Scientific Papers. Series D. Animal Science. Vol. LX, 2017
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

DETERMINATION OF CHEMICAL CONTENT AND DRY MATTER DIGESTIBILITY OF SOME UNDER- UTILIZED FEEDS IN RUMINANTS FEEDING THROUGH TWO *IN VITRO* METHODS

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Abstract

Chemical analyses, enzymatic and Tilley and Terry digestibility were used to describe the feeding values of thirteen feed samples. Two samples were from common used feeds in ruminant animals feeding: alfalfa hay of two successive cuts and nine other feeds representing under-utilized ones in ruminant feeding like as cereal straw, faba bean straw and pea straw collected at the end of vegetation. All samples were analyzed for their chemical content of dry matter (DM), crude protein (CP), ash, crude fat, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) and enzymatic digestibility of dry matter. The under-utilized feed, pea straws and faba pods have similar chemical content for main Weende parameters. They have higher values for CP that cereal straws (31-34%) and lower value of CF (11.6%). The NDF and ADF content of pea straw and faba pod resulted lower that in cereal straw respectively 24% and 14%. The dry matter digestibility (DMD) determined with enzymatic and Tilley and Terry methods resulted to be higher in pea straw and faba pod in comparisons with that of cereal straw respectively 20% and 18%. The dry matter digestibility values determined with Tilley and Terry method for all feeds included in the study resulted higher than DMD determined with enzymatic method. The results of DMD determined by two "in vitro" methods were strongly correlated. According to R^2-value (0.99) the DMD determined by Tilley and Terry method could be predicted from enzymatic test as most convenient since it does not need animals.

Key words*: under-utilized feeds; leguminous straw; chemical content; in vitro digestibility; ruminant feeding*.

INTRODUCTION

Among all feed resources that are frequently used in ruminant nutrition some of them are under-utilized like as cereals straw and leguminous straw due to lack of information on their chemical composition and nutritive value. Cereals straws are partly used during the scarcity of feeds and most of their production is burned on fields causing environmental pollution. The leguminous straws are rarely used in ruminant feeding. Leguminous grains like as pea bean and faba bean, are well used in human diets and recently there is an emphasizing of their increasing in animal nutrition as a healthy vegetable protein resources. Under severe shortage of hay, straw can become valuable low-cost forage that can be used effectively, especially in extensive ruminant production systems based on low inputs (López et al., 2005). To provide balanced diets that include straw, it is important to know the nutritive value of this roughage and its variability, as straw sources vary in their nutrient content and digestibility. Cereal straws have been characterized for their chemical content and "*in vivo*" digestibility in other previous studies in Albania like as (Papa et al., 2000). There are data available for nutritive value of cereal straw and recommendations for their uses in ruminants feeding. There are not data about chemical characterization and digestibility of leguminous straw. Component chemical analyses can provide important biochemical information leading to a better understanding of the factors that may limit the animal performances. Chemical characterization methods cannot give a direct estimate of nutritive value, but rather relay on statistical association to measure digestibility and intake (Cherney, 2000). Different methods are used to describe the digestibility of forages and roughages. The attempts to develop enzymatic assays are due to the undesirability cost that has

keeping rumen fistulae animals for experimental intentions. Variations in the cellulase method have been studied, mainly focusing on enzyme concentration and incubation time (Jones and Hayward, 1975, Aufrere, 1982, Aufrère, Baumont et al., 2007, Nousiainen, Rinne et al., 2003). Although the predicted *in vivo* digestibility derived from this methods may be less accurate than that from the Tilley and Terry method (Tilley and Terry, 1963), they are simpler, less time consuming, more convenient and reproducible and don't require fistulated animals.

The aim of the study is to provide scientific information and characterization of locally available feed resources that are often under-utilized due to lack of information on their chemical composition and nutritive value. Providing knowledge on feed resources, including unconventional and lesser known ones, we hope to contribute the development and use of innovative and appropriate feeding options and strategies.

