Asian-Aust. J. Anim. Sci.
Vol. 22, No. 4 : 520 - 527
April 2009

www.ajas.info

Correlations among Shearing Force, Morphological Characteristic, Chemical Composition, and *In situ* Digestibility of Alfalfa (*Medicago sativa* L) Stem

L. Liu, Z. B. Yang*, W. R. Yang, S. Z. Jiang and G. G. Zhang

Department of Animal Sciences and Technology, Shandong Agricultural University, Tai-an, Shandong, 271018, China

ABSTRACT : Alfalfa (*Medicago sativa* L) is a high-quality forage for ruminants and the main stem is the dominant morphological component contributing to the forage nutritive value in mature alfalfa forage. Shearing force, a fracturing property of plant stem, is an important indictor of forage value. The objectives of this study were to investigate the effects of morphological characteristic on shearing force, the relationship between shearing force and chemical composition, and the relationship between shearing force and *in situ* digestibility of alfalfa stem. The results showed that linear density (weight per unit length of stem) was more important than chemical composition in affecting shearing force. There was a positive relationship between lignin content and shearing force (r = 0.78). Correlations were not found between shearing force and other chemical components such as neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose and hemicelluloses. *In situ* digestibility (of dry matter and NDF) was related to shearing force. A negative correlation was found between shearing force and dry matter (DM) digestibility (r = -0.70), and there was also a negative correlation between shearing force and NDF digestibility (r = -0.87). When shearing force was standardized for stem diameter or stem linear density, the relationship between shearing force and digestibility was consistent regardless of stem diameter and stem linear density. Shearing force was significantly correlated with lignin content and *in situ* digestibility (of DM and NDF), and was a more direct indicator for estimating forage nutritive value related to animal performance, so it can be used to predict the forage value of alfalfa. (**Key Words :** Alfalfa Stems, Shearing Force, Stem Diameter, Stem Linear Density, Chemical Compositions, *In situ* Digestibility)

INTRODUCTION

Forage intake and digestibility of ruminants are major determinative factors of animal performance. Forage intake was affected by the action of thirst-controlling peptides and related to saliva secretion (Sunagawa et al., 2007). Ba et al. (2008) reported that forage intake declined as concentrate intake increased. Meanwhile, there are many factors affected forage digestibility. Recently studies show that forage digestibility were significantly affected by meal frequency and decreased as the number of meals increased (Assoumaya et al., 2009). Variable feed supply would also decrease forage digestibility (Habib et al., 2008). Forage quality was an important factor which could affect forage intake and digestibility equally. Iwaasa et al. (1996) found that the physical properties of forages are related to voluntary feed intake of ruminants. There is a need for physical measurement which can explain the differences in forage breakdown during mastication and may account for the variation in dry matter consumption not fully explained by the chemical composition of the forage. Shearing force describes the force required to fracture a plant when applied at 90 degrees to the plant surface and has been used in the past to physically characterize forages (Mackinnon et al., 1988; Inoue et al., 1994). This physical strength characteristic of forage denotes the required force when an animal bites the forage and animals may prefer low shearing-force plants. Mackinnon et al. (1988) found that low shear-strength plants had a faster breakdown rate during chewing thus increasing dry matter consumption on a per animal basis. This technique is suitable for routine work not only because it is a simple measurement of plant resistance properties, but the shearing technology also provides a more direct indicator than chemical constituents for estimating forage nutritive value related to animal performance, and can be used in evaluation of forage nutritive value.

* Corresponding Author: Z. B. Yang. Tel: +86-0538-824-1257, Fax: +86-0538-824-9371, E-mail: yangzb@sdau.edu.cn
Received August 26, 2008; Accepted December 5, 2008

Linear density is an important morphological characteristic of forage. Different anatomical tissue components have different relative weights and cellular densities (Wilson, 1994). Hughes et al. (2000) reported that density parameters may be of interest for inclusion in strength measurement because once the material is consumed these parameters may play a role in the ease of particle breakdown and fermentation during chewing and rumination. In order to study the effect of stem linear density on shearing force, the relationship between shearing force and stem linear density was investigated in this study.

Alfalfa stem diameter could affect shearing force, which has been confirmed by Iwaasa et al. (1996, 1998), but whether diameter would influence the correlations of shearing force with chemical composition and *in situ* digestibility was uncertain. Stem diameter was measured in order to standardize shearing force and correlations of shearing force, modified by stem diameter, with chemical constituents and digestibility were studied.

