

## Forages and Pastures: Harvested Forages, Ensiling and Forage Utilization

**663 Can bacterial inoculants improve the quality of rust-infested corn silage?** O. C. M. Queiroz\*, A. T. Adesogan, and S. C. Kim, *University of Florida, Gainesville*.

Southern rust is an aggressive disease caused by *Puccinia polysora* which may provide ideal conditions for the growth of undesirable opportunistic fungi that adversely affect forage quality. Little is known about the effects of the disease on the nutritional value of corn silage. Less is known about whether microbial inoculants can improve the quality of rust-infested corn silage. This project aimed to determine how inoculant treatment affects the fermentation, nutritive value and aerobic stability of corn silage containing varying levels of southern rust infestation. Corn plants with no rust (NR), or medium (MR), or high rust (HR) infestation were harvested at random locations from a field, chopped and ensiled alone (Control) or after applying  $1 \times 10^6$  cfu/g of *L. buchneri* and *P. pentosaceus*. Each treatment was prepared in quadruplicate in 20 l mini silos and ensiled for 97 days. As the level of rust infestation increased, concentrations of DM and NDF increased, whereas DM digestibility decreased by up to 13%. Control, HR silages also had lower NDF digestibility (NDFD; 36.2% of DM) than Control, MR (39.8%) or NR silages (38.1%). Inoculation increased the NDFD of NR (43.4%) and MR silages (45.7%) but not HR silages (33.0%). Concentrations of lactate and VFA decreased with increasing rust infestation in NR silages, but this trend was absent in inoculated silages. In HR silages, inoculation reduced mold counts (3.4 log cfu/g vs 0.95 log cfu/g), increased aerobic stability by 75% (77.3h vs 44 h), and prevented production of aflatoxin (5.2 vs. 0 mg/kg). The concentration of aflatoxin in uninoculated, HR silages exceeded action levels stipulated by the US Food and Drug Administration. In conclusion, rust infestation reduced the nutritive value and fermentation of corn silage. Inoculation reduced adverse effects of rust infestation on the fermentation, increased NDFD of NR and MR silages, and decreased mold growth, aerobic spoilage, and aflatoxin production in HR silages.

**Key Words:** *Puccinia polysora*, inoculant, corn silage

**664 Amaferm level and form on digestibility of forage differing in quality.** J. Nocek\*<sup>1</sup> and H. Jensen<sup>2</sup>, <sup>1</sup>*Spruce Haven Research Center, Auburn, NY*, <sup>2</sup>*Biozyme Inc, St Joseph, MO*.

The objective was to determine effects of Amaferm level and form on ruminal DM and NDF digestion of hay and corn silage of different quality. Four hay and corn silage samples with low and high NDF, low and high NDF digestibilities (NDFd, invitro analysis) were utilized to represent ranges in forage quality. Three lactating ruminally cannulated cows were used to determine rumen digestibilities. The treatments: Control: 0, 5, 15, 30g dry Amaferm/d or 10ml of liquid Amaferm/d were introduced into the rumen of each cow daily. Forage was mashed prior to bag insertion. Duplicate determinations were used per time point (0, 2, 4, 6, 12, 24, and 48 h). Dry matter and NDF determinations were conducted on each residue, each time in order to calculate relative pool fractions and rate of insoluble potentially digestible (A = water soluble, B fraction =  $100 - (A + C)$ , KdB, C = 48h ruminal extent) DM and NDF. There was no consistent effect of forage quality within hay or corn silage on DM or NDFd. For hay DM, B fraction was highest ( $P < .05$ ) for 10ml compared to the Control or 5g with the 15 and 30 being intermediate. The extent of digestion was greatest ( $P < .05$ ) for 10ml and 15g compared to other treatments. The KdB was also highest ( $P < .01$ ) for the 15g treatment compared to other treatments. Extent of hay NDFd was highest ( $P < .05$ ) for 10ml compared to other treatments. Doses of 15g and 10ml

