Validation of the *in vitro* Ruminant Digestibility Method Applied on Daisy Incubator

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Abstract
Determination of digestibility for ruminant feeds is important for nutritive value evaluation, and for practical use the in vitro methods are widely applied. The new version of Tilley-Terry in vitro method was used on Ankom Daisy Incubator and validated by testing repeatability, reproducibility and correlation with previous method version. All these validation parameters have been attended and the new version of method is indicated for current use.

Keywords: in vitro ruminant digestibility

1. Introduction

Diets for ruminants are established after knowing the nutritive value of the included feeds. It is established by determination of chemical composition, digestibility and degradability of these feeds. The reference method for digestibility evaluation is the in vivo method but it is time-consuming, labor and expensive. Consequently, alternative in vitro methods have been created for practical use. Applying the results of the in vitro method in a prediction equation, the in vivo digestibility can be obtained. The in vitro method of Tilley-Terry (1963) [1] is widely used because it has the smallest error of digestibility prediction. Using the new technology from Ankom and the methodology established in the lab, Martín-García (2004) [2] tested the dry matter digestibility of olive by-products. Also, in vitro enzymatic methods like Pepsin-Cellulase of Aufrere (2007) [3] are more often applied by using the Ankom technology. This method steps are very simple and easy to execute as many authors mentioned, i.e. Huhtanen (2006) [4], Selmi (2010) [5], Pastierik (2014) [6].
The aim of this study was to validate the new version of the Tilley-Terry method which uses the Ankom technology (Daisy Incubator and filter bags) and to confirm if more rapid and also, reproducible results of digestibility can be obtained.

2. Materials and methods

The in vitro method of Tilley-Terry is applied for determination of feeds organic matter digestibility (OMD) trials. The method is briefly described.

Tilley-Terry method-traditional version (in tubes)
0.5 g of the feed samples is weighed into 100 ml glass tube and 50 ml of diluted/buffered rumen fluid are added and gassed with carbon dioxide. The tube is closed with rubber plug. All the samples tubes are incubated 48 h at 39^{o}C in a water bath, further centrifuged, the sediment retained, and 50 ml of pepsin solution (2‰ in HCl 0.1N) are added. Upon the incubation for 48 h at

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39°C and centrifugation, the sediment which represents the residue of digestion is collected in crucible, then dried at 103°C and weighed. The crucibles with residues are incinerated at 550°C and also, weighed. Calculations for the digested part of the samples are based on difference between initial (sample) and final (residue) organic matter.

Tilley-Terry method-new version by Daisy Incubator

The *in vitro* digestibility was measured according to producer technology. 0.5 g of feed sample was weighed to F57 Filter bag (Ankom, USA) and the sealed bags have been incubated in the digestion jar of the DaisyII Incubator (Ankom, USA) under carbon dioxide coat. The stages of the method were similar as for the traditional version: the first stage with buffered rumen liquid and the second stage with pepsin-HCl 2‰ solution. Finally the bags with digestion residues were dried at 103°C, weighed, and further included in crucibles, burned at 550°C and the resulted ash was also weighed.

An alfalfa hay sample was tested for the OMD by the *in vivo* method. This hay was considered as an internal control feed with known digestibility and chemical composition (dry matter is 87.76% and organic matter is 83.42g/100 g dry matter). This feed was tested by the new version of the *in vitro* method and corrected by factor 1.105 for the final OMD value.

Validation parameters

In order to evaluate if this new version of the *in vitro* method can be applied for current use the validation was proceeded. The validation parameters are further described.

Repeatability

For the alfalfa hay it was settled that the intra-laboratory coefficient of variation (CV %) of the mean shall not exceed 2%. In order to evaluate the repeatability six replicates of the feed were tested and the CV was calculated.

Reproducibility

The maximum coefficient of variation to be met is 2% and settled as a within-laboratory reproducibility condition for the method. The alfalfa sample was tested for 6 repeated times (at 7 days interval) and the CV was calculated.

Accuracy

For monitoring the digestibility testing, the control chart was plotted (Figure 1). This chart was built with the OMD values already settled in laboratory for alfalfa hay. The central line was set as the average value of OMD digestibility (*Xavr*=60.99%), and the warning limits were set at ± 2*s*, while the action limits were set at ± 3*s*, where *s* is the standard deviation of reproducibility (1.06%). Using this chart the results of the new version method (*Xi_Daisy*) were controlled on 12 repeated times of testing (at 7 days interval).

3. Results and discussion

Repeatability and reproducibility

In Table 1 can be observed that CV % for repeatability was 1.11 and that for reproducibility was 1.85, and both of them are smaller than the settled value of 2%. Consequently the precision of the new version method is demonstrated.

Table 1. The OMD (%) values for precision testing of the method

	Repeatability testing	Reproducibility testing
	59.92	62.44
	61.31	61.85
	60.03	60.21
	60.27	60.82
	60.91	61.23
	61.5	59.32
average	60.80	60.69
standard deviation	0.67	1.12
CV%	1.11	1.85

Accuracy

The results obtained for the OMD determination by new version method (*Xi_Daisy*) is shown in the Figure 1. The diagram in this figure can monitor the systematic and random effects in an analytical method. Also, in the same diagram are presented OMD values obtained by traditional version method (*Xi_Tube).*

It can be observed that *Xi_Daisy* values were within the warning limits along all the times of testing and were distributed on both sides of the central line. The distribution of *Xi_Daisy* values is similar with the distribution of *Xi_Tube* values. In conclusion, the new version of the method fulfills the accuracy requirement as previous version does.

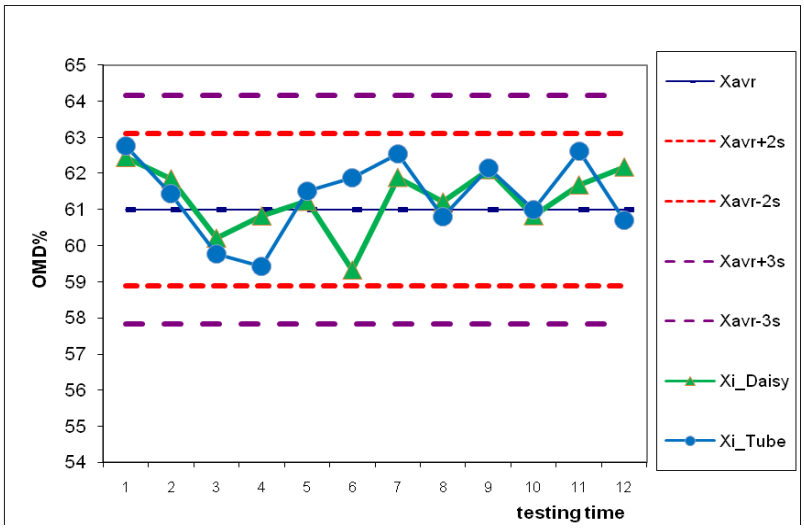

Figure 1. Control chart for OMD determination on alfalfa hay

4. Conclusions

The new version of the Tilley-Terry method is validated and had the required accuracy and reproducibility to be applied for digestibility testing. There is no difference between the results of the traditional (in tubes) and the new version of the *in vitro* digestibility method (on Daisy Incubator).

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