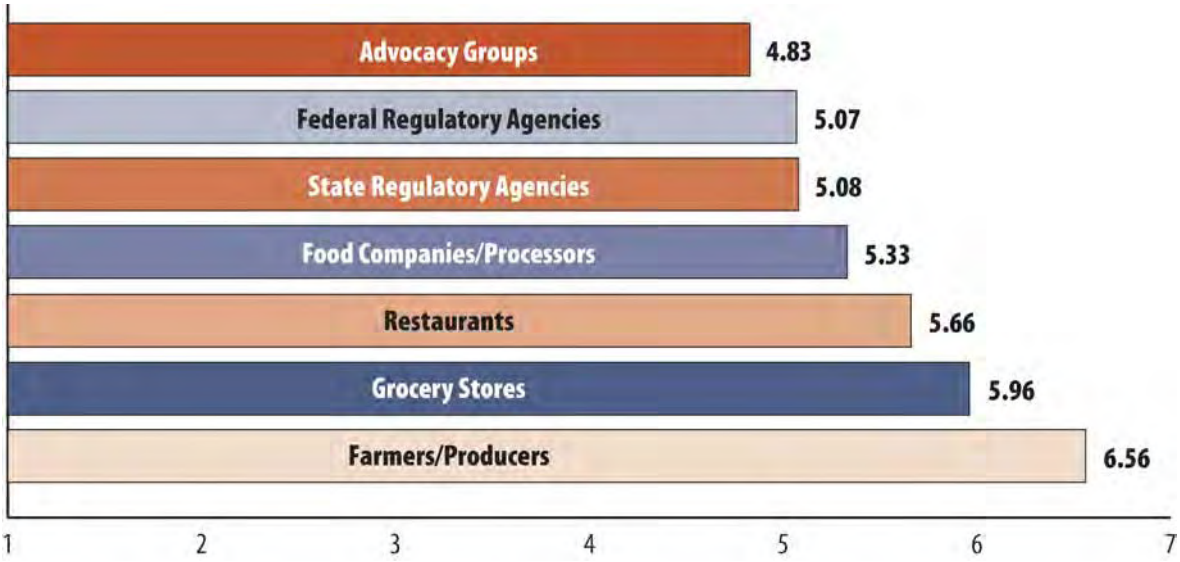


Though research shows most consumers aren't overly concerned about food safety, when asked to share potential worries, 50 percent cite disease and contamination. In contrast, only 1 percent cite biotechnology as a food safety concern.

Research also shows that most people are not greatly concerned about food safety, nor about modern food production technologies. U.S. and international consumer research, involving a total of 45 focus groups conducted in 2001, 2004 and 2008—and including a quantitative survey of 741 Americans taken in 2008—revealed that most consumers (nearly 70 percent in 2008) assume the meat and poultry they buy is safe. The research also showed that consumers care little about the origin of meat they purchase. And only 17 percent of the consumers surveyed in 2008 expressed a strong interest in knowing about modern food animal production, while nearly 60 percent had little or no interest, preferring instead to trust the food supply chain to ensure the food they consume is safe.

Whom do consumers trust most to ensure science-based food safety? Perhaps not surprisingly, it's the food producers—those who rely on modern technologies to help them grow food safely and efficiently. Interestingly, consumers trust producers to help maintain food safety to a much greater degree than they trust advocacy groups (Figure 4).

Figure 4. Whom Do Consumers Trust to Ensure Food Safety?
(1 = Trust Least, 10 = Trust Most)



With regard to ensuring food safety, consumers put the most trust in farmers and food producers.

Protecting the confidence and trust consumers place in the food supply chain is critical. Although consumer confidence remains relatively strong, research shows it is decreasing slightly. High-profile food recalls almost certainly helped to erode this confidence. But is the emergence of genetically modified (GM) foods also to blame? Probably not.

Research reveals that, unprompted, consumers do not put GM products high on their list of food worries. Moreover, in the EU—an area of the world that typically champions organic farming—few consumers actually avoid GM foods when shopping. In fact, regardless of what consumers say about GM foods in opinion polls, the vast majority of them readily buy the few available GM foods without apparent hesitation. It should be noted, however, that global demand for organic products continues to grow. Worldwide sales of organic products doubled from 2000 to 2006, with the EU emerging as one of the top three import markets for organic goods.

CONSUMERS WANT HIGH-QUALITY, AFFORDABLE FOOD

So if most consumers trust their food to be safe and accept GM foods with little concern, what do they worry about? When asked open-ended questions about what

they want most in their food, consumers consistently say they want it to be high-quality and affordable. As one example, recent polling in the U.S., U.K., Germany, Argentina and China found that taste, quality and price were the top considerations when choosing food products.

Of these, affordability continues to move to the forefront as the global economy remains in a state of heightened volatility. According to an October 2008 survey by the Center for Food Integrity, 60 percent of respondents are more concerned about food prices than they were just one year ago—"the highest level of concern … since World War II" according to the Center's CEO, Charlie Arnot (Figures 5, 6).

Figure 5. Consumer Agreement That Today's Food Supply Is Safer Than It Was During Their Childhood

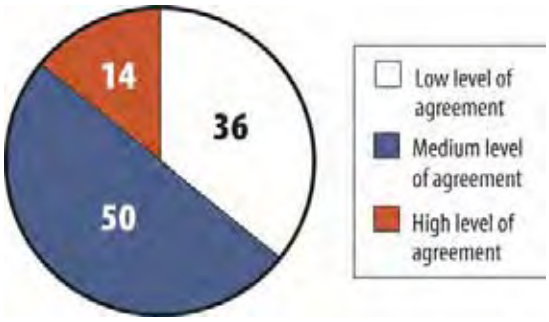
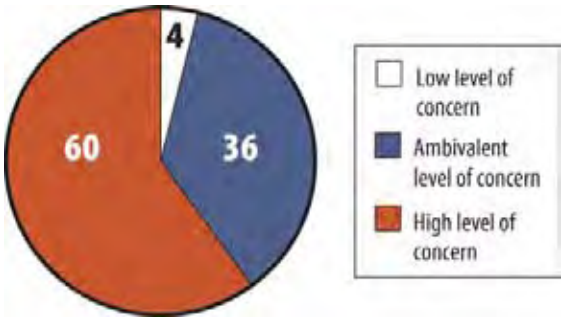


Figure 6. Consumer Concern About Food Prices



Sixty-four percent of Americans believe today's food supply is even safer than it was when they were young, though 60 percent express a high level of concern about food prices.

CONSUMERS WANT CHOICE

Of course, affordability matters less to some consumers, particularly those in affluent W1 countries where food costs account for only 10 percent of the average income. This includes consumers who prefer foods that are produced organically, i.e., with the use of few (if any) modern agricultural tools and technologies. Organic food production, however, typically requires *more* resources and produces *less* food—which currently makes it a questionable solution to meeting the world's growing food supply needs. As we prepare to enter the second decade of the 21st century, most organic foods remain a high-cost luxury that three-quarters of the world's population cannot afford, particularly those in developing nations where food costs consume 50 percent of the average income.

Needless to say, consumers who desire organic foods—which help the food industry satisfy demand and capture more value—should have that choice. Likewise, consumers who need an abundance of efficiently produced, high-quality and affordable food deserve that choice as well. All consumer preferences can and should be protected. Most of all, the undernourished in developing nations who are improving their diets by

increasing consumption of animal proteins, deserve the affordable foods that can be produced with carefully monitored, efficiency-improving agricultural technologies.

LESSONS FROM THE EUROPEAN CENTER OF COMPETITIVE EXCELLENCE

In 2003, a think tank called the Center of Competitive Excellence was assembled to assess a number of challenges. One of these was to evaluate the European meat industry and develop strategies for enhancing its competitive position across Europe and in the global marketplace. Surveys and panel discussions by highly respected agricultural experts, veterinarians and food producers from across Europe were conducted by the Center. Three key insights emerged:

1. It's crucial to have a credible, authoritative regulatory body.

The model for this is the U.S. Food and Drug Administration (FDA), a regulatory body that, despite some criticism, remains a respected authority by consumers in the U.S. and around the world. A central authority such as the FDA helps maintain consumer confidence—something Europeans recognized the need for as they addressed food contamination and animal disease issues. Ultimately, they created the centralized Food and Veterinary Office (FVO) and the European Food Safety Authority (EFSA).

2. Allow use of approved technologies and modern techniques to continue.

As an example, U.K. farmers learned in the 1990s that rewriting laws to appease the political demands of a vocal minority is a recipe for economic disaster. A decade after yielding to pressures to ban (or not approve) growth enhancers, biotech products, GMOs and certain production practices, the U.K. has transformed from a key global leader and competitor to a high-cost, low-productivity domestic producer that now relies on poultry and beef imports to meet consumer demand.

3. Food producers should avoid "differentiating on the negative."

Labeling food products with claims such as NO additives, NO this, NO that, etc., results in a costly contest among manufacturers to "out-NO" each other while only confusing consumers who neither understand, nor prefer these types of foods. Further, this practice can create an unfounded fear among consumers that products without such labels are less safe when, in fact, they can be even safer to consume. In any case, the consumer should make the final decision about which food products to purchase.

HIGH FOOD PRICES WILL WORSEN THE GLOBAL FOOD CRISIS

The question of how food is grown became even more relevant in 2008, when the entire world saw pressures on food production accelerate as never before. According to the International Monetary Fund (IMF), world market prices for food commodities rose more than 75 percent from early 2006 to July 2008. Of course, any increase in grain prices inevitably causes meat, egg and dairy costs to rise, because grain is used to feed livestock. As painful as these increases are in industrialized (W1) nations, they can be

devastating in poor nations where even modest increases in food prices can mean the difference between sustenance and starvation.

Josette Sheeran, head of the World Food Programme, reports that from 2002 to 2007 the cost of procuring basic foods for her program increased by 50 percent—and then by another 50 percent only one year later. As a consequence of these unprecedented cost increases, Sheeran warns that "high food prices are not only causing a humanitarian crisis but also putting at risk the development potential of millions of people."

The challenge of helping these millions of people requires us to ask ourselves: Can we afford *not* to use the technologies at our disposal to produce food as efficiently as possible?

WHY IS TECHNOLOGY SUCH AN IMPORTANT KEY TO MEETING THE GLOBAL DEMAND FOR FOOD AND CONSUMER CHOICE?

There are a wide variety of answers to this question, and here are three of the most important:

1. Technology enables food producers to provide more high-quality grains and protein sources using fewer resources.

Ironically, those who believe "all-natural" farming techniques (e.g., pre-1950) were superior to those used today could not, in many ways, be more mistaken. For example, a combination of modern feeding practices and efficiency-enhancing feed additives enables today's cattle growers to use two-thirds less land to produce a pound of beef as it takes to produce a pound from "all-natural" grass-fed cattle. In addition, we can now produce at least 58 percent more milk with 64 percent *fewer* cows than dairy farmers could produce in 1944. Researchers have also found that nationwide use of an FDA-approved swine feed additive could enable the U.S. to maintain pork production levels while raising 11 million fewer hogs. This would also reduce demand for cropland used to grow feed grains by more than 2 million acres.

Similarly, for every million dairy cows managed with another widely used technology, the world saves 2.5 million tons of feed that would have required 540,000 acres of land to produce. This increase in efficiency saves enough electricity to power 15,000 households and can substantially lower milk prices.

Technology has also played an important role in the poultry industry, which has seen a four- to six-fold increase in the slaughter weight of broiler chickens since 1957. Researchers attribute this increase to genetic selection and improvements in nutrition.

2. Technology can help keep food affordable while ensuring maximum consumer choice—especially in developing nations.

Organic foods are a fine option for people who can afford to pay a premium for them.

According to USDA researchers, these premiums can average 100 percent or more for vegetables, 200 percent for chicken and nearly 300 percent for eggs. On a global scale, however, most consumers can't afford to pay such premiums and instead demand less expensive food choices.

It bears noting that not all organic production methods are less efficient and provide foods that cost more. According to a U.N. FAO report, in some countries, well-designed organic systems can provide better yields and profits than traditional systems. In Madagascar, for example, farmers have increased rice yields fourfold by using improved organic management practices. In Bolivia, India and Kenya, farmers have shown that yields can be double or triple those obtained using traditional practices.

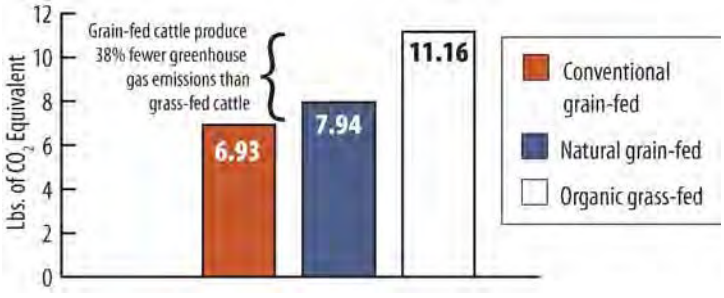
Nonetheless, the report also recognizes the need for more research to solve technical problems faced by organic growers, and suggests that organic agriculture could become a realistic alternative to traditional agriculture over the next 30 years, but only on a local level.

Still, given the magnitude of the food crisis the world faces in the coming decades, efforts to maximize choice and achieve high production efficiencies (and lower costs) for all foods—including organic products—deserve the support of all constituencies in the global food chain.

3. Technology can help minimize the global environmental impact of increased food production.

Modern production methods and technologies not only help produce more high-value protein from less land, but can also have a net positive impact on the environment. For instance, what today's beef producers call "conventional" (i.e., modern) production techniques can actually reduce greenhouse gas emissions per pound of beef by 38 percent compared with an "all-natural" production method (Figure 7).

Figure 7. Total Greenhouse Gas Emissions per Lb. of Beef (excludes NOx)



Today's conventional production methods help reduce total greenhouse gas emissions compared to organic methods.

Moreover, technology can help significantly reduce animal waste production that can threaten vital water resources in developing nations where modern pollution-control standards and technologies are not in use. Case in point: use of an FDA-approved feed

additive for swine can reduce manure production in pigs by 8 percent. Feeding this additive to every hog harvested in the U.S. in 2002 would have reduced annual production of swine manure by more than 3.4 billion gallons—or enough to fill about 5,600 Olympic-size swimming pools.

CONCLUSIONS

1. The global food industry needs technology.

Without advancements in agricultural technology, humanity would likely not have progressed through the 20th century without major famines or devastating food wars. Will we be able to say the same thing at the end of this century, given that a food crisis is already here?

I believe the answer is *yes,* because I concur with the U.N. that 70 percent of this food must come from the use of new and existing technologies and methods. And these technologies and methods must have no negative impact on the environment, animal welfare or food safety.

2. Consumers deserve the widest possible variety of safe and affordable food choices.

In general, consumers trust food producers to keep the food supply safe, and they're more concerned about food contamination than about technology used on the farm. Instead, one of the most pressing human concerns about food is affordability.

For this reason, consumers from all classes and geographies—from those who can afford organic foods to those who struggle to maintain a diet that sustains them—must be allowed to choose from an abundance of safe, nutritious and, most importantly, inexpensive food options.

3. The food production system can mitigate the food economics challenge and achieve an "ultimate win."

Facing a global food crisis, the world is at risk through the midpoint of this century. We already see the signs: our population consumed more grain than we produced during seven of the last eight years.

The good news: an "ultimate win" is still possible. What will it look like? Five key achievements will mark its success:

1. Improving the affordability of food by using new and existing technologies and optimal productivity practices.

2. Increasing the food supply by instituting a vastly improved degree of cooperation across the entire global food chain.

3. Ensuring food safety with a combination of technology and high-quality standards

and systems, coupled with a greater measure of worldwide collaboration.

4. Increasing sustainability through a highly productive and efficient system that simultaneously protects the environment by means of sensitive and efficient use of natural resources.

5. Producing more biofuels to reduce dependence on fossil fuels while creating no negative effect on global food supplies.

In summary, three key concepts—**collaboration, choice** and **technology**—emerge as the pathway to success. Not only will they provide the direction, they will be necessary requirements for an ***"ultimate win"*** in the food economics challenge.

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DEMYSTIFYING THE ENVIRONMENTAL SUSTAINABILITY OF FOOD PRODUCTION

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INTRODUCTION

Meat, eggs, and dairy products play significant roles in supplying high-quality protein, vitamins, minerals and essential fatty acids as part of a nutritionally balanced diet (Huth et al., 2006; USDA, 2005). According to FAO data for 2007, the U.S. is the leading producer of cow's milk, beef, chicken and poultry and second for pork, eggs and game meat worldwide (United Nations Food and Agriculture Organization, 2009). This is primarily achieved through the adoption of highly efficient agricultural practices that allow for considerable improvements in productivity (Capper et al., 2009).

The global population is predicted to increase to 9.5 billion people in the year 2050 (U.S. Census Bureau, 2008). Total food requirements will increase by 100% (Tilman et al., 2002) as a function of both the 50% increase in population and the additional global demand for animal protein as people in developing countries become more affluent (Keyzer et al., 2005). The resources available for agricultural production are likely to decrease concurrently with population growth due to competition for land and water and depletion of fossil fuel reserves. Livestock industries therefore face the challenge of producing sufficient safe, affordable animal protein to meet consumer demand, using a finite resource base – a challenge which is exacerbated by political and social concerns relating to the environment.

