USING A NEW IN VITRO METHOD AND FIBER MODEL (TTNDFD) TO IMPROVE ESTIMATES OF DIGESTIBILITY OF ALFALFA FOR DAIRY CATTLE

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National Institute of Food and Agriculture

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Non Technical Summary
The objectives of this project are to (1) Validate a new in vitro method (TTNDFD) for predicting in vivo total tract NDF digestibility of forages and diets for lactating dairy cattle; and (2) Incorporate TTNDFD into several cooperative extension tools that are widely used to evaluate forage quality and market alfalfa: milk/ton, milk/acre and relative forage quality (RFQ). We are also going develop an energy equation based on TTNFD so that we can price forages with another extension tool that calculates the economic value of feeds based on the value of nutrients contained in that feed (Feedval).

Animal Health Component

Research Effort Categories

Basic

Applied

Developmental

Classification

Knowledge Area
302 - Nutrient Utilization in Animals;

Subject Of Investigation
1640 - Alfalfa; 3410 - Dairy cattle, live animal;
Field Of Science

1060 - Biology (whole systems);

Keywords
alfalfa
dairy cattle
forage
ttnfd

Goals / Objectives
The objectives of this proposal are to (1) Validate a new in vitro laboratory method (TTNDFD) for predicting in vivo NDF digestibility of forages and diets for lactating dairy cattle; and (2) Incorporate TTNDFD into several extension tools that are widely used to evaluate forage quality and market alfalfa: milk/ton, milk/acre, and relative forage quality (RFQ). We are also going to develop an energy equation based on TTNFD so that we can price forages with another extension tool that calculates the economic value of feeds based on the value of nutrients contained in that feed (FeedVal). The objectives of this project addresses priority number four of the FY 2014 AFRP call for proposals: "Improving estimates of alfalfa forage quality as an animal feed to increase forage usage in animal feeds. This project outlines a 3-year plan of collaborative research and extension activities between University Wisconsin, University of Nebraska and the William H. Miner Agricultural Research Institute.