yielded higher ( $P < .05$ ) KdB compared to other treatments. Corn silage DMA and B fraction was not influenced by treatment ( $P > .10$ ). However, C fraction was lowest ( $P < .01$ ) for cows receiving 10ml compared to 5g with Control, 15 and 30g being intermediate. Extent of corn silage NDFd was highest ( $P < .05$ ) for cows receiving 10ml, intermediate for 15 and 30g and lowest for Control and 5g. Rates were not influenced by dose. Amaferm fed at 15g/cow/d in a dry form, or 10ml/cow/d in a liquid form, increased ruminal DMd of hay by increasing the extent and rate of digestion for both DM and NDF. The extent of corn silage DM and NDFd was increased when the 10ml/h/d Amaferm was included in the rumen.

**Key Words:** NDF digestibility, forage

**665 The ability of enterococci to survive the ensiling process.** S. N. Masiello\* and C. S. Petersson-Wolfe, *Virginia Polytechnic Institute and State University, Blacksburg*.

The objective of the current study was to determine the ability of enterococci to survive a 3-wk ensiling process. Harvested grass and corn crops were respectively divided into 3 treatment groups consisting of 2 commercially available silage inoculants and 1 negative control group. Within 24 h of harvest, a uniform amount of forage was added to each of 18 vacuum sealable freezer bags. Inoculants 1 and 2 were applied (according to manufacturer directions) to each of 6 bags and the remaining 6 bags were not inoculated and served as negative controls. An industry grade vacuum sealer was used and 90% nitrogen gas was pumped into the bag to create a pillow pack. Dry matter and bacterial enumeration were performed on the forage prior to ensiling as well as after each week of ensiling. Bacterial enumeration was conducted according to standard bedding sampling procedures with the addition of the Kanamycin Esculin Azide Agar for the enumeration of enterococci. At wk 1, 2, and 3 of the ensiling process, a total of 6 bags were opened; 2 from each of the 3 treatment groups. Preliminary data suggest an increased number of enterococci on inoculated grass samples compared with the negative control after the 3-wk ensiling process. Inoculant 2 ( $7.3 \pm 0.1$  log cfu/g DM) displayed a greater count than Inoculant 1 ( $4.2 \pm 1.7$  log cfu/g DM) after a 3-wk process and both were greater than the negative control ( $1.2 \pm 1.2$  log cfu/g DM). Inoculation of corn silage did not appear to change the enterococci count following the 3-wk ensiling. Enterococci counts for Inoculant 1, Inoculant 2, and the negative control group were  $4.3 \pm 0.1$  log cfu/g DM,  $4.4 \pm 0.1$  log cfu/g DM, and  $4.0 \pm 0.1$  log cfu/g DM, respectively. The addition of a silage inoculant led to greater levels of enterococci in grass silage compared with the negative control at the end of a 3-wk ensiling period. Enterococci levels did not show a marked difference in the inoculated corn silage samples. These preliminary data suggest that enterococci are able to survive the harsh conditions of an ensiling period.

**Key Words:** mastitis, silage inoculant, *Enterococcus*

**666 Expression of genes related to cell wall digestibility of tropical forages.** S. S. Stabile<sup>1</sup>, L. Jank<sup>2</sup>, A. P. Bodini<sup>1</sup>, N.S. Oliveira<sup>1</sup>, L. V. Marçó<sup>1</sup>, and L. F. P. Silva\*<sup>1</sup>, <sup>1</sup>*Universidade de São Paulo, Pirassununga, SP, Brazil*, <sup>2</sup>*EMBRAPA, Campo Grande, MS, Brazil*.