All food production has an environmental impact and livestock production has been singled out as a major contributor to climate change (Koneswaran and Nierenberg, 2008; Steinfeld et al., 2006). However, consumer and governmental perceptions of strategies and production systems used to reduce environmental impact are often simplistic and appear to be based on misconceptions that do not consider potential negative trade-offs. This paper aims to discuss some of the most commonly heard misconceptions relating to the environmental impact of food animal production and transport systems.

THE ROLE OF PRODUCTIVITY IN REDUCING ENVIRONMENTAL IMPACT

The dichotomous challenge of producing more food from a dwindling resource base often leads to the suggestion that adopting low-input production systems is the key to sustainable agriculture. However, this defies a fundamental principle of physics, the First Law of Thermodynamics which states that 'energy can neither be created nor destroyed, it can only change form'. Carbon is the key unit of currency of energy use of

living organisms. Just as we balance our checkbook every month, energy (carbon) inputs and outputs must be balanced against each other.

When assessing environmental impact, it is essential to use a standardized assessment tool and to express impact per functional unit of food, e.g. resource use and waste output per liter or kg of product (Schau and Fet, 2008). This ensures that the production system meets total demand for the product. Thus, greenhouse gas (GHG) emissions should not be simply assessed as per animal or per facility but rather based on system productivity using a life-cycle assessment (LCA) approach. This approach is prescribed by the EPA for environmental impact assessment, incorporating all inputs and outputs within the production system boundaries. This is particularly important when making comparisons across differing production systems. For example, Thomassen et al. (2008) reported greater ammonia volatilization per acre from conventional Swedish dairy farms than their organic counterparts. However, ammonia volatilization per unit of milk produced was greatest in organic systems due to reduced stocking rates and the increased number of animals required to produce the same quantity of milk. The purpose of dairy systems is to produce milk, thus the correct functional unit of the LCA analysis in this example is the unit of milk produced, not the acre. The productivity of the system must also be taken into account, to assure that demand is being met and total supply is maintained.

It is worth noting that a recent analysis from the Organic Center (Benbrook, 2009), intended to demonstrate the advantages of moving from conventional to organic dairy production, is based on a flawed premise, namely that productivity (milk yield per cow) does not differ between conventional and organic systems. Productivity is demonstrably lower under organic management with a reduction in milk yield per cow ranging from 15-27% (Nauta et al., 2006; Sato et al., 2005; USDA, 2007; Zwald et al., 2004). When differences in productivity are accounted for, organic dairy production requires considerably more resources (feed, land, water etc) per unit of milk produced and has a greater environmental impact (Capper et al., 2008).

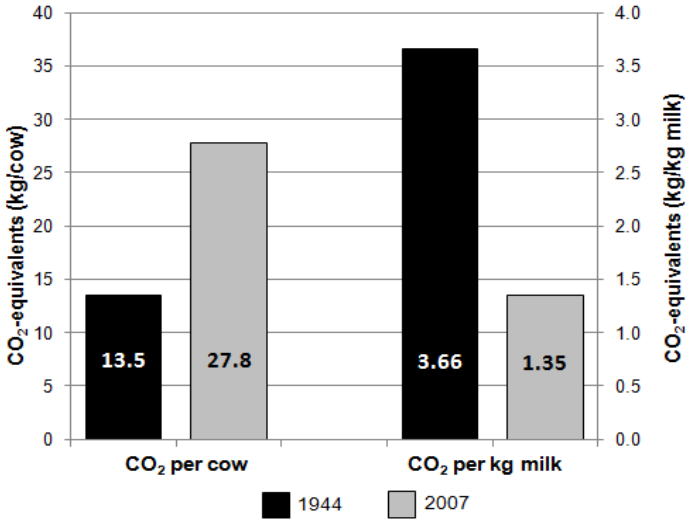

Figure 1. Carbon Footprint per Cow and per Kilogram of Milk for 1944 and 2007 U.S. Dairy Production Systems (Capper et al., 2008)

The 'good old days' of dairy production

The agrarian vision of U.S. dairy farming involves cows grazing on pasture with a gable-roofed red barn in the background – a traditional low-input system. By contrast, the image of modern dairy production propounded by anti-animal agriculture activists is synonymous with "filthy and disease-ridden conditions"[1] and 'industrialized warehouse-like facilities that significantly increase GHG emissions per animal' (Koneswaran and Nierenberg, 2008). The United Nations Food and Agriculture Organization (FAO) report 'Livestock's Long Shadow' (Steinfeld et al., 2006) concluded that intensification of livestock production is essential to mitigate environmental impact. However, this conclusion has often been overlooked in favor of more sensationalized data cited from the study. Despite the demonstrable need for further technological advances to increase future food production (Roberts, 2000; Waggoner, 1995), further intensification of food production is regarded by some as a profane suggestion (Koneswaran and Nierenberg, 2008).

As shown in Figure 1, daily GHG emissions per cow (expressed in CO_2-equivalents) have increased considerably over the past 65 years: the average dairy cow now produces 27.8 kg CO_2-equivalents compared to 13.5 kg CO_2-equivalents back in 1944 (Capper et al., 2009). However, expressing results on a 'per head' basis fails to take the entire system into account. When analyzed using LCA, GHG emissions per kg of milk produced have declined from 3.7 kg in 1944 to 1.4 kg in 2007. This has been achieved through considerable improvements in productivity. Milk yield per cow more than quadrupled between 1944 (2,074 kg) and 2007 (9,193 kg), allowing 59% more milk

[1] Comment from Danielle Nierenberg (Animal Agriculture and Climate Change Specialist, Humane Society of the United States) at the Hudson Institute's Conference on Food for the 21st Century: Challenging the Conventional Wisdom, Washington DC, September 10th, 2008.

(84.2 billion kg *vs*. 53.0 billion kg) to be produced using 64% fewer lactating cows (9.2 million *vs*. 25.6 million). As described in Capper et al. (2009), this improvement in productivity facilitates the 'dilution of maintenance' effect, by which the proportion of daily nutrients apportioned to maintenance is reduced. This effect is not confined to the nutrition of lactating cows, but also applies to non-productive animals within the population (dry cows, replacement heifers and bulls) that serve to maintain the dairy herd infrastructure. Increasing productivity therefore reduces both the number of dairy animals required and the resources required to produce a given amount of milk.

The resource use and waste outputs per unit of milk for 1944 and 2007 production systems are shown in Figure 2. The 4.4-fold increase in milk yield per cow drove a 79% decrease in total animals (lactating and dry cows, heifers, mature and adolescent bulls) required to produce a given quantity of milk. Feed and water use were reduced by 77% and 65% respectively, while land requirements for milk production in 2007 were reduced by 90% compared to 1944 due to improved crop yields and the shift from pasture-based to TMR systems. Manure output from the modern system was 76% lower than from the 1944 system, contributing to a 63% decrease in the carbon footprint per unit of milk. To put this into context, the carbon footprint of the entire dairy industry was reduced by 41% by the adoption of technologies and modern management practices that improved productivity between 1944 and 2007.

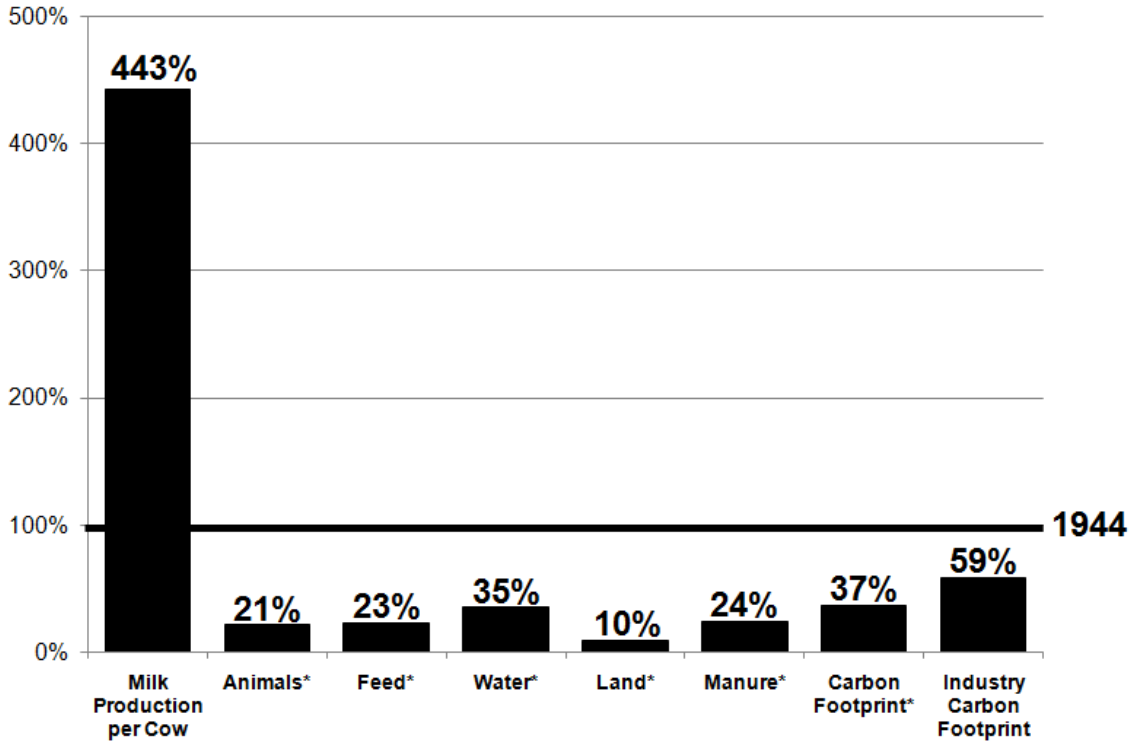

Figure 2. 2007 U.S. Milk Production, Resource Use and Emissions Expressed as a Percentage of the 1944 Production System (Adapted from Capper et al., 2009)

‘Grass-fed’ beef production

The environmental mitigation effect arising from improved productivity is a function of either output per animal (meat, milk or egg yield) or the time taken to produce the finished product. Average beef-carcass yield per animal has increased over the past 30 years from 266 kg in 1975 compared to 351 kg in 2007 (USDA, 1976; USDA/NASS, 2008), which, in combination with reduced time to slaughter over the same time period (19 mo *vs*. 18 mo), reduces resource use per unit of meat. Time to slaughter is primarily affected by growth rate, thus this is a primary productivity measure by which to mitigate the environmental impact of meat production.

Approximately 50-75% of a conventionally-reared beef animal’s life is spent on pasture, however ‘grass-fed’ or ‘grass-finished’ beef is often touted as a more environmentally-friendly option for the consumer than conventional (corn-finished) beef. If a superficial view is taken, considering only the comparative energy inputs required to produce and harvest corn in conventional systems, compared to the animals ‘harvesting’ the pasture through grazing, the suggestion that grass-fed beef has a lower environmental impact appears to be correct (Pimentel and Pimentel, 2007). However, this suggestion relies on three underlying erroneous assumptions that animals within both systems: 1) have equal energy requirements, 2) take the same time to finish and 3) produce the same quantities of GHGs from enteric fermentation. Accounting for the animal’s daily maintenance requirement (nutrients needed to maintain the vital functions and minimum activities in a thermo-neutral environment) becomes crucial for accurate analysis.

As shown in Table 1, animals finished on pasture have an additional energy requirement for grazing activity, thus increasing total daily maintenance requirements. The growth rate of beef animals on pasture is also lower than that of animals fed corn. Each day added to the finishing period adds an extra daily maintenance cost, which must be accounted for in the environmental impact of the final product. Finally, pasture-based diets promote greater ruminal acetic acid production (Johnson and Jonhson, 1995), increasing enteric methane production. Both energy use (MJ/kg gain) and methane emissions (kg/kg gain) are thus considerably increased in pasture-based systems.

Table 1. Comparison of energy inputs, methane output and cropland required to finish beef steers in corn-fed or pasture-fed systems

	Corn-fed	Pasture-fed
Start weight (kg)	254	254
Finished weight (kg)	635	635
Growth rate (kg/d)[a]	1.61	0.87
Finishing period length (d)	237	438
Daily energy for maintenance (MJ)	26	33
Daily energy for growth (MJ)	30	15
Total energy used during finishing (MJ)[b]	40,934	118,308
Total methane emissions during finishing (kg)[c]	53	149
Energy MJ/kg gain	107	310
Methane kg/kg gain	0.14	0.39
Total land required (ha)[d]	0.21	2.70

[a] Based on corn or pasture diet fed *ad libitum* during the finishing period, calculated according to NRC (2000) by Hereford x Angus steers weaned at 207 days (USDA, 2000)
[b] Includes energy for maintenance and growth (NRC, 2000) plus energy inputs for corn grain and pasture from Pimentel and Pimentel (2007).
[c] Calculated using the model described in Capper et al. (2009) adapted for beef production
[d] Corn yields from USDA (http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/) and pasture yields from Brink et al. (2008)

A significant proportion of land used to graze cattle is not suitable for growing crops for human consumption (Steinfeld et al., 2006). Furthermore, U.S. beef and dairy industries use considerable quantities of by-products from human food, fiber and biofuel production (e.g. citrus pulp, flaxseed oil, corn distiller's grains) that would otherwise be discarded and become a GHG source within landfill. The conversion of indigestible plant material and human food by-products into high-quality milk and meat protein provides an invaluable source of human nutrients, and should be offset against the environmental impact of livestock production.

To make the most efficient use of resource inputs it is essential to match nutrient supply and demand within individual components of the production system. Extensive rangeland systems provide sufficient nutrients to support the cow-calf component of the U.S. beef production system (NRC, 2000) while maintaining biodiversity (Steinfeld et al., 2006). Finishing cattle on intensively-managed pasture offers an opportunity to reduce GHG emissions per unit of beef compared to traditional, extensive grass-finishing systems (DeRamus et al., 2003). However, the resource inputs and greenhouse gas outputs generated by finishing the current U.S. population of 9.8 million fed cattle on intensively-managed pasture would require an extra 24.2 million ha of pastureland and 1.15×10^{12} MJ of energy. The increases in resource use per unit of output associated with 'traditional' dairy and beef production systems demonstrate that the popular perception of low-input sustainable systems does not align with true sustainability when trying to meet a static or increasing demand for food.

RECONCILING GLOBAL AND NATIONAL EMISSIONS DATA

The FAO (Steinfeld et al., 2006) reported that livestock are responsible for 18% of global anthropogenic GHG emissions. This statistic has been adopted by various groups as evidence that converting to a wholly-vegetarian diet would have a beneficial environmental impact (Walsh, 2009). As previously discussed, one major benefit conferred by livestock systems is the conversion of inedible plant species (e.g. pasture) into high-quality meat protein for human consumption. Indeed, Peters et al (2007) evaluated the ability of New York state agriculture to support a human population consuming one of 42 different diets, each containing 0-381 g/d animal protein (meat and eggs), and concluded that the diet that best optimized resource use contained 63-127 g of animal protein per day. This is further evidence that food production systems must be matched to available resources to improve productive efficiency.

A recent report from the U.S. EPA (2009) quantified the primary anthropogenic GHG sources within the US, concluding that total agriculture (livestock and crops) contributed 5.8% of national GHG emissions. Of this 5.8%, approximately 3.4% can be apportioned to animal agriculture (total emissions from manure and enteric fermentation, plus an estimate of the contribution made by animal feed production) and the remaining 2.4% to food crops consumed directly by humans. To reconcile the considerable difference between the global (18%) and national (3.4%) estimates of livestocks' contribution to GHG emissions it is necessary to explore the data in more detail.

Partitioning out the components of the global FAO figure reveals that almost half (48%) of the total is attributed to changes in land use pattern, specifically the carbon released by clearing forestland (a carbon sink) to grow animal feed. The potential for reduced cropland availability to lead to further deforestation on a global basis is exacerbated by the use of formerly food-producing agricultural land to grow biofuel crops (Sawyer, 2008). Deforestation therefore needs to be taken into account when analyzing the environmental impact of agricultural systems where a considerable portion of animal feed is imported, e.g. imports of soy from Brazil and Argentina into Europe. The majority of U.S. animal feedstuffs are produced domestically; available cropland area has remained stable (USDA, 2002) with increased crop yields compensating for an increase in feed and food crop production required to meet demand. In contrast to the deforestation occurring in South American countries, the U.S. is actively reforesting, with an average increase in forestland area of 0.2%/y over the past 30 years (Smith et al., 2005). Reforestation increases the amount of carbon sequestered from the atmosphere into plant tissue and soil, with an average of 6.4 kg carbon sequestered annually per tree (Sampson and Hair, 1996). The mitigating effect of carbon sequestered by new forest growth is not accounted for in the U.S. EPA (2009) calculations and would further reduce the estimate of agriculture's contribution.

Even after the component of total GHG emissions attributed to deforestation in the FAO report is disregarded, the global estimate remains nearly 3 times higher than the U.S. national estimate (9.4% *vs*. 3.4%). As demonstrated by the historical milk production (Figure 2) and beef production (Table 1) examples, environmental impact is

directly affected by the system productivity (food output per unit of resource input). By its very nature, the global average includes a wide range of system efficiencies. For example, U.S. agriculture is characterized by highly-efficient production systems, with the average dairy cow producing 9,219 kg milk per year in 2007. By contrast, the 2007 average annual yield for the top six milk-producing counties in Europe is 6,362 kg milk per year, while annual production in New Zealand and Canada averages 3,801 kg milk/cow and 8,188 kg milk/cow respectively (FAO, 2009).

