PRECISION OF AN EQUATION TO ESTIMATE DRY MATTER DEGRADABILITY OF *Clitoria ternatea*

[PRECISION DE UNA ECUACIÓN PARA ESTIMAR LA DEGRADABILIDAD DE LA MATERIA SECA DE *Clitoria ternatea*]

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SUMMARY

The objective of this study was to evaluate the precision of an equation developed to estimate the dry matter digestibility (DMD) of alfalfa (*Medicago sativa*) when used on the tropical legume Clitoria (*Clitora ternatea*). Acid detergent fiber from Clitoria was determined and its DMD estimated using the equation: %DMD = 88.9 - (0.779 x %ADF), and subsequently *in situ* and *in vitro* degradability (DMDE) of the mentioned legume was determined. Mathematical, *in situ* and *in vitro* estimates were compared by ANOVA as a completely randomized design and precision of the equation as DMDE estimator of Clitoria hay was determined if P>0.05. Mathematical DMD estimates were not different (P>0.05) to those obtained by *in vitro* method, and both were significantly different (P<0.05) and lower than *in situ* estimates. It can be concluded that the equation initially developed to estimate the DMD of alfalfa was a closer estimator of *in vitro* DMDE of Clitoria, whereas underestimated the *in situ* parameter by 8.6%.

Key words: Tropical legumes; *in vitro* degradability; *in situ* degradability.

RESUMEN

El objetivo del presente trabajo fue evaluar la precisión de una ecuación desarrollada para estimar la digestibilidad de la materia seca (DMS) de la alfalfa (*Medicago sativa*), al ser utilizada en la leguminosa tropical Clitoria (*Clitora ternatea*). Se determinó el contenido de fibra detergente ácido de la Clitoria y se calculó su DMS utilizando la ecuación: %DMS = 88.9 - (0.779 X %FDA) para posteriormente determinarse la degradabilidad (DEMS) *in situ* e *in vitro* de la mencionada leguminosa. Los estimados obtenidos por los tres métodos fueron comparados por ANOVA usando un diseño completamente al azar y se consideró como criterio para declarar que la ecuación cumplió con precisión como estimador de la degradabilidad si P>0.05. Los estimados matemáticos de la DMS fueron similares (P>0.05) a los obtenidos por el método *in vitro* y ambos fueron significativamente diferentes (P<0.05) y menores a los obtenidos por el método *in situ*. Se concluye que la ecuación desarrollada para estimar la DMS de la alfalfa resultó un estimador preciso de la DEMS de la Clitoria obtenida por el método *in vitro*, en tanto subestimó en un 8.6% la DEMS obtenida por el método *in situ*.

Palabras clave: Leguminosas tropicales; degradabilidad *in vitro*; degradabilidad *in situ*.

INTRODUCTION

Forage quality is an expression of the potential of livestock to produce meat, milk, and other products from forage through the utilization of its available nutrients. The level of animal production is controlled nutritionally by the daily intake of digestible nutrients and by the efficiency with which such nutrients can be metabolized and used for body processes. Both quality and amount of digested nutrients available per unit time are important to the production of specific animal products. Thus, forage quality may be defined as the

type and amount of digestible nutrients available to the animal per unit time (Barnes and Marten 1979; Bochi-Brum *et al*. 1999).

An accurate and precise assessment of quality before forage is fed to animals will have a marked effect on the economic feasibility. Measurement of forage quality becomes increasingly more important as one intensifies the production system (Barnes and Marten 1979). Several methods are used to estimate ruminal digestibility of dry matter, between which in tropical countries, the most frequently used methods are the *in situ* (nylon bag) technique (Harris *et al.* 1967) and the *in vitro* method of Tilley and Terry (1963).

In situ method has achieved the widest use and is routinely used for studying effects of the rumen environment. However, this method requires a large number of nylon bags to be ruminally incubated for each feed sample and, in turn, a substantial amount of human work (Olaisen *et al.* 2003). *In vitro* Tilley and Terry technique is accepted as the most appropriate and utilized laboratory methodology to estimate the digestibility of feedstuffs for ruminants, and has been used extensively because of a high degree of correlation to *in vivo* digestibility (Marten and Barnes 1980). Over the years, the technique has been modified to improve the precision of *in vitro* digestibility dry matter estimates as well as improve labor and time efficiency of assays (Holden 1999). In addition, both *in situ* and *in vitro* determinations are expensive, rendering these techniques impractical for routine analyses (De Figueiredo *et al*., 2000; Giraldo *et al*., 2007).