Several studies had linked shearing force to forage chemical constituents. Iwaasa et al. (1996) found that shear strength was significantly correlated with diameter, linear density and, to a lesser extent, with the cell chemical constituents of the stem. Chen et al. (2007) reported that shearing force had positive relationships with cellulose and lignin content. However, there is no consistent data about the relationship between shearing force and chemical constituents. For example, Evans (1967) reported that increased lignin could increase shearing force of ryegrass leaves, whereas Kokubo et al. (1991) reported that there was no relationship between lignin and shearing force in barley. Furthermore, in these studies, strength parameters had not been standardized for morphological characteristics, and whether morphological characteristics influenced the relationship between shearing force and chemical composition was not clear. The study of Hughes et al. (2000) showed that shearing strength had high positive correlations with structural constituents such as NDF, ADF and lignin, but these relationships were lost when strength parameters were standardized for morphological characteristics which included width, linear density and area density of leaf. In this study, we studied the correlations of shearing force with chemical composition. Since there are clear morphological differences between alfalfa stems, an attempt was made to standardize measures of shearing force for morphological characteristics, including stem diameter and stem linear density; the correlation of standardized shearing force with chemical composition was also studied.

Shearing force may influence digestibility of fresh stem tissue and also the extent of degradation of these samples. Modified strength parameters which were not related to the resistance of stem material to harvest by the animal may be more relevant to *in situ* digestibility. However, there is not much information about alfalfa stem shear power and its digestibility. A study was designed to investigate the relationship between shearing force and *in situ* digestibility.

MATERIALS AND METHODS

Shearing force and morphological measurements of stems

Alfalfa (*Medicago sativa* L) was grown in an experimental field in the livestock research center at Shandong Agricultural University. Full-bloom stage alfalfa was harvested and 150 plants were randomly selected from the field. All floral appendages, leaves and branch stems were removed from the main stem. Each stem was weighed and stem length was measured. Linear density was the weight per millimeter of stem length.

Only the shearing force of stem was measured because it is the dominant morphological component contributing to nutritive value, particularly as forge matures (Pritchard et al., 1963; Buxton et al., 1987). Each stem was cut into three 16 cm segments for measuring stem diameter and shearing force of top, middle and bottom segments of stem. The top segment was measured from the stem apex, the bottom from the harvest base and the middle segment extended 8 cm above and below the midpoint of the stem. Diameters were measured at the approximate midpoint of each segment and an average was calculated as the stem diameter. Each segment was sheared at the approximate midpoint ensuring that the location was between two nodes to prevent any influence of nodes on shearing force. Shearing forces of the three segments were averaged as the shearing force of a stem.

Shearing force was measured with a C-LM3 meat shear made by the Mechanics Research Center of Dongbei Agricultural University and commonly used to measure tenderness of meat tissue. Range of shearing force was 0-25.0 kg and deformation speed was 5 mm per second.

Chemical analysis

Stems that had been measured for morphological characteristics were crushed according to Feng (2004). 10 stems of 150±30 g fresh weight were merged into one sample. Stem was cut into segments shorter than 5 mm and then milled by high speed beater for 30 s. One part of the crushed sample was used for *in situ* digestibility and the other part was used for chemical analysis. Moisture content was calculated as the difference in weight between fresh sample and dry matter which was determined by oven-drying at 60°C to a constant weight. NDF, ADF and lignin were analyzed according to Goering and Van Soest et al. (1970); sodium sulfite was omitted from the NDF analysis

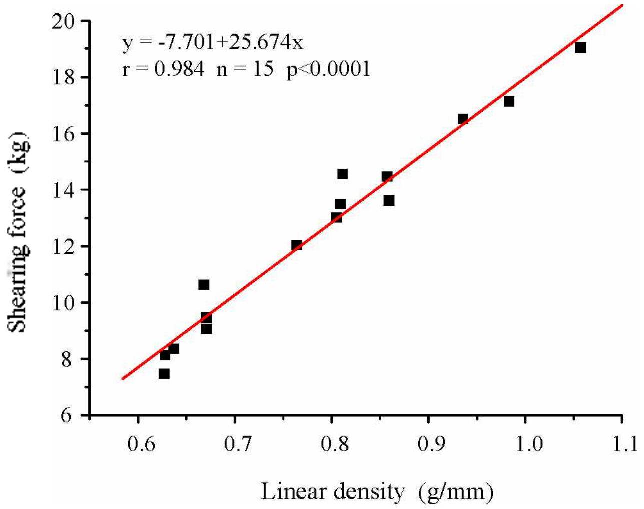

Figure 1. Correlations of shearing force with stem linear density.

and decalin was omitted from the ADF analysis (Van Soest et al., 1970). Hemicelluloses concentration was calculated as the difference between NDF and ADF concentrations, and cellulose content was assumed to be the difference between ADF and lignin concentrations.

In situ digestion

Three Xiao Wei sheep, fitted with a permanent rumen fistula, aged 2 years and of 30.8 kg live weight (S.D. 4.17), were used to determine *in situ* digestibility of DM and NDF for alfalfa stems. Animals were housed in a room in which temperature was maintained constant and had free access to drinking water. Sheep were fed with a diet of 55% sweet potato vines, 15% alfalfa hay and 30% corn-based concentrate. Daily ration was 1.6 kg DM, given in two equal portions (07:00 and 16:00). Sheep were adapted to their diet for 10 d before the trial.