Cell wall lignification with advanced maturity is the main limiting factor for ruminant production in tropical systems. Our objective was to iden-

tify genes related to rapid decline in cell wall digestibility with advanced maturity, in order to identify targets for future genetic manipulations. Eleven genotypes of *Panicum maximum* were harvested at 30, 60 and 90 days of regrowth. Samples were divided in three fractions: leaf blades, stems and senescent material. After drying and grinding, the fractions were incubated for 30h at 39°C in ruminal fluid and MacDougall solution for determination of in vitro cell-wall digestibility (NDFD). There was no difference among genotypes for NDFD of leaves, but there was a significant genotype and genotype × maturity interaction for NDFD of stems. From the 11 genotypes, we selected 3 with the slowest decline in stem NDFD and 3 with rapid decline of stem NDFD with advanced maturity for gene expression evaluation. Total RNA was isolated, treated with DNaseI and converted to cDNA for PCR quantification. Real-time PCR was used to quantify the expression of genes from the lignin biosynthesis pathway, using oligonucleotide primers specific for *P. maximum* phenylalanine ammonia lyase (PAL), caffeic acid O-methyltransferase (COMT), cinnamate 4-hydroxylase (C4H), cinnamoyl-CoA reductase, cinnamyl alcohol dehydrogenase and 4-coumarate CoA ligase. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control gene. We were interested in existing treatment x maturity interaction, which would indicate that the referred gene was expressed differentially between the rapid and the slow group of genotypes. There was a significant interaction for the genes C4H, COMT and PAL, where there was larger increase in expression from 30 to 60 days in the rapid group and no difference with 90 days of regrowth. These results indicate that the larger increase in expression of these genes from 30 to 60 days of regrowth could explain the faster decline in NDFD.

**Key Words:** Guineagrass, lignin, COMT

**667 Effect of citrate synthase genes transformed into alfalfa on aluminum tolerance of its cells.** F. Fan\*, J. J. Li, Y. M. Wu, and J. X. Liu, Zhejiang University, Hangzhou, P. R. China.

This study was conducted to investigate the effect of bivalence citrate synthase genes transformed into alfalfa (*Medicago sativa* L.) on aluminum tolerance of its cells. Two different kinds of citrate synthase genes (CS) were cloned from *E. coli* (CSI, 1284bp) and *Oryza sativa* L (CSII, 1425bp), respectively. Bivalence expression vector containing CSI, CSII and the phosphomannose isomerase gene isolated from *E. coli* as the selectable marker gene, was constructed and transferred into *Agrobacterium tumefaciens* LBA4404 strain through electrotransformation method. The expression vector containing CSI alone was also constructed with the same method. Leaf disks of *Medicago sativa* L cultivar Youke were inoculated with *Agrobacterium tumefaciens* LBA4404 and selected on the mannose (30g/L). The callus were grown on the solid SH medium for 6 weeks in a chamber at 25°C with 75% relative humidity, and exposed to 14 h of light and 10 h darkness. Integration of the transgene was detected by PCR with genomic DNA of the callus as template. Transcription of the transgene was determined by cDNA dot blotting analysis. The callus tissues of transgene were cultured in the liquid SH medium under the shake at 150 × g and 25°C for 2 days. The activity of the cells and their ATPase were detected when stressed under the different concentrations of Al<sup>3+</sup>. The specific PCR products of expected size (1284bp and 1425bp) were observed in the genomic DNA from all transformed callus, but not occurred in the non-transformed callus, indicating that two CS genes were integrated into the transformed callus. The cDNA dot blotting of the transformed callus revealed that the interested genes were transcribed. The activity of both the cells and their ATPase was significantly ( $P < 0.05$ ) higher in the transformed than that in the non-transformed cells, with significantly ( $P < 0.05$ ) higher

activity in both CSI and CSII transformed than in the CSI transformed cell. It is indicated that expression of the CS gene in the transformed cells could improve the aluminum tolerance of alfalfa cells.

**Key Words:** citrate synthase gene, aluminum tolerance, alfalfa

**668 A survey of condensed tannin concentrations in vegetative and mature legume forages in western Canada.** N. Berard<sup>1</sup>, K. Ominski\*<sup>1</sup>, K. Wittenberg<sup>1</sup>, D. Krause<sup>1</sup>, T. McAllister<sup>2</sup>, and Y. Wang<sup>2</sup>, <sup>1</sup>University of Manitoba, <sup>2</sup>Agriculture and Agri-Food Canada.

