

production of Holstein heifers in Iran has been due to environmental rather than genetic improvement.

**Key Words:** Genetic evaluation, Covariance function, Iranian Holsteins

**M37 Phenotypic study of lactation curve in Iranian Holsteins.** H. Farhangfar\* and H. Naemipour, *Birjand University, Birjand, Iran.*

A total of 136250 monthly test day milk records collected from 13625 Iranian Holstein heifers (3x milking) calved between 1991 and 2001 and distributed in 264 herds, was used to study the effects of some environmental factors influencing lactation curve parameters as well as production characteristics. Wilmink function ( $Y_t = W_0 + W_1t + W_2e^{-0.05t}$ ) was fitted to individual lactations. The results of least squares analysis of variance indicated that herd, year and month of calving had a very high significant effect on all traits under consideration. Correlation analysis showed that parameter  $W_0$  had a negative and significant relationship with parameters  $W_1$  and  $W_2$  while it was positively significantly correlated with milk at peak time and 305-day milk yield. Simple linear regression analysis of adjusted means of 305-day milk yield, days to reach peak yield as well as peak milk yield on year of first calving also revealed that there was a phenotypic increase of 137.152 Kg, 0.535 day and 0.434 Kg per year respectively.

**Key Words:** Lactation curve, Wilmink function, Iranian Holsteins

**M38 Estimation of genetic trends for milk production traits in Iranian Holsteins.** H. Farhangfar\*, H. Naemipour, and M. R. Asghari, *Birjand University, Birjand, Iran.*

In order to estimate genetic trends for milk production traits in Iranian Holsteins a total of 18989 adjusted 305d-2X-ME first lactation records collected from 18989 heifers calved between 1995 and 2001 and distributed in 202 herds over the country were used. The traits were milk, fat and protein yields as well as fat and protein percentages. A multiple trait animal model was utilized. The effects included in the animal model were fixed contemporary group of province-herd-year-season of calving, linear covariate of Holstein genes and random additive genetic effect. Restricted maximum likelihood estimates of variance and covariance components and best linear unbiased prediction of breeding value of individual cows for each trait were obtained by using MTC and MTJAAM softwares respectively. Genetic trends for the traits under consideration were estimated based upon simple linear regression analysis of BLUP solutions on year of first calving. The results obtained in this study revealed that the average breeding value of cows had a significant increase of +33.178 Kg per year ( $P < 0.01$ ) for milk yield. At the same time, fat and protein yields had positive genetic trends of +245 g ( $P > 0.05$ ) and +789 g per year ( $P < 0.05$ ) respectively. In contrast to yield traits, the genetic trends calculated for fat and protein percentage showed an annual decrease of -0.011 % ( $P < 0.001$ ) and -0.003 % ( $P < 0.05$ ) respectively which were significant statistically.

**Key Words:** Genetic trend, Milk production traits, Iranian Holsteins

## Forages and Pastures: Forage Quality

**M39 Direct or sequential determination of ADF in legume forages.** M. J. Marichal\*, M. Carriquiry, and A. I. Trujillo, *Facultad de Agronomía, Montevideo Uruguay.*

Objectives of this study were to compare ADF values of legume forages obtained from samples treated directly with acid detergent solution (direct, DP) or after extraction with NDF solution (sequential, SP) and the effect of the ADF extraction method on lignin quantification. Pastures (n=26) corresponding to nine harvest dates (HD) of alfalfa (AA), ten HD of birdsfoot trefoil (BT), and seven HD of red clover (RC) were evaluated. For each specie and HD, ADF and Lignin concentrations were determined in triplicate. The ADF was determined by filter bag technique (ANKOM Technology), corrected for ash. In SP,  $Na_2SO_3$  was not included in NDF solution. Lignin was determined by extraction with 72%  $H_2SO_4$  (Ligsa). Data were analyzed in a completely randomized design and the model included specie, extraction procedure, and their interaction. There was an interaction ( $P < 0.02$ ) of specie with extraction procedure for ADF concentration as ADF concentration determined by DP tended ( $P = 0.10$ ) to be greater than SP for AA ( $27.9$  vs  $26.4 \pm 1.5$ ) and lower ( $P < 0.01$ ) for BT ( $29.7$  vs  $32.1 \pm 1.5$ ) whereas ADF for RC ( $28.7$  vs  $29.4 \pm 1.5$ ) did not differ ( $P = 0.55$ ) between extraction procedures. There was an effect of ADF extraction method on Ligsa quantification as Ligsa was greater ( $P < 0.01$ ) for SP than DP for all legume species ( $8.57$  vs  $6.75 \pm 0.44$ ). It would be advisable to always specify the procedure followed in ADF quantification. Additional studies are required to establish equivalency between ADF determined by DP and SP, and the affects on nutritional fractions such as Ligsa and ADIN.

**Table 1. Number of determinations resulting on equal or different concentrations for ADF or Lignin**

| Specie            | Number | ADF   |           | Lignin |           |
|-------------------|--------|-------|-----------|--------|-----------|
|                   |        | Equal | Different | Equal  | Different |
| Alfalfa           | 9      | 4     | 5         | 7      | 2         |
| Birdsfoot trefoil | 10     | 3     | 7         | 5      | 5         |
| Red clover        | 7      | 6     | 1         | 3      | 4         |

**Key Words:** ADF, Lignin, Extraction procedure

**M40 Direct verses sequential analysis of acid-detergent insoluble nitrogen in forage legume hays.** J. H. Grabber\* and D. R. Mertens, *USDA-Agricultural Research Service, US Dairy Forage Research Center, Madison, WI.*

