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Original article

Rumen degradability of some feed legume seeds

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Abstract — The aim of this work was to determine the effective degradability (ED) of CP for different feed legume seeds and the possible relationship with their physical and chemical characteristics. The ED was measured using nylon bags and rumen outflow rate techniques on three rumen cannulated wethers fed at 40 g DM·kg$^{-0.75}$, with a 2:1 (on DM basis) hay to concentrate diet. Nine seed samples of the following legume species were tested: lupin (*Lupinus albus* L., cultivar multolupa), dwarf chickling (*Lathyrus cicera* L.), common chickling (*Lathyrus sativus* L.), common vetch (*Vicia sativa* L.) hairy vetch (*Vicia villosa* Roth.), bitter vetch (*Vicia ervilia* L.), monantha vetch (*Vicia monantha* Desf.) and field bean (*Vicia faba* L., variety minor Beck). Two different cultivars of this last species were studied: P69 (sample 1) and Jasper (sample 2). Estimates of ED of dry matter (DM) and crude protein (CP) were obtained for rumen outflow rates of 5.04 ± 0.31%·h^{-1}, determined for the diet concentrate. The variation of the ED of DM was moderate (from 58.8 to 69.2%) and correlated with the proportion of nitrogen linked to the acid detergent fibre (r = –0.847). Values of ED of CP of these seeds (in the above indicated order) were 79.4, 71.3, 80.7, 75.3, 75.4, 69.3, 76.6, 77.0, and 73.8%. Significant correlations between the ED of CP and the chemical composition or CP buffer solubility were not found.

legume seeds / rumen degradability / protein

Résumé — **Dégradabilité ruminale de certaines graines de légumineuses.** Ce travail avait pour but d'étudier la dégradabilité théorique (DT) dans le rumen des matières azotées totales (MAT) de différentes graines de légumineuses en essayant de les mettre en relation avec leurs caractéristiques chimiques ou physiques. La DT a été estimée à partir de la méthode des sachets de nylon couplée à la technique de passage des particules hors du rumen. Les mesures ont été effectuées sur trois béliers porteurs de canules du rumen, alimentés avec une ration de foin et de concentré (2:1, sur la base de la MS) à un niveau de 40 g MS·kg$^{-0,75}$. Neuf échantillons de graines de légumineuses ont été testés : lupin (*Lupinus albus* L., cultivar multolupa), gesse chiche (*Lathyrus cicera* L.), gesse commune (*Lathyrus sativus* L.), vesce commune (*Vicia sativa* L.), vesce velue (*Vicia villosa* Roth.), lentille bâtarde (*Vicia ervilia* L.), lentille d'Auvergne (*Vicia monantha* Desf.) et féverole (*Vicia faba* L., varieté minor

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Beck). Deux cultivars différents de cette variété ont été étudiés : P69 (échantillon 1) et Jasper (échantillon 2). Les estimations de DT ont été calculées avec des taux de sortie du rumen de 5,04 ± 0,31 %·h^{-1}, déterminés pour le concentré de la ration. Pour la MS, la variation de la DT a été modérée (de 58,8 à 69,2 %) et a été corrélée à la proportion d'azote lié à la lignocellulose (r = –0,847). Les valeurs de DT des MAT de ces graines (citées dans l'ordre précédent) ont été de 79,4, 71,3, 80,7, 75,3, 75,4, 69,3, 76,6, 77,0 et 73,8 %. Aucune corrélation n'a pu être observée entre la DT des MAT et la composition chimique ou la solubilité des MAT.

graines de légumineuses / dégradabilité ruminale / protéine

1. INTRODUCTION

The rotation of non-irrigated annual legumes for forage or grain production with cereals is a traditional and extended cropping practice in the Mediterranean and west Asian countries. The production of legume seeds represents in parts of these areas an important resource to meet the needs of energy and protein of ruminants. In addition, legume crops have positive ecological functions when rotated with cereal grains such as reducing soil nitrogen depletion and breaking the pest and disease cycles [4].

These legume seeds have a high protein content, but they may contain high levels of tannins or other antinutritive factors. On the other hand, studies on the nutritive value of most of the legume seeds are scarce. In particular, there is an important lack of information concerning their rumen protein degradability, which is a necessary parameter in order to establish the feed protein value. The objectives of this work were (i) to obtain information on the rumen degradability of crude protein (CP) of these legume seeds and (ii) to study the possible relationship between their physical and chemical characteristics and their rumen degradation.