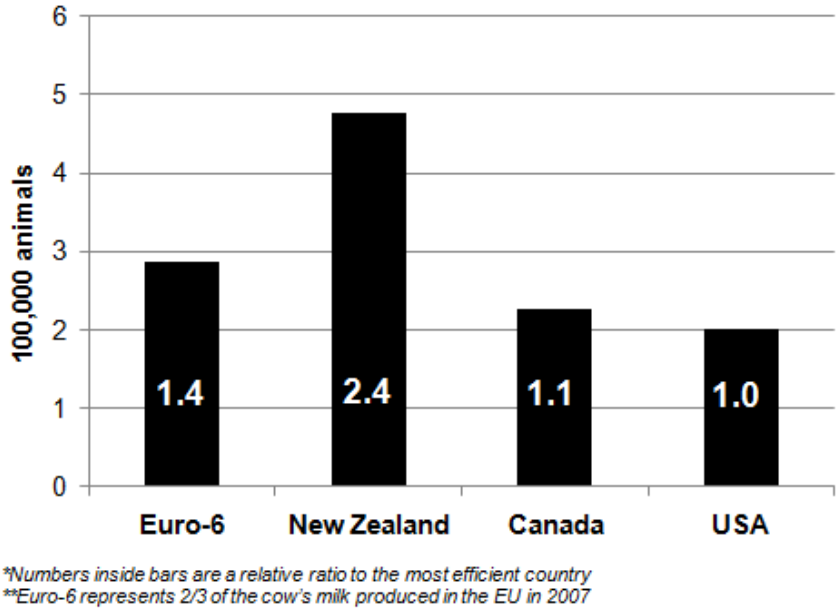

Figure 3. Dairy Animals (Cows, Heifers and Bulls) Required to Produce One Billion kg of Milk in 2007

Differences in productivity between countries means that the dairy population (lactating and dry cows, heifers and bulls) required to produce an equivalent amount of milk is extremely variable (Figure 3). Compared to the U.S. (indexed as 1.0), Canada requires a 1.1x population increase, Europe requires a 1.4x population increase and New Zealand requires a 2.4x population increase. The nutrient requirements and waste output associated with the dietary maintenance requirement for each population therefore varies considerably, with a significant increase in both resource use and GHG emissions per unit of milk in the systems with lower productivity.

When elucidating disparities between global and national GHG emissions, it is essential to understand the effects of differences in system productivity and efficiency. The global average for livestock's contribution to GHG emissions cannot be assumed to be representative of all agricultural systems.

CARBON SEQUESTRATION AS A MITIGATION STRATEGY

As previously discussed within the beef example, pasture-based systems are only sustainable when they are able to provide sufficient nutrients for meat or milk

production, without negatively impacting yield or increasing resource use per unit of food. This is a serious consideration when assessing the environmental impact of pasture-based animal production as it is associated with increased maintenance costs (due to activity) and decreased yields, thus more animals or more days to market (and associated resources) are required to produce the same amount of animal protein.

Carbon sequestration (long-term storage of carbon in soil or plant biomass) is often quoted as a major environmental advantage of pasture-based systems. This suggestion is based on the assumption that pasture sequesters carbon indefinitely and at a constant rate. However, carbon sequestration into soil can only be significantly altered with a change in land use, and only occurs over a finite time period (Post and Kwon, 2000; Schlesinger, 2000).

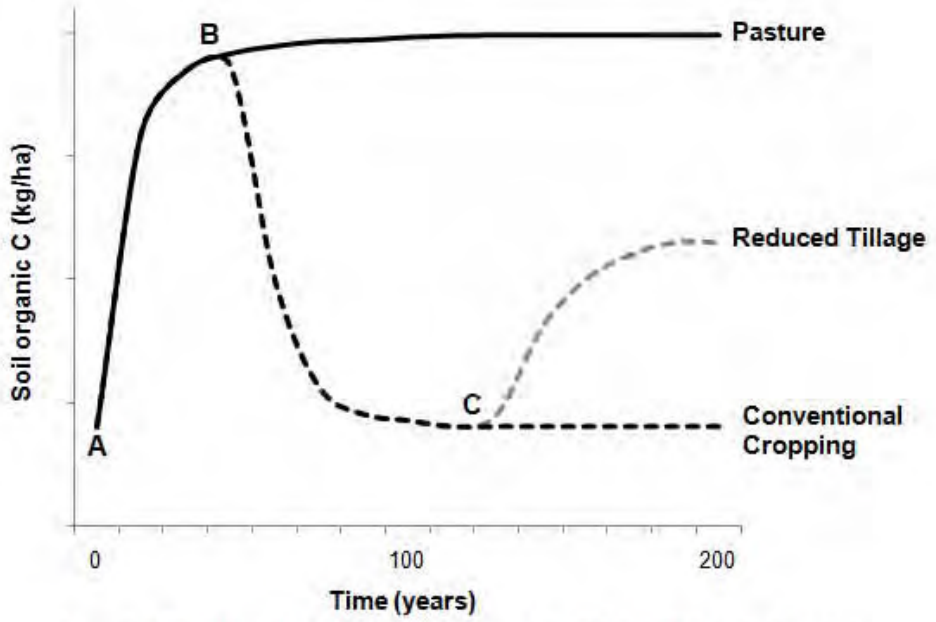

Figure 4. Variation in Soil Organic Carbon Profile According to Changes in Management Practice

The effects of changing from conventional cropping to pasture (point A), pasture to conventional cropping (point B) and conventional cropping to reduced-tillage (point C) on soil carbon reserves are shown in Figure 4. Converting cropland to pasture, or pasture to forestland, leads to increased sequestration and an improvement in soil carbon status. Conversely, changing land use from forest or pasture to cultivated crops increases emissions and reduces soil carbon status. These alterations in carbon sequestration or emissions only continue until an equilibrium point is reached after about 20 years, with the majority of soil sequestration/emissions occurring within the first 10 years following land management change (Smith et al., 2007). Land subjected to the same land use practices over 20+ years is considered to have a net carbon balance of zero, i.e. the amount of carbon sequestered into soil is equal to carbon lost to the atmosphere and the soil is at equilibrium. The environmentally positive effects of sequestration therefore only occur in land recently converted from cropping to pasture – negligible additional carbon is sequestered into permanently-established pasture and if

pasture is tilled or converted to cropland, sequestered carbon may be lost to the atmosphere.

At a superficial level, carbon sequestration appears to be a relatively easy strategy for offsetting the environmental impact of livestock production. However, as noted by a recent U.S. Congress report (2007), this is a temporary (and easily reversible) mitigation strategy, capturing a limited amount of carbon. Sequestration potential therefore does not compensate for the comparative inability of pasture-based systems to support intensive livestock production.

‘FOOD MILES’ AND THE TREND TOWARDS CONSUMING ‘LOCAL’ FOOD

The term “Food Miles” is simply defined as the distance that food travels from its place of origin to its place of final consumption. Food miles have become a common topic of discussion in the social media debate over the merits of modern intensive agriculture *vs*. locally grown food. Often, “locally grown” is touted as preferable because consumption of remotely-grown food is responsible for extra atmospheric carbon emissions due to the excessive distance it must travel. As energy prices undoubtedly increase in the future, debate will continue as to the wisdom of transporting food over long distances.

This section demonstrates how to evaluate the most efficient use of fossil fuels to move food to its point of consumption. Many factors must be considered, including supporting the local economy, energy availability, food safety, freshness, and security, cultural preferences and climate. Potentially the most important factor is the agronomic ability of the local land and resources to supply sufficient food in a healthy balanced diet to the indigenous population.

A common but naïve method for evaluating food miles is to measure the linear distance food travels from point-of-origin to point-of-consumption. Intuitively, it seems logical that if a local source of a certain food (e.g. eggs) is available, then purchasing ‘local’ eggs is more energy-efficient and eco-friendly than purchasing eggs that originated from some distance away. However, as discussed by Watkiss (2005) and Saunders et al. (2006) this approach fails to consider the productivity of the transportation system. The following scenario comparisons demonstrate that linear travel miles are not indicative of total energy use and therefore not necessarily a valid measure of the environmental impact of moving food over long distances. Rather this must be evaluated through appropriate measures of fuel efficiency based on cargo capacity and energy use per unit of food moved.

An illustrative example was developed comparing three typical scenarios for a consumer purchasing a dozen eggs: 1) the local chain grocery store supplied by a production facility some distance away; 2) a farmer’s market supplied by a source much closer than the grocery store’s source; or 3) directly from a local poultry farm. Only the impact of energy use to transport food is examined and eggs at each facility are

assumed to be produced with similar egg production practices. As a result, the carbon footprint of a dozen eggs leaving the production facility is similar for all three scenarios. The example illustrates the basic LCA process required to appropriately assess food miles' environmental impact and is not meant to provide the definitive answer as to which food transportation system is consistently superior. To provide some realism to the example, an area of the country known to the authors (Pacific Northwest) was chosen for the farmers' market and farm scenarios in order to develop a plausible example. For the grocery store example, the home is located in the Pacific Northwest but the eggs were transported from California. At least three data inputs are critical to accurately assess the impact of food transportation: distance traveled, fuel use, and cargo capacity of the transport vehicles. Intermediate distances between egg source to store and store to home are shown in Table 2. The total distance traveled by the eggs in scenario 1 (grocery store) is 1,293 km, for scenario 2 (farmer's market) is 150 km, and for scenario 3 (local farm) is 44 km.

Table 2. Linear Road Distances for Transportation Segments

One-way distance (km)	The home	Grocery store	Farmers' market	Local farm	Farmers' market source	Grocery store source
The home		2.4	11	44		
Grocery store	2.4					1,291
Farmers' market	11				138	
Local farm	44					
Farmers' market source			138			
Grocery store source		1,291				

It is not sufficient to simply examine the distance between source and consumption point because in some cases vehicles must make a round-trip. Total miles assigned to each scenario are shown in Figure 5. In all cases, the personal auto must make a round-trip from the home to the place of purchase. In order to simplify the examples we assume that no other business will be conducted during the trip, thus all miles travelled by the auto are assigned to egg transport. The same is true for the pick-up truck used to transport eggs to the farmer's market. Eggs are transported from the source in California to the grocery store using a tractor hauling a refrigerated trailer (reefer). Under these conditions, backhauls are used as much as possible – for example, a load of apples might be backhauled from Washington to California. Situations both with and without backhauls have therefore been included in the analysis. As shown in Figure 5, examining total miles for each scenario seems to reinforce the preliminary conclusion that purchasing local eggs is by far the most eco-friendly option.

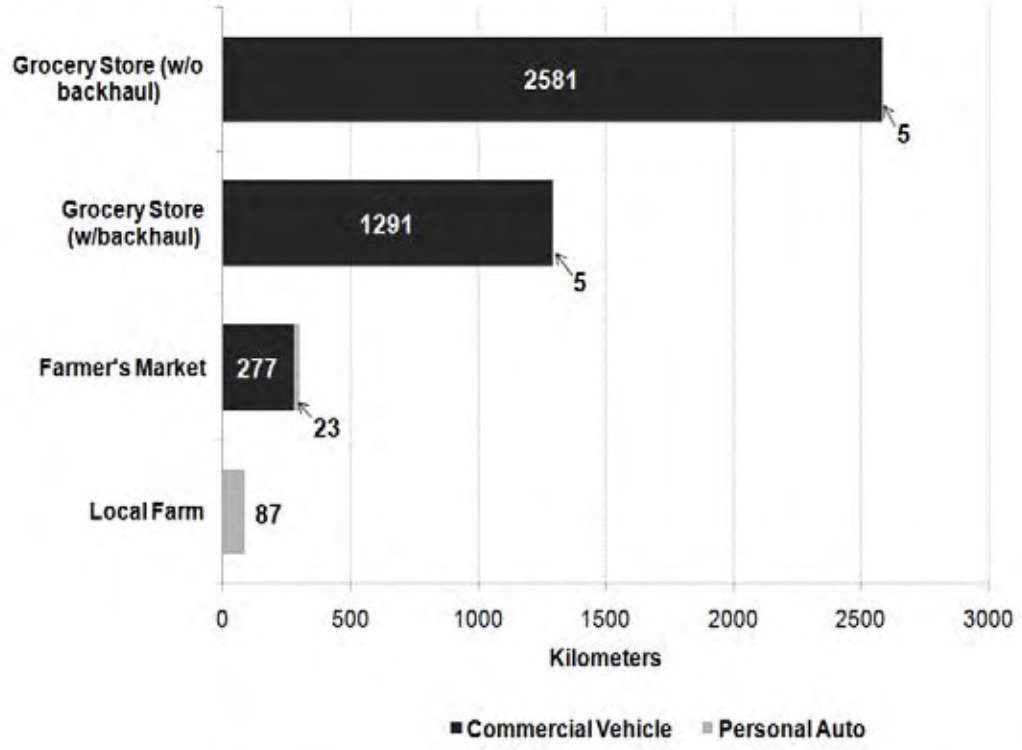

Figure 5. Vehicle Distance Traveled to Move Eggs from Source to Home

Up to this point, the implicit assumption is that each vehicle carries equivalent numbers of eggs and uses equivalent fuel. Data relating to fuel efficiency and vehicle capacity is summarized in Table 3. High fuel efficiency again appears to favor the automobile as the most environmentally-friendly form of egg transport, however, the tractor-trailer moves 23,400 dozen eggs in one trip. Because of the enormous quantity of eggs that can be moved in a single trip by one tractor-trailer (another form of productivity), the fuel use efficiency per dozen eggs is greatly increased over the automobile. Fuel use (total distance divided by fuel efficiency, plus fuel use for egg refrigeration by the tractor-trailer) and egg-carrying capacity were used to estimate total fuel use per dozen eggs for each vehicle within the three scenarios to determine the most fuel efficient method for transporting eggs from the source to the home refrigerator.

Results summarized in Figure 6, provide a very different, perhaps non-intuitive, conclusion as to the most energy-efficient method for moving eggs to the consumer, namely the tractor-trailer from a remote location. Even if a backhaul is not used and therefore the fuel efficiency is halved, fuel expended per egg is still far superior to either the farmer's market or the local farm. This a direct result of the enormous number of eggs moved by the tractor trailer compared to the other two vehicles. Over 90% of fuel consumption is contributed by the automobile in each scenario because the auto only carries one dozen eggs. Using the vehicle fuel efficiency and cargo capacity in these scenarios, eggs could actually be transported across the entire North American

continent by the tractor trailer, and the grocery store model would remain the most fuel-efficient, eco-friendly option.

Table 3. Vehicle Fuel Efficiency and Cargo Capacity

	Fuel efficiency (km/l)	Egg capacity (dozen)
Auto	9.5[a]	1
Pick-up truck	7.7[a]	1,740[d]
Refrigerated tractor-trailer	2.3[b]	23,400[e]
Refrigeration unit	1.9[c]	N/A

[a] Bureau of Transportation Statistics (2009)
[b] Langer (2004)
[c] Anon (2006)
[d] Estimated according to egg crate dimensions and pick-up carrying capacity
[e] Personal Communication, Dr. Robert Taylor, Jr., University of New Hampshire, May 2009

To test the robustness of this example, three additional modifications were examined (also depicted in Figure 6): the purchase of two dozen eggs from the grocery store; improving fuel efficiency; and reducing distance traveled from the home to the local farm source. One of the most effective means to reduce fuel consumption per dozen eggs is to purchase two dozen eggs. Because so much of the fuel use is by the auto, purchasing 2 dozen eggs, doubling the carrying capacity of the auto, cuts the fuel consumption per dozen eggs by almost 50%. Using the farmer's market scenario, we examined the impact of improving fuel efficiency for the automobile and pickup truck to 56 km/l and 35 km/l respectively (representative of some of the more fuel-efficient vehicles available on the market today) *vs*. average fuel efficiency used in the baseline scenarios. This improved the overall fuel use per dozen eggs by 36%. Finally, given the fact that the automobile only carries one dozen eggs, distance traveled becomes an extremely important factor in determining how close to home 'local' eggs have to be in order to be more fuel-efficient and eco-friendly to obtain than grocery store eggs. As shown in Figure 6, even when the local eggs are only 0.8 km (3.2 km) further away than the grocery store (2.4 km), the grocery store eggs are still more eco-friendly. Similar results were reported by Coley et al. (2009) in a comparison between large-scale vegetable box delivery *vs*. consumers driving to purchase vegetables from an on-farm store.

This example demonstrates that as a result of high capacity cargo volumes in modern transportation systems, food can be efficiently moved over long distances and remain highly fuel efficient and thus environmentally friendly compared to locally-grown food. This has important consequences when considering how to feed people in high-density population centers (e.g. large cities) where buying locally-produced food is not an option. These results also strongly suggest that food should be grown where the agricultural resources and capacity are most suited to efficient food production rather than converting low-yielding land that is better suited for other purposes such as human occupation or wildlife habitat. It is not sufficient to judge miles travelled to determine the cost, fuel efficiency, and eco-friendliness of food transport. A much more detailed LCA

is required. The approach illustrated in this paper only demonstrates the most basic of considerations that must be considered.