Project Methods
Objective 1: Validate the TTNDFD in vitro method for predicting in vivo total tract NDF digestibility of forages and diets for lactating dairy cattle. In vitro rumen and total tract NDF digestibility (TTNDFD) prediction. For Sub-objectives 1-1, 1-2 and 1-3 the following in vitro procedure (as cited in Goeser and Combs, (2009)) and model (as cited in Combs (2013)) will be used to predict TTNDFD. Dried, ground (1 mm) samples (0.5 g) are sealed in Ankom F-57 bags, which are then placed in 125-mL Erlenmeyer flasks and incubated in 40 ml of in vitro media and 10 ml rumen fluid. The rumen fluid is collected from cannulated cows fed a high forage diet, strained through cheese cloth, then combined with the in vitro media. The media consists of buffers, reducing solution, and priming nutrients. The flask containing rumen fluid/media inoculum is then sealed and placed in a 39°C water bath. Gas pressure within the flask is monitored and when the pressure reaches 46.7 mmHg, 50 ml primed inoculum is added to the 125 ml flasks containing the samples. Samples are incubated in a shaking water bath (39°C) for 24, 30, 48 or 240 h. The residues are then analyzed for neutral detergent fiber with an ANKOM forage fiber analyzer. Fiber digestibility is determined as: NDFD (% of NDF) = 100-[(NDF0h - NDF residue at time 24, 30, 48 or 240 h)/(NDF0h)]. The TTNDFD in vitro model is based on the concept that fiber digestion is a two-step process beginning on the rumen, followed by fiber digestion in the hindgut. Ruminal digestion of NDF is described as a time-dependent, competitive process that is affected by the rate of passage of fiber, and the rate of degradation (kd) of potentially digestible NDF (pdNDF), similar to the cellulose digestion model described by Waldo et al. (1972). The TTNDFD model inputs include fraction of pdNDF, and the rate of degradation of pdNDF (kd) the rate of passage of fiber from the rumen. The rate of fiber degradation is calculated from pdNDF residue measurements taken at 24, 30 and 48 h of in vitro incubation in rumen fluid using a first order kinetics model with an indigestible fraction as
described by Mertens (1993). The passage rate of pdNDF (kp) is predicted from a regression model (Krizsan et al., 2010) for iNDF which is adjusted to account for the selective retention of pdNDF (Lund et al., 2007) determined using the flux/compartment pool method described by Ellis et al. (1994). In the TTNDFD model, the predicted NDF digestibility is indexed to a 630 kg dairy cow consuming 23.4 kg DM/d of a diet containing 30% of NDF. This index sets the kp of pdNDF at 2.67 %/h. The TTNDFD model calculates rumen and total tract NDF digestion according to the following equations: (1) TTNDFD = (ruminal NDF digestion) + (hindgut NDF digestion) (2) Ruminal NDF digestion = pdNDF * [kd/kp + kp] (3) pdNDF = NDF - iNDF (4) iNDF = (NDF residue after 240h in vito incubation / NDF) (5) Hindgut NDF digestion = ruminal NDF digestion * .10 The TTNDFD value is the digestibility coefficient for NDF for cows consuming diets at approximately 3X maintenance intake. Our hypothesis is that this digestibility coefficient (TTNDFD) will accurately predict fiber digestibility in dairy cattle (objective 1). The TTNDFD coefficient could then be used to replace the 48 h in vitro NDF digestibility coefficients (NDFD48) used in the equations to calculate RFQ, milk/acre or milk/ton (see objective 2). Sub-objective 1-1: Diet samples, and forage samples (if available) used in the diets will be submitted to the lab of Dr. Combs by Dr. Grant and Dr. Kononoff. Samples selected will be from feeding studies in which total tract NDF digestibility of the diets can be or have been determined. The feeding studies will have been conducted with dairy cattle and total tract digestibility measured by either total fecal collection or by use of digesta markers and spot sampling of feces. All samples will be coded prior to submission to Dr. Combs; lab so that Dr. Combs; group cannot cross reference the TTNDFD analysis to in vivo digestibility measurements until the in vitro analysis have been completed. Samples will be analyzed for NDF, iNDF, kp pdNDF and TTNDFD, and the TTNDFD values will be compared to the in vivo digestibility values. Sub-objective 1-2: Document the variability in NDF, iNDF, kd pdNDF, and TTNDFD of alfalfa hays produced by commercial hay growers in Nebraska. Dr. Kononoff’s lab will coordinate collection of approximately 75 samples of alfalfa hay from commercial forage producers in Nebraska. The forage samples will be composited from multiple samples collected within identified lots of hay. The hay samples will be sent to Dr. Combs lab and analyzed for DM, NDF, iNDF, kd pdNDF and TTNDFD. RFQ, and milk/ton will be determined from established equations currently in use. The modified RFQ and milk/ton equations (modified by incorporating fiber digestibility as determined by TTNDFD assay) will also be calculated and compared. Feedval prices of hays as determined with a standard book value; of NEI or with a modified estimate of NEI from the TTNDFD method will also be compared. The comparisons of RFQ, and milk/ton values will provide useful insight into if and by how much a modified energy equation would affect ranking and evaluation of alfalfa forage quality. The comparisons of economic values of hays with the original Feedval and the modified Feedval equations would also provide important information about how the economic value of forages could be affected if TTNDFD were used to predict forage fiber digestibility. Sub-objective 1-3 Document the variability in NDF iNDF, kd pdNDF and TTNDFD of alfalfa forages produced on alfalfa research plots at the University of Wisconsin and the William Miner Agricultural Research Institute. Dr. Dan Undersander and Dr. Rick Grant will collect samples from research plots of alfalfa and alfalfa/grass mixtures at the University of Wisconsin and the William Miner Agricultural Research Institute, respectively. Alfalfa variety, grass specie and variety, cutting number, and forage maturity of samples will be documented. Samples will be oven-dried, ground and submitted to Dr. Combs lab for analysis of DM, NDF, iNDF, kd pdNDF and TTNDFD. Goal two. Incorporate the NDF digestibility coefficients from the TTNDFD model into several
extension tools (Milk/acre, Milk/ton, Milk 2006, Feedval) that are widely used to evaluate and index forages and develop extension programming tools to train extension agents, dairy producers, forage industry professionals, forage testing laboratories and dairy nutritionists. Study results will be disseminated via programming efforts of Extension Forage, Dairy and Beef Teams. Extension programming efforts will include conference presentations and proceedings, website information, radio tapes, articles in newspapers and magazines, e-newsletter articles, posts on social media sites (Facebook and YouTube), presentations at in-person meetings and field days, and webinars.

