RESEARCH ARTICLE

Comparision of Digestibility Estimation fFor Omd Through two In Vitro Methods for some Feeds, Used in Ruminant Animals

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Abstract

Two in vitro procedures were used to assess the digestibility of some feeds used in ruminant feeding in Albania. In total nine samples from common used feeds in ruminant animals feeding, from which six cereal grains, two cereal straws and one ryegrass straw were analyzed for their chemical content of ash, crude protein (CP), crude fat (CF), neutral detergent fiber (NDF), acid detergent fiber(ADF), acid detergent lignin (ADL), acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN). All the samples were estimated for organic matter digestibility (OMD) through both Tilley&Terry and Pepsin-cellulase methods. Average protein content of cereal grains varied from 10.1 % for oat to 10.8% for barley. The ADF and ADL content varied respectively 3.90% -.17.6% and 0.80% -3.70%. The straws have similar chemical content for main Weende parameters and as expected average protein content was lower and varied from 2.60% for wheat to 3.60% for ryegrass while the ADF and ADL content varied respectively 53.8% (oat) - 56.3 (wheat) and 7.70% (ryegrass) - 8.70% (oat). The OMD determined with Tilley and Terry and pepsin cellulase methods resulted to be similar in straw samples while for cereals, the oat grain presented the lowest value obtained from both methods, 69.7% and 65.0%, respectively for TT and PC method.Tilley and Terry procedure gave higher values of OMD for all feed samples in comparison with enzymatic method, 68.4% vs 58.6%respectively for TT and PC method.The study revealed that digestibility results obtained by these two methods were highly correlated (r=0.99). According to R^2-value (0.98) the OMD determined by Tilley and Terry method could be predicted from enzymatic test as most convenient since it does not need animals.

Keywords: Tilley and Terry; Pepsine-celulase; organic matter digestibility; feeds.

1. Introduction

The determination of nutritional values of feeds for ruminant nutrition is very important because it affects not only animal production but also the economic level. Determining the chemical composition of these feeds would help to formulate the most appropriate diets, meeting the nutritional requirements, improving the health and productivity of animals and at the same time reducing environmental pollution. Determination of feeds digestibility through in vivo methods can be achieved by knowing their chemical composition. Several in vitro methods have been used to determine feed digestibility for ruminants among which, the most used method is that of Tilley and Terry (1963). Although this method can provide better determination of feed digestibility and is greater similarity to in vivo trials, its implementation requires the use of fistulated animals, which can create ethics problems and also affect animal's health. To overcome these limitations, over the years a number of other in vitro methods have been developed, simpler to use, such as the enzymatic Pepsin-Cellulose

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(PC) method, which aims to use commercial enzymes aiming to replace the in vivo trials and rumen fluid techniques. The purpose of this study is to compare the Tilley and Terry method with the Pepsin-cellulose enzymatic method in organic matter degistibility and whether the enzymatic method is capable of replacing Tilley and Terry as the most practical method to be used in laboratory and especially in Albania, as one of the countries with limited laboratory capacities.

