A PRELIMINARY STUDY ON SOME LESS-USED FEEDS FOR PREDICTING THEIR RUMEN DEGRADABILITY BY A LABORATORY TECHNIQUE

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Abstract

A laboratory technique (in vitro), based on protease of Steptomyces griseus, was preliminary investigated to determine if it could accurately predict the rumen degradation of feed intake protein for some plants by-products. The evaluated feeds were some less-used ones but they can be used as sources for ruminants feeding: grape marc (7 types), camelina meal (3 types), pumpkin meal, poppy meal, wheat germs meal, linseed meal. Results from laboratory technique were correlated and linearly regressed with the in sacco nylon bag method results.

Key words: rumen degradability, protease *Streptomyces griseus*

INTRODUCTION

For high efficiency ruminants feeding it is important cu determine their protein requirements. Their particular digestion is based on rumen step which settle the rumen degradability, the microbial protein production, and the quantity of feed protein reaching the small intestine [1]. If the protein feed is rapidly degraded it is mainly converted to ammonia, which is then utilized with varying efficiencies by the rumen microbes. If the production of ammonia exceeds the microbes capacity, a large quantity of it is absorbed from the rumen, and in the end, the feed protein (or nitrogen) utilization will be reduced. Consequently, it is important to know the characteristics of protein degradation in the rumen, by a method which has the ability to predict it.

The rumen protein degradation is usually evaluated by *in sacco* method (also, called *in situ* nylon bag method) which is most closely related with the *in vivo* rumen environment and in the present is the basis for calculation of the digestible proteins in the intestine in the Romanian feeding system, similar to French system. This technique simulates the rumen environment and reflects the true degradation process. However, this approach requires the rumen cannulated animals, and is time-consuming. The need for rapid, and also trusted results, induced the further developing of cheaper, simpler rapid methods. One of these, widely applied and/or modified, was created by Aufrere (1989) [1], based on enzymatic *in vitro* activity on feed protein of protease from *Streptomyces griseus*. But the use of correction factors for each group of related feedstuffs makes the method less suitable for application on a large range of rations ingredients [4] and also, is little information about the prediction obtained by this method.

The purpose of this paper was to apply the enzyme Aufrere method for 14 less-used feeds that will be introduced in ruminant rations and to compare the calculated degradability measured by this method and by the *in sacco* method.

MATERIALS AND METHODS

In this study 14 less-used feeds but that could be sources for ruminants feeding were investigated: grape marc (7 types), camelina meal (3 types), pumpkin meal, poppy meal, wheat germs meal, linseed meal. The dried feedstuffs were grounded to pass a 1 mm screen before analysis. The dry matter (DM) content was determined after 4 h at 103°C (ISO 5984) and the crude protein (CP),

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respectively the nitrogen (N) content, was determined using the Kjeldahl method (ISO 5983). The methods were applied also, to the feeds respective residues left after *in sacco* and *in vitro* processes as described in the following sections.

In sacco determinations for degradability

This method for feed N degradation was determined according to [6] and [5]. Briefly, the N disappearance of the feed samples (3 g DM) was determined after the 0, 2, 4, 8, 16, 24 and 48 hours incubations of nylon bags (internal size 6 x 11 cm, closed with two stitches, nylon pore size 46µm) in the rumen of cannulated non-dairy cows fed with a diet containing 70% hay and 30% concentrate on a DM basis. The contents of incubated bags were pooled per incubation time and their dry matter and nitrogen values were determined. The results are from 3 repetitions.

The rumen theoretical degradability (D_T, % feed CP) was calculated by the Orskov and McDonald (1979) model [7] : $D_T = a + b * (1 - e^{-c*t})$, where t = incubation time (hours) and a, b, c are three regression coefficients.

Laboratory (in vitro) technique for degradability

The technique of Aufrere (1991) [2] was applied for measuring *in vitro* degradability by using the protease enzyme extracted from *Streptomyces griseus* (type XIV, Sigma P5147) and feeds hydrolysis for 1 h in a borate-phosphate enzyme buffer at pH 8 (20 mg enzyme/ liter).