**Progress** 09/01/15 to 08/31/16

**Outputs**
Target Audience: Dr. Combs was an invited speaker at several conferences in North America that were targeted to dairy producers, forage producers, dairy nutritionists and forage agronomists. The general topic in each meeting was how total tract NDF digestibility affects intake and production of dairy cattle, with an emphasis on alfalfa. Conferences included: October 1, 2015-World Dairy Expo Forage Superbowl seminar. (approx. 50 farmers, nutritionists and agronomists); November 6, 2015 - IEFAC meeting in Lacrosse, WI. (approx. 25 extension agents and industry alfalfa seed producers); California Alfalfa Symposium, Reno, NV. (approx. 200 alfalfa hay growers, and forage agronomists); January American Forage and Grasslands Conference, Baton Rouge, LA, (approx. 150 University and Private sector research agronomists; January 26, 2016 Midwest Forage Association Meetings, Wisconsin Dells, WI; March 8, 2016- Western Canadian Dairy Conference, Red Deer, Alberta, CA. (approx. 150 University ruminant nutrition researchers, dairy consultants and veterinarians; July 12, 2016-North American Alfalfa Improvement Conference, Madison, WI. (approx. 120 University and Industry forage agronomists); Manitowoc County Forage Council Field Day, Newton, WI. (approx. 50 dairy producers and forage consultants) ; August 28, 2016- Alforex/Dow Diamond Showcase, Fresno, CA (approx. 80 alfalfa producers and forage agronomists). Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? We have presented the concepts of using TTNDFD in ration formulation and forage evaluation to several groups of dairy nutritionists and forage agronomists in the past year. (See Target Audience). We are also working with the UW Forage Testing Lab in Marshfield, Wisconsin to provide TTNDFD analysis of alfalfa. How have the results been disseminated to communities of interest? Results pertaining to this project have been shared with target audiences at major dairy nutrition and agronomy conferences (Western Canadian Dairy Conference, California Alfalfa Symposium, American Forage and Grasslands Conference, North American Alfalfa Improvement Conference and Midwest Forage Association). What do you plan to do during the next reporting period to accomplish the goals? Goal 1. (Validate the in vitro TTNDFD method with in vivo total tract digestibility measurements for lactating cattle). We will complete in vitro analysis of rations from previous studies where in vivo NDF digestibilities have been measured as described in the original proposal. If the reduced lignin alfalfa forages and the conventional forages harvested in 2016 are different in fiber digestibility, we will conduct a feeding experiment to validate that in vitro TTNDFD measurements can be used to formulate diets for lactating cows. Goal 2. (Incorporate TTNDFD into extension tools for evaluating forage quality). We plan to release a new version of Milk 2006 for alfalfa in early 2017. We have a beta version of Feedval (a decision making tool for pricing feeds) that we plan to release in early 2017 that uses TTNDFD of forages to adjust the economic value of hays and silages for fiber digestibility.
Impacts
What was accomplished under these goals? Under objective 1. We conducted a feeding study in which we fed an alfalfa/corn silage based forage diet supplemented with processed and un-processed corn stover. This was another validation study to test our hypothesis that an in vitro measurements of forage NDF digestibility could predict in vivo total tract fiber digestibility in dairy cattle. We also have harvested in the summer of 2016 reduced lignin and conventional lignin alfalfas that were planted in the spring of 2016 at the Arlington research station. We are currently evaluating NDF digestibility of the harvested forages. If they are significantly different in in vitro NDF digestibility, we will be conducting a feeding study to test our hypothesis that in vitro measurements of NDF digestibility in forages can predict in vivo fiber digestion in ruminants. We have doubled our capacity to run in vitro NDFD analyses and are now ready to test diets and forages provided by the University of Nebraska and the Miner Institute this spring. Objective 2. We continue to work on a two extension tools, (Milk 2006, and Feedval) to incorporate TTNDFD as a measure of forage fiber digestibility. We expect to release new versions of the tools Milk and Feedval shortly after January, 1, 2017.

Publications

- Type: Conference Papers and Presentations Status: Published Year Published: 2015 Citation: Combs, D. 2015. Fiber digestibility and dairy cattle performance. October 1, 2015-World Dairy Expo Forage Superbowl seminar.
- Type: Conference Papers and Presentations Status: Published Year Published: 2016 Citation: Combs, D. 2016. Reduced lignin alfalfa for dairy cattle. August 28, 2016-Alforex/Dow Diamond Showcase, Fresno, CA.
- Type: Conference Papers and Presentations Status: Published Year Published: 2016 Citation: Combs, D. 2016. Low lignin alfalfa for dairy cattle. Manitowoc County Forage council Field Day, Newton, WI.
- Type: Conference Papers and Presentations Status: Published Year Published: 2016 Citation: Combs, D. 2016. Using a New in Vitro Method and Fiber Model (TTNDFD) to Improve Estimates of Digestibility of Alfalfa for Dairy Cattle. North American Alfalfa Improvement Conference, Madison, WI.
- Type: Conference Papers and Presentations Status: Published Year Published: 2016 Citation: Combs, D. 2016. Fiber digestibility and dairy cattle performance. Western Canadian Dairy Conference, Red Deer, Alberta, CA.
- Type: Conference Papers and Presentations Status: Published Year Published: 2016 Citation: Combs, D. 2016. Low lignin alfalfa and its affect on dairy cattle performance. Midwest Forage Association Meeting, Wisconsin Dells, WI.
- Type: Conference Papers and Presentations Status: Published Year Published: 2016 Citation: Combs, D. 2016. Impacts of forage fiber digestibility on Dairy Cattle performance. Forage and Grasslands Conference, Baton Rouge, LA.
- Type: Conference Papers and Presentations Status: Published Year Published: 2016 Citation: Combs, D. 2016. Forage Quality and Utilization, Total Tract NDF digestibility. California Alfalfa Symposium, Reno, NV.
**Progress 09/01/14 to 08/31/15**