2. Material and Methods

Nine feed samples were tested for this study. All feed samples were analyzed for their chemical parameters according Weende Proximate Analysis (AOAC 2000) for dry matter (DM), ash, crude fiber (CF), crude protein (CP), etherextract (EE). *Van* Soest detergent system (Van Soest and Robertson 1985; Robertson and Van Soest 1981) was used to determine neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Hemicellulose was calculated as NDF – ADF and cellulose as ADF – ADL (Rinne et al., 1997a). Acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) were determined according (Van Soest, Robertson et al. 1991). Two "in vitro" methods were used to determine the OMD of selected feeds: enzymatic (cellulose and pepsin) and Tilley and Terry method. The two stage Tilley and Terry (1963) procedure modified by Van Soest, Wine et al, (1966) was used to determine the in vitro digestibility of feeds. Briefly, 0.25 g DM of each experimental sample ground at 1 mm screen was added to each of 50 mL conical centrifuge tubes (Corning Inc., New York, NY, USA). In each tube 50 mL buffer-inoculum mixture as described by Marten and Barnes (1980) was added under purging with CO_2, rumen fluid was obtained from two fistulated non-pregnant and non-lactating Holstein cows, fitted with rumen cannulae, after a two weeks adaptation period to the diet with continuous access to fresh drinking water. Two total mixed rations (TMR) were used to feed the cows administered twice a day. After two weeks adaptation to the diet, the rumen contents were collected to a pre-warmed (39 ◦C) thermos container and transported to the laboratory. After the rumen inocula collection, diets were exchanged between cows and a new two weeks adaptation period started. All samples and two tubes used as blanks for the experiments were incubated in a water-bath at 39 ◦C. Incubations were stopped after 48 h. Blanks and samples were incubated in duplicate per inoculum and per incubation, incubations being replicated in two separate runs, resulting in eight replicates for each feedstuff. The calculations for organic matter digestibility (OMD), were made based on the weight after its incineration (at 500 ºC).The enzymatic method used to assess the *in vitro* digestibility of 37 feeds is the one according to Aufrère, Baumont *et al,*(2007). The enzymatic digestion was carried out by adding the sample with 50 mL pre-heated solution of 2% pepsin with 0.1 N HCl in a water bath at 39 ºC for 24 h. After 24 h incubation, the tubes were transferred into a water bath at 80°C for an acid hydrolysis for 30 min and again incubated for another 24 h in a water bath at 39°C with 50 mL pre-heated cellulase-buffer. Blanks and samples were incubated in triplicate. The OMD was estimated from the weight after its incineration at 500 ºC.

3. Results and Discussion

The data of organic matter digestibility measured through two in vitro methods were elaborated according to linear regress analyses with least square method. MINITAB software was used to perform the t- test on differences between OMD-TT and OMD-PC and for assessing regression between TT and PC data to develop prediction equations for Tilley and Terry OMD. Chemical analyses will continue to be an indispensable part of feed evaluation as they give general information of the main chemical parameters present in the feeds. Such information can improve and provide a better balance of nutrients and also reduce anti-nutritive factors. Chemical characterization methods can not give a direct estimate of nutritive value, but rather relay on statistical association to measure digestibility and intake (D.J.R. Cherney 2000). All feeds presented similar content for main Weende parameters according to each group of feed. The highest crude protein (CP) content for cereal grains was found in barley grain (10.8%) and for straws the highes value (3.60%) was for ryegrass. Between straw samples the average values of NDF, ADF and ADL fractions appear at approximately the same levels with very small differences respectively 80.1, 54.8 and 8.13%.The lowest values of these fractions were in grain samples (respectively 21.0, 8.02 and 2.60%). Lower values for NDF, ADF and ADL content grains were expected based on their structure, with lower content of cell walls.The average values for NDIN

and ADIN content in grain samples was similar (respectively 1.79 and 0.32%) meanwhile for straws respectively 1.57 and 1.60 %). Anyway is important to notice that both these fractions resulted lower in all samples which indicate the quality of feeds and their level of use in the ruminant organism.

The digestibility data for all feeds included in the study (n=9) show that the extent of in vitro organic matter digestibility obtained by Tilley and Terry assay was greater than that measured by the Pepsin-Cellulase method with average values respectively 68.4 vs. 58.6% for OMD, in agreement by previous comparisons done from other authors, (Jones and Hayward, 1975; Andrighetto, 1992; Forejtová, Lád *et al,* 2005), which may be related to rumen-liquor microorganism and their ability to break down cell walls. The average values of OMD determined by both in vitro methods of group of feeds showed that grain samples had higher OMD than straws, respectively 86.4 and 82.3 vs. 44.4 and 27.0, determined by both TT and PC method.There was an excelent correlation (Pearson coificient 0.99 for OMD) determined by two in vitro methods. The linear regression of Tilley and Terry and Pepsin-Cellulase procedure, produce an adequate prediction equation for all feeds ($Y = 1.287x - 294.6$, $R^2 = 0.98$) for OMD. The values for organic matter digestibility obtained by TT method were regressed against the values obtained by PC method suggesting that enzymatic method may provide good estimations of feeds for OMD in the absence of cannulated animals and limited laboratorial conditions. Based on the approximate results with the rumen fluid technique the enzymatic method may be the most effective method in terms of time and practical application in the laboratory for rapid evaluation of feeds for less developed countries like Albania, that for lack of laboratory capacity and personnel doesn't have the ability to use the rumen fluid technique as a routine technique to assist the nutritional values of different feeds.