In situ digestibility was determined during the collection phase using Dacron bags (Shanghai Quancheng Environmental Protection Technology Co.). Dimensions of the bags were 17 cm×9 cm with a pore size of 37 μm (Mehrez, 1977). An average of 10 g of green forage that had been milled by high speed beater was weighed into the Dacron bags. All bags containing samples were soaked in distilled water at 38°C for 10 min. A pair of bags was also incubated in distilled water at 38°C for 10 min to estimate washout at time zero for each sample. Three Dacron bags with different samples were tied to a plastic leader, which was attached to a nylon line, and incubated in the rumen. After 48 h, samples were removed from the rumen of each sheep and analyzed. Nylon bags removed from the rumen were thoroughly washed under running tap water, and then dried to a constant weight in a forced-air oven at 100°C. DM losses were determined by differences between the initial dry matter incubated and the dry matter that remained after incubation corrected for the zero time weight. NDF losses were determined in a similar manner.

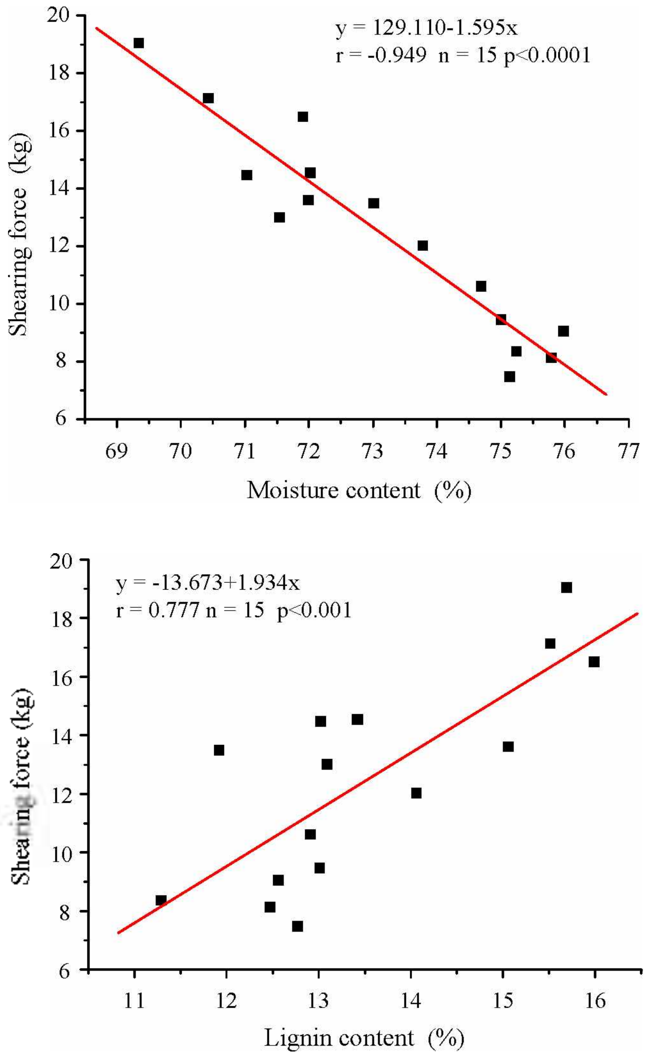

Figure 2. Correlations of shearing force with moisture, lignin content.

Statistical analysis

An average was calculated for shearing forces of 10 plants in one group; similarly for stem diameter and stem linear density. Values obtained from individual replicate samples were used as units for statistical analysis. Pearson correlation coefficients (r) between shearing force and linear density, chemical constituents and *in situ* digestibility were calculated using the PORC CORR procedure (SAS, Institute, Inc. 1999). Significance was declared at the 0.05 level of probability. According to the Pearson correlation coefficients, factors which affected shearing force significantly were found. Linear regression analysis was done to determine the correlation of shearing force with linear density, moisture and lignin content. Linear regression analysis was also done to determine the relationship between shearing force and *in situ* digestibility of alfalfa stem.

RESULTS

Correlations between shearing force and morphological characteristic

As one important morphological characteristic of forage, linear density was positively related to shearing force as shown in Figure 1; p value was lower than 0.0001, while r was 0.98. Correlation between stem shearing force and linear density is shown in Figure 1.

Correlations between shearing force and stem chemical composition

The relationship between shearing force and moisture content is shown in Figure 2 and Table 1. There was a negative relationship between shearing force and moisture content. The relationship was significant ($p<0.0001$), while r was -0.95. Moisture content of stem decreased with advancing maturity, and shearing force increased with advancing maturity. As shown in Figure 3, 4 and Table 1, there were also significant correlations between shearing force and moisture content when shearing force was standardized for morphological characteristics such as stem diameter or stem linear density.