MATERIALS AND METHODS

Samples

Thirteen feed samples were used in this study. Two samples were from common used feeds in ruminant animals feeding: alfalfa hay of two successive cuts and nine other feeds representing under-utilized ones in ruminant feeding like as cereal straw, faba bean straw and pea straw collected at the end of vegetation. All these representative samples had an identification number and have been brought from Albania to Animal Science Laboratory of Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto, Portugal, in vacuum small bags, previously air-dried and ground in a sieve 4 mm. Thereafter, they have been milled again at ICBAS Institute in a sieve 1 mm as rapid as possible, to avoid their exposure to the atmosphere, set in small container labelled with an identification code and type of material and left in the room temperature for their evaluation according to the procedure for its analysis.

Chemical analysis

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), ether extract (EE). DM was determined after drying at 105°C, and ash after combustion at 550°C (Regulation No. 497/2004, 2004). Crude fat was extracted for 6 h with petroleum ether, whereas the Kjeldahl method was used to determine nitrogen (N) (AOAC, 1990). CP was calculated as N × 6.25. Van Soest detergent system (Van Soest and Robertson, 1985; AOAC, 2000) 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).

Digestibility triels

Two "*in vitro*" methods were used to determine the DMD of selected feeds: enzymatic (celulase and pepsin) and Tilley and Terry method.

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 digestibility of feeds. Briefly, rumen fluid obtained from two fistulated non-pregnant and non-lactating Holstein cows after a two weeks adaptation period to the diet with continuous access to fresh drinking water, was diluted anaerobically with four parts of (Marten and Barnes 1980) buffer solution, under O_2-free CO_2 and dispensed anaerobically into 50 mL conical centrifuge tubes (Corning Inc., New York, NY, USA) containing 250 mg DM of each sample, ground at 1 mm screen and 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 DM digestibility (DMD), were made based on the weight of the dry residue at 103ºC.

Pepsin-cellulase procedure

The feedstuffs were analyzed for DDM according to the pepsin-cellulase method (Aufrère, Baumont et al., 2007). The enzymatic digestion was carried out by adding fifty millilitres pre-heated solution of 2% pepsin-HCl solution (Pepsin 1:10.000, Biotechnology,

VWR AMRESCO, LLC, Fountain Parkway) in a 50 mL capped conical centrifuge tubes (Corning Inc., New York, NY, USA) with 300 mg DM of each sample (ground to pass a 1 mm screen and incubated in a water bath at 39ºC for 24 h, shaking some time during 24 h. After 24 h incubation, the tubes were transferred into a water bath at 80°C for an acid hydrolysis for 30 min. The residue was then washed with 300 mL hot distilled water at 40°C and vacuum filtered in a Dosi-Fiber equipment (JP Selecta S.A., Spain) to glass crucibles that were then incubated for another 24 h in a water bath at 39°C with 50 mL pre-heated cellulase-buffer from *Trichoderma viride* (Onozuka R-10, Yakult Pharmaceutical, Japan). At the end of second incubation the residue was washed again with 300 mL hot distilled water and vacuum filtrated in the Dosi-Fiber, dried for 48 h in an air-forced ventilated oven at 103°C, cooled to room temperature and weighed. Blanks and samples were incubated in triplicate. The DMD was estimated from the weight of the dry residue.

Statistical analysis

The data of dry matter digestibility measured through two in vitro methods and ADF and ADL content were elaborated according to linear regress analyses with least square method. MINITAB software was used to perform the t- test on differences between DMD-TT and DMD-PC and for assessing regression between TT and PC data to develop prediction equations for Tilley and Terry DMD.