Acid-detergent insoluble nitrogen (ADIN) is thought to represent nitrogen that is not degraded during ruminal and post-ruminal digestion of forages by cattle. Forage ADIN can be determined following direct acid detergent extraction or following sequential extraction with neutral and acid detergents. In this study, we evaluated how direct verses sequential detergent extraction influenced the ADIN content of legume hays differing in polyphenol composition and degree of conditioning at harvest. In 2002 and 2003, first and second cuttings of polyphenol-free alfalfa, low to high tannin birdsfoot trefoil, and red clover with

o-diphenols and polyphenol oxidase were conditioned by rolls or by maceration and then dried as hay. ADIN, as a proportion of total N, was determined by combustion after direct or sequential detergent extraction by the ANKOM filter bag system, without the addition of sulfite or amylase to neutral detergent solution. The forage X conditioning method X ADIN method interaction was highly significant ( $P < 0.0001$ , SEM 3.7 g/kg). In hays conditioned with rolls, ADIN concentrations by direct and sequential methods averaged 41 and 34 g/kg for alfalfa, 55 and 32 g/kg for low tannin trefoil, 50 and 38 g/kg for moderate tannin trefoil, 57 and 41 g/kg for high tannin trefoil, and 48 and 33 g/kg for red clover. In hays conditioned by maceration, ADIN concentrations by direct and sequential methods averaged 41 and 34 g/kg for alfalfa, 41 and 36 g/kg for low tannin trefoil, 33 and 40 g/kg for moderate tannin trefoil, 40 and 55 g/kg for high tannin trefoil, and 52 and 40 g/kg for red clover. Thus, analysis method, forage type, and severity of conditioning differentially influenced ADIN concentrations. The potential impact of shifts in ADIN and other nitrogen fractions on milk production and nitrogen utilization by dairy cattle will be evaluated using nutrition models.

**Key Words:** Acid detergent insoluble nitrogen, Forage conditioning, Polyphenols

**M41 Ruminant dry matter, crude protein, neutral detergent fiber and acid detergent fiber degradation parameter kinetics of *Agropyron tauri*, *Agropyron trichophorum*, and *Bromus tomentellus*.** P. Shawrang<sup>1</sup>, A. Nikkhah<sup>\*1</sup>, and A. A. Sadeghi<sup>2</sup>, <sup>1</sup>Tehran University, Karaj, Iran, <sup>2</sup>Science and Research Campus, Islamic Azad University, Tehran, Iran.

This study was carried out to determine rate and extent of DM, CP, NDF and ADF degradation parameter kinetics of *Agropyron tauri* (ATA), *Agropyron trichophorum* (ATR), and *Bromus tomentellus* (BT). Forage samples were collected at the preflowering stage in spring by hand. Dried forage samples were incubated for 0, 6, 12, 24, 48, 72 and 96 h in the rumen of four Varamini wethers. Immediately after submersion of the 0 h bags of substrate into the ruminal fluid, all bags were removed and rinsed with an automatic washing machine. Bags were then freeze dried, weighed and analysed for chemical composition. Data were fitted to non-linear degradation model to calculate effective rumen degradation (ERD), and analysed (DM, CP, NDF and ADF degradability parameters of forages) using GLM proc of SAS (1996) as CRD design. Crude protein contents of ATA, ATR and BT were 123, 72, and 143 g/kg, NDF contents were 604, 638, and 451 g/kg and ADF contents were 348, 378 and 273 g/kg DM, respectively. There were differences ( $P < 0.05$ ) between effective DM, CP, NDF and ADF degradability of these pasture species. The ERD of DM, CP, NDF and ADF for ATA at rumen outflow rate of 0.05/h were 473, 630, 239 and 395 g/kg, for ATR were 393, 498, 331 and 337 g/kg, and for BT were 548, 760, 298 and 237 g/kg, respectively. Constant degradation rate of DM, CP, NDF and ADF for ATA were 6.0, 11.8, 2.7 and 5.7%/h, for ATR were 3.8, 8.6, 5.8 and 3.5 %/h and for BT were 4.2, 10.0, 1.8 and 3.7%/h, respectively. The differences between pasture species in the rate and extent of DM, CP and fiber degradation are likely to lead to major differences in forage intake, therefore these characteristics must be considered as main parameters in developing models and ration formulation of grazing ruminants.

**Key Words:** Pasture species, Chemical composition, Ruminant degradation

**M42 Voluntary feed intake, rumen fermentation characteristics and nitrogen retention in Iranian Balouchi sheep fed halophyte forages.** A. Riasi<sup>\*1</sup>, M. Danesh Mesgaran<sup>2</sup>, H. Nassiri Moghaddam<sup>2</sup>, and A. Heravi Moussavi<sup>2</sup>, <sup>1</sup>University of Birjand, Birjand, Khorasan, Iran, <sup>2</sup>Ferdowsi University of Mashad, Mashad, Khorasan, Iran.