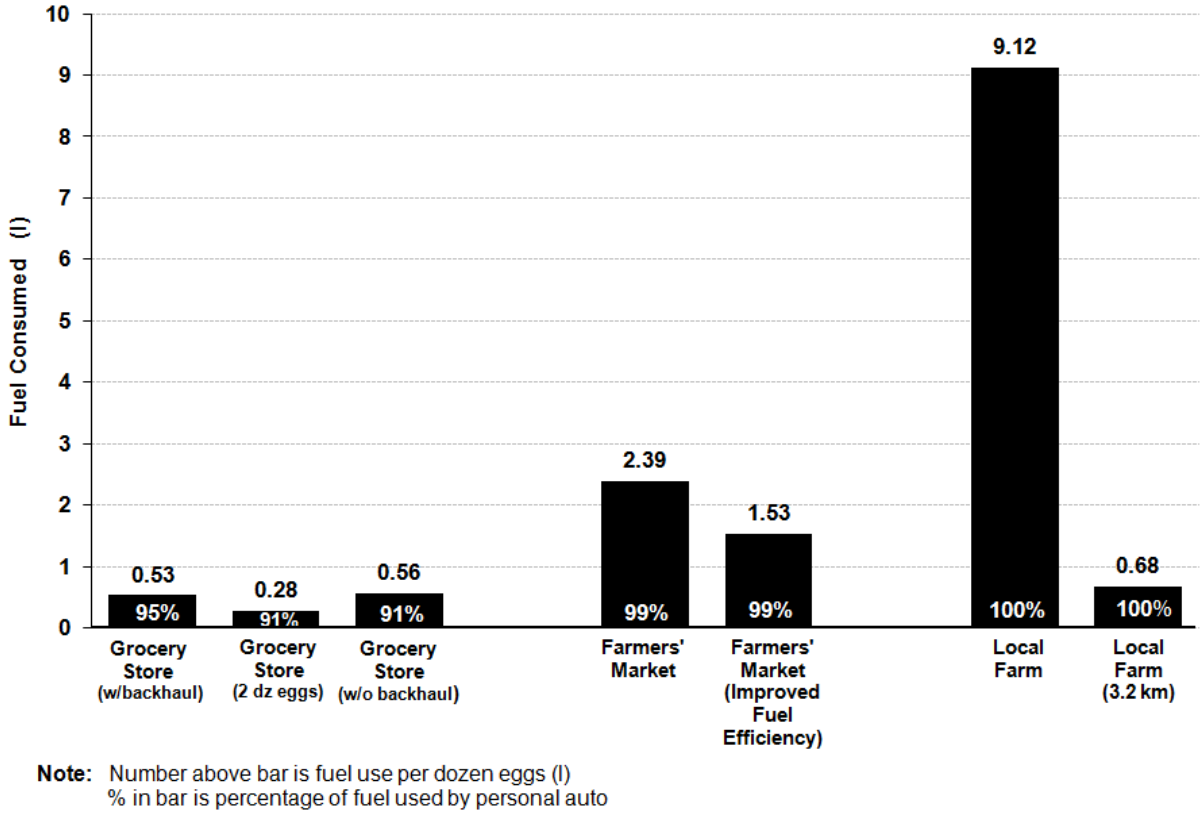

Figure 6. Fuel Consumed per Dozen Eggs to Move Eggs from Source to Home

CONCLUSION

The environmental impact of livestock production is an issue that will remain high on the consumer, producer and political agendas for the foreseeable future. This will be of particular importance as the population continues to increase, leading to a greater dichotomy between the amount of food required to meet the nutritional needs of humans and the resources available for food production. Environmental impact and options must therefore be evaluated using whole-system approaches based on productivity, rather than allowing ideological principles, based either on naïve or incomplete misinformation or a lack of understanding, to direct food production practices. All attempts to mitigate environmental impact are laudable in intent. However, attention should be focused on strategies that make a long-term, positive contribution to enhancing sustainability, rather than focusing on ‘quick-win’, low impact solutions.

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AN EVALUATION OF MILK FATTY ACID COMPOSITION OF WHOLE FLUID MILK IN THE UNITED STATES

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INTRODUCTION

Milk and dairy products are excellent dietary sources of essential nutrients including high quality protein, energy, and many minerals and vitamins (Huth et al., 2006). Fat is the most variable component of milk and its fatty acid (FA) composition accounts for many of the physical properties, manufacturing characteristics and organoleptic qualities of milk and dairy products. However, milk fat is considered "bad" by many consumers because the saturated fatty acids (SFA) and *trans* fatty acids (TFA) in milk fat are associated with perceived negative effects on human health, especially coronary heart disease (CHD). On the other hand, milk fat is an excellent source of oleic acid and recent research indicates that conjugated linoleic acid (CLA) and the long chain omega-3 fatty acids may have potential beneficial effects in health maintenance and the prevention of chronic diseases (Bauman and Lock, 2006a).

Consumers are increasingly aware of the connection between diet and health, and scientists are being asked to clarify the role of specific food components in health maintenance and disease prevention. From these investigations it has become clear that broad generalizations about fat are inappropriate because they are often misleading or inaccurate (Bauman and Lock, 2006a). Rather, one must consider the biological effects and nutritional value on the basis of individual FA. The most recent survey of the FA composition of the U.S. retail milk supply was in 1984 (Barbano, 1990). In the interval since that survey, there have been changes in the management and feeding of dairy cows and some of these may impact milk fatty acid composition, for example the increased use of total mixed rations (TMR) and the increased feeding of fat supplements and byproducts (Jensen, 2002).

The fatty acid composition of milk fat provides valuable information that is used in nutritional assessments and the development of U.S. dietary recommendations. For example, data from the National Health and Nutritional Examination Survey (NHANES) is used by U.S. Department of Health and Human Services, Centers for Disease Control and Prevention to estimate the dietary intake of fats and fatty acids for the U.S. population (Ervin et al., 2004). The fat type and FA composition of foods is also of interest to consumers in comparing different food sources. The modern dairy case includes milk products labeled on the basis of production practices. Specialty labeling of milk is gaining attention and many consumers assume some labeled milks are more nutritious and healthier than others; however, actual nutritional and fatty acid differences among these retail labeled milks have not been adequately evaluated. In the following sections we will first provide background on milk fatty acids. We will then present preliminary results from two surveys that detail the fatty acid composition of the U.S.

retail milk supply. The first represents a survey that examines the FA content of conventionally produced milk that was obtained from processers across seasons and geographic regions in the contiguous 48 United States. The second survey examines the FA content of specialty labeled milk obtained from grocery shelves across the U.S., specifically comparing retail milk labeled as “organic” or “rbST-free” with unlabeled, conventional milk.

BACKGROUND

The FA in milk fat originate from two sources, *de-novo* synthesis and the uptake of preformed FA from circulation. *De-novo* synthesis occurs in the mammary gland and this process produces the short and medium chain FA (C4 to C14 plus one-half of the C16 carbon FA). The preformed FA, representing the other half of C16 and all FA >16 carbons, originate from gut absorption or mobilization from body fat stores. The milk FA content and composition is easily influenced by diet and this has been extensively studied using a variety of dietary components including lipid supplements, byproduct feeds, and forage sources (see reviews by Palmquist et al., 1993; Chilliard et al., 2001; Jensen, 2002). The main focus for many of these feeding studies was to increase the PUFA content of milk fat including CLA and omega-3 FA. The rumen environment is sensitive to changes in dietary components due to the dynamic response of microbial populations to the toxic properties of PUFA (Palmquist et al., 2005). Therefore, extensive biohydrogenation of PUFA occurs in the rumen so that rumen outflow of lipid is predominately SFA. However, some biohydrogenation intermediates escape the rumen without being completely hydrogenated; CLA and it’s endogenous precursor, vaccenic acid (*trans*-11 18:1; VA) are two examples. In the mammary gland, VA is converted to CLA via Δ-9 desaturase, and this endogenous conversion is responsible for 70-90% of CLA secreted in milk fat (Bauman and Lock, 2006b).

Nearly all of the human dietary intake of CLA originates from ruminant milk and meat products. When consumed as a natural component of the diet, CLA has been shown to have potent anti-carcinogenic and anti-atherogenic effects in biomedical studies using animal models of human disease as well as human epidemiological studies (Bauman et al., 2006). The CLA content in bovine milk is affected by a number of physiological factors such as breed and stage of lactation, but the major factor is diet composition (Bauman and Lock, 2006b). Over the past few decades, researchers have worked to develop methods that will sustain an enhanced CLA content in milk fat. Some dairy and beef food products currently in the marketplace have label claims of enhanced CLA, even though the ‘baseline’ value of a typical, current CLA content in the overall U.S. milk supply is unknown. Without this knowledge, comparisons across food products and research results with enhanced CLA content cannot be accurately or appropriately compared. Thus, human dietary recommendations and labeling for all dairy products would benefit from a detailed analysis of the FA composition of current U.S. retail milk.

Omega-3 FA have also been studied in milk fat due to their bioactive properties in the prevention of acute and chronic diseases. Alpha-linolenic acid (ALA) is the predominant omega-3 FA in milk fat, and an essential nutrient in the human diet.

Elongation and desaturation of ALA provides synthesis of very long chain omega-3 polyunsaturated fatty acids (VLC n-3 PUFA), of which eicosapentaenoic acid (20:5; EPA) and docosahexaenoic acid (22:6; DHA) are involved in the prevention of CHD and metabolic and neurological dysfunction (Yashodhara et al., 2009). Current U.S. consumption of omega-3 FA is less than half that recommended by the American Dietetic Association (Gebauer et al., 2006; Kris-Etherton et al., 2009). Thus, increasing the omega-3 content in dairy products may provide an additional food source of these essential FA in the human diet and increase total omega-3 consumption (Whelan and Rust, 2006).

Not all fatty acids in milk are considered favorable. Saturated FA and TFA have been implicated as negative factors in the occurrence of CHD, However, recent investigations have challenged this perception (e.g. see reviews by German et al., 2009 and Parodi, 2009). Milk and meat products contain ~60% SFA and those of concern for human health are lauric (12:0), myristic (14:0) and palmitic (16:0) acids. These SFA were directly studied for individual effects on cholesterol and CHD risk in animal models of human disease, as well as epidemiological and cohort studies. When using updated measures of CHD risk, results indicate that these SFA once considered strongly detrimental to health are now neutral to CHD risk (Mensink et al., 2003; Parodi, 2009).

In the 1970s, when the 'detrimental' effects of SFA were first reported, the food industry developed partially hydrogenated vegetable oil (PHVO) as a replacement for butter and lard. PHVO contains high levels of TFA and it was assumed they would have a lower risk of causing CHD than SFA. However, over the last two decades, research has clearly established that intake of industrial TFA are strongly correlated with increased CHD risk (Willett and Stampfer, 1993; Mensink et al., 2003). Ruminant fat also contains TFA and initially it was assumed this natural source of TFA would also be associated with an increased risk of CHD. These two sources differ in their TFA isomer profile and recent work has indicated that the natural TFA source from the intake of ruminant derived foods does not represent a risk for CHD (Jakobsen et al., 2008; Stender et al., 2008). Nonetheless, *trans* fats have been highly publicized as fats to avoid, and this recommendation unfortunately affects dairy and meat products, as they contain the natural *trans*-11 isomer, which has anti-carcinogen properties and is the precursor to CLA.

SURVEY OF MILK FATTY ACID COMPOSITION IN THE U.S.

Assessment of dietary intake is an important part of monitoring the nutritional status of the U.S. population and this includes fats and FA (Ervin et al., 2004). Thus, the composition of milk fatty acids is of interest because of human dietary considerations, as well as the effect of milk FA profile on manufacturing properties of dairy foods. The last survey of the U.S. milk supply utilized retail milk samples obtained in 1984; a portion of these results were reported at the 1990 Cornell Nutrition Conference (Barbano, 1990) and the detailed milk fatty acid results were reported in a review by Palmquist et al. (1993). Jensen (1999) recognized the lack of comprehensive data for the fatty acid composition of U.S. dairy products and concluded "we cannot make

reliable recommendations about the health aspects of milk fat unless we know the kinds and amounts of fatty acids therein". More recently, Jensen (2002) summarized the milk fatty acid composition data reported in Journal of Dairy Science publications over the period of 1995 to 2000; this summary, however, reflected data from various experimental treatments rather than the FA profile of the retail US milk supply.

Since the 1984 survey, there have been a number of changes in industry practices that may impact milk fat (Palmquist et al., 1993; Chilliard et al., 2001; Jensen, 2002). Holsteins are the predominate breed in today's dairy herds and the use of TMR and dietary lipid supplements have become common place. By-products from the feed and food industries are routinely used in least cost ration formulations and their type and availability can vary by geographic region and season. In addition, analytical procedures for lipid analysis have improved, allowing greater detail of the fatty acid profile, especially for CLA and TFA. Knowledge of the current U.S. milk fat composition will also provide valuable information to address consumer interests and concerns about saturated fat, *trans* fats, and bioactive FA such as the omega-3 fatty acids and CLA. Hence, our objective was to survey the FA composition of U.S. fluid milk supply and provide an updated profile of retail milk fat composition. We were also interested in possible seasonal and regional effects on the U.S. retail milk supply and these aspects were included in our experimental design.

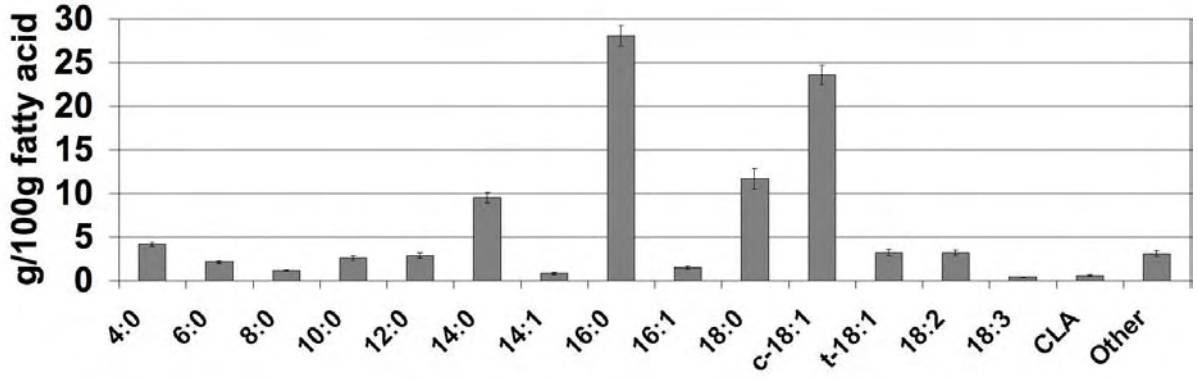
To address our objective, we collaborated with Dr. David Barbano (Department of Food Science, Cornell University) and obtained whole fluid milk samples from 56 milk processing plants across the contiguous 48 United States. All milk samples were homogenized, pasteurized and packaged for transport to retail stores. Milk samples were conventionally produced; UHT milk and specialty labeled milk, such as 'rbST-free' or 'organic' were excluded. Processing plants were selected based on the criteria that they represented the major volume of milk produced in that area, and samples were obtained from each plant every 3 months for one year to capture seasonal variation. Milk was shipped on ice overnight to Cornell University and immediately analyzed for fatty acid composition. Following procedures routinely used in our laboratory, milk fat was extracted, fatty acids were methylated and the resulting fatty acid methyl esters were analyzed by gas chromatography. Statistical analysis was performed using JMP 7.0 (SAS Institute Inc., Cary, NC); FA means and SD are reported.

The overall fatty acid composition for the retail milk samples is presented in Figure 1A. We found statistical differences for some individual FA among geographic regions and seasons (data not presented), but these were uniformly of a small magnitude that represented no consequence for human nutrition or manufacturing characteristics. Dietary fat is often classified based on the predominant types of FA that make up the food. For the overall profile of the milk samples in the present study, SFA, monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) represented 63.7, 29.1 and 4.1% of total fatty acids, respectively (Figure 1B). Oleic acid averaged 23.6% and total TFA represented 3.2% of total milk FA with *trans*-11 18:1 (vaccenic acid; VA) as the major TFA isomer (Figure 1C). VA is the endogenous precursor to *cis*-9, *trans*-11 CLA, and it represented ~47% of the *trans*-18:1 isomers. Alpha-linolenic acid

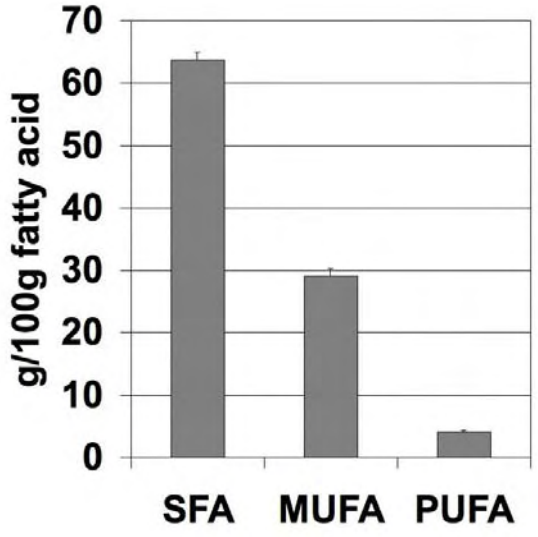
(0.39g/100g FA) was the predominant omega-3 FA and *cis*-9, *trans*-11 CLA (0.56g/100g FA) was the predominant CLA isomer. The omega-6 to omega-3 ratio was 8.5, which compares favorably with the recommended ratio of >10:1 (Simopoulos, 2002).

Figure 1. Whole fluid milk fatty acid composition in the U.S.