RESULTS AND DISCUSSIONS

Chemical composition of the investigated feedstuffs for different group of feeds is presented in Table 1. The average values of chemical content of analyzed feeds of similar group are presented in the Figure 1. The aim of the study was to evaluate the feeding value of under-utilized feed like as legume straw and cereals straw. Alfalfa hay is included just to make comparisons with under-utilized and not well known feeds. The under-utilized feed, pea straw and faba pod have similar chemical content for main Weende parameters. They have higher values for CP that cereal straw (31-34%) and lower value of CF (11.6%). The NDF and ADF content of pea straw and faba pod resulted lower that in cereal straw respectively 24% and 14%. Makkar et al., 1996 found that chemical composition of chickpea straw differs from typical cereal straw in that it general contains more protein and metabolize energy concentrations and lower neutral detergent fiber (NDF) contents that cereal straw. Higher values of lignin content in both peas straw and faba pods and their differences with other feeds especially with alfalfa hay was expected based on their structure. According to Aufree et al. (1996) legumes are less rich in cell wall material, and composition and structure of their lignin are different and resulting in lower effect on cell wall digestibility. There are not significant differences in insoluble lignin between different varieties of pea straw and faba pod. The higher values were found in Belshi VF (Albania) faba pod with 11.6% followed by pea straw Belshi (Albania) with 10.7%. These values for lignin content are comparable with the one found in official feed tables of Feedipedia.

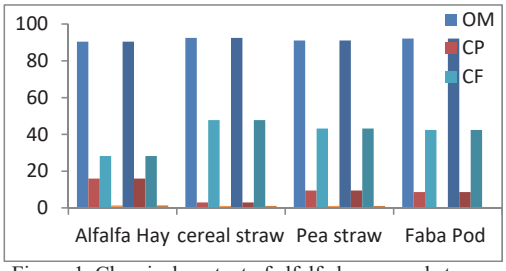

Figure 1. Chemical content of alfalfa hay, cereal straw, pea straw and faba pod (% of DM)

The dry mater digestibility data (DMD) with two "*in vitro*" methods are presented in Table 2. The average values of DMD determined by both in vitro methods of group of feeds showed that pea straw and faba pod had higher DMD than cereal straw, but lower than alfalfa hay. There were differences between DMD determined with two in vitro methods ($p \leq 0.05$). For pea straw and faba pod there were small differences in digestibility prediction by the two methods used, indicating good correlation between the linear function and the experimental values. The pepsin-cellulas method produced consistently lower DMD contents than Tilley and Terry method as more similar to in vivo digestibility. Similar results have been noticed by (Papa et al., 2011) where

pepsin-cellulas method produced lower values of DMD for straws than gas-test method. Within-species variability was noticed also in pea straws and faba pods. The pea straw of Belshi (Albania) varieties showed the highest digested DM than other varieties in both in vitro methods (50.8 and 65.65%). The faba straw of Aguadulce (Italy) varieties showed also the highest DMD (43.4 and 70.32%). The DMD of Aguadulce faba pod was close to DMD of alfalfa hay of second cut (70.46%). Similar results have been reported by (Haddad et al., 2001) where the nutritive value of lentil straw was close to nutritive value of alfalfa hay. The regress linear model used to describe the relations between two in vitro methods is shown in Figure 2.

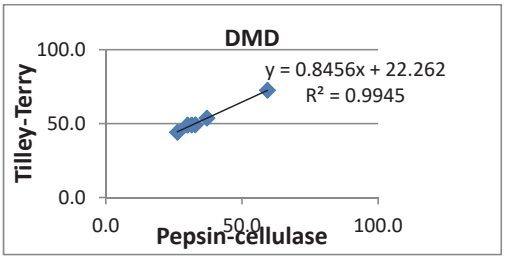

Figure 2. Linear regression between pepsin-celulas and Tilley and Terry method

According to R^2-value (0.99) for p≤0.001 the DMD determined by Tilley and Terry method could be predicted from enzymatic test as most convenient since it does not need animals. R^2=0.99 (p≤0.001).

Chemical components ADF and ADL are very good predictors of Tilley and Terry dry matter digestibility.

The multiply regression equation gives a reliable prediction judging from R^2-value = 0.97 and residual mean square error = 2.006.

Y=96.045-1.241ADF+2.395ADL

In accordance with the present study, Nousiainen et al., 2003; Huhtanen et al., 2006b, found ADL as the best single predictor of *in vivo* organic matter digestibility for grass silages (R^2 = 0.62) and for a set of forage samples (grasses, legumes and whole crops) (R^2 = 0.43), respectively.