Halophyte forages (*Kochia scoparia* and *Atriplex dimorphostegia*) were harvested from different regions of central Iran desert (with average annual rainfall of 170 mm). These forages are halophytic plants typically grown on salty land that are a significant part of the local flora in central Iran. During unfavorable environmental conditions, these forages can provide supplemental or emergency feed for ruminants. Each sample ( $n=20$ ) was immediately chopped and composited before air-drying. Dried forages were analyzed for total N, NDF, ash, Na, Cl and K. Nine cannulated Balouchi ewes ( $48 \pm 2$  kg) were transferred to metabolism cages and randomly allocated to three dietary treatments [T 1: kochia + alfalfa (1:1), T 2: atriplex + alfalfa (1:1), T 3: alfalfa] in a Latin Square design with three periods of 21 d each. Animals had ad libitum access to feed and water during each period. The results showed that the voluntary dry matter intake of T 1 and T 2 were lower ( $P < 0.05$ ) compared with T 3 (468 and 486 g/d, respectively v 628 g/d). Mean ruminal pH (T 1= 7.53, T 2= 7.52, T 3= 7.46) and ammonia-N concentrations (T 1= 10.25, T 2=9.89, T 3= 9.71 mg/100ml) were similar for all treatments at different times after feeding (0, 0.5, 1, 2, 3, 4, 6, 8 hours) ( $P > 0.05$ ). The N retention was higher ( $P < 0.001$ ) in ewes fed T 3 (2.03 g/d) compared with T 1 (1.07 g/d) and T 2 (0.93 g/d). Urine volume for T 2 was higher ( $P < 0.01$ ) than for the other two treatments. Sheep fed an Atriplex-based diet excreted more Na in the urine daily ( $P < 0.01$ ) whereas sheep fed kochia excreted more urea-N in the urine daily ( $P < 0.001$ ). Results showed that the diets containing kochia or atriplex have good potential as ruminants' feeds.

**Key Words:** Feed intake, Rumen fermentation, Halophyte forages

**M43 Effective neutral detergent fiber supply to dairy grazing cows by alfalfa pasture.** R. Gregoret<sup>\*1</sup>, M. Gallardo<sup>1</sup>, P. Ludueña<sup>2</sup>, and M. Cagnolo<sup>2</sup>, <sup>1</sup>INTA Rafaela Experimental Station, Rafaela, Santa Fe, Argentina, <sup>2</sup>Villa Maria National University, Villa Maria, Cordoba, Argentina.

The objective of this trial was to evaluate the effectiveness of the neutral detergent fiber (NDF) supply by an autumn alfalfa pasture and their effect on milk composition. Six lactating Holstein cows (100 DIM) were used in a cross-over design with two 28 days periods. The treatments were: Alfalfa hay coarsely chopped (LH), and Alfalfa pasture grazed (AG). In LH, penned cows were fed ad-libitum coarsely chopped alfalfa hay. In AG, cows daily grazed alfalfa in strips that provided approximately 40 kg DM/d. Pasture available herbage was  $1655 \pm 182$  kg/ha. All cows received 2.7 kg DM/d of ground corn grain, half at each milking. Pasture DMI was estimated by difference between pre- and postgrazing pasture mass. Chewing activities of the six cows were monitored visually every 5 min for a 24-h period in each experimental period. Alfalfa hay particle size was 44%  $> 13$  mm and 49%  $> 2$  mm. Cows receiving alfalfa hay had significantly higher DMI than AG cows (22.8 vs. 18.1 kg DM/d,  $P = 0.023$ ). The NDF intake was also higher for the LH cows (10.6 vs. 5.4 kg DM/d,  $P < 0.001$ ). The time used for rumination was 447 and 318 min/d for LH and AG respectively ( $P = 0.051$ ). Total chewing activity per kilogram of NDF was significantly higher for grazing cows (83.95 vs. 135.17 min/kg NDF,  $P = 0.026$ ). No significant differences were detected in milk production (16.5 vs. 17.8 kg/d,  $P = 0.445$ ). Milk fat content was lower for the grazing (3.42%) than for the hay feed cows (3.99%), ( $P =$

0.018). Concentration of short and middle chain fatty acids (C4 – C17) was lower in milk from grazing cows ( $P < 0.05$ ). On the other hand C18 chain fatty acids concentration was significantly higher for grazing cows ( $P < 0.05$ ). Concentration of C18:1 trans and CLA were four times as high in AG milk, as compared to LH treatment ( $P < 0.01$ ). Under the condition of the present work the NDF of the grazed alfalfa was not effective enough to support the milk fat of grazing dairy cows. Grazing could enhance the production of milk fat synthesis inhibitors.

**Key Words:** Alfalfa grazing dairy cows, Milk fat depression, eNDF

**M44 Forage conservation effects on conjugated linoleic acid and trans-C18:1 production by rumen microbes when incubated with soybean oil and fish oil in continuous culture.** R. Buckles, A. AbuGhazaleh\*, and G. Apgar, *Southern Illinois University, Carbondale*.

The objective of this study was to evaluate the effect of forage conservation method (freeze-dried vs. oven-dried) on ruminal production of trans-C18:1 and cis-9, trans-11 conjugated linoleic acid (CLA) when incubated with soybean oil (SBO) and fish oil (FO) in continuous culture. Forages (70% Pasja, 30% oat) were grown in paddocks, and a 30 kg sample was collected. One portion was rapidly frozen  $-5^{\circ}\text{C}$  and then freeze-dried (T1) while the other portion was oven-dried (T2) at  $45^{\circ}\text{C}$  for 48 h. Four dual flow continuous culture apparatus, designed to simulate ruminal digestion and solid and liquid outflow, were used in this experiment in two 10 d periods using a cross over design. Treatment diet (50% forage, 50% concentrate) was fed (120 g/d DM basis) in three equal portions during the day. The concentrate mix contained 1% FO and 2% SBO (DM basis). Concentration of trans-C18:1 was not affected ( $P < 0.15$ ) by conservation method, but was numerically higher with T2 (22.0 mg/g of overflow) than T1 (20.4 mg/g of overflow). The concentration of cis-9, trans-11 CLA tended ( $P < 0.08$ ) to increase with T2 (0.32 mg/g of overflow) compared with T1 (0.18 mg/g of overflow). Concentration of linolenic acid was higher ( $P < 0.05$ ) with T2 (2.12 mg/g of overflow) than T1 (1.72 mg/g of overflow). Based on these results, forage conservation method has limited effect on ruminal production of trans-C18:1 and cis-9, trans-11 CLA in continuous culture.

