Determining Genetic Factors That Influence Forage Quality In Alfalfa

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Abstract

This integrated project will use 200 diverse alfalfa varieties to determine molecular markers and other genetic information that predict hay quality based on the linkage of these traits with Total Tract Neutral Detergent Fiber Digestibility (TTNDFD). Our overall goal is to implement the resulting data into breeding programs so that new varieties of alfalfa can be generated that maximize that availability of nutrients in ruminant digestion – this will necessarily involve: i) quantifying alfalfa genetic diversity in forage quality in a diverse germplasm, ii) quantifying the relationship between alfalfa quality and other possible confounding and important agronomic parameters; and iii) identifying genetic information associated with forage quality. To accomplish these goals we have developed four objectives: 1) Determine quality and stage of maturity at first harvest of 200 alfalfa varieties at four locations in the PNW. Fall dormancy and yield for all cuttings will be determined to identify and reduce confounding factors with quality. 2) Quantify the genetic diversity of alfalfa that is related to forage quality to understand the potential to breed new alfalfa varieties for higher forage quality. 3) Identify molecular markers associated with forage quality (NDF, NDFD24, NDFD30, NDFD48, kd, iNDF, TTNDFD) traits in alfalfa to aid alfalfa breeders. 4) Extend the knowledge gained from project to positively impact alfalfa producers, breeders and others in the alfalfa industry. Breeding programs from industry stakeholders are cooperators on this project which will impact both alfalfa and dairy producers by improving the reliability of access to more digestive alfalfa feed sources.

Objectives

1) Determine quality at first harvest of 200 alfalfa plant introductions and varieties at three locations in the PNW
2) Quantify the genetic diversity of alfalfa that is related to forage quality
3) Identify genetic areas associated with forage quality
4) Extend the knowledge gained

Materials and Methods

- Grow 200 varieties from diverse germplasm at three locations in PNW in an augmented design using vernal and Alforex 360 as checks in 11 blocks per location
- DNA analysis of germplasm
- Fiber analysis including NDF, NDFD24, NDFD30, NDFD48, kd, iNDF, TTNDFD
- Determine genetic regions and molecular markers related to fiber quality using deep sequencing system

Figure 1. Diverse Germplasm including 148 PI and Std. cultivars from USDA National Plant Germplasm database and 52 varieties from S&W Seed Co., Alforex Seeds, Legacy Seeds and Blue River Hybrids

Figure 2. All three locations are planted and include: Prosser, WA, Union OR, Kimberly, ID with results to follow

Item	Definition
NDF	Neutral detergent fiber. Lab assay that measures total cell wall content of plant material. Composed primarily of cellulose, hemicellulose and lignin. NDF affects animal performance by restricting feed intake and reducing energy value (TDN) of forage
iNDF	Proportion of NDF that is indigestible in ruminants. Measured as the NDF remaining after long-term (240 h) in vitro incubation in rumen fluid. We hypothesize that the iNDF component of fiber is affected in part by biosynthesis of lignin, cellulose, and hemicellulose.
pdNDF	Potentially digestible NDF. Calculated as pdNDF= NDF-iNDF. Proportion of plant fiber that can be digested by bacteria. The absolute amount of pdNDF digested depends on its rate of digestion and time exposure to fiber digesting bacteria.
kd	Rate of digestion of pdNDF. Measured by calculating the change in pdNDF remaining after 24, 30 and 48 hours of incubation in rumen fluid. We hypothesize that the rate of fiber digestion is influenced by the biosynthesis of lignin, cellulose and hemicellulose.
NDFD	NDF digested after a defined period of time. Example NDFD48 is the measured proportion of total NDF remaining after 48 hours incubation in rumen fluid. Typical incubation time measurements are 24, 30, 48 and 240 hours.
TTNDFD	Total tract NDF digestibility. A calculated estimate of in vivo NDF digestion in ruminants. TTNDFD integrates pdNDF, kd and rate of passage into a single digestibility coefficient for NDF.

Table 1. Laboratory measurements of fiber related to forage quality.

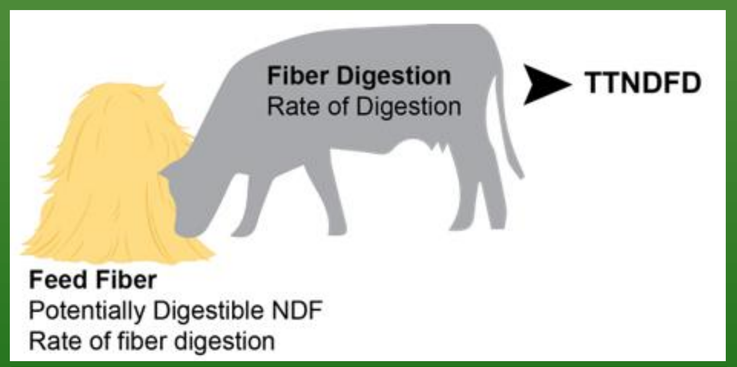

Figure 3. TTNDFD model. Total Tract NDF Digestibility is an indexing tool that predicts how fiber will be utilized in a cow consuming 24 kg of DMI/d of a typical dairy diet (28-30% NDF).

Time point	Responsible PI	Scope of Work
W '17/18	Norberg	Seed is gathered for spring planting
W '17/18	Norberg (2 locations), Wang, Shewmaker	Land is identified and prepared for planting
Sp '18	Norberg, Wang, Shewmaker	Planting of 200 varieties and care of emerged crop
Sp '18	Yu	Collects samples from plots for DNA analysis
1st Harvest	Norberg, Wang, Shewmaker	Mean stage by count and harvest is accomplished with moisture, yield and sample collection, drying and grinding to 2 mm. Send samples to Rock River Lab.
2nd Harvest and others	Norberg, Wang, Shewmaker	Yield, fall dormancy recorded compared to checks
Su/F '18	Norberg, Combs	Rock River Labs sends results to David Combs and Steve Norberg for review and analysis and results forwarded to Yu for use
F/W '18	Yu	Starts genetic process of developing molecular markers from first year's data
W '18	Llewellyn, and Norberg	Start extension activities sharing field results and quality data from four locations
As Needed	Norberg	Attend training, submit progress reports
1st Harvest	Norberg, Wang, Shewmaker	Mean stage by count and harvest is accomplished with moisture, yield and sample collection, drying and grinding to 2 mm. Send samples to Rock River Lab
2nd Harvest and others	Norberg, Wang, Shewmaker	Yield, fall dormancy recorded compared to checks
Su/F '19	Norberg, Combs	Rock River Labs sends results to David Combs and Steve Norberg for review and analysis and results forwarded to Yu for use
F/W '19	Yu	Starts genetic process of developing molecular markers from second year's data
W '19	Llewellyn and Norberg	Continue extension activities sharing field results and quality data from four locations
As Needed	Norberg	Submit progress reports
W '19	Norberg	2 year grant ends submit Final Report

Table 2. Summary of work to be completely by research/extension team.

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