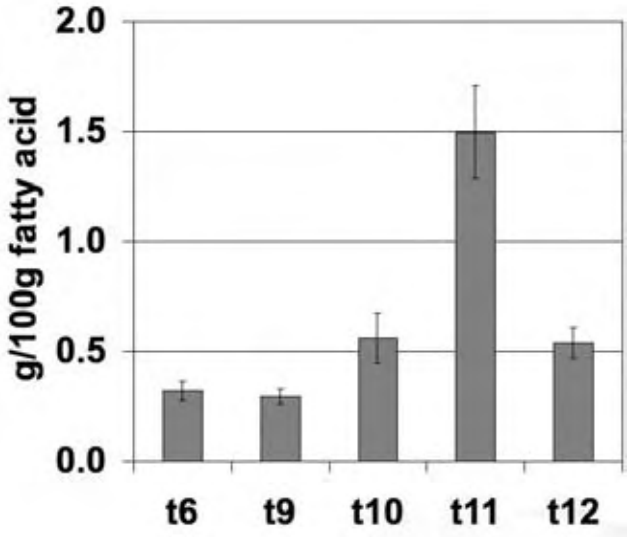
A. Overall milk fatty acid profile



B. Degree of saturation



C. *trans*-18:1 fatty acids



Diet is a major factor affecting the fatty acid composition of milk fat and based on the relatively constant FA composition we observed, the increased use of TMR, lipid supplements and by-product feeding must be well established in all regions and across all seasons. Some differences between individual farms would be expected, but the milk from any farm implementing unique feeding or management practices would be diluted by the pooling of milk at the processing plant. Our focus was on retail milk and for this reason we purposefully obtained processed and packaged milk samples to more accurately reflect milk available to the everyday consumer.

The values we observed for SFA, MUFA and PUFA were remarkably similar to the values reported for the milk samples obtained in 1984 (63.8, 33.5 and 2.8% of total FA, respectively; calculated from Palmquist et al., 1993). In the current study, SFA was

nearly identical whereas MUFA was decreased slightly and PUFA was increased slightly. There were also minor differences in proportions for individual FA isomers, and overall *de novo* fatty acids (C4 to C16) were lower compared to 1984, suggesting increased use of lipid supplementation and byproduct feedstuffs. However, it is important to note that analytical techniques were not as advanced to identify 18:3 unsaturated FA or longer chain FA. This would likely result in an under-reporting of PUFA and a corresponding overestimation of the proportion of SFA and MUFA. In addition, the co-elution of several FA probably occurred with the techniques used in analyses of the 1984 samples; for example, 14:1 with 15:0, and 16:1 with 17:0, probably co-eluded thereby increasing the estimate of MUFA. Nevertheless, our results indicate that the fatty acid composition of milk samples in the current survey was relatively constant across seasons and geographic regions and similar to the milk FA profile in 1984.

SURVEY OF SPECIALTY LABELED MILK: CONVENTIONAL, rBST-FREE AND ORGANIC

There is a growing trend to label foods according to agriculture management or production practices. For example in the dairy case, milk may be labeled as “certified organic” or “rbST-free”. This type of labeling represents an effort to increase market share, and these specialty labeled products are generally more expensive. However, to some consumers the specialty label implies the food product is more nutritious or of higher quality. Thus, labeling creates a consumer perception based on retail marketing rather than on actual differences that can be documented by scientific tests. All milk produced in the U.S. is regulated by strict production and processing standards as set forth by the Food and Drug Administration. These regulations were established so that all milk, regardless of the production system, is safe, natural and nutritious. The Pasteurized Milk Ordinance and the Hazard Analysis and Critical Control Point system are designed to maintain milk quality and identify any potentially harmful contaminants in milk before shipment for human consumption. Multiple-stage testing occurs for antibiotics and there are additional tests for pesticides and other foreign contaminants.

While the existence of organic or rbST-free milk does not represent an improvement in the safety of milk products, it does provide an alternative method of dairy production for niche market consumers. Organic milk producers are required to follow management guidelines restricting use of antibiotics for the treatment of sick animals, use of pesticides in the production of feedstuffs, and other management technologies including transgenic products such as recombinant bovine somatotropin (rbST) or genetically modified crops (USDA Organic Guidelines, www.ams.usda.gov/nop/NOP/standards/ProdHandReg.html). rbST is a biotechnology product that increases the productive efficiency by diluting maintenance costs so that dairy cows produce more milk with less feed resource input and lower animal waste output per unit of milk (Bauman, 1999). Since its approval for commercial use in 1993, over 30 million U.S. cows have received rbST supplements. However, there is no test or milk difference that can detect rbST use; thus, the “rbST-free” label is based on a farmer affidavit indicating that this supplement is not used.

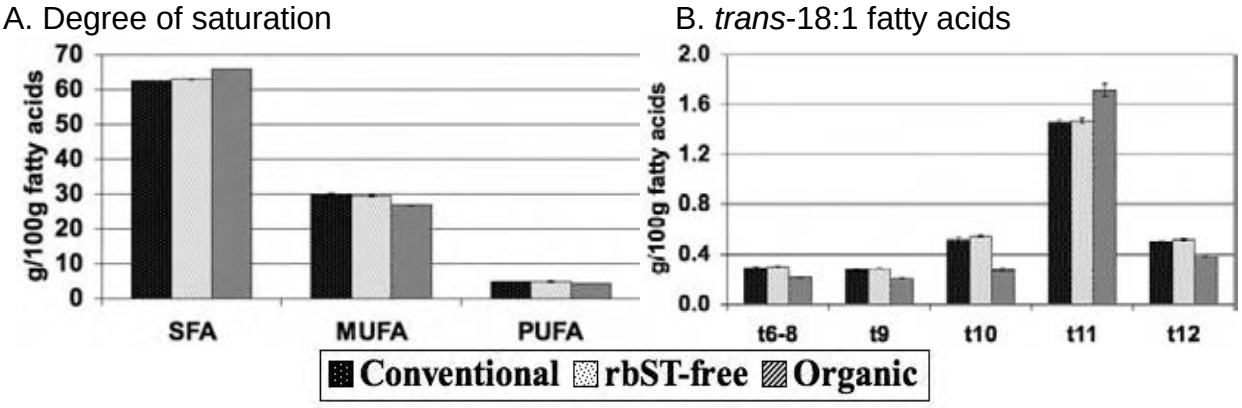
Very few peer-reviewed scientific studies have investigated the potential nutritional or quality differences for specialty labeled food products. An exception was the recent study by Dangour et al. (2009) that involved a systematic review of the nutritional quality of organic foods, including meat and dairy products. They found that there was no evidence of a difference in nutrient quality between organically and conventionally produced foodstuffs. Comparisons involving samples at the retail level are of special interest and recently Vicini et al. (2008) evaluated retail milk labeled as organic, rbST-free and conventional as to quality (antibiotic and bacterial counts), nutritional value (protein, fat and solids-not-fat) and hormone composition (somatotropin, insulin-like growth factor-1, progesterone and estradiol). They found the milks were similar in composition with no meaningful differences related to label claims. We were interested in extending these investigations to the FA content because some milk FA are bioactive and can effect human health maintenance and the prevention of chronic diseases. Our specific objective was to analyze the FA content of retail milk labeled as organic or rbST-free and compare results with unlabeled conventional milk.

To address our objective we collaborated with Dr. John Vicini (Monsanto Company) and utilized milk samples reported in the study by Vicini et al., (2008). Briefly, milk samples (n = 292) were obtained from stores within the 48 contiguous United States during a 3 week period in October and November 2006. The retail milk samples were purchased in blocks that consisted of one of each label type: conventional, rbST-free, or organic. More details on the selection of stores and sampling are given by Vicini et al., (2008). Milk samples referred to as conventional represent retail milk samples that had no label claims about rbST-supplementation or organic production practices. Milk labeled “rbST-free” included a statement on the label that cows received no supplements of rbST. Milk labeled as “organic” came from farms that were certified to meet the USDA standards for organic production practices. Aliquots from each retail milk sample were frozen at -20°C and shipped overnight on ice. Following routine procedures in our laboratory, milk fats were extracted and methylated; fatty acid methyl esters were then analyzed by gas chromatography. Statistical analysis (JMP 7.0, SAS Institute Inc., Cary, NC) included treatment (conventional, rbST-free or organic) as the main effect and block as a random effect. Least-squares means are reported using Tukey’s test for detecting differences. In the case of unequal variances as detected by Levene’s test, variables were analyzed by SAS 9.0 (SAS Institute Inc., Cary, NC) to allow reporting of individual variance estimates for each treatment group. Significance was declared at $P < 0.05$.

Milk fat from the three retail groups of samples was nearly identical in composition. Conventional and rbST-free milk fatty acids were not statistically different, thus values presented in the text represent an average of these two milks, compared to organic milk. Milk labeled as ‘organic’ was significantly higher in SFA (65.9% vs 62.8%) and lower in MUFA (26.8% vs 29.7%) compared to conventional and rbST-free milk. This pattern is less desirable from a public health perspective, but the differences were relatively minor (Figure 2A). Overall the milk fat content of TFA was similar across milk label type with TFA representing about 3.0% of total FA. There were minor differences among the milks in the *trans* 18:1 isomer distribution with organic milk having a slightly

higher *trans*-11 18:1 and lower content of the other *trans* 18:1 isomers (Figure 2B). However, differences among the TFA isomers were minimal indicating the relative proportion of different isomers was conserved across label type. Thus, for these fatty acid groupings all milk was similar regardless of production practice; from a practical use or public health relevance any differences were minor and of no physiological importance. Recommendations in the Dietary Guidelines for Americans are to limit the intake of SFA and TFA because these FA have been associated with a higher risk of CHD (NRC, 1989; USDHHS/USDA, 2005). To a large extent this has been the basis for the perception that milk and dairy products are associated with a higher risk of CHD. However, as discussed earlier, recent epidemiological studies and dietary intervention trials challenge this perception and available evidence does not support the concept that consumption of dairy products adversely affects the risk of CHD (Bauman and Lock, 2006a; 2008).

Figure 2. Milk fatty acid composition of organic, rbST-free and conventional milk.

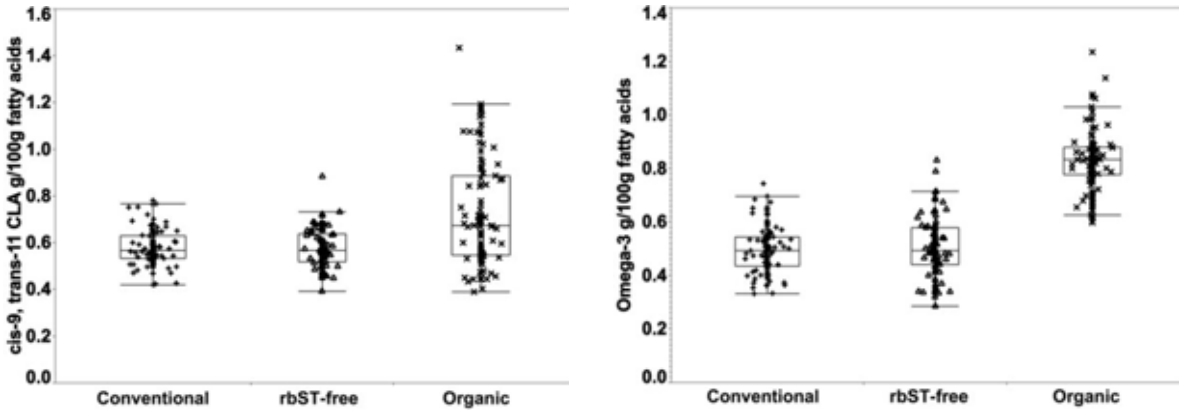


There were statistical differences in the milk fat content of CLA and omega-3 FA with values being greater in the organic labeled milk (Figure 3); however, differences were too small to have any human health implications. In the case of CLA, extrapolations from the cancer studies with animal models indicate that the CLA level needs to be 2- to 5-fold greater than that found for the organic, rbST-free or conventional milks to achieve the cancer preventative effects (Bauman et al., 2006). Likewise, the milk levels of omega-3 FA are too low to be an important source of omega-3 in human diets and differences are of no consequence in meeting the daily requirement for omega-3 FA (Kris-Etherton et al., 2009). Furthermore, the health benefits from omega-3 FA are related to the VLC n-3 PUFA, specifically EPA and DHA (Yashodhara et al., 2009; Lavie et al., 2009). The major omega-3 FA in all of the milk samples was alpha-linolenic acid and unfortunately humans have a very limited ability to convert this FA to VLC n-3 PUFA (Arterburn et al., 2006; Brenna et al., 2009).

There are several previous studies that have compared the CLA and omega-3 FA content of organic and conventional milk, but these have typically involved comparisons for a few specific farms rather than an examination of the consumer supply of retail milk. Some of these investigations have reported greater CLA content in organic milk

(Bergamo et al., 2003; Kraft et al., 2003), while others report differences in the milk content of omega-3 FA but not in CLA (Ellis et al., 2006; Molkentin, 2009). These inconsistent results are most likely related to the use of controlled, farm-level experiments rather than retail milk that would reflect consumer supply. In practice, organic farms as well as conventional farms can vary widely in feeding and diet formulations. As diet of the dairy cow is a major determinant of milk fatty acid composition, variation in diet among farms may provide an explanation of differences reported between organic and conventional milk. This was clearly demonstrated in a study by Molkentin (2009) who compared the omega-3 FA milk fat content of retail organic and conventional milk, as well as one individual organic farm to show variation on a biweekly basis for 18 months. The organic farm sample provided the highest and lowest omega-3 FA value for all organic retail samples over the duration of the study, illustrating the immense variation that exists with single-farm sampling. Molkentin also concluded that omega-3 FA differences in retail samples are related to the seasonal change in feed composition caused primarily by the temporary availability of fresh pasture, and the use of corn silage and concentrates by conventional producers. Nevertheless, across all studies that have compared organic with conventional milks, any differences that were observed in FA content were small and not of magnitude to have human health implications.

Figure 3. Range of variation among milk types for CLA and omega-3 FA



An interesting observation in the current study was the presence of unequal variances for several fatty acids in the organic group. This was particularly evident for CLA and omega-3 FA as shown in Figure 3. As mentioned previously, these two fatty acids are highly responsive to changes in the feed quality and composition of dairy cow rations. Considering the changing seasons and differences related to geographic region over the period when samples were obtained, the variation may be explained by differences in feedstuffs and reduced availability of fresh pasture for some organic producers. Butler et al., (2008) recognized the need for organic or low-input systems to find a way to increase concentrations of bioactive fatty acids during the indoor/winter feeding period if year-round grazing is not available. Organically produced milk is marketed with the idea that cows are continuously grazed on pasture, thus increasing CLA and omega-3 in the milk. However, it is evident from the range of variation that

diets varied on organic farms and some probably had greater access to lush pasture than others.

SUMMARY

The current U.S. milk supply consisted of 63.7, 29.1 and 4.1% SFA, MUFA, and PUFA, respectively. Compared to 1984, FA composition was remarkably similar, even considering analytical differences. Although feeding practices have changed significantly to include new feedstuffs and management tools, milk FA composition was quite uniform. In addition, we found seasonal and regional differences were minimal, suggesting consistent use of TMR and feed supplements on a national level. We also found that the FA composition of milk labeled as organic, rbST-free and conventional was similar with no differences of physiological importance among these specialty labeled milks. However, there was greater variation in the CLA and omega-3 FA content among the organic milk samples, and this presumably relates to variation in the proportion of grazing versus other feed components utilized by organic certified farms.

While there were no nutritionally important differences in the milk fatty acids composition among the retail milks labeled as organic, rbST-free, and conventional, it is possible to produce milk with enhanced CLA and omega-3 FA concentrations that would be physiologically significant for the human nutrition and health industry. The dietary components to achieve these enhanced levels are available to dairy producers and can be used as TMR ingredients. However, application will require a group of producers to follow similar practices to ensure a consistent FA enhancement. In addition, marketing and pricing of this milk will need to provide incentives to the dairy farmer to off-set the increased costs of production. Lastly, and most importantly, caution is warranted against labeling milks in such a manner that may lead to a consumer perception that some milk is ‘bad’ while others are ‘good’. All milk is wholesome and nutritious, and specialty products should exist with the goal to increase overall dairy consumption.

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USING FORAGES IN DAIRY RATIONS: ARE WE MOVING FORWARD?

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INTRODUCTION

Forage was the main ingredient of dairy cow diets until it was discovered in the 1960's that cows responded to increased concentrate feeding by producing a lot more milk. In addition grain surpluses and low grain prices of the past often dictated that milk be produced with as little forage as possible. Welcome to the present. Diversion of grain to ethanol has eliminated grain surpluses. A growing population and a population that demands higher protein diets has increased demand for grains world-wide. Impact of animal wastes on the environment has called for more sustainable systems to be developed, which will result in fewer imported nutrients being allowed onto the farm. Detailed attention to all aspects of the dairy operation has become critical, and forage quality and use have become more important than ever. Managing for high quality forage with proper harvesting and fertilization is one way of mitigating rising costs of doing business and as potential solutions to environmental management challenges now encountered on intensively managed dairy farms.

FORAGE MANAGEMENT FOR QUALITY

Higher milk production has placed an emphasis on ration balancing and the use of forage and feed testing. Fortunately improved forage quality evaluation techniques and tools have improved along with higher milk production and have demonstrated that cows can produce relatively large volumes of milk on diets with a high proportion of quality forage. Forage quality definitely has a relevant role in the future of the dairy industry.