**Key Words:** Forage, Trans FA, Continuous culture

**M45 The effect of fatty acid source and forage source on trans-C18:1 and conjugated linoleic acid production by ruminal microbes in batch culture.** R. Buckles, A. AbuGhazaleh\*, and G. Apgar, *Southern Illinois University, Carbondale*.

Previously, docosahexaenoic acid (DHA) was identified as the active component in fish oil that enhances trans-C18:1 production. The objective of this study was to evaluate the effect of combining DHA with linoleic acid (C18:2) and linolenic acid (C18:3), using three different dried forages (oats, pasja, or alfalfa) on production of trans-C18:1 and conjugated linoleic acid (CLA) in batch culture. Treatments were as follows: 1) Forage (T1), 2) Forage + 100mg C18:2 (T2), 3) Forage + 80mg C18:2 + 20mg DHA (T3), 4) Forage + 100mg C18:3 (T4), and 5) Forage + 80mg C18:3 + 20mg DHA (T5). Treatments (15 in total) were incubated in triplicate in 500 ml flasks containing 2g finely ground forage, 40 ml strained ruminal fluid, 160 ml

media, and 8 ml reducing solution. A 5-ml sample of culture contents was taken at 24 h for fatty acid analysis by gas liquid chromatography. After 24 h of incubation, the concentration of trans-C18:1 was higher ( $P < 0.01$ ) in cultures incubated with C18:2 than C18:3. Addition of DHA further increased the concentration of trans-C18:1 and the increase was greatest ( $P < 0.01$ ) with T3. Forage type had no effect ( $P > 0.05$ ) on trans-C18:1 concentration. Cis-9, trans-11 CLA was detected only in cultures incubated with C18:2 (T2 and T3) and concentration was higher ( $P < 0.05$ ) with T3 than T2. The concentration of cis-9, trans-11 CLA was higher ( $P < 0.05$ ) when pasja and alfalfa were used as forage sources compared with oats. In conclusion, production of trans-C18:1 and cis-9, trans-11 CLA in rumen cultures were greatest when DHA was incubated with linoleic acid. In addition, forage type used in this study had a minimal effect on trans fatty acids formation.

**Key Words:** Forage source, FA source, Trans fatty acids

**M46 Using the Synchrotron (SRFTIRM) to Reveal Molecular Structural-Chemical Differences between Two Types of Forages Seeds –Winterfat (*Krascheninnikovia lanata*).** P. Yu\*, R. Wang, and Y. Bai, *University of Saskatchewan, Saskatoon, Canada*.

Winterfat (*Krascheninnikovia lanata*) (forage seed) is a long-lived native shrub with superior forage quality for livestock and wildlife. The objectives of this study were to use the advanced synchrotron technology (SRFTIRM) as a novel approach to reveal protein molecular structural-chemical differences between the two types of winterfat (forage) seeds which showing physiological differences in low temperature tolerances. This experiment was performed at beamline U10B at Brookhaven National Laboratory. Statistical analyses were performed using SAS with the model:  $Y = m + \text{seeds (S)} + \text{fitting methods (F)} + S \times F + \text{error}$  (where, S= Large, Small; F= Gauss, Lorentz). The results showed that with the synchrotron SRFTIRM, the molecular structural-chemical makeup and characteristics of the winterfat seed tissues could be imaged and revealed. The protein secondary structures differed between the large and small seed tissues. The large type of seeds contained no significant differences ( $P > 0.05$ ) in percentage of  $\beta$ -sheet (average 37.0%) and  $\alpha$ -helix (average 24.1%). However, the large type of seeds contained lower ( $P < 0.05$ ) percentage of  $\beta$ -turn (18.1 vs. 20.1%) and lower ( $P < 0.05$ ) ratio of  $\beta$ -turn to  $\alpha$ -helix (0.8 vs. 0.9) and  $\beta$ -turn to  $\beta$ -sheet (0.5 vs. 0.6). The results indicated that: 1) the large and small type winterfat seeds have subtle differences in protein secondary structure profile, which implicating differences in structural chemical make-up and features of the two types of winterfat seeds. These subtle differences may affect winterfat seed quality, germination behaviors and growth characteristics. Keeping in mind current research on 'Prion Diseases', perhaps only subtle changes in secondary structure are needed to provide big consequences. Our results demonstrate the potential of highly spatially resolved synchrotron-based FTIR microspectroscopy to reveal differences of structural molecular chemistry and protein secondary structures, which are associated with seed size variation and may affect germination behaviors.

**Key Words:** Synchrotron FTIR microspectroscopy, Forage seed, Molecular chemistry



**M47 Assessment of forage production and nutritional value of subtropical grasses in north-western Argentina.** M. V. Cornacchione<sup>1</sup> and J. I. Arroquy\*<sup>1,2</sup>, <sup>1</sup>*Instituto Nacional de Tecnología Agropecuaria, Santiago del Estero, Argentina*, <sup>2</sup>*Consejo Nacional de Investigaciones Científicas y Técnicas, Santiago del Estero, Argentina*, <sup>3</sup>*Universidad Nacional de Santiago del Estero, Santiago del Estero, Argentina*.