2. MATERIALS AND METHODS

2.1. Tested samples

Nine seed samples of the following legume species were tested: lupin (*Lupinus albus* L., cultivar multolupa), dwarf chickling (*Lathyrus cicera* L.), common chickling (*Lathyrus sativus* L.), common vetch (*Vicia sativa* L.) hairy vetch (*Vicia villosa* Roth.), bitter vetch (*Vicia ervilia* L.), monantha vetch (*Vicia monantha* Desf.) and field bean (*Vicia faba* L., variety minor Beck). Two different cultivars of this last species were studied: P69 (sample 1) and Jasper (sample 2). All of the samples were ground with a blade mill through a 2 mm screen for nylon bag assays and through a 1 mm screen for the analysis of chemical composition and CP solubility. All these analyses were carried out in triplicate.

2.2. Experimental procedure

The rumen degradability of DM and CP of these samples was measured in three Manchega wethers fitted with rumen cannulas, fed with a diet of grass-legume hay and concentrate (0.515 barley grain, 0.16 corn grain, 0.14 wheat bran, 0.125 sunflower meal, 0.04 soybean meal, 0.02 minerals) in the ratio 2:1 (DM basis), at a DM intake level of 40 g·kg$^{-0.75}$, which represents 1.1 times the energy maintenance requirements. The diet was offered in two equal meals (at 8:00 and 16:00 h), starting 15 days before the experimental periods. Additional details of this diet have been previously published [11].

For each sample, two series of incubations were carried out over different days in order to have two bags per wether and incubation time. The bags (11 × 7 cm inner dimensions), with a pore size of 46 μm, were filled with approximately 3 g (air-dry basis) of sample and incubated in the rumen of each animal for 2, 4, 8, 16, 24 or 48 h. After collection from the rumen, the bags were rinsed under tap water and deep-frozen (–20 ºC). After thawing, the bags were machine-washed (3 times for 5 min), dried for 48 h at 80 ºC in a forced-air oven and analysed for DM and CP. The disappearance of material from the bags with incubation time was described for each animal using the model proposed by Ørskov and McDonald [23]. To obtain the effective degradability (ED) of DM and CP values, the rumen outflow rate (k_p) of the diet's concentrate labelled with ytterbium was determined in this experiment. Previously, this feed was washed with a commercial detergent in an automatic washing machine to eliminate the soluble components. Labelling was carried out by immersion for 24 h using a dose of 20 mg $Yb \cdot g^{-1}$ of feed. Then, a pulse dose (50 g) was supplied in the stall of each animal immediately before the first morning meal and a total of 18 samples of faeces for each animal were taken from the rectum, the first before supplying the marker and the rest between 12 and 120 h afterwards. The concentration of ytterbium in the faeces was determined by atomic absorption spectrometry. Then, its evolution with time was fitted with the model proposed by Grovum and Williams [12] and the rate constants derived from the decreasing phase of concentrations were used as k_p values. Additional details on this method have been described by González et al. [11]. The ED of the tested samples was estimated for each animal using their values of k_p and rumen degradation parameters.

2.3. Analytical

The analyses of chemical composition and CP solubility in the McDougall buffer were performed as indicated by González et al. [11] and Alvir et al. [2], respectively. The content of the tannins was evaluated through a tannin index determined by spectrophotometry [9].

The degradation kinetics were fitted using a non-linear regression programme. The degradation parameters of the different legume species were analysed by a variance analysis with feeds and animals as factors in the model, and, when significant effects were detected, they were compared by an LSD (least significant difference) test. Correlation analyses of data were also used to establish the possible effects of physical and chemical characteristics of these seeds on their rumen degradation. All the statistical analyses were performed using the Statistical Analysis System for Windows software v 6.12 (SAS Institute Inc., Cary, NC, USA).

3. RESULTS

These seeds showed a high concentration of CP (Tab. I), which varied from 222 to 397 $g \cdot kg^{-1}$ DM (bitter vetch and lupin, respectively). Solubility of CP was also high. Excluding monantha vetch, the soluble CP represented more than a third of total CP and for field bean and lupin about two thirds. The proportion of NDIN was also variable, with high values for bitter vetch (11.5%) and the field bean 2 (19.1%). This last sample also provided the highest proportion of ADIN (7.62%). The values of NDIN and ADIN showed a close correlation ($r = 0.854$, $P < 0.01$; $r = 0.785$, $P < 0.05$, respectively) with the tannin index, which showed a high variability (from 0.01 to 0.62; Tab. I). Large differences for the values of NDF, ADF, CP, NDIN, ADIN and the tannin index were recorded between both cultivars of the field bean.