Condensed tannin (CT) concentrations of legume forages and the potential benefits of inclusion in ruminant diets have been well-documented in semi-arid or tropical regions. To date, there has been limited effort to examine the CT concentration of legume forage species grown in temperate climates such as the prairie region of western Canada. As such, CT concentrations were measured using the butanol-HCl technique in *Medicago sativa*, *Trifolium hybridum*, *Trifolium ambiguum*, *Dalea purpurea*, *Trifolium pratense*, *Onobrychis vicifolia*, *Lotus corniculatus*, and *Trifolium repens* varieties grown in research plots across the prairies. Above ground plant biomass was harvested at vegetative and mature stages for two growing seasons. Statistical analyses were performed using SAS. Significant interactions, LS means, SE and SD for stage of maturity and growing season were determined using PROC MIXED. *Dalea purpurea* (purple prairie clover), a native legume, had the highest mean condensed tannin concentration of 68.7 ± 22.6 g/kg DM, with minimum and maximum values ranging from 37.9 to 92.9 g/kg DM. *Onobrychis vicifolia* (sainfoin) had the second highest mean CT concentration of 46.0 ± 19.3 g/kg DM with a range of 16.3 to 94.4 g/kg DM. The third highest mean CT concentration of 15.1 ± 6.3 g/kg DM was found in *Lotus corniculatus* (birdsfoot trefoil) with a range from 0.0 to 25.7 g/kg DM. No condensed tannin was found in *Medicago sativa* (alfalfa) or *Astragalus cicer* (cicer milkvetch). Forage CT concentrations were higher ( $P < 0.05$ ) when in the mature stage compared to vegetative stage for all species except sainfoin. Growing season did not have a significant effect on plant CT concentration. Potential benefit of inclusion in ruminant diets at the identified concentrations requires further exploration.

**Key Words:** condensed tannin concentration, forage, western Canada

**669 Development of prediction equations to estimate hay intake of beef cows under limited access feeding times.** T. S. Dennis\*<sup>1</sup>, T. D. Nennich<sup>1</sup>, R. P. Lemenager<sup>1</sup>, C. J. Fleenor<sup>1</sup>, S. L. Lake<sup>2</sup>, and L. J. Unruh-Snyder<sup>1</sup>, <sup>1</sup>Purdue University, West Lafayette, IN, <sup>2</sup>University of Wyoming, Laramie.

Livestock producers are often faced with limited feed supplies due to drought, disaster, or other shortages and are forced to restrict intake of available feeds. The objective of this study was to develop prediction equations to determine the DMI of forages with differing qualities at limited access feeding times. Forty-five mature, mid-gestation Angus crossbred cows (BW = 602.7 kg; BCS = 5.13) were randomly assigned to pens of five cows by BW, BCS, and breed type. Pens of cows were assigned to one of three limited access times (1, 2, or 4 h) with three replications each. Actual pen intake of LOW (10.9% CP, 60.3% NDF, 59% TDN), MID (12.3% CP, 57.5% NDF, 58% TDN), and then HIGH (18.1% CP, 45.9% NDF, 63% TDN) quality hay was determined three times/wk. On non-sampling days, cows were allowed limited access, according to assigned time, to large round bales of hay (12.8% CP,

56.1% NDF, 60% TDN). All cows were supplemented with soybean hulls and a vitamin-mineral mix to meet NRC requirements for maintenance. Data collected from this study were combined with data from Fleenor et al., 2008 (Abstr.) to develop regression equations. Increased daily access feeding times and/or hay quality resulted in greater hay DMI. Regression equations developed to estimate hay DMI for limited access fed cows were Hay DMI (kg/d) =  $1.80 \times \text{Hours access} - 0.12 \times \text{Hay NDF\%} + 8.34$  ( $n = 72$ ,  $R^2 = 0.82$ ) and Hay DMI (% of BW) =  $0.30 \times \text{Hours access} - 0.02 \times \text{Hay NDF\%} + 1.34$  ( $n = 72$ ,  $R^2 = 0.82$ ). These regression equations should provide livestock producers with a valuable tool for formulating diets with differing forage qualities and limited daily access feeding times. Further research is necessary to validate these equations.