The aim of this trial was to evaluate forage yield and quality of subtropical grasses in the Semiarid Chaco of Argentina. In 2002, ten cultivars were planted in a completely randomized block design; *Brachiaria brizantha* (cv. Marandú; BBM), three cultivars of *Chloris gayana* (cv. Callide [CGC], cv. Katambora [CGK], and cv. Finecut [CGF]), two cultivars of *Cenchrus ciliaris* (cv. Biloela [CCB] and cv. Molopo [CCM]), *Panicum maximum* (cv. Gatton panic [PMG]), *Panicum maximum* var. *Trichoglume* (cv. Green panic [PMTG]) and two *Panicum coloratum* (cv. Klein [PCK], and cv. Bambatsi [PCB]). During 4 seasons, three experimental plots (10 × 20 m) per cultivar were clipped once a year at the end of the growing season (May; mature forage). Cultivar × year interaction was significant for forage production ( $P < 0.01$ ). In a group of cultivars (CGC, CGK, CGF, BBM, and CCM) forage production linearly decreased ( $P < 0.01$ ) in response to sward maturity. But other species (PMG, PMTG, PCB, PCK, CCB) had a quadratic response ( $P < 0.01$ ; initially peaked, and then decreased in DM production) to maturity. BBM had more forage accumulation ( $P < 0.01$ ; 7445 kg DM /ha) than *Chloris* cultivars (5698 kg DM /ha) and *Panicum* spp. (4310 kg DM /ha), but did not differ from *Cenchrus* cultivars (6306 kg DM /ha). Cultivars of *Cenchrus* accumulated more forage than *Panicum* spp. ( $P < 0.01$ ), and they had similar DM production than *Chloris* cultivars. *Chloris* cultivars were superior to *Panicum coloratum* ( $P < 0.01$ ; 2980 kg DM /ha) but they were similar to *Panicum maximum* (4975 kg DM /ha). *Panicum maximum* had more forage production than *Panicum coloratum* ( $P < 0.01$ ). Crude protein and NDF content did not differ among cultivars ( $P < 0.10$ ; average CP =  $4.75 \pm 0.46$ , NDF =  $77.5 \pm 6.3$ ). The content of CP in the forage linearly decreased ( $P < 0.01$ ) because of advancing maturity of the sward, while NDF increased ( $P < 0.01$ ) in response to sward maturity. In conclusion, *Brachiaria* and *Cenchrus* cultivars had higher forage productivity in the Semiarid Chaco than the other species, whereas forage quality was similar among cultivars.

**Key Words:** Subtropical pastures, Forage production, Forage quality

**M48 In vitro ruminal degradation of anthocyanidin-containing alfalfa transformed with the maize *Lc* regulatory gene.** Y. Wang\*<sup>1</sup>, T. A. McAllister<sup>1</sup>, and M. Y. Gruber<sup>2</sup>, <sup>1</sup>*Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta, Canada*, <sup>2</sup>*Agriculture and Agri-Food Canada Research Centre, Saskatoon, Saskatchewan, Canada*.

To reduce the propensity of alfalfa to cause pasture bloat in grazing animals, researchers sought to transform this popular forage with a *bHLH* anthocyanin regulatory gene (*Lc*) from maize. Expression of the *Lc* (leaf color) gene causes accumulation of anthocyanidin and proanthocyanidins in alfalfa tissues, giving rise to novel 'purple-green' forage. In this study, the effects of *Lc* expression on ruminal degradation of the forage were studied in vitro. Field-grown parental and three transgenic forages were harvested at the pre-bud stage, freeze-dried and ground (1.0 mm) for use as substrate. Anthocyanidin content in the transgenic genotypes ranged from 130 to 136 µg/g DM. The ground forages (500 mg in F57 bags) were incubated anaerobically for 0, 2, 4,

8, 12, 16, 24, and 48 h in a DAISY<sup>II</sup> Incubator (ANKOM Technology, Macedon, NY) for determination of DM and N degradabilities ( $n = 9$ ). A second incubation was conducted (sampling at 0, 4, 8 and 12 h) with <sup>15</sup>N included as a marker for microbial protein. Compared to the non-transgenic parental alfalfa (NT), the *Lc*-transgenic genotypes contained less ( $P = 0.034$ ) rapidly soluble DM, but their slowly degradable DM fractions, rates of degradation and lag times for digestion were similar ( $P \geq 0.122$ ) to NT. At 0 h, the true disappearances of N and DM were lower ( $P < 0.001$  and  $P = 0.027$ , respectively) from the *Lc* genotypes than from NT alfalfa, but they did not differ ( $P \geq 0.137$ ) at 4, 8, or 12 h. Solubility of DM and N were both negatively correlated ( $r^2 \geq 0.563$ ) with the concentration of anthocyanidins measured in the forage. *Lc*-transformation reduced the initial rate, but not the extent of DM and N digestion of alfalfa in the rumen. These properties could improve the utilization of protein and possibly reduce the risk of bloat in ruminants consuming fresh alfalfa.

**Key Words:** *Lc*-transformation, Forage quality, Ruminal degradation

**M49 Antiherbivory compounds on the leaf surface of intact and resprouted tarbush.** R. Estell\*<sup>1</sup>, E. Fredrickson<sup>1</sup>, and M. Remmenga<sup>2</sup>, <sup>1</sup>*USDA-ARS Jornada Experimental Range, Las Cruces, NM*, <sup>2</sup>*New Mexico State University, Las Cruces*.