**Outputs**

Target Audience: Dr. Combs was an invited speaker at several major conferences that were targeted to forage producers, dairy producers, professional dairy nutritionists and forage agronomists. Information pertaining to this project was presented at the following conferences. In November (11/13/15) the conceptual framework of the TTNDFD model was presented at the Penn State Nutrition Conference in the main conference proceedings and in a workshop. The audience for this meeting includes professional nutritionists, academia, veterinarians and dairy producers. In February of 2015 (11/25/15), the PD spoke at the Idaho Hay and Forage Conference (Burley, ID) and spoke about the concept of pricing forages based on the TTNDFD model. This conference was attended by over 200 commercial alfalfa growers, agronomists and dairy producers. Dr. Combs also spoke at the Tri-State Dairy Nutrition Conference (5/20/15) in Fort Wayne, IN, and presented the conceptual framework of the TTNDFD model and its implications for using alfalfa in diets of dairy cattle. Lastly, the PD also was an invited speaker at the Missouri Forage Conference (6/8/15) in Springfield, MO, where validation data of the TTNDFD model was presented to approximately 150 veterinarians, dairy nutritionists and dairy producers.

**Changes/Problems:** Nothing Reported

What opportunities for training and professional development has the project provided? Nothing Reported

How have the results been disseminated to communities of interest? Research results pertaining to this project have been shared with target audiences at major dairy nutrition conferences (Penn State Dairy Nutrition Conference, Tri-State Dairy Nutrition Conference, Missouri Forage Conference), and at a major conference attended by commercial alfalfa growers (Idaho Hay and Forage Conference). What do you plan to do during the next reporting period to accomplish the goals? Goal (1). We will continue to validate the TTNDFD in vitro method as a tool to predict alfalfa quality. Samples of alfalfa that have been fed to cows and for which we have in vivo measurements of fiber digestion will be evaluated with the TTNDFD in vitro method to compare our estimates of fiber digestion to what has been directly measured. Goal (2). An extension publication on the TTNDFD model and presentation materials are being prepared. Our goal is provide these materials to county level extension agents. Agent training on the conceptual basis of TTNDFD and how to instruct dairy producers on how to use TTNDFD to evaluate alfalfa or formulate rations for dairy cows is planned for 2016.

**Impacts**

What was accomplished under these goals? Goal (1). The present study investigated how reduced lignin alfalfa affects in vitro digestibility of fiber (NDF). Two alfalfa cultivars (Roundup Ready (RR) and double stacked RR and Reduced Lignin alfalfa (RR/RL)) were harvested at intervals of 28d, 33d, and 35d from research plots. All replicated plots were established in June of 2014 and harvested for two regrowth periods in July and August in 2014. Statistical analysis was 2x3 factorial design analyzed using SPSS 22. Crude Protein (CP), Neutral Detergent Fiber (aNDF), Lignin, and NDF Digestibility were analyzed. CP content was not significantly different between RR and RR/RL (27.5 vs 28.0, P>0.05) and declined with advancing maturity (P<0.05). aNDF content of RR was significantly higher than RR/RL (31.6 vs 30.1), though differences among harvest intervals were not significant (30.8 vs 30.5 vs 31.1). Lignin content was not significantly different between RR and RR/RL (5.6 vs 5.5) or among harvest intervals (5.6 vs 5.6 vs 5.5). Indigestible NDF (iNDF), rate of fiber digestion (Kd), and total Tract NDF Digestibility (TTNDFD) were analyzed. The reduced lignin alfalfa tended to be lower in iNDF and higher in Kd though differences were not significant. The TTNDFD was significantly higher for the reduced-lignin alfalfa. It appears that RR/RL in alfalfa reduced the aNDF content and improved TTNDFD. Goal (2) we began to
incorporate TTNDFD into extension tools that can be used to more accurately evaluate alfalfa quality and to price alfalfa. The concepts of this approach were shared with hay growers at the Idaho Hay and Forage Conference. Feedback from the target audience is being used as we develop extension tools. We have started working to incorporate TTNDFD into two major extension tools that are widely used to assess alfalfa quality: FeedVal and Milk2006. We anticipate that we will be releasing versions of the FeedVal and Milk extension software that utilize TTDNFD in 2016.