**Open Access**
Asian-Australas J Anim Sci
Vol. 30, No. 12:1718-1723 December 2017
https://doi.org/10.5713/ajas.17.0077
pISSN 1011-2367 eISSN 1976-5517

AJAS
Asian-Australasian Journal of Animal Sciences

# Dynamics of shearing force and its correlations with chemical compositions and *in vitro* dry matter digestibility of stylo (*Stylosanthes guianensis*) stem

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Submitted Feb 13, 2017; Revised Apr 10, 2017; Accepted Jun 2, 2017

**Objective:** The study explored the dynamics of shearing force and its correlation with chemical compositions and *in vitro* dry matter digestibility (IVDMD) of stylo.
**Methods:** The shearing force, diameter, linear density, chemical composition, and IVDMD of different height stylo stem were investigated. Linear regression analysis was done to determine the relationships between the shearing force and cut height, diameter, chemical composition, or IVDMD.
**Results:** The results showed that shearing force of stylo stem increased with plant height increasing and the crude protein (CP) content and IVDMD decreased but fiber content increased over time, resulting in decreased forage value. In addition, tall stem had greater shearing force than short stem. Moreover, shearing force is positively correlated with stem diameter, linear density and fiber fraction, but negatively correlated with CP content and IVDMD.
**Conclusion:** Overall, shearing force is an indicator more direct, easier and faster to measure than chemical composition and digestibility for evaluation of forage nutritive value related to animal performance. Therefore, it can be used to evaluate the nutritive value of stylo.

**Keywords:** Goat; Stylo Stem; Shear Force; Chemical Composition; *In vitro* Dry Matter Digestibility

## INTRODUCTION

Legume forages play very important role in ruminant feeding worldwide because they possess high crude protein (CP) content, high gross energy (GE), moderate fiber content, high dry matter intake and high dry matter digestibility (DMD). *Stylosanthes guianensis* (Stylo) grows naturally in the tropical, subtropical and temperate regions of the Africa, Australia, Southeast Asia and South Americas [1]. As a popular forage legume in the tropical areas, it has been widely used as feed for pigs, goats, sheep and cattle in some developing countries and has positive effects on their feed intake, digestibility and growth performance [2-5].

In general, the forage quality of stem is measured using its chemical compositions. It has been shown that higher lignification degree and fiber content are correlated with lower digestibility. However, physical properties of forage have also been linked to ruminants' feed intake, which is a major determinant factor of animal performance [6,7]. As one of the most important physical properties of plants, shear force, which is defined as the maximum force required for breaking down forage in laboratory experiments, represents the force required by animals to bite off the forage [8] and is directly related to animal performance. Recently, shearing force has been used as a more direct indicator than chemical compositions to estimate the nutritional value of forage and assess the physical characteristics of forage stems, such as wheat stalks, maize plant and crop straws. The results have shown that it can be used to measure the easiness of stems to be broken-down

during mastication [6,9-13]. Some studies have shown that shearing force is related to grass maturity, and morphological characteristics, chemical compositions, and degradability of forage stem. Therefore, the shearing force measurement is considered to be an alternative means of estimating forage nutritional value related to animal performance [14,15].

Although shearing force can be measured more easily and directly than chemical composition, little information is available about the relationship between shearing force and nutritional value of stylo. The objectives of the study were to investigate the dynamics of shearing force and its correlations with chemical compositions and *in vitro* dry matter degradability of stylo stem.

## MATERIALS AND METHODS

### Study site

This study was conducted at the experiment base of the Tropical Animal Research Center (TARC) of Chinese Academy of Tropical Agricultural Sciences (CATAS) in 2012. TARC is located in Danzhou, Hainan, China (longitude 109°30′E, latitude 19°30′N, altitude 149 m), and has an average annual rainfall and temperature of 1,815 mm and 23.3°C, respectively.

### Sample preparation and experimental methods

A total of 270 stylo plants with height of approximately 40, 50, 60, 70, 80, 90, 100, 110, and 120 cm, with 30 repeats at each height were randomly selected and cut at 5 cm above the ground. After leaves were removed from the stem, the shear force, weight, diameter and length, moisture content (MC), and chemical composition of the stem, the dominant part contributing to stylo nutritive value, were measured. Linear density is defined as the weight per millimeter of stem length. Each stem was cut into 3 segments for measuring diameters and shearing forces at bottom, middle and top, respectively. Each segment was cut at the approximate midpoint and between two nodes in order to avoid the influence of the nodes on shear force. Diameters were measured with Vernier caliper at the approximate midpoint. The shearing force was measured using a TMS-PRO texture analyzer (Food Technology Corporation, Stering, VA, USA) with the accuracy of 0.01 N. Deformation speed was maintained at 5 mm/s in all shearing experiments.