Shrub encroachment into desert grasslands of the southwestern United States is a serious problem resulting in loss of forage and rangeland productivity. *Flourensia cernua* (tarbush) is one such shrub contributing to the decline of Chihuahuan Desert grasslands. Our previous research has shown tarbush consumption by sheep and goats to be negatively related to leaf surface concentration of individual terpenes and epicuticular wax. Concentrations of antiherbivory compounds such as terpenes change with plant age and phenology. Our objective was to alter the vegetative state and potential palatability of tarbush by clipping intact plants and generating resprouts. We hypothesized resprouts would invest fewer resources into antiherbivory compounds and therefore have lower concentrations of volatile chemicals such as terpenes than intact plants. Ninety tarbush plants were randomly selected and all biomass within 10 cm of the soil surface was removed from 45 plants during winter dormancy. Leaves were collected the following summer during active growth from the canopy of intact controls and resprouts. Leaf surface volatiles were extracted in ethanol and analyzed by gas chromatography-mass spectroscopy. Data were transformed to natural logarithms and subjected to univariate analysis of variance and stepwise discriminate analysis. Of the 87 compounds present on tarbush leaves, 35 were greater in canopy samples and 16 were greater in regrowth samples based on univariate analysis ( $P < 0.05$ ). Mean concentration of total volatiles on canopy leaves tended to be less ( $P = 0.0622$ ) than that of regrowth (3642 vs 4684 µg/g DM). Nine compounds in the discriminate analysis ( $\alpha$ -muurolene, iso-borneol, unknown#6, *p*-cymen-8-ol, unknown#7, sabinene,  $\beta$ -caryophyllene,  $\delta$ -cadinene, and  $\alpha$ -copaene) explained 95% of the variation between canopy and regrowth samples. In contrast to our hypothesis, lower cumulative concentration of volatile compounds in canopy than regrowth samples suggests resprouts may be less vulnerable to herbivory than old growth tarbush. However, animal studies are needed and effects on other classes of secondary compounds must be examined.

**Key Words:** Regrowth, Shrub, Terpene

### M50 Chemical composition, intake and *in vitro* gas production of the forage selected by cattle in a grassland of Northern Mexico.

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The study was performed to determine the voluntary intake, nutritional value and *in vitro* gas production of the diet of grazing cattle during two periods of the year: wet (Jul-Dec), and dry (Jan-Jun). Four esophageal cannulated steers ( $320 \pm 5.0$  kg BW) were utilized to obtain extrusa samples. The collection was carried out at 700 during 45 min for a period of 4 days during Jul, Sept, Oct, Dec, Jan, Mar, Apr and Jun. The OM voluntary feed intake was determined from total fecal collection and the indigestible fraction of the forage after 48h incubation in the rumen. Chromium sesquioxide (8g/anim/14d) was dosed to the four steers to estimate total fecal production. The sampling was performed morning (700) and evening (1600) for 4 days during the months mentioned above. Estimations of CP, NDF, ADF and lignin were performed. *In vitro* gas production determinations were carried out by incubating 500 mg DM samples in calibrated 100 ml glass syringes using rumen fluid from two steers fed alfalfa hay *ad lib*. Gas production was recorded at 0, 3, 6, 15, 24, 36, 48, 72 and 96h. Data were fitted to the exponential equation:  $Y = a + b(1 - e^{-ct})$ . The variables were then analyzed using PROC GLM according to a completely randomized design. Nutritional level was increased ( $P < 0.05$ ) during the wet season compared with that observed during the dry season. Dry matter intake was increased 82%, CP by 128%, and the gas production rate constant  $c$  by 35% during the wet vs. dry season. Potential degradability ( $a + b$ ) was not affected ( $P > 0.05$ ). Based on these data a supplementation schedule is warranted during the dry season to improve the body condition score of cattle.

**Table 1. Voluntary intake, Nutritional value and parameters from *in vitro* gas production**

| Item                                  | Sampling period    |                    | Mean  | SEM   |
|---------------------------------------|--------------------|--------------------|-------|-------|
|                                       | Dry                | Wet                |       |       |
| <b>Intake</b>                         |                    |                    |       |       |
| kg OM/d                               | 5.0 <sup>b</sup>   | 9.1 <sup>a</sup>   | 7.0   | 0.270 |
| Percentage BW                         | 1.5 <sup>b</sup>   | 2.8 <sup>a</sup>   | 2.1   | 0.048 |
| g/kg BW <sup>0.75</sup> (g OM)        | 66.8 <sup>b</sup>  | 123.3 <sup>a</sup> | 95.0  | 0.127 |
| <b>Nutritional value (% DM)</b>       |                    |                    |       |       |
| CP                                    | 6.6 <sup>b</sup>   | 15.1 <sup>a</sup>  | 10.8  | 0.61  |
| NDF                                   | 74.7 <sup>a</sup>  | 60.6 <sup>b</sup>  | 67.6  | 0.73  |
| ADF                                   | 48.6 <sup>a</sup>  | 40.5 <sup>b</sup>  | 44.5  | 0.32  |
| Lignin                                | 6.7 <sup>a</sup>   | 3.5 <sup>b</sup>   | 5.1   | 0.27  |
| <b><i>in vitro</i> gas production</b> |                    |                    |       |       |
| $a + b$ (ml/500 mg DM)                | 106.8 <sup>a</sup> | 100.1 <sup>a</sup> | 103.5 | 7.69  |
| Digestion rate constant (%/h)         | 3.4 <sup>b</sup>   | 4.6 <sup>a</sup>   | 4.0   | 0.60  |

Means between rows with different superscripts differ ( $P < 0.05$ )

**Key Words:** Range cattle, Nutritional value, Gas production

**M51 Effect of nitrogen-fertilizer application on chemical compositions and *in vitro* rumen digestibility of corn stovers.** Q. Meng<sup>\*1,2</sup> and G. Yan<sup>1,2</sup>, <sup>1</sup>State Key Laboratory of Animal Nutrition, Beijing, China, <sup>2</sup>Beef Cattle Research Center and College of Animal Science & Technology, China Agricultural University, Beijing, China.