The relationship between shearing force and stem lignin content is shown in Figure 2. Correlation between shearing force and lignin content was high ($r = 0.78$, $n = 15$, $p< 0.001$). When strength parameters were standardized for stem diameter or stem linear density, the correlations still existed, as shown in Figure 3, 4 and Table 1, and showed that the relationship between shearing force and chemical composition had not been influenced by morphological characteristics. This result indicated that cell wall chemical constituents such as moisture and lignin were important determinants of shearing force.

However, correlations were not found between shearing force and chemical components such as NDF, ADF, cellulose and hemicelluloses, and when shearing forces were standardized for morphological characteristics

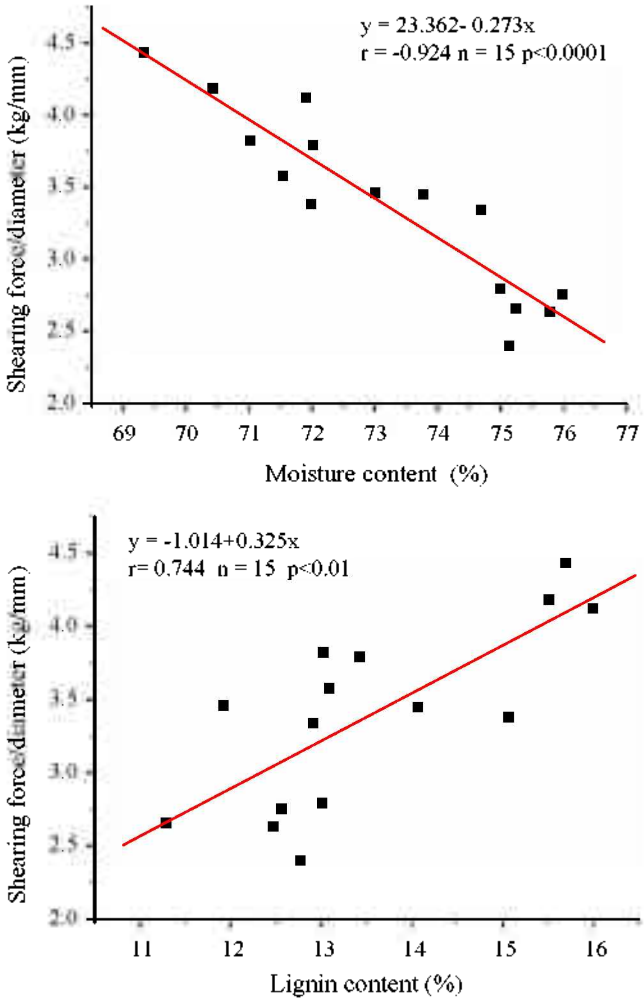

Figure 3. Correlations of shearing force standardized for stem diameter with moisture and lignin content.

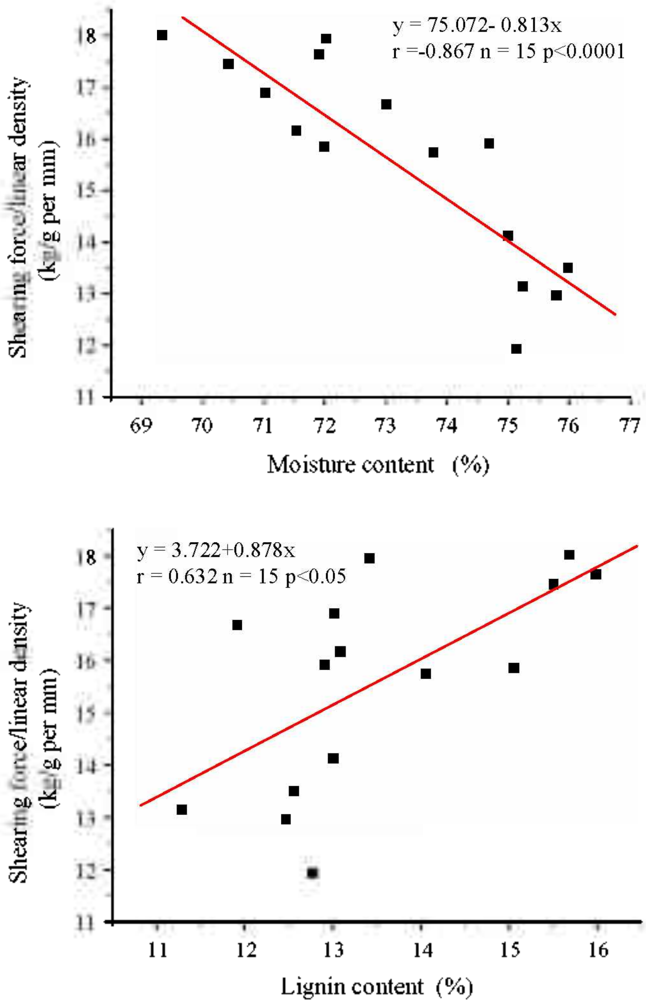

Figure 4. Correlations of shearing force standardized for stem linear density with moisture and lignin content.