### Chemical analysis

Stylo samples were oven dried at 65°C for 48 h and milled through a 1.0 mm screen prior to chemical analysis and *in vitro* studies. Samples were analyzed for MC and ether extract according to the guidelines of Association of Official Analytical Chemists (AOAC [16]). The GE was measured using a bomb calorimeter (Parr6300, Parr Instrument Co., Moline, IL, USA). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the method of Van Soest et al [17]. Heat-stable amylase and sodium sulfite were used in the NDF procedure, and the results were expressed without residual ash. Chemical composition analyses were performed in triplicate.

### *In vitro* digestion

Rumen fluid was collected from three healthy mature Hainan black goats with permanent rumen cannulae (Ø = 50 mm) for measurement of *in vitro* dry matter digestibility (IVDMD). The collected rumen fluid was filtered through a layer of gauze and mixed with buffer solution as described by Menke and Steingas [18] with proper improvement. In brief, 0.2 g of stylo samples were placed into 100 mL calibrated glass syringes that have been pre-warmed at 39°C and mixed with 30 mL of rumen fluid-buffer mixture. After 96 h digestion in a water bath at 39°C, the samples were gently rinsed with cold tap water and dried at 65°C for 48 h to determine IVDMD. Each sample was measured in triplicate.

The metabolizable energy (ME) value was calculated using the equation ME = GE×IVDMD×0.815 (ARC [19]), where GE is gross energy (MJ/kg) and IVDMD is *in vitro* dry matter digestibility.

### Data analysis

Data analysis was carried out using the general linear model procedure [20] to determine the effect of cut height and diameter on shear force. Linear regression analysis was done to determine the relationships between the shearing force and cut height, diameter, chemical composition, or IVDMD. Pearson correlation coefficients (r) for these relationships were calculated using the PORC CORR procedure [20]. Differences between groups with $p<0.05$ or $p<0.01$ were considered statistically significant.

## RESULTS

### Dynamics of shear force, diameter and linear density

The dynamics of shear force, as well as diameter and linear density of stylo stem are shown in Table 1. The stem had shear forces

**Table 1.** Shearing force, diameter and linear density of stylo (*Stylosanthes guianensis*) stem at different cut heights

| Height (cm) | Shearing force (N) | Diameter (mm) | Linear density (g/mm) |
|---|---|---|---|
| 40 | 79.03$^{f}$ | 2.66$^{d}$ | 0.46$^{c}$ |
| 50 | 100.03$^{e}$ | 2.74$^{d}$ | 0.46$^{c}$ |
| 60 | 120.55$^{de}$ | 3.01$^{cd}$ | 0.52$^{c}$ |
| 70 | 130.88$^{d}$ | 3.16$^{c}$ | 0.48$^{c}$ |
| 80 | 157.52$^{c}$ | 3.14$^{c}$ | 0.52$^{c}$ |
| 90 | 196.97$^{bc}$ | 3.51$^{b}$ | 0.66$^{b}$ |
| 100 | 240.43$^{b}$ | 3.38$^{b}$ | 0.62$^{b}$ |
| 110 | 268.85$^{a}$ | 3.87$^{a}$ | 0.93$^{a}$ |
| 120 | 274.40$^{a}$ | 3.91$^{a}$ | 0.85$^{a}$ |
| SEM | 24.58 | 0.15 | 0.06 |
| p value | <0.001 | 0.01 | 0.03 |

SEM, standard error of means.
Values within a column with different superscripts in lowercase letter differ significantly from each other at $p<0.05$.