The samples, each of 0.5 g in triplicate, were weighed in centrifuge tubes, followed by the addition of 50 ml of enzyme buffer. The tubes were stoppered and were placed in a water bath at 39°C for 1 h under agitation. After incubation, the tubes were placed in ice water for 10 min., then the residues were filtered, dried and transferred to Kjeldahl tubes for N analysis.

The feed enzyme degradability was calculated as: dE1= (Nt – Nr) *100/ Nt, where Nt=total N in original sample (g/kg DM); Nr=N in the sample residue after enzyme incubation (g/kg DM). The equations for calculation of degradability in vitro $D_{T(in\ vitro)}$ are from [1] and will be mentioned in the Results section.

RESULTS AND DISCUSSIONS

The N content of the feed samples is shown in Table 1. Also, in Table 1 are presented their rumen degradability results by *in sacco* method ("a" and "b" coefficients are expressed as percentage of original feed sample N (or CP), and "c", the degradation rate, in h^{-1}; the assumed rumen passage rate was 6% h^{-1}, for concentrates).

The results for in vitro degradability method were summarized in Table 2 including N content in the samples residues. For each feed the applied Aufrere's equation [1] for degradability calculation was $D_{T(in\ vitro)} = 1.48*dE1-0.0076*(dE1)^2+21.1$ because our feeds cannot be included in already settled Aufrere's categories and this equation was the only recommended [1].

In our testing procedures we included the soybean meal as a concentrate feed reference for the method. Only for soybean meal, the equation was $D_{T(in\ vitro)} = 0.36*\ dE1 + 47.9 + \Delta$, where $\Delta = +3.6$ (indicated for soybean cakes). As it can be seen in Table 2, dE1 is 26.29% and is in the same range with values of 24.30% from [3] and 36% from [2].

Relationships between in sacco and in vitro results for degradability

Comparing the D_T data between Table 1 and 2, we observed only 4 feeds that have relatively close degradability values: camelina meal 2 (80.94 vs. 77.73), camelina meal 3 (77.98 vs. 78.80), grape marc 5 (35.86 vs. 40.36) and grape marc 6 (31.51 vs. 35.65). But the correlation coefficient was $R^2 = 0.77$ for all 14 feeds D_T values from Table 1 and Table 2. This correlation can be improved if another enzyme incubation time is selected. We tested enzymatic degradation for 1 h as [2] recommended. But other authors found increased correlation for other incubation times: *e.g.* Cone (1996) [4] has $R^2 = 0.80$ for 24 h incubation. The explanation by [4] is that no enzyme preparation will mimic exactly the properties of rumen fluid; enzyme has a limited duration for high activity, which gradually decreases. But the microorganisms in the rumen fluid grow during incubation and stay active for the entire incubation.

In a ration not the percentage, but the amount of protein undegraded in rumen is important [4] because it will be available to the stomach and intestine. Consequently, we calculated the correlation between the disappeared N amount - g/100 g DM – for both methods, as presented in Table 3. The resulted value was an improved one, $R^2 = 0.87$.

At the end of our study we conclude that feeds should be tested *in vitro* for at least 2 more incubation times (*e.g.,* 8 h and 24 h) and search for the highest correlation between data of this method and *in sacco* method. Also, the correction equations/factors should be adapted for obtaining results much closer to the reference method - the *in sacco* method or *in vivo* feed testing, whenever it is possible. The degradability values obtained by applying the *in vitro* method could be useful for less common feeds which were not tested by reference methods.