Table 1. Chemical content of feeds.

Feeds	No. of samples	OM	CP	CF	NDF	ADF	ADL	Hemicelulose	Celulose	NDIN	ADIN
Oat grain	2	96.4	10.1	15.6	35.3	17.6	3.70	17.7	14.0	1.72	0.62
Wheat grain	2	98.1	10.6	2.90	14.9	3.90	2.30	11.0	1.60	2.39	0.17
Barley grain	1	97.1	10.8	5.50	21.1	6.70	3.60	14.4	3.10	1.6	0.35
Yellow corn	1	98.5	10.4	3.40	12.8	3.90	0.80	8.9	3.10	1.48	0.16
Oat straw	1	92.1	2.70	46.5	78.9	53.8	8.70	25.1	45.1	1.25	1.38
Wheat straw	1	93.1	2.60	48.5	83.4	56.3	8.00	27.1	48.3	1.47	1.59
Ryegrass straw	1	92.2	3.60	48.3	78.0	54.3	7.70	23.7	46.6	2.01	1.83

Table 2.OMD (%DM) digestibility by Tilley and Terry and Pepsine-celulasemethod .

Feeds	No. of samples	TT	PC
Oat grain	2	69.7	65
Wheat grain	2	92.3	93.1
Barley grain	1	89.3	84.8
Yellow corn	1	94.1	86.3
Oat straw	1	46.0	29.9
Wheat straw	1	41.5	22.7
Ryegrass straw	1	45.8	28.3

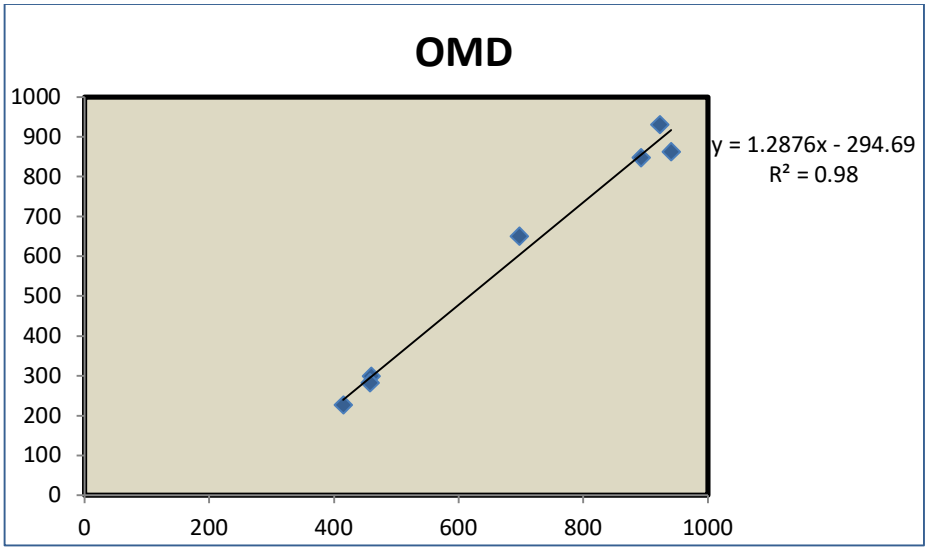

Figure 1.Linear regression between Pepsin-celulase and Tilley and Terry method.

4. Conclusions

Proximate analysis plays an important role in assessing the suitability of feedstuff for different ruminant's requirement. All these data indicate the quality of these feedstuffs and they give useful information on their feeding value. These data will be added to the existed information of Albanian feeds value and the most important will help the farmers to create effective feeding strategy's improving animals performance. The two in vitro methods showed high correlation and OMD results with pepsin cellulase could be used to predicted Tilley and Terry digestibility.Based on the approximate results with the rumen fluid technique the enzymatic method may be the most effective method in terms of time and practical application in the laboratory for rapid evaluation of feeds for less developed countries like Albania, that for lack of laboratory capacity and personnel doesn't have the ability to use the rumen fluid technique as a routine technique to assist the nutritional values of different feeds.

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