**Table 2.** Chemical compositions, IVDMD and ME of stylo (Stylosanthes guianensis) at different cut heights

| Height (cm) | Moisture (% DM) | CP (% DM) | ADF (% DM) | NDF (% DM) | GE (MJ /kg DM) | IVDMD (%) | ME (MJ/kg DM) |
|---|---|---|---|---|---|---|---|
| 40 | 48.66$^{a}$ | 7.62$^{a}$ | 54.06$^{e}$ | 56.04$^{d}$ | 15.73$^{b}$ | 35.41$^{a}$ | 4.54$^{a}$ |
| 50 | 43.52$^{b}$ | 7.3$^{a}$ | 54.86$^{e}$ | 60.64$^{cd}$ | 15.91$^{b}$ | 33.28$^{a}$ | 4.32$^{a}$ |
| 60 | 43.55$^{b}$ | 6.51$^{b}$ | 57.37$^{d}$ | 63.92$^{cd}$ | 15.91$^{b}$ | 33.27$^{a}$ | 4.31$^{ab}$ |
| 70 | 40.66$^{c}$ | 6.19$^{bc}$ | 61.05$^{c}$ | 65.13$^{c}$ | 16.05$^{b}$ | 31.86$^{ab}$ | 4.17$^{ab}$ |
| 80 | 40.39$^{c}$ | 5.75$^{c}$ | 63.01$^{bc}$ | 70.81$^{b}$ | 16.34$^{ab}$ | 31.35$^{ab}$ | 4.17$^{ab}$ |
| 90 | 39.62$^{c}$ | 5.78$^{c}$ | 66.86$^{b}$ | 73.58$^{ab}$ | 16.87$^{ab}$ | 29.31$^{b}$ | 4.03$^{b}$ |
| 100 | 37.46$^{cd}$ | 5.27$^{d}$ | 67.28$^{b}$ | 74.89$^{ab}$ | 17.16$^{a}$ | 30.01$^{bc}$ | 4.20$^{ab}$ |
| 110 | 32.65$^{d}$ | 5.42$^{cd}$ | 71.48$^{a}$ | 75.92$^{a}$ | 17.47$^{a}$ | 28.69$^{c}$ | 4.08$^{b}$ |
| 120 | 30.53$^{d}$ | 4.98$^{d}$ | 70.61$^{a}$ | 76.47$^{a}$ | 17.78$^{a}$ | 25.91$^{d}$ | 3.75$^{c}$ |
| SEM | 1.86 | 0.30 | 2.19 | 2.47 | 0.25 | 0.96 | 0.07 |
| p value | 0.01 | 0.01 | 0.03 | 0.01 | 0.02 | 0.02 | 0.03 |

IVDMD, *in vitro* dry matter digestibility; ME, metabolizable energy; DM, dry matter; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; GE, gross energy; SEM, standard error of means.
Values within a column with different superscripts in lowercase letter differ significantly from each other at $p < 0.05$.

ranging from 79.03 N at 40 cm cut height with a diameter of 2.66 cm and linear density of 0.46 g/mm to 274.40 N at 120 cm cut height with a diameter of 3.91 cm and linear density of 0.85 g/mm. Clearly, the shear force, diameter and linear density increased with plant height increasing.

### Dynamics of chemical composition, IVDMD, and ME

The chemical composition, IVDMD and ME of stylo stem are shown in Table 2. The MC of stylo stem ranged from 30.53% at cut height of 120 cm to 48.66% at cut height of 40 cm. The CP contents at the above two cut heights were 4.98% and 7.62%, respectively. The ADF and NDF contents were 54.06% and 56.04%, respectively, at cut height of 40 cm, and 70.61% and 76.47%, respectively, at cut height of 120 cm. The GE content changed from 15.75 MJ/kg to 17.78 MJ/kg at cut height of 40 cm and 120 cm, respectively. The IVDMD values and ME contents were 25.91% and 3.75 MJ/kg, respectively, at cut height of 120 cm, and 35.41% and 4.54 MJ/kg, respectively, at cut height of 40 cm, as shown in Table 2. Overall, the contents of ADF, NDF, and GE increased with plant height increasing, while the MC and CP contents as well as the IVDMD values decreased with plant height increasing, though less dramatically.

### Relationships between shearing force and diameter, linear density, chemical composition or IVDMD

The correlations between shearing force and diameter, linear density, chemical composition or IVDMD are shown in Figure 1 to 4, respectively. The shearing force was positively correlated with the diameter ($R^2 = 0.9185$, $p<0.05$) and linear density ($R^2 = 0.835$, $p<0.05$) of stylo stem, but negatively correlated with MC ($R^2 = 0.885$, $p<0.01$). Moreover, the correlations between shearing force and chemical compositions (*i.e.*, CP, ADF, and NDF) were also analyzed. The results showed that shearing force was negatively correlated with CP content ($R^2 = 0.8507$, $p<0.05$) and IVDMD ($R^2 = 0.8801$, $p<0.05$) of stylo stem, but positively correlated with ADF content ($R^2 = 0.8959$, $p<0.01$) and NDF content ($R^2 = 0.9053$, $p<0.01$).