Therefore, a need exists for less expensive, time-efficient and animal friendly techniques, in particular for routine analysis, within which mathematical approaches have been proposed. In this context, the aim of this study was to evaluate the precision of one equation developed to estimate the dry matter digestibility (DMD) of alfalfa (*Medicago sativa*), when was used on Clitoria (*Clitora ternatea*), one of the most promissory legumes for tropical and subtropical regions.

MATERIAL AND METHODS

Site

The study was conducted at the Laboratory of Nutrition, University of Guadalajara located in Ciudad Guzmán, Jalisco, México at 19°42'10'' north latitude and 113°27'45'' west longitude. The site has a warm climate with an average temperature of 20.2°C and 732 mm of annual average rainfall.

Experimental procedure

Acid detergent fiber from 30 samples of ground early flowering Clitoria hay, were determined by the method of Goering and van Soest (1970), and used to calculated Clitoria DMD according to the equation proposed to estimate DMD of alfalfa by Linn and Martin (1989) in which, %DMD = 88.9 - (0.779 x %ADF).

According to the procedure proposed by Harris *et al.* (1967) to estimate *in situ* dry matter degradability (DMDE), 30 nylon bags, 52 µm pore size; 5 x 10 cm (33 mg/cm^2 ratio of sample size to bag surface area) containing 5 g of Clitoria hay were incubated for 48 h in the rumen of a mature Holstein cow fed corn silage, alfalfa hay and Clitoria hay and fitted with ruminal cannulae. After removal, bags were hand-rinse until colorless, dried at 60°C in a forced-air oven for 48 h, and the residual material weighed.

Finally, *in vitro* DMDE was estimated, using the first stage of the Tilley and Terry technique (Tilley and Terry, 1963). Thirty samples of 0.5 g of Clitoria hay were placed into 100 ml plastic test tubes and incubated at 39°C with 10 ml of cow ruminal fluid and 40 ml of McDougal´s artificial saliva buffer (McDougal 1948). After 48 h, tubes were removed from the *in vitro* bath, hand-rinsed until colorless, dried at 60°C in a forced-air oven for 48 h, and the residual material weighed.

Statistical Analyses

Mathematical DMD estimates were compared with those DMDE obtained by *in situ* and *in vitro* methods by ANOVA as a completely randomized design using the general linear model procedure of SAS (1989). Significant differences were determined using Tukey test and equation precision as DMDE estimator of Clitoria was declared if $P>0.05$. In addition a Pearson Correlation test was performed to set a relationship between estimators.

RESULTS AND DISCUSSION

Average ADF content of Clitoria hay was 35.74 ± 5.06% with minimum and maximum values of 29.06 and 47.07% respectively. Results presented in Table 1 show that mathematical DMD estimates were not different ($P>0.05$) to those obtained by *in vitro* method; however, both were significantly different ($P<0.05$) and lower than *in situ* estimates. Mathematical method underestimated the *in situ* parameter by 8.6%, while the *in vitro* technique underestimated it by 11.71% (Table 1).

Table 1. Mathematical, *in vitro* and *in situ* dry matter digestibility (DMD) of *Clitoria ternatea* (n=30).

Method	DMD (%)	SEM
In situ	66.32[a]	0.08
Mathematical	61.06[b]	0.13
In vitro	59.37[b]	0.20

[ab] Means within columns followed by different letters differ (P<0.05).
CV = 6.95

DMD estimates obtained by mathematical method and DMDE obtained by *in vitro* and *in situ* techniques showed no significant correlations (P>0.05), being positive among *in situ* and *in vitro* techniques and negative among mathematical and the previous mentioned techniques (Table 2).

Table 2. Correlation coefficients of the relationship between mathematical, *in vitro* and *in situ* dry matter digestibility of *Clitoria ternatea.*

	Mathematical	*In situ*	*In vitro*
Mathematical	- - -		
In situ	-0.33[NS]	- - -	
In vitro	-0.25 [NS]	0.18 [NS]	- - -

NS=Non-significant (P>0.05).