The k_p values determined for the concentrate of the diet were $5.04 \pm 0.31\% \cdot h^{-1}$ (mean ± standard error). The mean values of degradation kinetic parameters and ED of DM and CP are shown in Tables II

Table I. Chemical composition (g·kg^{-1} DM) of legume seeds.

Sample	OM	EE	CP	NDF	ADF	ADL	NDIN[1]	ADIN[1]	CPS[2]	TI[3]
Lupin	967	97.2	397	217	169	33.9	2.06	2.71	64.0	0.01
Dwarf chickling	972	11.9	308	297	118	33.5	5.37	4.61	40.9	0.29
Common chickling	956	15.1	290	277	93.3	17.6	4.62	1.56	49.5	0.16
Common vetch	967	12.6	258	261	124	39.8	6.31	2.36	40.9	0.24
Hairy vetch	963	14.5	304	343	184	49.9	9.25	4.82	33.6	0.18
Bitter vetch	970	14.1	222	308	95.4	33.7	11.5	3.97	38.9	0.32
Monantha vetch	963	13.4	258	360	90.1	17.6	7.80	2.62	23.7	-
Field bean 1	965	19.8	370	242	109	18.7	5.53	3.59	69.3	0.35
Field bean 2	968	24.6	326	340	137	18.5	19.1	7.62	67.4	0.62

OM: organic matter, EE: ether extract, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, NDIN: neutral detergent insoluble nitrogen; ADIN: acid detergent insoluble nitrogen, CPS: crude protein solubility, TI: tannin index.
[1] % on total N.
[2] % on total CP.
[3] Absorbance units.

Table II. Degradation kinetics and effective degradability (ED) of dry matter of legume seeds.

	a (%)	b (%)	r (%)	k_d (%·h^{-1})	ED (%)
Lupin	30.3	69.7	0.0	5.80	67.6
Dwarf chickling	25.6	70.0	4.4	5.98	63.3
Common chickling	32.1	67.5	0.4	6.03	69.2
Common vetch	30.3	65.0	4.7	7.30	68.5
Hairy vetch	23.7	63.6	12.7	8.22	63.2
Bitter vetch	29.2	59.7	11.1	6.43	62.7
Monantha vetch	26.3	70.9	2.8	7.69	69.1
Field bean 1	26.2	53.6	20.2	8.84	60.2
Field bean 2	24.6	52.9	22.5	9.46	58.8
L.S.D.	4.59	5.81	4.12	2.19	3.14

a, b, and r represent soluble, non-soluble degradable, and undegradable fractions, respectively; k_d: fractional degradation rate of fraction b; ED: effective degradability (calculated with individual rumen outflow rates (k_p) averaging 5.04%·h^{-1}); L.S.D.: least significant difference at $P < 0.05$.

and III, respectively, with an indication of the significance of the differences between the samples ($P < 0.05$). No animal effects were observed for the parameters of the degradation kinetics of DM or CP. In both cases, this effect was significant ($P < 0.05$) for the ED values, as a consequence of the above indicated variability of the k_p values.

For DM degradation (Tab. II), the undegradable fraction (r, calculated as

Table III. Degradation kinetics and effective degradability (ED) of crude protein of legume seeds.

	a (%)	*b* (%)	*r* (%)	k_d (%·h^{-1})	ED (%)
Lupin	45.9	54.1	0.0	8.22	79.4
Dwarf chickling	30.7	69.3	0.0	7.14	71.3
Common chickling	40.3	59.7	0.0	10.9	80.7
Common vetch	35.5	64.1	0.4	8.35	75.3
Hairy vetch	33.0	64.0	3.0	9.83	75.4
Bitter vetch	30.0	66.9	3.1	7.16	69.3
Monantha vetch	33.0	65.0	2.0	10.3	76.6
Field bean 1	38.0	59.5	2.5	9.65	77.0
Field bean 2	33.5	61.3	5.2	9.93	73.8
L.S.D.	7.60	7.58	1.85	3.26	3.12

a, *b*, and *r* represent soluble, non-soluble degradable, and undegradable fractions, respectively; k_d: fractional degradation rate of fraction *b*; ED: effective degradability (calculated with individual rumen transit rates (k_p) averaging 5.04%·h^{-1}); L.S.D.: least significant difference at $P < 0.05$.