## DISCUSSION

The plant height is one of the important indexes to measure the morphological characteristics because it could reflect plant maturity and yield. Therefore, it is necessary to investigate the relationship between plant height and chemical compositions. Previous studies have shown that i) plant height could affect the chemical compositions and nutritional value of dairy cows [21], ii) tall plants had lower CP content, but higher fiber content than short plants

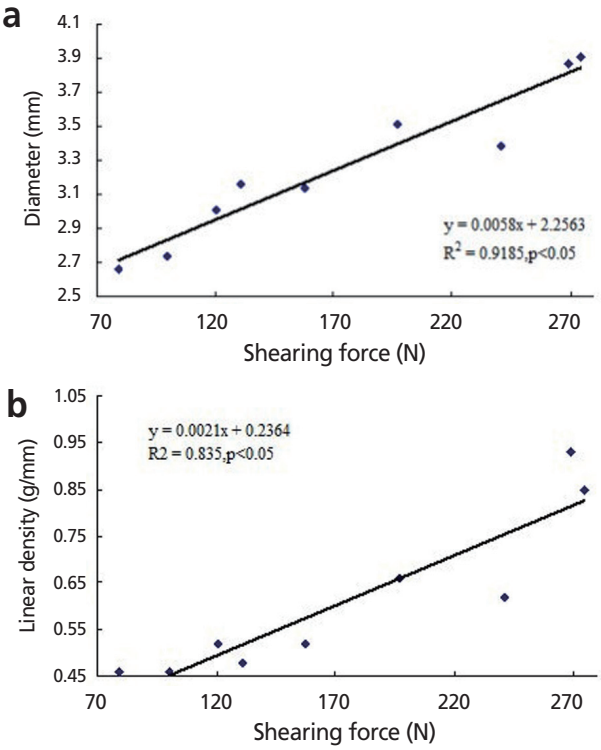


**Figure 1.** Correlation of shearing force with (a) diameter and (b) linear density.

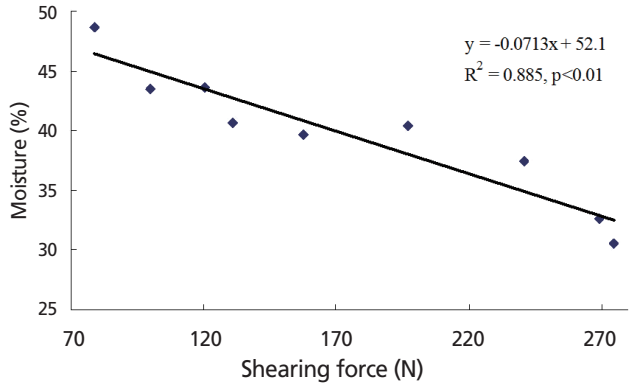


**Figure 2.** Correlation between shearing force and moisture.

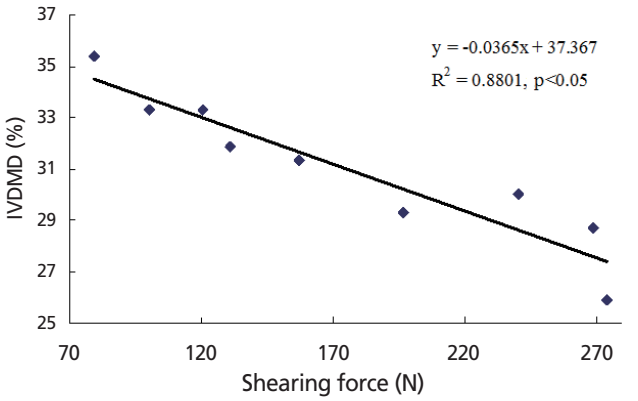


**Figure 4.** Correlation between shearing force and *in vitro* dry matter digestibility (IVDMD).

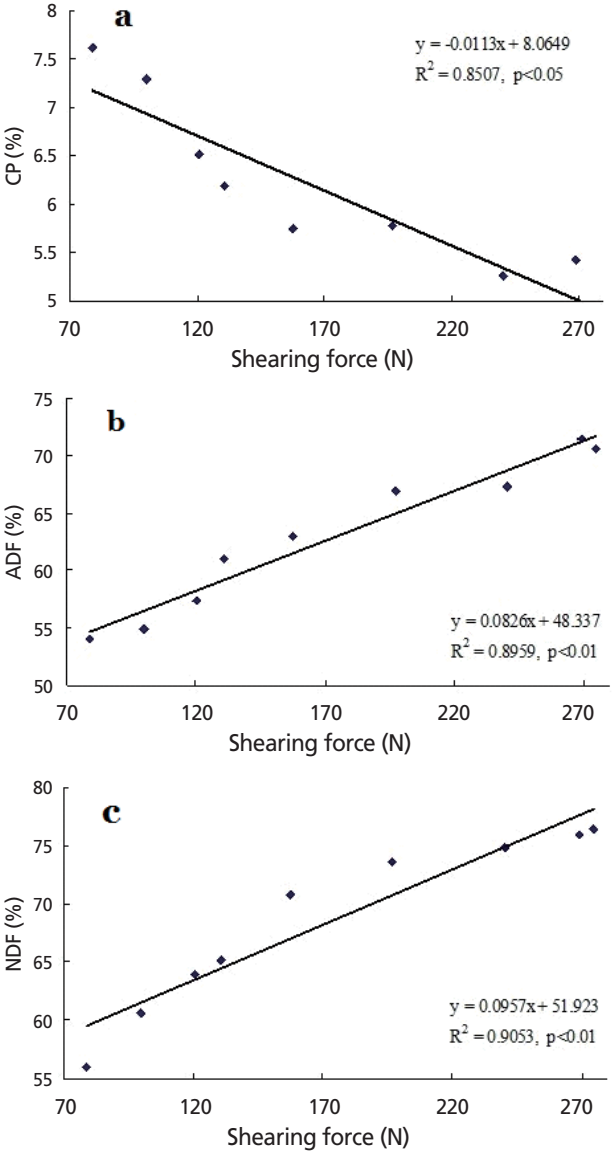


**Figure 3.** Correlations of shearing force with chemical compositions (a) crude protein (CP) content, (b) acid detergent fiber (ADF) content, and (c) neutral detergent fiber (NDF) content.

[22], iii) plant height affects the kinetics of *in vitro* rumen fermentation and nutritional value [23] and plants with different height have different CP, ADF, and IVDMD contents [24]. These results are consistent with the present study, and could explain the differences in feeding value of forages with different heights. With the progress of plant maturation, the stem becomes harder and has increased shearing force.

Our data show that the shearing force of stylo stem increases with its diameter increasing. Diameter is one of important factors affecting shearing force. Previous studies have shown that shearing force of crop stems is mainly influenced by stem diameter [6,10,14]. These studies found that shearing force increases with stem diameter increasing, which is consistent with our results. The linear density of X has been reported to have positive linear relationship with shearing force [12-14,25]. In the present study, a similar relationship was observed.

Chemical composition of stem is also a factor affecting shear force. As shown in Table 2, shearing force of stylo stem increased with MC decreasing and decreased with CP content increasing. These results are consistent with previous studies showing that shearing force increased with MC decreasing and fiber tensile strength was possibly negatively related to MC. Meanwhile, the CP content decreased with maturity increasing, resulting in a negative relationship between shearing force and CP content [7,12,14]. Previous studies also reported that lignin or cellulose contents were largely affected