We need to harvest forage grasses and legumes to optimize the fiber content for the class of livestock being fed; therefore neutral detergent fiber (NDF) is the most useful harvest date target. There is a relatively small range in optimal NDF for lactating dairy cows, making correct harvest management decisions critical relative to quality. A reliable method to estimate the fiber content of grass and alfalfa-grass mixtures would help producers in timing harvesting operations to optimize the quality of the harvested forage. Once the forage is harvested and stored, an accurate forage quality analysis is needed prior to ration balancing (Cherney and Cherney, 2003).

Alfalfa-grass mixtures do and will continue to have large impacts on forages in NY. A study was undertaken recently to develop a system for estimating standing NDF of alfalfa-grass mixtures (Cherney et al., 2006) and for estimating alfalfa NDF in mixtures (Parsons et al, 2006). Figure 1 shows the estimated optimum NDF of standing forage at harvest for mixtures and pure alfalfa or pure grass. This is based on the assumption that

the optimum standing NDF is 38% for alfalfa and 50% for grass. Selecting different values would change the slope of the line in Figure 1. These goals assume a 10-15% decline in forage quality due to harvest, storage and feedout.

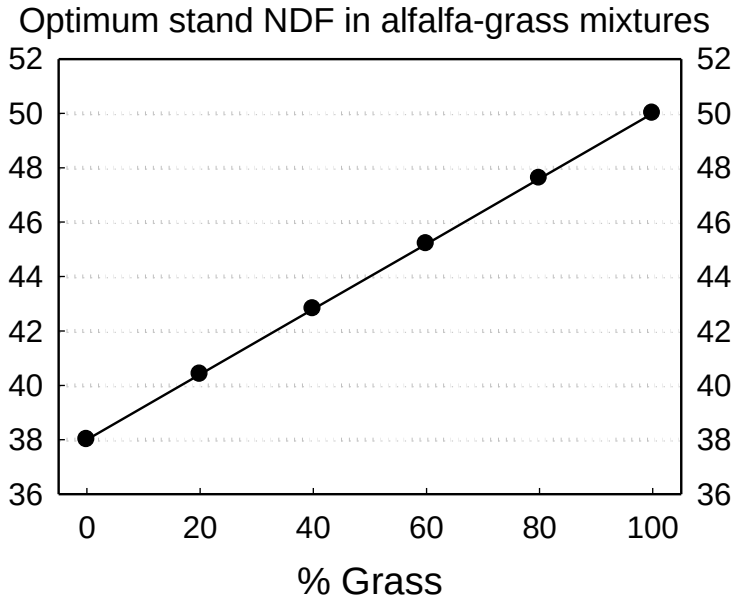

Figure 1. Optimum NDF of standing forage, assuming pure stand optimums of 38% for pure alfalfa and 50% for pure grass.

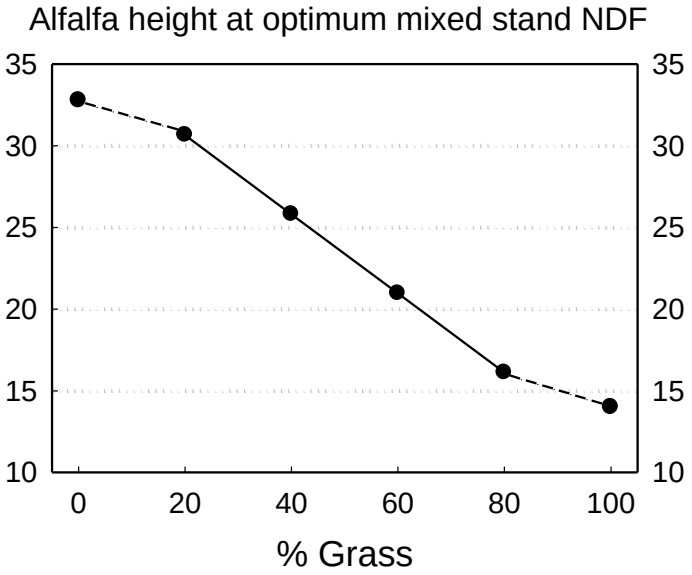

Figure 2. Alfalfa height for optimum NDF of standing forage, assuming pure stand optimums of 38% for pure alfalfa and 50% for pure grass.

FORAGE QUALITY ASSESSMENT

Models, such as the Cornell Net Carbohydrate and Protein System, and ration balancing programs are increasingly used by consultants and producers to predict animal performance. These models and programs rely heavily on chemical and in vitro characterization of feeds. Because forage plays such a large role in dairy rations, animal performance could be improved through timely and improved forage analyses.

Animal performance is a function of intake and digestibility. Attempts to accurately predict intake routinely have met with a lot of frustration, in part because routine laboratory methods do not measure the characteristics of forage which are the true determinants of differences. So what forage analyses would measure those forage characteristics that relate most closely to intake and digestibility? Chemical fractions that have been associated with intake and digestibility include fiber and lignin.

A symposium held at the recent Joint Meetings of the ASAS, ADSA and CSAS discussed some of these emerging techniques for predicting forage quality in terms of fiber and lignin. Methods to analyze for NDF and ADF have been around for about 50 years while methods to analyze for lignin and protein have existed for more than 100 years. So what's new with these analyses that can help improve animal performance?

Because different factors affect intake and digestibility, and intake and digestibility are not always well related, we really need separate equations for both intake and digestibility. New equations for intake and for total digestible nutrients, like those used in the Dairy NRC, have shown promising results. These equations relay on accurate analysis of NDF digestibility. NDF digestibility can be estimated by lignin concentration and in vitro NDF digestion.

Lignin's value is in its relationship to digestibility or indigestibility. Understanding the causal factors of the negative relationship of lignin to digestibility has proven difficult. Advances in understanding this causal relationship will help to improve predictions of intake, which will improve performance. NDF fermentation can be broken down into three components: a fast digestion pool, a slow digestible pool and an indigestible pool. Van Soest et. al., (2005). observed that estimation of NDF, lignin and fermentation at 24 hours would enable the prediction of extent of digestion at any time of fermentation from a single fermentation at 24 hours.

In vitro gas production techniques have received a lot of attention lately. They have been used with success for digestibility kinetics, but to date have not been shown to be any better than traditional measures for predicting intake. New research with combined traditional measures of digestibility with information on gas production rates show increased accuracy of prediction over either newer gas methods or conventional in vitro methods for predicting intake (Pell and Schofield, 1993). The initial gas box methods of Pell and Schofield have been commercialized and are being used world-wide to evaluate forages.

It is clear that fiber and lignin assays will continue to be important, due to their strong association with factors affecting animal performance. Improved understanding of relationships of chemical constituents to digestibility and intake will improve models used to predict performance and Cornell will continue to be at the forefront of these developments.

ANIMAL TRIALS

We have conducted a number of studies in recent years that have evaluated the role of grass in high producing dairy cow diets. In one set of studies cows fed alfalfa or late-cut orchardgrass of similar ADF and indigestible NDF content had similar DMI when diets were formulated for similar NDF, ADF, and CP (Cherney et.al., 2002b). This supports previous research that orchardgrass can be an acceptable component of dairy cattle rations, although diets containing mature orchardgrass will necessarily require higher levels of concentrate to maintain milk production. Early-cut high quality orchardgrass had lower fiber than late-cut orchardgrass and higher fiber digestibility. Despite the similarity of NDF, ADF, lignin, and indigestible NDF contents in the diets of cows, those cows fed diets containing early-cut orchardgrass had higher DMI and milk production. This illustrates the importance of managing forages for high quality. Milk production decreased linearly as diet forage content increased from 50 to 80%. The NDF intake remained constant as forage content increased from 50 to 80%, suggesting that when forage source is constant, NDF intake is a reliable predictor of DMI and milk production.

The agronomic yield potential of tall fescue (*Festuca arundinacea* Schreb.) in NY averaged >20% higher than orchardgrass (*Dactylis glomerata* L.) (Cherney et al., 2002a), but there was reluctance to switch to tall fescue, because of potential endophyte problems. We observed that dairy cows consumed the first-cutting endophyte-free fescue TMR readily and performed as well as those on alfalfa or first-cutting, orchardgrass-based TMR in terms of lactation performance, but fescue and orchardgrass rations will require more concentrate in the ration than alfalfa.

Milk production per cow continues to be a major factor in determining dairy farm sustainability/profitability. The inclusion of non-fibrous carbohydrates (NFC) in the range of 35 to 42% of dietary dry matter is seen as a popular way to increase energy. Balance of carbohydrates in the diet impacts milk production because it affects amount and ratios of ruminal volatile fatty acids produced, which in turn alters metabolism and partitioning of nutrients (Cherney 2008). Forages differ in rate of passage and buffering capacity, and this will influence response to level and source of NFC in the diet (Moore et al., 1990). Although there have been few studies in the USA with cows fed predominantly grass-based TMR's, there are a number of European studies regarding how dairy cows on predominantly grass-based TMR's will respond to changes in levels and sources of concentrates. Many of these studies either dealt with perennial ryegrass (*Lolium perenne* L.) as the forage source or included less concentrate in diets than would be required for high-producing dairy cows in the USA (Fitzgerald and Murphy, 1999; Keady et al., 1998). With this in mind, we determined the influence of altering the

level of NFC and replacing some of the high-moisture corn grain with sucrose on milk production and composition of high producing dairy cows (>35 kg/d) fed a fescue-based TMR (Cherney et. al., 2003).

Cows offered the higher level of NFC had milk with lower fat, higher protein and lactose, and lower MUN than those cows offered the higher fiber diets. Feeding diets with higher fiber levels resulted in lower efficiency of N utilization (expressed as milk N/intake N) than did feeding lower fiber levels. Replacing 10% of high-moisture corn with sucrose had little influence on milk production and composition, other than increasing lactose and MUN levels. The higher sucrose diets did, however, have a trends towards lower efficiency of N retention than did the lower sucrose diets (P=0.06). This was observed even with the higher fiber diet. The implications of this research suggest that replacing high-moisture corn with sucrose is not an effective strategy for increasing NFC in the diets of cows fed grass silage TMR. The practice could also have negative environmental impacts, due to a reduction in N use efficiency and N retained.

Here is a summary of results from our Cornell feeding trials with grass over the last 10 years:

- Variations in fiber digestibility of grasses can account for differences in intake and milk production of cows fed diets similar in chemical composition but varying in ingredient composition (Cherney et al., 2002a).

- Grass-based TMRs produced similar quantities of milk as those fed alfalfa-based TMRs (Jonker et al., 2002).

- Dry matter intake increased as the portion of concentrate in diets increased, resulting in higher milk production in high tall fescue diets compared to high alfalfa diets (Cherney et al., 2002b).

- Comparing starch vs. sugar supplementation of grass-based diets, the NFC source did not influence intake or milk production, but sucrose feeding lowered N utilization efficiency, when replacing a portion of the high moisture corn in the diet (Cherney et al., 2003).

- Tall fescue and orchardgrass TMRs performed as well as alfalfa, but grass will require more concentrate in the ration than alfalfa (Cherney et al., 2004).

Using the results from one of our trials above, we evaluated the impact of price on the economic return per cow per day for diets varying in forage to concentrate ratio (Figure 3). All other factors were held constant. Clearly the more forage in the diet, the less impact corn and other off-farm commodities will affect return per cow.

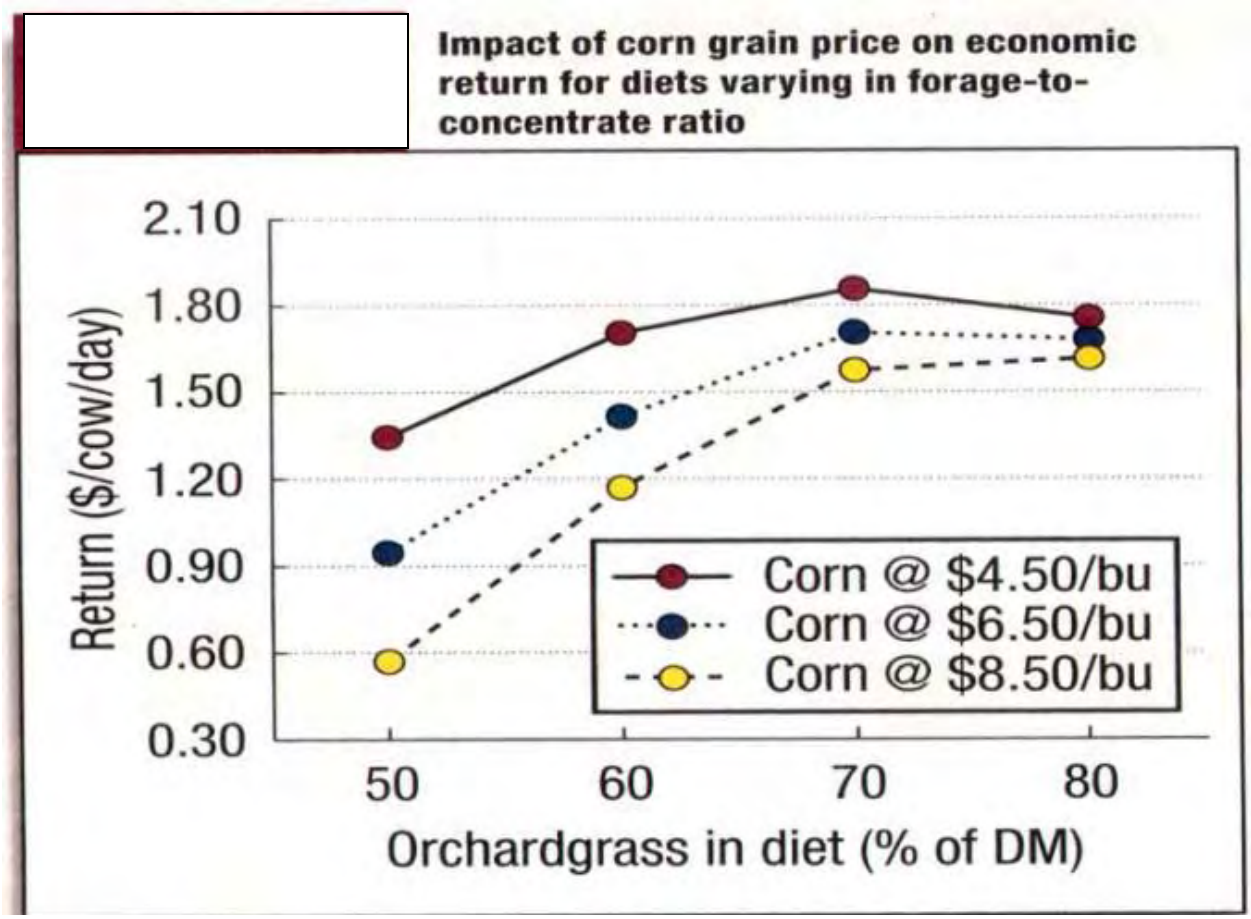

Figure 3. From: Progressive Dairyman, Issue 12, Aug. 11, 2009, pg. 16.

CONCLUSION

Improved management for high quality has made grass and grass-legume mixtures attractive options for dairy producers. Grasses and grass-legume mixtures also have significant nutrient management benefits, particularly regarding manure management. Grass species and variety evaluation should be focused on maximum yield at optimum quality. Once proper species and varieties are selected, harvest management will determine the success or failure of grass as high producing dairy cow forage. Differences in milk production and composition will vary with forage quality and inclusion in diet. Differences in DMI can also be attributed to differences in fiber digestibility and indigestible residue, resulting from lignin differences. So in answer to my opening question, we have come a long way. As our industry continues to change, we will continue doing the type of research that will result in management solutions they can use.

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AMMONIA EMISSIONS FROM DAIRY BARNS: WHAT HAVE WE LEARNED?

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Research, extension, the feed industry and veterinarians have long advocated dairy cow diets that maximize milk production while assuring good animal health and reproduction. Under practical conditions, only 20 to 30% of the crude protein (CP) fed to a dairy cow is converted into milk protein. The remaining feed nitrogen (N) is excreted about equally in urine and feces, although this can be highly influenced by diet. Three-fourths or more of the N in urine can be in the form of urea. Urease enzymes, which are present in feces and soil, rapidly convert urea to ammonia, which is in equilibrium with ammonium. Depending on pH, ammonium is converted into ammonia gas and lost to the atmosphere. Loss of N as ammonia is thought to range from 20 to 55% of total N excreted in manure. The main factors that affect ammonia N loss from dairy barns are dairy cow diets, the type of bedding used, barn ventilation and temperature, and manure handling practices. After release, ammonia is re-deposited as ammonium containing dust particles, which can adversely affect human health, and as acid rain and nitrates which can be detrimental to natural ecosystems. The Clean Air Act amendments of 1990 required USEPA to establish National Ambient Air Quality Standards for pollutants considered harmful to human health. The Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) requires the reporting of the release of a hazardous substance in excess of threshold levels, for example 45.5 kg of ammonia over a 24-h period (Aillery et al., 2006). Although CERCLA is focused on emissions of hazardous wastes from industrial plants, the increased size and geographic concentration of animal feeding operations make their ammonia emissions subject to the notification provisions. We examine impact of dairy cow diets on milk production, feed N use efficiency (proportion of feed N transformed into milk), urine N excretions and ammonia N emissions from dairy barns. We compare the results of various scale methods to estimate ammonia emissions from dairy barns, as well as recent field study results that reveal dairy cattle management impacts on ammonia emission, manure N capture and recycling through crops. We conclude with a synopsis of management practices that enhance feed N use efficiency (FNUE), reduce urine N excretions and ammonia loss from dairy barns.