Table 1. Chemical content of feeds

Feeds	Varieties	DM	Ash	OM	CP	CF	EE	NDF	ADF	ADL	NDIN	ADIN
Alfalfa Hay	Second cut	**91.8**	**10.2**	**89.8**	**12.2**	**29.6**	**1.2**	**47.5**	**33.4**	**6.4**	**0.58**	**0.21**
Alfalfa Hay	Third cut	**91.2**	**8.9**	**91.1**	**19.7**	**26.8**	**1.5**	**38**	**31.4**	**7.6**	**0.40**	**0.23**
Oat straw		**92.4**	**7.9**	**92.1**	**2.7**	**46.5**	**1.5**	**78.9**	**53.8**	**8.7**	**0.12**	**0.14**
Wheat straw		**92.6**	**6.9**	**93.1**	**2.6**	**48.5**	**0.8**	**83.4**	**56.3**	**8**	**0.15**	**0.16**
Ryegrass straw		**91.9**	**7.8**	**92.2**	**3.6**	**48.3**	**0.9**	**78**	**54.3**	**7.7**	**0.20**	**0.18**
Pea straw	Belshi (Albania)	**91.7**	**8.4**	**91.6**	**7.3**	**45.9**	**1.5**	**64.1**	**50.4**	**10.7**	**0.37**	0.26
Pea straw	Belshi (Albania)	**91.8**	**9.8**	**90.2**	**9.3**	**36.3**	**1.2**	**53.3**	**42.3**	**8.1**	**0.37**	0.19
Pea straw	Vitra* (Ltonia)	**91.8**	**9.8**	**90.2**	**11.1**	**44.1**	**1.9**	**61**	**48.5**	**9.9**	**0.36**	0.25
Pea straw	Alderman* (Italy)	**91.5**	**7.6**	**92.4**	**10.1**	**46.3**	**1**	**65.4**	**48.5**	**9.9**	**0.34**	0.24
Fava pod	Belshi VF (Albania)	**91.4**	**6.9**	**93.1**	**7.5**	**47.9**	**0.5**	**64.8**	**54.8**	**11.6**	**0.27**	0.25
Fava pod	Aguadulce* (Italy)	**90.9**	**8.5**	**91.5**	**9.5**	**37**	**0.5**	**55.8**	**41.6**	**7.6**	**0.36**	0.25
Fava pod	Jogeva* (Letonia)	**91.7**	**8.1**	**91.9**	**7.4**	**45.4**	**0.6**	**63.8**	**49.2**	**10.4**	**0.34**	0.23
Fava pod	Skrapari* (Albania)	**91.2**	**8**	**92**	**10.0**	**39.3**	**0.6**	**56**	**49.6**	**9.8**	**0.32**	0.28

Table 2. Dry matter digestibility with enzymatic and Tilley and Terry methods

	Enzymatic digestibility			Tilley and Terry		
Feeds	Mean ±SD	Minimum	Maximum	Mean ±SD	Minimum	Maximum
Alflalfa hay	59.3±6.1	55.0	63.7	72.48±2.9	70.46	74.50
Cereal straw	29.7±3.3	26.3	32.9	47.4±2.9	44.06	49.15
Pea straw	42.7±6.1	36.0	50.8	58.76±5.0	54.17	65.65
Faba pod	38.6±3.9	35.5	43.4	61.01±7.2	53.84	70.32

CONCLUSIONS

The data of the study confirm that the under-utilized feeds pea straw and faba pod have, have similar chemical content for main Weende parameters. They have higher values for CP that cereal straws, higher protein content and and lower value of CF. The NDF and ADF content of peas straw and faba pod resulted lower that in cereal straw. There are not significant differences in insoluble lignin between different varieties of pea straw and faba pod. The average values of DMD determined by both in vitro methods of group of feeds showed that pea straw and faba pod had higher DMD than cereal straw but lower than alfalfa hay. There were differences between DMD determined with two in vitro methods. The pepsin-cellulase method produced consistently lower DMD contents than Tilley and Terry method. Pea straw and faba pods could be good feed resources for ruminant animals and must not be under valuated.

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