Corn stovers supply a major source of roughage feeds of ruminant animals in China. Chemical compositions and ruminal fermentation of

stover generally determines the animal performance and the product quality. Because emphasis has been placed on improvement of corn grains rather than nutritive values of stover, this experiment was conducted to ascertain the effect of nitrogen-fertilizer application on chemical composition and *in vitro* digestibility of corn stovers. Traditional corn (*Zea mays* L.) plants were fertilized under five levels of urea (0, 75, 150, 300, 450 kg/ha) during middle growth stage. After ear harvest, stover samples were collected and measured for chemical components, *in vitro* digestibility and gas production characteristics. Increasing N-fertilizer application levels linearly increased ( $P < 0.001$ ) the contents of crude protein (CP), total amino acids and Ca, but linearly decreased ( $P < 0.01$ ) the total water soluble saccharides. Fertilizer rate had little or no influence ( $P > 0.08$ ) on the contents of ether extract, ash, P, NDF, ADF and lignin. Increasing N-fertilizer application levels resulted in quadratic ( $P < 0.05$ ) changes in digestibilities of dry matter, CP, NDF and ADF with the highest digestibilities achieved at the urea application level of 300 kg/ha. As urea application levels increased, potential degradability ( $a + b$ ) of stovers linearly decreased ( $P < 0.001$ ), but gas production rates linearly increased ( $P < 0.001$ ). Results indicated that application rate of N-fertilizer during middle growth stage of corn plants can influence the chemical composition and rumen fermentation of corn stover.

**Key Words:** Nitrogen fertilizer, Corn stover, Chemical composition and rumen fermentation *in vitro*

**M52 *In vitro* evaluation of various energy supplements for tropical and temperate forages.** R. D. L. Pacheco<sup>\*1</sup>, D. D. Millen<sup>1</sup>, N. DiLorenzo<sup>2</sup>, and A. DiCostanzo<sup>2</sup>, <sup>1</sup>FMVZ/UNESP, Botucatu, Sao Paulo, Brazil, <sup>2</sup>University of Minnesota, St. Paul.

The objective of this study was to evaluate the effects of various energy supplements (Corn Grain, CG; Citrus Pulp, CIP; or Soy Hulls, SH) on IVDMD in two types of forages: *Brachiaria decumbens* (BD), a tropical forage, or *Poa pratensis*, (PP) a temperate grass, hay. Energy supplementation level was based on providing 0.2 % corn per kg BW for a 450-kg cow expected to consume a total of 8.1 kg DMI daily. Energy-supplemented diets were isoenergetic and formulated based on the following TDN values: CG = 92%, CIP = 81% and SH = 74%. Forage only (FOR) or forage mixed with respective supplement (DIET) in Ankom fiber bags were incubated in jars in a Daisy<sup>II</sup> Incubator (Ankom Technology Corp.). Jars containing FOR bags only were added respective supplement in the media. A set of control bags (CTL) contained forage only. Supplementation had no effect on IVDMD of the forage ( $P > 0.05$ ) regardless of source. Temperate grass hay had greater ( $P < 0.01$ ) IVDMD values than BD. A supplement type X forage source interaction was observed ( $P = 0.04$ ) for total diet IVDMD. No differences ( $P > 0.05$ ) in total diet IVDMD were observed for BD supplementation (CTL = 41.58%, CG = 40.19%, CIP = 42.38%, SH = 42.67%; SEM = 2.42), but greater ( $P < 0.05$ ) total diet IVDMD values were observed for CTL and CG when compared to SH (CTL = 55.20%, CG = 55.02%, CIP = 53.00%, SH = 51.21%; SEM = 2.42). In conclusion energy supplementation effects on total diet and forage fraction IVDMD were dependent on forage type and supplemental source. At the supplementation level tested, no effect on IVDMD was observed in the forage fraction of the diet for either PP or BD. No effects were observed in IVDMD when supplementing tropical forage (BD) based diets. A reduction in total diet IVDMD was observed when supplementing a PP with soy hulls, when compared to no energy supplementation or supplemented with corn grain.

**Key Words:** Forages, IVDMD, Energy supplementation

**M53 Neutral detergent fiber digestibility of cool-season grasses.** P. D. French\*, T. W. Downing, B. D. Frisch, J. L. Chamberlain, and J. L. Wartjes, *Oregon State University, Corvallis.*

Forage grass production is an important component of profitable dairying along the Pacific Northwest coast. Neutral detergent fiber digestibility (NDFD) is increasingly being used in ration formulation and forage benchmarking. However, data on differences in NDFD between and within cool-season grass species is limited. Therefore, the objective of this study was to determine if NDFD differs between and within species. In experiment 1, 15 cool-season grass varieties were replicated 3 times each in 6 m<sup>2</sup> randomized field plots. Included were 5 varieties each of perennial ryegrass (*Lolium perenne*), orchardgrass (*Dactylis glomerata*), and tall fescue (*Lolium arundinaceum*). In experiment 2, three diploid and three tetraploid cool-season ryegrass varieties (*Lolium perenne*) were replicated 3 times each in 6 m<sup>2</sup> randomized field plots. Plots were mechanically harvested six times at approximately 28 d intervals beginning in March. Forty eight-hour in vitro neutral detergent fiber (NDF) digestibility was determined using 0.3 g of sample in F57 bags in a Daisy<sup>II</sup> Incubator (Ankom Technology, Fairport, NY). In experiment 1, yield (2124±79 kg DM/ha/cutting) was similar across species. Neutral detergent fiber was less for ryegrass compared to orchardgrass (47.1 vs 48.5±0.4%;  $P < 0.01$ ), but similar to both of these grasses was tall fescue (48.0%). Neutral detergent fiber digestibility was greater for ryegrass compared to orchardgrass and tall fescue (80.4 vs 77.5±0.6% of NDF;  $P < 0.01$ ). Digestible NDF did not differ and was 37.4±0.3% of DM or 799±32 kg/ha/cutting. Neutral detergent fiber increased (linear;  $P < 0.01$ ) and NDFD decreased (quadratic, cubic;  $P < 0.05$ ) as the growing season progressed. In experiment 2, yield (2220±110 kg DM/ha/cutting), NDF (47.2±0.5%), NDFD (80.7±0.5%), and digestible NDF (38±0.4% of DM and 851±47 kg DM/ha/cutting) were similar for diploid and tetraploid ryegrasses. These results indicate species, when managed intensively, has little effect on yield and digestibility.