Table 1. Pearson correlation coefficients (r) between shearing force and chemical composition parameters of alfalfa stem

Measurements (%)	Moisture	NDF	ADF	Lignin	Cellulose	Hemicelluloses
Shearing force (kg)	-0.93***	0.4	0.33	0.78**	0.23	0.3
Shearing force/diameter (kg/mm)	-0.92***	0.42	0.31	0.74*	0.17	0.37
Shearing force/linear density (kg/g per mm)	-0.87***	0.42	0.31	0.63*	0.19	0.38

* $p<0.01$; ** $p<0.001$; *** $p<0.0001$.

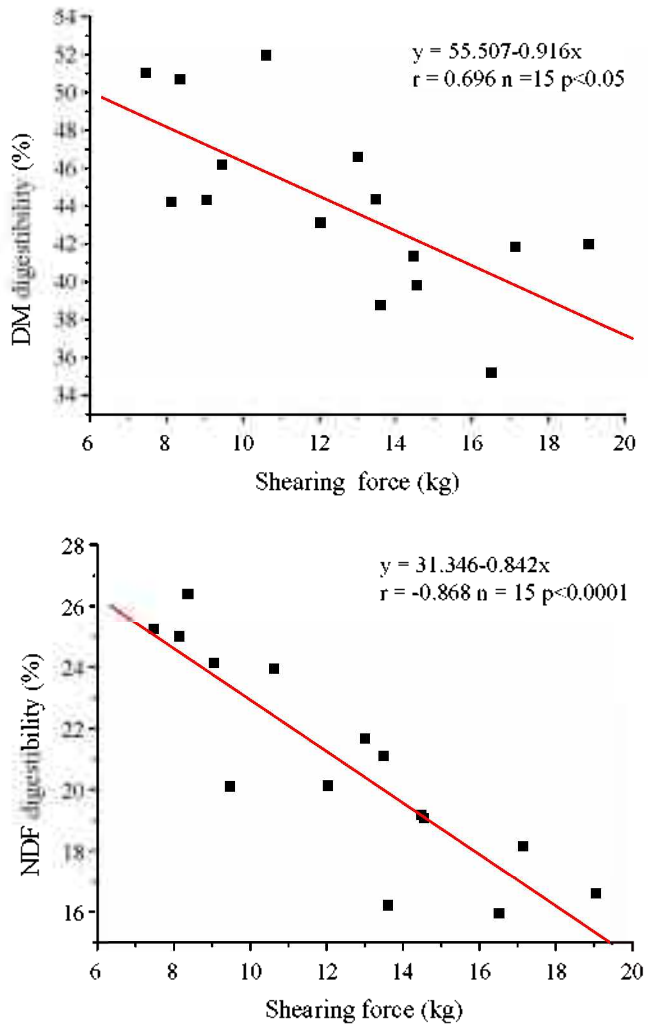

Figure 5. Correlations of shearing force with *in situ* digestibility of stem.

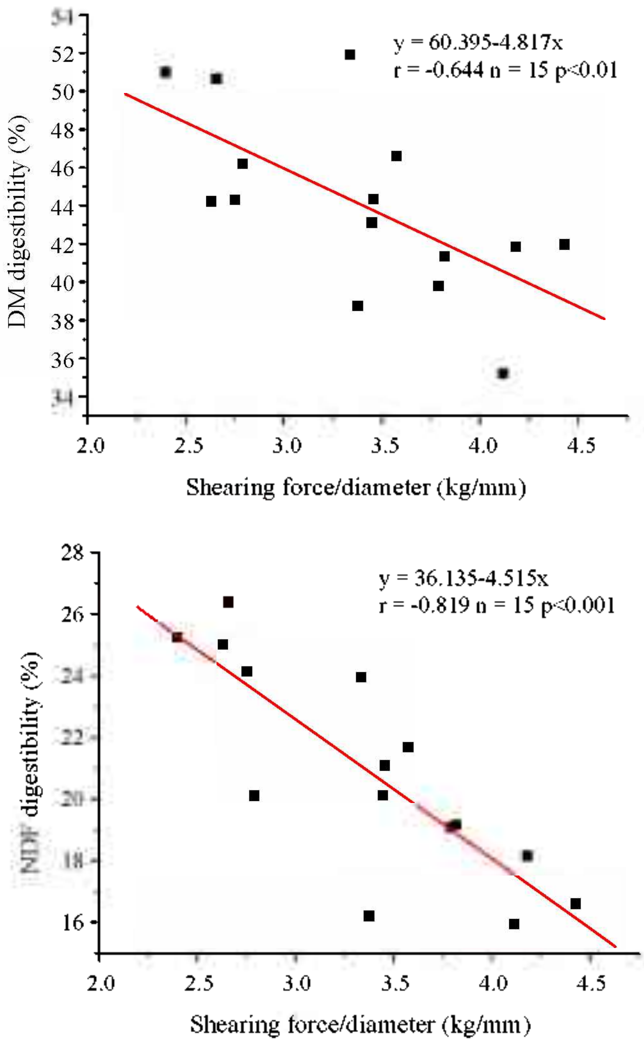

Figure 6. Correlations of shearing force standardized for stem diameter with *in situ* digestibility of stem.

correlations were not found either, as shown in Table 1.