**Key Words:** cows, hay intake, limited access time

**670 Whole plant barley NDF digestibility and its relationship with chemical constituents and dry matter yield.** M. L. Swift<sup>\*1</sup>, M. Oba<sup>2</sup>, P. E. Juskiw<sup>1</sup>, and J. H. Helm<sup>1</sup>, <sup>1</sup>Alberta Agriculture and Rural Development, Lacombe, AB, Canada, <sup>2</sup>University of Alberta, Edmonton, AB, Canada.

The objective of this study was to determine the relationships of 30-h in vitro NDF digestibility (IVFD) of whole plant barley with yield, chemical constituents, and growing environment. Concentrations of ADF, NDF and lignin, and IVFD were determined for 173 samples of whole plant barley harvested in 2005 ( $n=56$ ), 2006 ( $n=58$ ) and 2007 ( $n=59$ ). Correlation coefficients ( $r$ ) were computed using Proc Corr (SAS) to evaluate the relationships of IVFD with lignin (%DM or %NDF), ADF, NDF, dry matter yield (DMY), growing degree days (GDD) and precipitation during the growing period. To evaluate the effect of hull type (hulled versus hull-less) and row type (2 versus 6) the data were analyzed using Proc Mixed (SAS) with year as a random variable. The IVFD was best related to lignin (%NDF), regardless of hull or row type as shown by  $r$  values of -0.80 (hulled 2-row), -0.85 (hulled 6-row), -0.82 (hull-less 2-row) and -0.91 (hull-less 6-row). The  $r$  values of IVFD with NDF content were 0.11 and 0.46 for hulled and hull-less barleys, respectively regardless of row. Similarly, there was not a strong relationship between IVFD and DMY (-0.28, 0.22, -0.35, -0.22), GDD (-0.30, -0.13, 0.25, -0.04) or precipitation (-0.39, -0.47, -0.16, -0.30) for hulled 2-row, hulled 6-row, hull-less 2-row and hull-less 6-row, respectively, during the growing period. The hull type ( $P=0.883$ ) or row type ( $P=0.545$ ) of barley had no effect on IVFD. The IVFD values ranged from 43.5 to 57.4% for hulled 2-row, 37.9 to 59.5% for hulled 6-row, 39.1 to 55.0 for hull-less 2-row, and 34.9 to 61.5 for hull-less 6-row whole plant barley. The large variation in IVFD within hull and row type of barley indicates that there is scope for genetic selection of cultivars with enhanced fiber digestibility.

**Key Words:** barley, NDF, digestibility

**671 Forage quality of biomass vs. conventional alfalfa cut at early bud or late flower maturity.** H. G. Jung<sup>\*1,2</sup>, K. P. Rock<sup>2</sup>, and J. F. S. Lamb<sup>1,2</sup>, <sup>1</sup>USDA-ARS, St. Paul, MN, <sup>2</sup>University of Minnesota, St. Paul.

Cellulosic bioenergy systems will result in large areas planted to biomass crops. An important question is whether biomass crops can also