**Key Words:** Neutral detergent fiber digestibility, Cool-season grass, In vitro digestibility

**M54 Effect of *Lactobacillus buchneri* applied to alfalfa hay treated at high moisture.** G. E. Higginbotham\*<sup>1</sup>, S. Mueller<sup>1</sup>, and R. Kuber<sup>2</sup>, <sup>1</sup>*University of California Cooperative Extension, Fresno,* <sup>2</sup>*Conner Marketing, Clovis, CA.*

The effectiveness of a microbial inoculant containing *L. buchneri* in high moisture alfalfa hay was evaluated. Third cutting alfalfa from alternate windrows was baled into six 900 kg bales at 75% DM (high moisture) without (HM-C) or with (HM-T) inoculant and at 85% DM (normal moisture) without inoculant (NM). Application rate was 1.2 million colony forming units (CFU) per gram of forage. Each bale (six per treatment) had three core samples collected per end at 0 and at 28 d of storage to determine chemical composition. Internal bale temperatures were measured throughout the 28-d storage period. Treatment effects on all quality attributes were analyzed using the NCSS statistics software. At baling, differences between high moisture treatments were not significant for percentages of CP, soluble protein, bound protein, ADF, NDF or 30-h NDF digestibility. After 28 d of

storage, no differences were detected between high moisture treatments for CP, soluble protein, ADF, NDF and NDF 30h digestibility. Bound protein was greater ( $P < 0.05$ ) for HM-T compared to HM-C and NM (1.48%, 1.09%, 0.98%, respectively). Both HM hays heated to between 37.8°C and 48.9°C. Temperatures of NM varied between 27.8°C and 32.2°C. After storage, differences in mold values for both HM groups were not different with yeast counts similar for HM hays but significantly lower than NM hay. Bales from each treatment were broken open and evaluated by an independent nutrition consultant for timber, color, leaf quality, aroma, mold, dustiness and overall quality using a scale from 1 to 5 where 5 is a high rating. No differences were detected for timber rating but both HM treatments had significantly lower ratings than NM treatment in color, leaf quality aroma, dustiness and overall quality. No differences were noted between the HM treatments. Based on this trial, the addition of *L. buchneri* to the high moisture alfalfa hay did not appreciably improve chemical or visual characteristics over non-treated controls or hay baled at normal moisture levels.

**Key Words:** Inoculant, Alfalfa, Hay

**M55 Effect of fibrolytic enzymes or ammonia treatment on the nutritive value of 6-wk and 8-wk regrowths of guineagrass hay.** D. B. Dean\*<sup>1,2</sup>, A. T. Adesogan<sup>1</sup>, E. Valencia<sup>3</sup>, and N. Krueger<sup>1</sup>, <sup>1</sup>*University of Florida, Gainesville,* <sup>2</sup>*Universidad del Zulia, Maracaibo, ZU, Venezuela,* <sup>3</sup>*Universidad de Puerto Rico, Mayaguez, PR.*

This study evaluated the effect of applying four fibrolytic enzymes or NH<sub>3</sub> on the nutritive value of 6- (6-wk) and 8-week (8-wk) regrowths of guineagrass (*Panicum maximum*) hay. The NH<sub>3</sub> was applied at 4% DM and enzymes Promote (Pr) Biocellulase X-20 (X-20), Fibrozyme (Fib) and Biocellulase A-20 (A-20) were dissolved in water and applied in a fine spray at 0, 0.5x, 1x and 2x manufacturer-recommended rates to 2 kg of hay in triplicate. Hays were stored for 3 weeks before determination of chemical composition and 6-h and 48-h in vitro digestibility of DM (IVDMD), NDF (IVNDFD), and ADF (IVADFD) using a completely randomized design. Crude protein concentration, 6-h and 48-h IVDMD, and 48-h IVNDFD and IVADFD values were greater ( $P < 0.01$ ), while ADF and NDF concentrations were lower ( $P < 0.01$ ) in 6-wk hays compared to 8-wk hays. Additive treatment did not affect ADF or NDF concentration of 6-wk hays, but in 8-wk hays, X-20 and Fib treatment slightly (<4%) reduced NDF and ADF concentrations (additive x maturity interaction,  $P < 0.1$ ). Ammoniation increased ( $P < 0.01$ ) CP concentration of 6-wk and 8-wk hays by 42 and 91%, respectively (additive x maturity interaction,  $P < 0.01$ ). Ammoniation increased 6-h IVDMD by 35% and increased 48-h IVDMD and IVNDFD by 10%, and there were no maturity x additive interactions for these measures. Additive treatment did not increase 48-h IVADFD. This study shows that guineagrass quality decreases as the duration between harvests increases. Certain enzymes produced small (<4%) decreases in the fiber concentration of 8-wk hays, but enzymes had no other effects on nutritive value. Ammoniation increased CP concentration and in vitro DM and NDF digestibility, therefore ammoniation was the only treatment that increased the nutritive value of guineagrass hays

**Key Words:** Guineagrass hay, Enzymes, Nutritive value