Relationship between shearing force and *in situ* digestibility of stem

In situ digestibility (of DM or NDF) was related to shearing force. As shown in Figure 5, a negative correlation existed between shearing force and DM digestibility (r = -0.70), while there was also a negative correlation between shearing force and NDF digestibility (r = -0.87). As shown in Figure 6 and 7, negative correlations were also evident between *in situ* digestibility (of DM or NDF) and shearing force per unit of stem diameter or linear density. Thus, when the shearing force of alfalfa stem was lower, the stem was easier to break down during ruminal degradation and the digestibility value was higher.

DISCUSSION

Linear density (weight per unit of stem length) has been used in attempts to relate morphological characteristic to shearing force. Stem density may be influenced by a combination of anatomical features and cell wall chemical constituents. Esau (1977) reported that lignin and hemicelluloses are important constituents of the cellulose

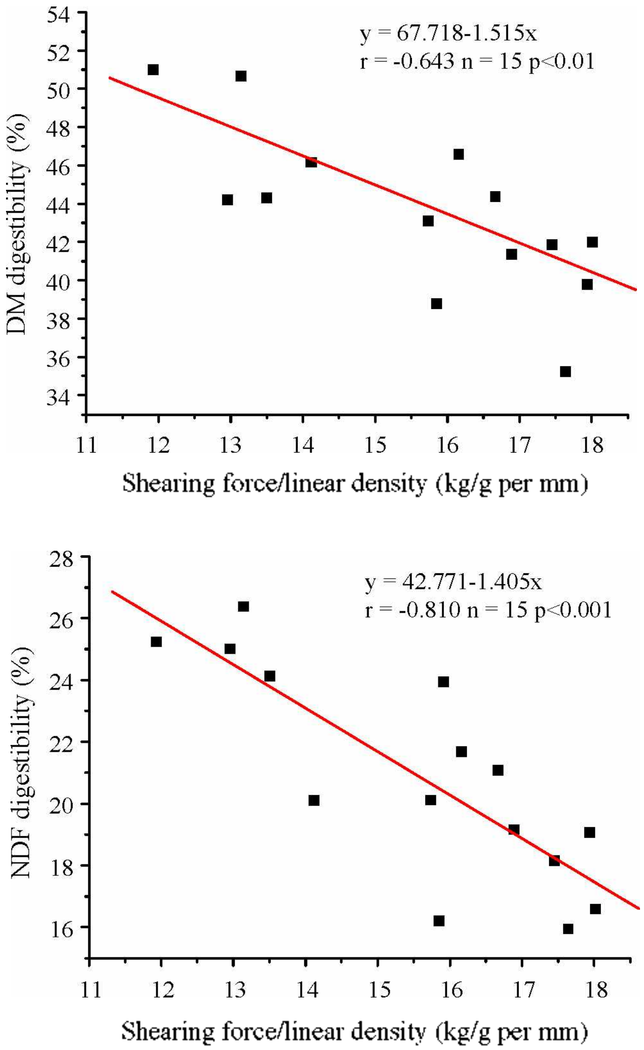

Figure 7. Correlations of shearing force standardized for stem linear density with *in situ* digestibility of stem.

framework of the cell wall, providing rigidity to the wall and influencing stem density. Linear relationships between shearing strength and linear density in forage species, such as ryegrass and timothy, have been found by a number of authors (McClellad and Spielrein, 1957; Iwaasa et al., 1961). In our study, shearing force of alfalfa stem also increased with increasing stem linear density. A similar relationship was observed by Herrero et al. (2001).

Moisture content of stem was also a factor that affected shearing force. This result was consistent with the report of Prince et al. (1965) that mechanical properties of alfalfa stems were sensitive to moisture content. Shearing force increased with decreasing moisture content, which is due to fiber tensile strength being related to moisture content; tensile strength was high when moisture content was low (Chen et al., 2007).