Table 1 The feeds total nitrogen content Nt (g/ 100 g DM) and their rumen degradability by *in sacco* mehod (D_T)

feed	Nt (g N/ 100 g DM)	a (%)	b (%)	c (/h)	In sacco degradability D_T
Grape marc 1	1.8840	20.89	32.49	0.0439	34.62
Grape marc 2	1.5884	15.79	50.52	0.0646	41.98
Grape marc 3	1.8768	48.07	32.92	0.0890	67.73
Grape marc 4	1.7129	17.36	32.42	0.0489	31.92
Grape marc 5	1.6204	22.00	20.83	0.1193	35.86
Grape marc 6	1.7689	11.90	35.21	0.0754	31.51
Grape marc 7	1.9414	16.84	30.20	0.0184	23.93
Camelina meal 1	6.1147	43.67	60.49	0.0445	69.43
Camelina meal 2	4.8540	57.64	39.34	0.0872	80.94
Camelina meal 3	6.9573	37.05	59.41	0.1329	77.98
Pumpkin meal	6.2487	70.75	19.94	0.0596	80.69
Poppy meal	5.6320	77.31	13.43	0.1813	87.40
Wheat germs meal	5.1839	72.31	26.28	0.1465	90.95
Linseed meal	5.4463	37.45	31.11	0.0910	56.20
Soybean meal (as reference)	7.6430	15.7	88.80	0.068	62.95

Nt = nitrogen in original sample
a, b, c = regression coefficients for D_T calculation (see text)

Table 2 The feeds *in vitro* degradability ($D_{T(in\ vitro)}$) by enzymatic method

feed	Nr (g N/ 100 g residue DM)	dE1 (% Nt)	*in vitro* degradability $D_{T(in\ vitro)}$
Grape marc 1	0.333	15.53	42.25
Grape marc 2	0.468	25.89	54.32
Grape marc 3	0.494	23.71	51.26
Grape marc 4	0.325	16.68	43.67
Grape marc 5	0.259	14.03	40.37
Grape marc 6	0.209	10.39	35.65
Grape marc 7	0.246	11.15	36.66
Camelina meal 1	4.334	62.32	83.81
Camelina meal 2	2.889	52.33	77.73
Camelina meal 3	4.266	53.92	78.80
Pumpkin meal	2.073	29.17	57.80
Poppy meal	2.662	41.56	69.48
Wheat germs meal	2.243	38.04	66.40
Linseed meal	3.407	55.00	79.51
Soybean meal (as reference)	2.286	26.29	60.96

Nr = nitrogen in the sample residue after enzyme incubation
Nt = nitrogen in original sample
dE1= (Nt – Nr) *100/ Nt

Table 3 The disappeared N feeds amount - g/100 g DM – after using *in sacco* and *in vitro* methods for degradability

feed	N disappeared after *in sacco* method (g N/ 100 g)	N disappeared after *in vitro* method (g N/ 100 g)
Grape marc 1	0.6522	0.293
Grape marc 2	0.6668	0.411
Grape marc 3	1.2712	0.434
Grape marc 4	0.5468	0.286
Grape marc 5	0.5811	0.227
Grape marc 6	0.5574	0.184
Grape marc 7	0.4646	0.216
Camelina meal 1	4.2454	3.811
Camelina meal 2	3.9288	2.540
Camelina meal 3	5.4253	3.751
Pumpkin meal	5.0421	1.823
Poppy meal	4.9224	2.341
Wheat germs meal	4.7148	1.972
Linseed meal	3.0608	2.995
correlation	$R^2 = 0.87$	

CONCLUSIONS

The real validation of the results can be fulfilled if the *in vitro* and *in sacco* data are compared with *in vivo* data. However, *in vivo* data are difficult to obtain for most individual feed ingredients because only complete rations can be tested. It can be concluded that *in vitro* enzymatic method, using protease from *Streptomyces griseus*, offers a rapid estimation of rumen degradation, especially for concentrate feedstuffs, and can be preferred since it is easy to apply. For our studied feeds, the correlation coefficients of this method with *in sacco* method are 0.77 (results expressed as percentage for degraded feed N) or 0.87 (results expressed as amount - g/100 g DM - for degraded feed N).

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