100-*a*-*b*) was the parameter with the highest variation, from 0 (lupin) to 20.2 and 22.5% (samples of field bean 1 and 2, respectively). The variability of the potentially degradable fraction (*b*) was also high (from 52.9 to 70.9%), as for its rumen degradation rate (k_d) (from 5.80 to 9.46%·h^{-1}). The variation of the soluble fraction between samples was also high (from 23.7 to 32.1%). The results of ED of DM, which varied between 58.8 and 69.2%, were mainly determined by the importance of the *r* fraction (r = –0.914, $P < 0.001$). A close correlation (r = 0.815, $P < 0.05$) was observed between the tannin index and the *r* fraction, as well as with the ED of DM (r = –0.788, $P < 0.05$). The best correlation recorded for ED was, however, when it was regressed upon ADIN (r = –0.848, $P < 0.01$).

The rumen degradation of CP of these seeds was characterised by (i) high values of the soluble fraction (from 30.0 to 45.9%), (ii) medium values of the fractional degradation rate (from 7.14 to 10.9%·h^{-1}), and (iii) low contents of undegradable CP (from 0.0 to 5.2%). Therefore, the values of ED of CP were high and were related mainly with the CP soluble fraction (r = 0.846, $P < 0.01$) and with the fractional degradation rate (r = 0.676, $P < 0.05$). Most samples showed ED values higher than 75%, whereas some reductions were observed in sample 2 of the field bean, as a consequence of its higher *r* fraction, and in dwarf chickling and bitter vetch which combined the minimum values of soluble CP and degradation rate. There were no significant correlations between the ED of CP and physical or chemical parameters. Significant differences ($P < 0.05$) in CP degradation were observed between both cultivars of the field bean. So, sample 2 showed a higher undegradable fraction and a lower ED of CP than sample 1.

4. DISCUSSION

The chemical composition of these legume seeds was variable in accordance with the inclusion of the different species. The correlation observed between the tannin index and NDIN and ADIN proportions showed that fibre values may also be influenced by tannins, since these compounds and tannin-protein complexes may apparently increase the feed contents of neutral detergent fibre and acid detergent fibre [25]. This explanation justifies, in part, the difference observed for these parameters between both samples of the field bean as a consequence of the differences recorded for NDIN and ADIN.

The high values of ED of DM of these seeds are indicative of a high rumen microbial activity, which is, however, diminished as a consequence of the lower fermentation level by the tannin content and the proportion of ADIN in total N. The effects of tannins are associated with their ability to combine with dietary proteins, cell wall polymers such as cellulose, hemicellulose and pectin, and minerals thus retarding or preventing their microbial digestion [20]. Although tannins are generally regarded as inhibitory to the growth of micro-organisms, the review of McSweeney et al. [20] does not show significant effects of tannins on microbial synthesis. Since the ED of CP was high in general for these seeds, the microbial synthesis derived from its rumen fermentation represented the main contribution to its protein value. So, assuming that the degradability of OM should be similar to that of DM and the hypothesis used in the PDI INRA system [28], the relation between the estimations of synthesised microbial CP and those of the rumen undegraded CP (both per kg of feed DM) varied from 1 (samples of field bean and dwarf chickling) to 1.7 (common chickling).

High values of ED of CP are usual in raw seeds as tested in the present work. Previous studies on CP degradability of the tested raw seeds are scarce, except for lupin. The available data shows evidence of a great variability for all these seeds (Tab. IV), which is attributable in one way to methodological differences in these works (animals and feeding conditions, sample granulometry, bag pore size, value interpretation, rumen outflow rate used, ...) and, in other ways, to differences between the tested samples.