Some studies showed that in addition to stem diameter, stem linear density and moisture, chemical constituents of stem also influenced shearing force (Mackinnon et al., 1988; Kokubo et al., 1989). Increasing levels of components of cell wall chemical constituents such as cellulose and lignin have been reported to increase shearing force (Evans, 1967). In our study, lignin was an important determinant of shearing force but cellulose and hemicelluloses, which are also cell wall chemical constituents, were not correlated with shearing force. This was consistent with the study by Iwaasa et al. (1996). This finding supported the conclusion of Evans (1967) that the strength of the cellulose, and thus stem shearing force, depended on the way cellulose was laid down in the cell wall and on the presence of other substances such as lignin. Since cellulose is an important component of secondary cell wall thickening, it should be correlated with the physical strength characteristics, but this was not the case. Cellulose was not significantly correlated with shearing force, contradicting other studies (Evans, 1967; Kokubo et al., 1989; Chen et al., 2007). Apparently, the filling and binding action of hemicelluloses and lignin to the cellulose matrix rather than increasing levels of cellulose, increased stem density and shearing force. Iwaasa et al. (1996) reported that the effect of cellulose on shearing force was influenced by the orientation of its fibrils or other chemical components like silica or lignin. However, it was not necessarily the amount of cellulose, lignin or hemicelluloses but their interaction that affects cell wall composition, density and shearing force.

The *in situ* digestibility of DM and NDF was the nutritive value index of forage. It was of great interest to determine whether *in situ* digestibility showed a relationship with a simple physical measure such as shearing force. However, there was not much information about the relationship between alfalfa stem shear power and its digestibility. In our study, shearing force influenced *in situ* DM and NDF digestibility, and the digestibility of DM or NDF was negatively related to it. Herrro et al. (2001) reported that there was a negative correlation between shear strength and plant *in vitro* digestibility, which supports the results of our study. These relationships could be explained by the changes in chemical concentration which were related to shearing force. Much of the variability in digestibility of herbage was closely associated with variation in cell wall concentration and components. In work reviewed by Wilkins (1966), lignin was more closely related to *in situ* digestibility of organic matter and dry matter and the potential digestibility of cellulose which was contained in NDF had significant negative correlations with both lignin content and lignified tissue (Wilkins, 1972). There was a positive relationship between shearing force and lignin content in this study. Increasing shearing force indicated more lignin contained in cell wall and lower *in situ* digestibility (of DM and NDF).

Shearing force had a negative relationship with stem digestibility, and was significantly influenced by stem diameter and stem linear density. Different anatomical tissue components have different relative weights and cellular densities (Wilson, 1994) and it is well known that these different tissues also have different rates of fermentation within the rumen (Wilson et al., 1989). However, studies of the effect of morphological characteristics on the relationship between shear power and stem digestibility have not been reported. In our study, when shearing force was standardized for stem diameter and linear density, the relationship between shearing force and digestibility was consistent regardless of stem diameter and linear density. Alfalfa stem diameter had significant correlation with shearing force (Iwaasa et al., 1996, 1998), but stem diameter had little effect on *in vitro* digestibility with vernal alfalfa (Mowat et al., 1967). In other words, stem diameter had no effect on the relationship between shear power and stem digestibility, which supported our conclusion.

CONCLUSION

Shearing force as measured by the G-LM3 meat shear was a more direct indicator than chemical constituents because it was easy to measure and it can be used as an indicator of forage nutritive value. Correlation between shearing force and linear density was highly significant. Moisture content also was an important factor which affected shearing force of alfalfa stem. Lignin, a cell wall chemical constituent, was correlated with shearing force. The results showed that the variance in stem shearing force was probably due to a combination of factors, including changes in chemical composition, morphological characteristics, and perhaps cellular structure and alignment also being involved. *In situ* digestibility of alfalfa stem had a negative relationship with shearing force and this relationship should be confirmed with a greater number of forages. Future research should evaluate the influences that shearing force has on breakdown of forage particles during mastication and on voluntary intake through animal feeding trials.

REFERENCES

Assoumaya, C., D. Sauvant, F. Pommier, M. Boval, B. Calif and H. Archimede. 2009. Effect of f requency of meals on intake and digestion of tropical grass consumed by rams. Asian-Aust. J. Anim. Sci. 22:72-81.

Ba, N. X., N. H. Van, L. D. Ngoan, C. M. Lwddin and P. T. Doyle. 2008. Effects of amount of concentrate supplement on forage intake, diet digestibility and live weight gain in yellow cattle in Vietnam. Asian-Aust. J. Anim. Sci. 21:1736-1744.

Buxton, D. R., J. S. Hornstein and G. C. Marten. 1987. Genetic variation for forage quality of alfalfa stems. Can. J. Plant. Sci. 67:1057-1067.

Chen, Y. X., J. Chen, Y. F. Zhang and D. W. Zhou. 2007. Effects of harvest date on shearing force of maize stems. Livest. Sci. 111: 33-44.

Esau, K. 1977. Anatomy of seed plants. Wiley, New York, p. 550.

Feng, Y. L. 2004. Ruminant nutrition. Science Technology Press, Beijing, p. 574 (In Chinese).

Evans, P. S. 1967. Leaf strength studies of pasture grasses: II. Strength, cellulose content and sclerenchyma tissue proportions of eight grasses grown as single plants. J. Agric. Sci. (Camb.) 69:175-181.

Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analyses. Agricultural Handbook No. 379, US Department of Agricultural, Washington, DC. p. 387.