FACTORS AFFECTING FNUE & URINARY N-EXCRETION

Protein Metabolism in the Rumen

Ruminants obtain the metabolizable protein (MP) required for maintenance and production from microbial protein synthesized in the rumen plus dietary protein that escapes the rumen undegraded. The amino acid (AA) pattern of this protein is better than the feedstuffs commonly fed to domestic ruminants (Schwab, 1996). Although more efficient than most other ruminants, dairy cows still excrete 2 to 3 times more N in

manure than they secrete in milk, even under optimal nutrition and management. Inefficient N utilization necessitates feeding additional protein, increasing costs and contributing to environmental pollution. Optimizing microbial protein formation in the rumen plus feeding only enough rumen-undegraded protein (RUP) to meet the cow's MP requirements are the best strategies for maximizing N transformation into milk.

Optimizing Formation of Microbial Protein in the Rumen

Ruminal bacteria utilize peptides, AA and ammonia for protein synthesis. In vitro research showed that microbial protein formation was not improved by ammonia-N concentrations greater than 5 mg/dL (Satter and Slyter, 1974). However, in situ studies suggested that ammonia-N levels as high as 20 mg/dL (Mehrez et al., 1977) may be required to maximize carbohydrate digestion in the rumen. Ammonia derives largely from deamination of AA released from rumen-degraded protein (RDP) and ammonia production parallels formation of peptides and free AA. The NRC (2001) feeding model assumes that RDP from nonprotein N (NPN) sources, such as urea, are equivalent to RDP from true protein. However, protein degradation products other than ammonia stimulate microbial protein synthesis. For example, Argyle and Baldwin (1989) found that adding only 1 mg/L of protein AA plus 1 mg/L of peptides (from trypsin-digested casein) more than doubled in vitro cell yield of mixed ruminal organisms. Recently, we demonstrated that replacing RDP from true protein (soybean meal; SBM) with that from urea depressed yields of milk, fat and protein; these responses were paralleled by reduced ruminal outflow of nonammonia N (NAN) and total AA that resulted from reduced microbial protein formation (Broderick and Reynal, 2009). The NPN content of the basal diet before urea supplementation in our study accounted for 20% of the total CP (largely from alfalfa silage).

There are substantial differences in ruminal fermentability among dietary sources of fiber (Coblentz and Hoffman, 2009), starch (Herrera-Saldana et al., 1990) and other carbohydrates. For example, effects of reducing corn particle size on extent of ruminal starch digestion are much greater than effects on total tract digestibility (Owens et al., 1986). We found that grinding high moisture corn through a 1-cm screen optimized ammonia uptake in vitro and feeding this high moisture corn ground (with 1.7 mm mean particle size) reduced in vivo ammonia concentration in the rumen and increased milk yield 2.4 kg/d and protein yield 120 g/d compared to control high moisture corn (with 4.3 mm mean particle size) (Ekinci and Broderick, 1997). Reducing mean particle size of dry shelled corn from 3.5 to 0.6 mm increased ruminal starch digestibility from 54 to 70% (Remond et al., 2004). Charbonneau et al. (2006) observed that replacing cracked corn with ground corn or ground corn plus cornstarch increased milk yield 10% and protein yield 14% in lactating dairy cows. Valadares et al. (2000) reduced the ratio of dietary alfalfa silage: concentrate from (DM basis) 80:20 to 65:35, 50:50, and 35:65. Quadratic responses indicated that maximal DM intake and yield of fat-corrected milk (FCM) occurred at 51% concentrate and maximal fat yield at 43% concentrate. However, milk and protein responses were linear rather than quadratic and both were still increasing at 65% concentrate despite low ruminal pH and milk fat depression. The

lactating cow's energy requirements are substantial and optimal dietary concentrate is dictated more by long-term rumen and animal health than by maximum milk production.

The high-energy forage corn silage can be fed to dilute the degradable protein in hay-crop silages. Dhiman and Satter (1997) replaced 1/3 or 2/3 of dietary alfalfa silage with corn silage. Compared to all forage from alfalfa, milk yield and N efficiency were 6% higher over the whole lactation when 2/3 of the dietary forage was alfalfa silage and 1/3 was corn silage. Brito and Broderick (2006) found the greatest improvement in N efficiency without loss of production at about 50% of the forage from alfalfa silage and 50% from corn silage. Part of these positive effects was due to improved microbial protein production in the rumen (Brito et al., 2006).

Optimizing Amount and Composition of Rumen-Degraded Protein

Dietary CP that is not utilized by the cow gets excreted largely as urinary N, regardless of whether the CP is digested in the rumen or the intestine. We fed diets with 3 energy densities (36, 32, and 28% NDF in dietary DM) at each of 3 levels of CP (15.1 to 16.7, and 18.4% of DM, added as solvent SBM) (Broderick, 2003). Cows responded to CP the same at all 3 energy levels: milk and protein yield increased from 15.1 to 16.7% CP, but there were no production differences between 16.7 and 18.4% CP. However, marked increases in urinary N excretion (virtually all as urea) were observed with added CP. In a later trial, dietary CP was increased, in steps of about 1.5 percentage units, from 13.5 to 19.4% CP (Olmos Colmenero and Broderick, 2006). Milk urea N reflected the linear increase in urinary N excretion, and linear decrease in milk N/N-intake, that occurred with elevated CP. Production was highest on 16.5% CP and quadratic responses indicated that milk and protein yields were maximal at, respectively, 16.7 and 17.1% CP. Greater than those levels of CP reduced yields, a surprising result in view of the common practice of feeding lactating cows diets with 18% or more CP (Shaver and Kaiser, 2004). Depressed production at higher CP may have occurred because high moisture corn was replaced with SBM, which diluted dietary energy, and because of the metabolic cost of excreting excess N as urea (NRC, 1989). Most of these trials were short-term reversal studies in which diets are switched after a few weeks. However, Wu and Satter (2000) found that the dietary CP regime supporting optimum yield of fat-corrected milk (FCM) over the whole lactation involved feeding 17.4% CP for the first 16-weeks after calving, followed by 16.0% CP for the remaining 28 weeks. Increasing CP to as much as 19.3% during the first-phase, or to 17.9% CP during the second phase, did not improve yield of FCM, but only increased N excretion.

Compared to an isonitrogenous diet supplemented with urea, we found much greater production when feeding 1 of 3 true proteins that differed in RUP and AA content (Brito and Broderick, 2007). Although flow of RUP and total protein from the rumen was greatest on cottonseed meal, intermediate on canola meal and lowest on SBM (Brito et al., 2007), protein and fat yield both were highest on canola meal, intermediate on SBM, and lowest on cottonseed meal. Enhanced production on different sources likely was related to the AA pattern of the RUP being complementary to

microbial protein (Broderick, 1994). Methionine and lysine are the essential AA most often limiting for lactating dairy cows (Schwab, 1996). Responses to rumen-protected methionine (RPM) have been more consistent than to rumen-protected lysine (Armentano et al., 1997). We obtained similar protein yield, and even greater yield of milk and FCM, when RPM was fed in diets containing 17.3 and 16.1% CP versus an 18.6% CP diet without RPM (Broderick et al., 2008). Moreover, production on 15.8% CP plus RPM was about equal to that on 17.1% CP without RPM in a recent study (Broderick et al., 2009). We also obtained similar improvement in another trial in which the dietary treatments were not reversed when feeding RPM (Broderick and Muck, 2009), giving us confidence that RPM supplements will correct the methionine limitation without adding dietary CP. Rulquin et al. (2006) also reported increased yields of milk protein when supplementing with 2 different sources of RPM.

METHODS USED TO MEASURE AMMONIA EMISSIONS FROM DAIRY BARNS

Measurements of ammonia emissions (Figure 1) were made in Wisconsin using (1) lab chambers to simulate barn floors (Misselbrook et al., 2005 and Misselbrook and Powell, 2005), (2) barn chambers to house dairy cows in a simulated tie-stall barn (Powell et al., 2008a,b), and (3) lasers positioned outside three commercial free-stall dairy barns (Harper et al., 2009). Lab chambers were constructed from plastic drainage pipe of 10 cm internal diameter and 19 cm height. Each chamber lid had 4 horizontally-positioned inlet and outlet ports to ensure good mixing of air within the chamber. The main body of the chamber was filled with cement to simulate a barn floor leaving a headspace of approximately 350 ml. Air was drawn through the system by means of a vacuum pump. An acid trap at the inlet port of each chamber removed any ammonia from inlet air and a second acid trap at the outlet port of each chamber collected any ammonia emitted during the measurement period. Results reported in the present study pertain only to ammonia emissions after slurry application to sand (bedding used on commercial dairy farms) or after application of slurry derived from lactating cows fed three different diets: two diets having CP concentrations of 13.6% and 19.4% of feed dry matter (DM) intake, and one alfalfa silage-based diet. For the purpose of the present study, cumulative ammonia emissions after 24 h were divided by the chamber surface area (78.5 cm^2) to arrive at daily emission rates from barn floors ($g/m^2/d$).

Figure 1. Methods used to measure NH_3 emissions from dairy barns

•Lab chambers •Barn chambers •Lasers

Four barn chambers (each approximately 6.0 m wide x 9.1 m long x 2.9 m high containing 165 m^3 of air space) to house four lactating dairy cows each were constructed at the end of an existing tie-stall barn. Airflow through each chamber was controlled by an intake fan, which pushed air through each chamber exiting from one

exhaust duct per chamber. Stainless steel cross-sectional (spider) samplers were constructed to sample air from chamber inlets and exhaust ducts. During each trial day, cows were milked, fed and chambers were cleaned from approximately 0600 to 0900 h. At approximately 0900 h, chamber curtains were lowered, curtain wall seams were sealed, and from 1000 h to 1500 h emission recordings were made. Cows were milked from 1500 h to 1700 h, and curtains were lowered again at approximately 1800 h and night-time emission measurements were made from approximately 1900 h to 0500 h the next morning. The chambers were used to evaluate seasonal ammonia emissions (1) from four bedding types (manure solids, chopped newspaper, pine shavings and chopped wheat straw), and (2) from lactating dairy cows fed four diets: corn silage- or alfalfa silage-based diets at low CP (15.7 to 16.1%) or high CP (17.2 to 17.3%) levels.

Ammonia emissions from free-stall dairy barns on three Wisconsin dairy farms were measured using inverse dispersion analysis (backward Lagrangian stochastic analysis, bLS) combined with open-path laser measurement technology. One farm housed approximately 900 lactating cows in two barns. The other farm had approximately 1400 lactating cows and 300 dry cows distributed in two barns. Downwind ammonia concentrations were measured using open-path lasers with inverse dispersion analysis to obtain ammonia emissions. Three-dimensional sonic anemometers provided the meteorological information for the inverse dispersion calculations. Dietary CP concentrations between 16.8 and 17.8% were fed over the course of the study.

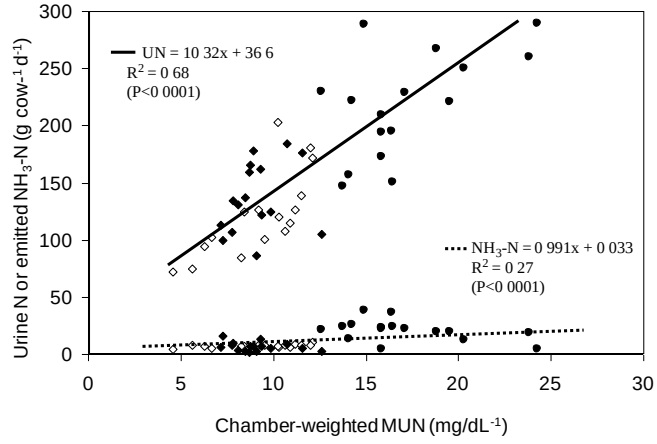
BEDDING IMPACTS ON AMMONIA EMISSIONS

An initial laboratory study revealed that the physical characteristics (urine absorbance capacity, bulk density) of bedding materials are of more importance than their chemical characteristics (pH, cation exchange capacity, carbon to nitrogen ratio) in determining ammonia emissions from applied dairy urine and feces (Misselbrook and Powell, 2005). Ammonia emissions were significantly lower from sand (23% of applied urine N), followed by pine shavings (42% of applied urine N), than from the other four (straw, newspaper, cornstalks and recycled manure solids) bedding types (mean 63% of applied urine N). A subsequent barn chamber study determined that ammonia emissions (g/heifer/d) from manure solids (20.0), newspaper (18.9) and straw (18.9) were similar and significantly greater than emissions using pine shavings (15.2). Relatively high chamber N balances (percent of feed N and bedding N inputs recovered in manure N, body weight N, and ammonia N outputs) of 89 to 105%, and favorable comparisons of study data with published values of ammonia emissions, feed N intake and manure N excretion provided confidence in the accuracy of barn chamber results.

DAIRY COW DIET IMPACTS ON MILK, MANURE AND AMMONIA EMISSIONS

In other barn chamber studies, there were seasonal diet impacts on milk production, FNUE, excreted manure N (ExN), urine N (UN), and ammonia N emissions. During the early-fall and winter trials, milk production (37.9 kg/cow/d) was similar in cows fed the low CP and the high CP diets, and also in cows fed the alfalfa silage- (AS) and the corn silage- (CS) based diet (37.8 kg/cow/d). During early-fall and winter FNUE (32.9%) of

Figure 2. Relationships between MUN, urine-N (UN) and emitted ammonia N



cows fed the low CP diets were significantly greater than cows fed the high CP diets (29.9%). There were no significant differences in FNUE of cows fed diets based on AS or CS. There was significantly less ExN (318 g/cow/d) by cows fed LP diets than cows fed HP diets (354 g/cow/d). Forage type did not significantly impact ExN and ammonia emissions, and only during the early-fall trial did dietary CP level impact ammonia emissions. During the early-fall, ammonia emissions (8.0 g/cow/d) from chambers containing cows fed the low CP diet were significantly less than emissions (8.8 g/cow/d) from cows fed the high CP diet. Ammonia-N emissions accounted for approximately 1% to 3% of N intake, 2% to 5% of ExN, and 4% to 11% of UN. Average ammonia emissions (across all diets) during winter (6.7 g/cow/d) were 20% less than during early-fall (8.4 g/cow/d). We discovered significant positive relationships between MUN, UN and emitted ammonia N (Figure 2). Confidence in these barn chamber results were derived from (1) the relatively high chamber N balances (91 to 95% of feed and bedding N inputs were accounted for in manure N, ammonia N and animal N outputs), and (2) the generally favorable comparisons between study results and published values of UN excretion and ammonia emissions.

CORRESPONDENCE AMONG METHODS TO MEASURE AMMONIA EMISSIONS

The barn chambers and commercial dairy barns provided a wide range in the number of dairy cattle, and the lab chambers, barn chambers, and commercial dairy barns provided a wide range of barn floor surface areas and temperatures, from which ammonia emission measurements were made (Table 1).

Table 1. Number of cows, barn floor area, dietary CP and temperature during seasonal measurements of ammonia emissions using different scale methods

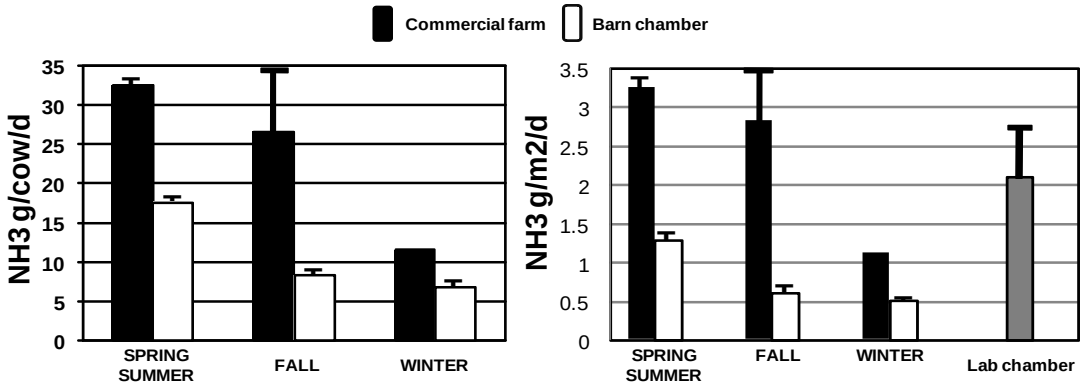
Parameter	Method	Season		
		Spring-Summer	Fall	Winter
Number of cows [a]	Barn chambers	16	16	16
	Commercial barns	3101 (376)	3698 (924)	2556 (269)
Barn floor area (m^2)	Barn chambers	54.6	54.6	54.6
	Commercial barns	30,350	30,350	26,500
Dietary CP (%)	Barn chambers	17.5	16.6	16.6
	Commercial barns	17.8	16.8	17.3
Temperature (^oC) [b]	Barn chambers	26.1	10.1	7.0
	Commercial barns	20.0	7.9	-2.4

[a] Most were lactating dairy cows. The number in parentheses, which is included in adjacent total, refers to dry cows and heifers that were housed in the commercial barns.