by plant species and their chemical composition and not closely related to shearing force [10,12,26]. The present study showed that shearing force of stylo increased with contents of crude fiber, ADF, and NDF increasing, possibly because that the lignin and cellulose contents could positively affect plant hardness and subsequently impact the shear force. Therefore, further study is necessary to investigate the effect of changes in shearing force on cattle digestibility and nutritional value of grass.

The degradability and shearing force are important indexes of forage nutritional value. But their relationship was not well

explored. In the present study, we found that there was a negative relationship between the shearing force and IVDMD, and IVDMD increased with shearing force of stylo stem decreasing. The results are consistent with the results found in other forages [14,26]. Wang et al [12] showed that stem shearing force could not influence the *in situ* DM digestibility of ryegrass and maize. However, the present study showed that shearing force was negatively related with IVDMD. In other words, shearing force of stylo stem decreased with IVDMD increasing. This discrepancy may be due to variation in physical properties and chemical compositions of different forages. According to our results, as the shearing force increased, the protein content, IVDMD decreased and the fiber and lignin contents increased. Therefore, it is possible that shearing force has a great influence on the nutritional value of grass. However, this need to be further examined.

The present study explored the relationships of shearing force with the chemical compositions, and *in vitro* dry matter degradability (IVDMD) of stylo. The results showed that with stylo stem height increasing, the shear force, diameter, linear density and fiber content increased, but CP and IVDMD decreased and stem shearing force had a positive correlation with stem diameter, linear density and fiber content, but negatively related with stem moisture, CP content and IVDMD. These findings indicted that shearing force could be used as a direct or easier indicator for evaluating the nutrition value of stylo.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

## ACKNOWLEDGMENTS

This work was supported by the Hainan Province Grant (No. ZDKJ2016017-03) and Central Public-interest Scientific Institution Basal Research Fund for Chinese Academy of Tropical Agricultural Sciences (No.17CXTD-18,1630032017033,1630032015044) and National Basic Research Program of the China Ministry of Science and Technology (No. 2014CB138706).

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