Habib, M., G. E. Pollott and J. D. Leaver. 2008. Effect of cattle genotype and variable feed supple on forage intake and digestibility. Asian-Aust. J. Anim. Sci. 21:1435-1440.

Herrro, M., C. B. do. Valle, N. R. G. Hughes, V. de O. Sabatel and N. S. Jessop. 2001. Measurements of physical strength and their relationship to the chemical composition of four species of Brachiaria. Anim. Feed Sci. Technol. 92:149-158.

Hughes, N. R. G., C. B. do. Valle, V. Sabatel, J. Boock, N. S. Jessop and M. Herrero. 2000. Shearing strength as an additional selection criterion for quality in Brachiaria pasture ecotypes. J. Agric. Sci. (Camb.)135:123-130.

Inoue, T., I. M. Brookes, A. John, W. F. Hunt and T. N. Barry. 1994. Effects of leaf shear breaking load on the feeding value of perennial ryegrass (*Lolium perenne*) for sheep: Ⅰ.Effects on leaf anatomy and morphology. J. Agric. Sci. (Camb.)123:129-136.

Inoue, T., I. M. Brookes, A. John, W. F. Hunt and T. N. Barry. 1994. Effects of leaf shear breaking load on the feeding value of perennial ryegrass (*Lolium perenne*) for sheep: II. Effects on feed intake, particle breakdown, rumen digesta outflow and animal performance. J. Agric. Sci. (Camb.) 123:137-147.

Iwaasa, A. D., K. A. Beauchemin, J. G. Buchanan-Smith and S. N. Acharya. 1996. A shearing technique measuring resistance properties of plant stems. Anim. Feed Sci. Technol. 57:225-237.

Iwaasa, A. D., K. A. Beauchemin, S. N. Acharya, S. R. Bowley and J. G. Buchanan-Smith. 1998. Shearing force of alfalfa stems as affected by seeding rate. Can. J. Plant. Sci. 78:273-280.

Kokubo, A., S. Kuraishi and N. Sakurai. 1989. Culm strength of barley. Correlation among maximum bending stress, cell wall dimensions, and cellulose content. Plant Physiol. 91:876-882.

Kokubo, A., N. Sakurai, S. Kuraishi and K. Takeda. 1991. Culm brittleness of barley (*Hordeum vulgare L.*) mutants is caused by smaller number of cellulose molecules in cell wall. Plant Physiol. 97:509-514.

Mackinnon, B. W., H. S. Easton, T. N. Barry and J. R. Sedcole. 1988. The effect of reduced leaf shear strength on the nutritive value of perennial ryegrass. J. Agric. Sci. (Camb.) 111:469-474.

McClellad, D. J. and R. E. Spielrein. 1957. An investigation of the ultimate bending strength of some common pasture plants. J. Agric. Eng. Res. 2:288-292.

Mehrez, A. Z. and E. R. Ørskov. 1977. A study of the artificial fiber bag technique for determining the digestibility of feeds in the rumen. J. Agric. Sci. (Camb.) 88:645-650.

Mowat, D. N., R. S. Fulkerso and E. E. Gamble. 1967. Relationship between stem diameter and *in vitro* digestibility of forage. Can. J. Plant. Sci. 47:423-426.

Prince, R. P., D. D. Wolf, Jr. and J. W. Bartok. 1965. The physical property measurements of forage stalks. Agric. Exp. Sta., University of Connecticut, Storrs, CT. Bull, pp. 1-27.

Pritchard, G. I., L. P. Folkins and W. J. Pigden. 1963. The *in vitro* digestibility of whole grasses and their parts at progressive stages of maturity. Can. J. Plant. Sci. 43:79-87.

Sunagawa, K., T. Ooshiro, N. Nakamura, Y. Ishii, I. Nagamine and A. Shinjo. 2007. Physiological factors depressing feed intake and saliva secretion in goats fed on dry forage. Asian-Aust. J. Anim. Sci. 20:60-69.

Sunagawa, K., T. Ooshiro, Y. Murase, R. Hazama and I. Nagamine. 2007. Mechanisms controlling feed intake in large-type goats fed on dry forage. Asian-Aust. J. Anim. Sci. 20:1182-1189.

Van Soest, P. J. and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wilkins, R. J. 1966. The digestibility of forage and its relationship to lignification. Ph.D. Thesis. University of New England.

Wilkins, R. J. 1972. The potential digestibility of cellulose in grasses and its relationship with chemical and anatomical parameters. J. Agric. Sci. (Camb.) 78:457-464.

Wilson, J. R., M. N. Mcleod and D. J. Minson. 1989. Particle size reduction of the leaves of a tropical and temperate grass by cattle: 1. Effects of chewing during eating and varying times of digestion. Grass and Forage Sci. 44:55-63.

Wilson, J. R. 1994. Cell wall characteristics in relation to forage digestion by ruminants - A Review. J. Agric. Sci. (Camb.) 122: 173-182.