be used for livestock feed. This study compared forage quality of an experimental alfalfa germplasm developed for a biomass production system with a conventional variety when harvested at early bud (four times annually) and late flower (three times annually) maturity stages. Replicated field trials were established at two locations and harvested in 2007 and 2008. Leaf percentage and stem NDF, ADL, and 16- and 96-h in vitro rumen NDF digestibility (IVNDFD) were determined. As expected, delaying harvest to late flower reduced leaf percentage (56.5 vs. 46.7%), and increased stem NDF (58.7 vs. 65.9% DM) and ADL (16.0 vs. 16.7% NDF) concentrations and reduced stem 16-h (22.0 vs. 20.0%) and 96-h (55.5 vs. 50.8%) IVNDFD. When harvested at early bud, biomass alfalfa had less leaf material (55.6 vs. 57.4%) and a lower stem ADL concentration (15.9 vs. 16.0% NDF), was lower in stem fiber digestibility (21.8 vs. 22.1% and 55.4 vs. 55.7% for 16- and 96-h IVNDFD, respectively), and had a higher stem NDF concentration in the first (58.3 vs. 55.7%) and last (61.3 vs. 59.4%) harvests, but not the two mid summer harvests. Harvesting at late flower, biomass alfalfa again had a lower leaf percentage (44.3 vs. 49.2%) and stem ADL concentration (16.6 vs. 16.8% NDF). Stem NDF concentration of the biomass alfalfa was higher (67.0 vs. 64.7% DM) for all three late flower harvests. Stem fiber digestibility was lower for biomass alfalfa at two of three harvests, the first (19.9 vs. 20.7% and 49.6 vs. 51.1% for 16- and 96-h IVNDFD, respectively) and last (19.1 vs. 19.6% and 49.2 vs. 50.3% for 16- and 96-h IVNDFD, respectively). When harvested at late flower for biomass, both alfalfa types were of low quality. While quality of the biomass alfalfa was lower than the conventional variety, the reductions were minor if harvested at early bud. Producers will have some flexibility in how they manage and utilize biomass alfalfa varieties.

**Key Words:** alfalfa, forage quality, biomass

**672 Nutritional evaluation of shrubs as fodder source for ruminants.** J. Sultan<sup>\*</sup>, I.-U. Rahim, M. Yaqoob, and H. Nawaz, *University of Agriculture, Faisalabad, Pakistan.*

The intent of the study was the nutritional evaluation of shrubs as fodder source for ruminants. Eight shrub species (*Debregeasia salicifolia*, *Indigofera gerardiana*, *Anisomoles indica*, *Marisina affricana*, *Desmodium* spp., *Impatiens bicolor*, *Dodonaea viscosa*, *Adhatoda vesica*) were selected and analyzed for dry matter (DM), organic matter (OM), ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemi-cellulose, and lignin contents. The mean percentage values for DM, OM, ash, CP, NDF, ADF, hemi-cellulose and lignin were  $31.4 \pm 2.16$ ,  $27.8 \pm 1.83$ ,  $11.3 \pm 0.67$ ,  $17.6 \pm 1.06$ ,  $59.1 \pm 2.05$ ,  $30.4 \pm 2.04$ ,  $28.8 \pm 2.33$  and  $5.7 \pm 0.46$ , respectively. The mean percentage values for Ca, P, K and Mg at were  $1.22 \pm 0.223$ ,  $0.044 \pm 0.006$ ,  $0.77 \pm 0.095$  and  $0.019 \pm 0.004$ , respectively and mean values for Cu, Zn, Mn and Co were  $18.42 \pm 1.556$ ,  $19.64 \pm 5.894$ ,  $11.01 \pm 1.103$  and  $0.042 \pm 0.007$ , respectively. In shrub species, the highest ( $p < 0.05$ ) potential intake rate (PIR) was observed for *Debregeasia salicifolia* ( $67.50 \pm 11.00$  g/4 minute) and lowest for *Adhatoda vesica* ( $8.80 \pm 1.54$  g/4 minute). However, the highest ( $p < 0.05$ ) relative preference (RP) was noted for *Debregeasia salicifolia* ( $83.20 \pm 1.22\%$ ) and lowest for *Adhatoda vesica* ( $3.63 \pm 0.88\%$ ). The mean in vitro dry matter digestibility (IVDMD) and metabolizable energy (ME) of fodder shrubs species were  $56.9 \pm 2.76\%$  and  $7.74 \pm 0.31$  MJ/kg DM, respectively. The IVDMD, RP and PIR values indicate that fodder shrubs be fed to livestock with some supplementation for different levels of production and types of livestock.

**Key Words:** shrubs, chemical composition, metabolizable energy