In situ, mathematical and *in vitro* Clitoria digestibility and degradability estimates obtained in the present study (66.32, 61.06 and 59.37% respectively) were higher than the *in vivo* value (56.9%) found by Juma *et al*. (2006). Variations among both studies results could be explained by differences in the method of estimation and in the ADF content of Clitoria samples. Some authors report that 48 h *in situ* and *in vitro* incubations of many feedstuffs overestimate *in vivo* digestibility and when the incubation period is reduced to 24 h, the *in vitro* technique accurately predict the *in vivo* digestibility (Holden, 1999; Damiran *et al*. 2008). Oba and Allen (2005) point out that 24 to 30 h of incubation is appropriate to evaluate feedstuff digestibility. On the other hand, Juma *et al*. (2006) reported an ADF content of 47.4*% versus* 35.74% obtained in this work. In agreement with the equation %DMD = 88.9 - (0.779 X %ADF) a lower ADF content results in a higher DMD estimate. Another study found an intermediate value of 60.40% (Ratan *et al*. 1982).

As happened in the present study, Varel and Kreikermeier (1995) and Torres *et al*. (2009) found that *in situ* method consistently provided a greater extent of digestion of legumes and grasses than the *in vitro* method. *In vitro* results can be affected by several factors such as grinding size, distribution of particle size in the sample (Judkins *et al*. 1990; Damiran *et al*. 2008) and smaller concentration of inoculum (Varel and Kreikermeier 1995).

Also, adaptation and development of new procedures to determine *in vitro* digestibility of feeds constitute an additional source of variation. The traditional Tilley and Terry method to determine the *in vitro* DMD of forages has been compared with new methods such Daisy[II] system with inconsistently results. Whereas Damiran *et al*. (2008) found that Daisy[II] *in vitro* DMD estimates were greater than traditional Tilley and Terry estimates, Holden (1999) reported that the method of analysis did not affect *in vitro* DMD of alfalfa hay. Different values for the conventional *in vitro* method and Daisy[II] technique seems to be related to incubation environment. In the traditional method sample particles are in direct contact with the inoculum and the buffer solution, the Daisy[II] system used filter bags placed into incubation jars (Spanguero *et al*. 2003). Nevertheless, authors concur that Daisy[II] system allows simultaneous incubation of a large number of samples compared with fermentation batch, giving advantages in terms of labor consumed and costs per determination.

Contrary to the results of the present study, Giraldo *et al*. (2007) found that *in vitro* DMD estimates of forages using the Daisy[II] system were significantly higher than those obtained using the *in situ* nylon-bag method. In that study differences were attributed to the size pore of the bags and to the sample size to bag surface area ratio (SS:SA). Despite studies that showed that a reduction of in SS:SA from 54 to 16 mg/cm^2 resulted in a dramatic increase of DM disappearance (Mehrez and Ørskov, 1977) and recommended SS:SA values ranging from 10 to 20 mg/cm^2 (Vanzant *et al*., 1998), the value used in this study (33 mg/cm^2) did not inhibit *in situ* DMD. Mehrez and Ørskov (1977) suggest that the inhibition of digestion in large SS:SA ratios was a result of inadequate mixing and removal of the digestion end products from bags. On the other hand, an appropriate pore size allowed sufficient influx of digestive microorganism, permitting adequate efflux of digestion end products, and minimizing influx of ruminal digest residues and efflux of small sample particles (Vanzant *et al*., 1998; Foster *et al*., 2007). While some evidence suggests that pore size ranging from 40 to 60 µm introduces large variation in measurements of digestion *in situ* (Nocek, 1985; Vanzant *et al*., 1998). In this study, standard error of *in situ* estimates and hence variability, was smaller than those obtained by mathematical and *in vitro* methods. Pore size used (52 µm) was similar to the one (53 µm) most reported in the literature (Vanzant *et al*., 1998).

Other factors that influence *in situ*-derived estimates of rumen degradation are bag material, sample processing, host animal, animal diet, feeding level and frequency, bag insertion, removal procedures, location of bags within the rumen and containment procedures for the bags, rinsing procedures, microbial correction and incubation times (Huntington and Givens 1995; Vanzant *et al*., 1998; Spanguero *et al*., 2003).

Due to technique-derived variability, some studies found significant correlations between *in situ* and *in vitro* digestibility estimates (Judkins *et al*. 1990; Kamalak *et al*. 2005). Others indicated that *in situ* and *in vitro* digestibility showed a positive and significant correlation only after 48 h of incubation (Ceballos *et al*. 2008), and as it was observed in the present study, Silveira *et al*. (2009) did not find significant correlations.

It can be concluded that equation %DMD = 88.9 - (0.779 x %ADF) developed to estimate the DMD of alfalfa was a closer estimator of *in vitro* degradability of Clitoria, whereas underestimated the *in situ* parameter by 8.6%. This equation could be used as an easier alternative reference of Clitoria DMDE. However, present results should be cautiously applied because of capability of the equation to predict *in vitro* data depend upon ADF content of Clitoria.

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