Measurements from barn chambers and commercial dairy barns provided similar patterns of ammonia emissions per cow during each of the three study seasons (Figure 3). As determined in the original studies, ammonia emissions from barn chambers (Powell et al., 2008 a,b) and commercial dairy barns

(Harper et al., 2009) are significantly lower during winter than during the other seasons of the year. Across all seasons, ammonia emissions per cow determined from tie-stall barn chambers were less than one-half emission rates from commercial free-stall barns, which correspond to differences in ammonia emissions from tie-stall barns and free-stall barns summarized in recent literature reviews (Powell et al., 2008a,b). The typical organic beddings (e.g., straw, shredded newspaper, wood shavings) used in tie-stall barns separate urine and feces, which reduces ammonia production and loss (Misselbrook and Powell, 2005). Also, whereas relatively narrow gutter scrapers remove manure from tie-stall barns usually once daily, wide alley scrappers constantly mix urine and feces and remove manure from free-stall barns. This results in large differences in the emitting surface area of tie-stall and free-stall barn floors.

Figure 3. Relative ammonia emission rates from barn chamber study and lab chamber study



Lower emission rates were determined in barn chambers relative to the commercial farms during fall than during the other two study seasons. Average ammonia emissions (g/m2/day) determined in lab chambers (2.1) were similar to average ammonia emissions determined on commercial dairy barns (2.9) during spring-summer and fall.

The lab chambers and barn chambers provided a similar ranking of ammonia emissions from a range of organic beddings, with lowest emissions measured from pine shavings, followed by shredded newspaper, straw and recycled manure solids (Powell et al., 2008b). The lab chambers, barn chambers and lasers provided valid estimates of ammonia emissions, depending on the measurement system. While the enclosure systems, such as our chambers are useful for comparison measurements, non-interference techniques [such as inverse dispersion analysis (bLS)] must be used to establish emission rates in commercial barn settings. Lab chambers can be useful in evaluating a wide range of potential animal and barn floor management practices for their relative ammonia emission potential. Barn chambers provide a useful tool for screening a range of management practices, such as animal feeding and bedding, for their potential impacts on ammonia emissions from commercial dairy barns.

DAIRY HERD MANAGEMENT IMPACTS ON AMMONIA EMISSIONS

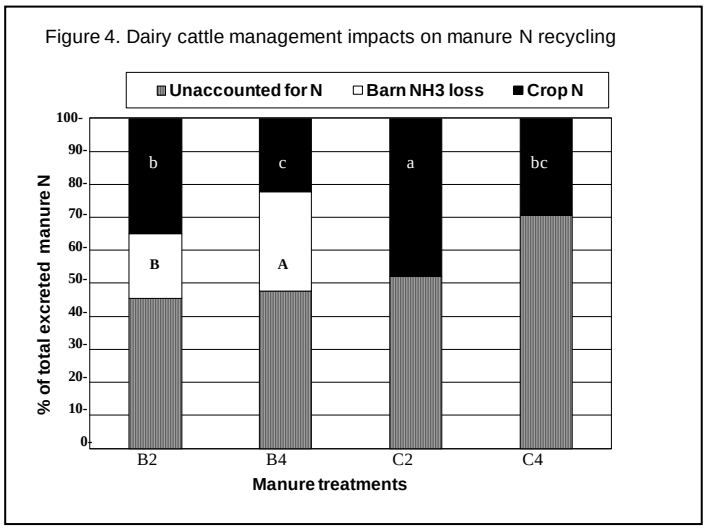


Figure 4. Dairy cattle management impacts on manure N recycling

We compared two dairy herd management practices on manure N capture and recycling through crops: the conventional practice of barn manure collection and land application, and corralling dairy cattle directly on cropland (Powell and Russelle, 2009). Heifers were kept in a barn for two (B2) or four (B4) days and manure was hauled to fields, or heifers were corralled directly on cropland for two (C2) or four (C4) days. Four successive manure application seasons, spring-summer (SS), fall-winter (FW), summer (S) and winter (W) were evaluated over two years. Each season was followed by three-year crop rotations: SS and S by wheat, sudangrass, winter rye, corn, winter rye, and corn; and FW and W by corn, winter rye, corn, winter rye, and corn. In-barn N losses (% of excreted manure N) were greater from B4 (30%) than B2 (20%). As a percent of excreted manure N, manure N recovery over the 3 yr rotation from C2 was 50%, B2 35%, C4 30% and B4 22% (Figure 4). Overall results demonstrated that corralling dairy cattle on cropland improves urine N capture, reduces ammonia loss and enhances manure N recycling through crops.

SUMMARY

Dairy cattle barns can be major sources of ammonia emissions to the atmosphere. Various feed strategies are able to reduce urinary N excretion. A close matching of diets to animal nutritional requirements, feeding only enough RUP to meet cows' metabolizable protein requirements, reducing particle size to increase ruminal digestion of grain starch and increase microbial protein formation (so long as ruminal pH is not depressed) optimizes microbial protein synthesis, maximizes feed N conversion into milk and minimizes urinary N excretion. Ammonia emissions from dairy barns can be reduced by using bedding materials and/or barn floor configurations that physically separate feces (which contains urease) and urine. It may be possible to further reduce ammonia emissions through alternative herd management. For example, corralling dairy cows directly on cropland captures greater amounts of urine, reduces in-barn ammonia N losses, and therefore recycles more excreted manure N through crops than the conventional practice of housing cattle in barns, hauling and mechanically applying manure to fields.

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FEEDING LOW CRUDE PROTEIN RATIONS TO DAIRY COWS – OPPORTUNITIES AND CHALLENGES

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Dairy producers should consider lowering ration crude protein (CP) levels in rations for two primary reasons. One is to improve profitability by increasing the efficiency of converting feed nitrogen (N) intake to milk N output while at least maintaining milk production. This usually reduces purchased feed cost and increases income over feed cost and/or income over purchased feed costs. A second reason is that feeding lower CP rations decreases the excretion of N to the environment. This can decrease the number of acres needed for land application of manure. When ammonia emission regulations are implemented, lower CP rations will decrease animal ammonia emissions. This provides a win-win situation for both the dairy industry and society. On many of farms, there is an opportunity to lower ration CP by 0.5 to 1.5 units with minimal risk of lowering milk production. This can have significant implications on both farm profitability and nutrient management practices. There are a limited number of farms that have already made this step by feeding lower CP rations. These farms may have limited opportunity to further lower ration CP. However, they demonstrate that lower CP rations can be used in herds while maintaining high levels of milk production.

NITROGEN USE IN THE DAIRY COW

Even though N metabolism in the dairy cow is complex, it can be broken down to a few key points. Nitrogen consumed in the feed is either used as a nutrient source to support productive functions (maintenance, growth, pregnancy, milk) or it is excreted via urine and feces. The dairy cow has a limited ability to store N compared with energy. Milk N efficiency (MNE) is one index that can be used to assess the efficiency of N use in the dairy cow. This index is simply the ratio of the quantity of N excreted via the milk as a percent of the quantity of feed N consumed. The MNE values observed in commercial dairy herds usually ranges between 20 and 35%. This implies that 65 to 80% of the consumed N is excreted in the manure. As ration CP increases, the MNE value tends to decrease. Table 1 contains information from a research trial in which rations ranging from 13.5 to 19.4% CP were fed (Olmos Colmenero and Broderick, 2006). The key points from this table are:

- The quantity of N excreted in the milk changed very little for all levels of ration CP used in this trial.
- Total manure N excreted per day increased as ration N intake went up.
- The portion of the total manure N found in the fecal portion varied little with increasing ration CP levels.
- As total N excretion went up with higher levels of ration CP, urinary N was the main route of excreting excess N.
- Milk N efficiency decreased as ration CP increased.

Table 1. Nitrogen intake and excretion from rations varying in CP levels

Ration CP,%	**13.5**	**15**	**16.5**	**17.9**	**19.4**
N intake, g/day	483	531	605	641	711
Milk N, g/day	173	180	185	177	180
Total manure N, g/day	309	316	376	410	467
Fecal N, g/day	196	176	186	197	210
Urinary N, g/day	113	140	180	213	257
Urinary N, % of manure N	36.5	44.3	47.8	52	55
Milk N, % of N intake	36.5	34	30.8	27.5	25.4

Source: Olmos Colmenero and Broderick, J. Dairy Sci. 89:1704, 2006

FIELD TRIAL DATA

Two dairy herds in New York were used in a field trial to examine the use of the Cornell Net Carbohydrate and Protein System (CNCPS 6.1) model to lower ration CP levels (Higgs, 2009). The changes made in the N portion of the CNCPS model used in this trial have been previously described (Van Amburgh et.al. 2007). The herds used were selected in cooperation with the nutritionist working with these herds. One herd used a nutritionist from a major feed company and the other herd used an independent consultant. The initial herd rations were evaluated and ration adjustments suggested that could be made to lower ration CP levels. Rations were adjusted a number of times over the 8-month trial period. This trial was done between September, 2008 and April, 2009. Ration changes were not made unless the herd nutritionist agreed with the changes suggested as a result of the CNCPS model runs. The goal of this trial was to lower ration CP, improve the efficiency of N use and validate field use of the CNCPS 6.1 model. Milk income and feed costs were calculated using constant prices typical of New York for April, 2009. There were a number of feed and forage changes made on both farms during the course of the trial as inventories, quality and silos changed. Both farms also replaced a portion of the purchased corn meal with farm produced high moisture corn during the trial. Monthly DHI and milk component data was obtained for each farm.

Table 2 contains an overview of the results for each farm. Farm A had about 400 milking cows while farm B had about 600 cows. Key points from this table are:

- There was an increase in % milk true protein in both herds. This most likely related to the decrease in ration fat levels and the increase in ration starch levels.
- Milk urea nitrogen (MUN) values decreased by about 2 units in these herds.
- Ration CP levels were lowered about 1 unit.
- Ration fat levels were lowered and ration starch levels increased as ration CP was lowered.
- Total ration metabolizable protein (MP) was decreased in Herd A.
- Manure N and urinary N decreased in both herds. This would decrease the ammonia emission potential of these herds.
- Milk N (as % of N intake) increased about 2-3 units. This is an index of improved efficiency of N use. The ratio of milk N to urinary N also increased. This is another indication of improved N use and less N excretion.
- Both total and purchased feed costs were reduced in both herds.
- Income over feed cost and income over purchased feed cost increased in both herds.
- There are additional opportunities to further improve N utilization in these herds. Balancing for amino acids would be the next logical step in both herds. However, there are some daily management considerations that also need addressed before trying to go the next step in these herds.

DAIRY HERDS FEEDING LOWER CRUDE PROTEIN RATIONS

As we started to select herds for the field trial, a number of herds already feeding lower CP rations were identified. It would be difficult to make additional decreases in ration CP or N in these herds. However, it was decided to put the information together for these herds to gain an insight into the rations being used. Table 3 contains data on a number of herds feeding lower CP (<16%) rations. The information on these herds was provided by the feed industry person working with the herds. The herds in Table 3 are from Wisconsin, Michigan, Pennsylvania and New York and were provided by 9 different individuals. All of these herds feed total mixed rations. Some key observations from the information in Table 3 are:

- Milk production is high (>80 lbs/day) for most herds. The ration for herd D is the high group ration for that farm.
- MUN values are consistently < 12 mg/dl except for herd N.
- Most herds feed high (>55%) forage rations.
- Corn silage is the primary forage except in herd H.
- Ration fat levels tend to be moderate while NFC and starch levels are at the upper end of the range found in most current rations.
- The efficiency of N use is high in these herds. Milk N ranges from 28 to 38% of the intake N.
- There is a wide variation in the amino acid balance in these herds. In most cases, this appears to be an area of opportunity for future consideration.

- This information does verify that lower CP rations can be fed in commercial dairy herds and will support high levels of milk production while improving the efficiency of N use and decreasing N excretion to the environment.

Table 2. Field Trial Results

Item	Herd A – Initial Ration	Herd A – Final Ration	Herd B – Initial Ration	Herd B – Final Ration
Milk, lbs	79	80	82	80
Milk fat, %	3.58	3.63	3.56	3.63
Milk true protein, %	3.03	3.11	2.96	3.07
MUN, mg/dl	14.8	12.5	14.5	12
Forage, % of ration DM	54	57	60	48
Corn silage,% of forage	59	71	53	60
Ration CP, %	17.5	16.6	17.7	16.9
NDF, % of DM	32.5	33.6	31.3	33.2
Starch, % of DM	23	27.6	23.6	26.3
Fat, % of DM	4.3	3.8	5.4	4.2
Total MP, g/day	2950	2769	2646	2690
N intake, g/day	697	641	655	629
Manure N, g/day	500	441	469	441
Fecal N, g/day	250	237	233	231
Urine N, g/day	250	204	236	210
Milk N, % of N intake	28	31	28	30
Milk N:Urine N	0.78:1	0.98:1	0.78:1	0.9:1
Feed cost, $/cow/day	5.88	5.43	6.14	5.97
Purchased feed, $/cow/day	3.55	2.96	3.73	3.42
IOFC, $/day	3.08	3.83	3.01	3.22
IOPFC, $/day	5.41	6.30	5.42	5.77

CHALLENGES TO LOWERING RATION CP IN DAIRY HERDS

There are always considerations and risks involved when altering rations and nutrition management programs on dairy farms. The size of the “safety factor” used in

formulating rations is a tool routinely used by feed industry professionals and consultants. They vary the safety factor based on their evaluation and assessment of the consistency of forages and daily feeding management practices. In 2006, we surveyed a number of New York feed industry personnel for the challenges they felt needed to be considered as ration CP levels are lowered. The primary factors they listed were:

- Consistency and quality of daily on-farm feed mixing and feeding management.
- Daily variations in forage quality and dry matter.
- Herds feeding total mixed rations versus component fed herds.
- Lack of on-farm forage dry matter determinations and the use of this information for adjusting the quantity of feeds added to the mixer wagon.
- Herd grouping and ration strategies.
- The increasing level of soluble protein in home-produced forages.
- The increased use of baleage on some farms.
- Accuracy of the forage samples and the forage lab analyses.
- Limited availability of milk urea nitrogen (MUN) values as a monitoring tool.
- Are our ration formulation tools accurate enough to crank down ration CP?
- The need to gain experience and a comfort level in lowering CP in rations and observing herd responses.
- Lack of “real” farm information from herds that have successfully adopted lower CP rations.

WHAT DO WE BALANCE FOR?

Crude protein is the term that has been used to formulate and evaluate dairy rations for many years. Dairy cattle do not have a CP requirement but do need absorbable amino acids to meet requirements for maintenance, growth, pregnancy and milk production. However, a number of refinements have been added over the years to increase the usefulness of the CP system. These include considering soluble protein, rumen degraded protein and rumen undegraded protein (RUP). The recent Dairy NRC publication proposes that metabolizable protein (MP) be used for ration formulation rather than CP (NRC, 2001). Metabolizable protein is basically the sum of microbial protein and RUP. The NRC committee examined the relationship between ration CP and milk production using 393 treatment means from 82 published research trials. Ration CP % accounted for only 29% of the variation in milk production in these studies. A more detailed rationale for using MP to balance rations is available (Varga, 2007).

Table 3. Commercial Dairy Herds Feeding Lower Crude Protein Rations

Item	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Cows	1550	108	270	920	140	100	700	60	180	45	220	45	250	53
Milk, lbs	88	88	85	116	89	85	89	60	95	80	75	85	85	72
Milk fat,%	3.6	3.6	3.8	3.2	3.65	4	3.5	4	3.6	3.6	3.85	3.7	3.56	3.64
Milk true protein,%	3.05	3.2	3.07	3	3	3	3.1	3.1	3.1	3.1	3.2	3.2	3.03	2.9
MUN, mg/dl	10.6	12	-	8	8-10	9	7-9	9	8-9	8-9	8-9	8-9	10	14
Ration CP, %	15.9	15.5	15.7	15.9	14.3	16	16.3	16.5	15.8	15.6	15	15.6	15.5	15.8
MP, g/cow	2625	2720	2961	3306	2599	3016	2792	1991	2744	2305	2256	2419	2739	-
Lysine, % of MP	6.6	6.23	6.4	6.74	6.42	6.17	6.64	5.63	5.77	6.32	6.23	6.31	6.29	6.4
Methionine,% of MP	1.94	1.96	2.05	2.71	2.1	1.77	2.79	1.78	1.85	1.91	1.88	1.91	1.93	1.9
Lys:Meth	3.4:1	3.18:1	3.12:1	2.5:1	3.05:1	3.5:1	2.38:1	3.16:1	3.12:1	3.3:1	3.3:1	3.3:1	3.3:1	3.3:1
NDF, %	28.9	30.8	30.7	30.9	31.4	31.5	32.2	30.5	32.3	29.3	31.5	29.3	31.5	33.7
Forage NDF, % of BW	0.88	0.86	0.86	0.94	0.99	0.91	0.88	0.78	0.99	0.89	0.78	0.89	1.02	0.94
NFC, %	43.4	41.9	40.6	41.5	42.4	38.1	39.1	40	39.3	41.3	40.7	44.4	42.5	40
Starch,%	28.5	27.1	31.6	28.7	29.3	24	27.6	26.3	28.7	28.6	27.6	29.5	28.6	29
Sugar, %	3.5	3.1	4.2	5.4	5	3.3	5.1	7	3.5	3.7	3.4	4.1	7.4	3.9
Fat, %	4.3	3.8	4.3	5.1	4.4	5.2	5.4	5.4	5.1	5.1	4.8	4	5.2	4.1
Forage, % of ration DM	57	60.4	48	60	59	57	53	50	51	59	52	59	55	60
Corn silage, % of forage	80	72	37	68	53	47	64	0	58	56	49	38	74	46
Milk N, % of N intake	35	35	32	38	36	28	35	28	35	35	35	36	31	32

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