All the works on lupin seed from the literature showed a very low content of undegradable CP, but a great variability in the CP distribution between the soluble and the insoluble fractions has been reported (Tab. IV). However, some of this variation should be related to differences in the loss of fine particles through the bag pores rather than to differences in true solubility [22]. In addition to the bag pore size, the loss of fine particles is mainly related to sample preparation (e.g. mill type and screen size) [21]. On the other hand, Moss et al. [22] showed that the increase of the screen size used in the preparation of lupin seed leads to an important reduction of the water solubility of both DM and CP, also observing effects between samples and for the interaction screen size × sample. Also, Freer and Dove [10], using a sample of *Lupinus angustifolius* seed at three levels of fineness (coarse, medium and fine (obtained by grinding three times without a screen, or once through a 4 mm or a 0.8 mm screen, respectively)), have shown that the reduction of the feed particle size increased both the soluble CP fraction (0, 33, and 74%, respectively) and the rate of degradation of the degradable fraction (5.9, 7.1, and 34.3$\%\cdot h^{-1}$, respectively). With some exceptions [24], this was the behaviour observed in the data of the soluble CP fraction compiled in Table IV. Most values of the CP degradation rate of Table IV are moderate (between 5.3 and 13.1 $\%\cdot h^{-1}$), although there are values as high as 74.8$\%\cdot h^{-1}$ [3]. Protein from raw lupin is usually considered as having high degradability. However, the important disparity between the data of degradation kinetic parameters derived in a high variability for the proportion of by-pass protein (from 4.6 to 37.7%), which makes it difficult to establish the protein value for this seed. Our reported value of ED of CP for this seed was very close to the mean value from the literature.

The literature results for the *Lathyrus* genus are scarce [13, 16, 27]. The results of

Valdes et al. [27] (which were obtained using samples ground through a 1 mm screen and assuming a k_p value of 5%·h^{-1}) were not included in Table IV, since the CP disappearance values were fitted to a logistic model and, therefore, the k_d values are not comparable with the remaining data. The present results for the dwarf chickling were lower than those of Guedes et al. [13]. Our results for the common chickling were also lower than those of Infascelly et al. [16], but similar to the 81.8% value observed by Valdes et al. [27].

Table IV. Degradation parameters of crude protein in some legume species.

Species	Animal	Ground through[1]	n	*a* (%)	*b* (%)	k_d (%·h^{-1})	k_p (%·h^{-1})	ED (%)	Ref.
Lupinus albus	sheep	2	1	36.3	62.5	9.8	2.2	87.2	[1]
	sheep	NI	1	63.5	34.5	74.8	6.0[2]	95.4	[3]
	sheep	3	1[3]	34.0/40.9	57.8/66.0	7.1/9.6	4.9/7.7	70.6/75.3	[5]
	cattle	1	1	80.7	18.3	22.1	6.0[2]	95.1	[7]
	sheep	none	1	3	96	13.1	5.0[2]	72	[15]
	cattle	1	1	75.3	23.7	19.2	6.0[2]	93.4	[17]
	cattle	8	4	17.6/35.5	64.2/82.3	6.7/8.8	5.0[2]	64.7/76.4	[22]
	cattle	3	1	80.5	18.3	8.9	8.0[2]	92.8	[24]
	cattle	38	1	6.9	92.8	8.3	5.6[2]	62.3	[26]
	cattle	NI	1	46.7	52.1	5.3	6.0[2]	71.0	[30]
Lathyrus cicera	cattle	4	1	38.9	60.6	20	4.4	79.8	[13]
Lathyrus sativus	buffalo	1	1	51.8	47.8	16.6	5.02	88.5	[16]
	sheep	1	1	62.5	36.6	14.0	5.02	89.4	[16]
Vicia sativa	sheep	2	1	21.7	78.1	4.3	2.2	73.0	[1]
	cattle	4	1	32.9	66.2	17.3	4.4	80.8	[13]
Vicia ervilia	sheep	2	1	26.8	72.4	6.3	2.2	80.4	[1]
Vicia monantha	cattle	4	1	31.1	66.0	31.2	4.4	79.6	[13]
Vicia faba	sheep	2	1	33.1	66.2	8.6	2.2	85.7	[1]
	cattle	1	1	67.9	31.1	13.1	6.02	89.2	[6]
	sheep	1	1	76	22	12.4	5.02	91	[15]
	sheep	none	1	35	64	11.0	5.02	79	[15]
	buffalo	1	1	79.3	18.2	9.9	5.02	91.4	[16]
	sheep	1	1	70.2	27.8	10.6	5.02	89.0	[16]
	cattle	3	1	64.2	34.0	7.4	6.02	82.7	[28]

[1]Seeds ground through a hammer mill without a screen or using the screen size (mm) indicated.
[2]Assumed value.
[3]A same sample tested with two different rations.
n: number of samples, NI: not indicated; for other abbreviations see Table II.

With the exception of *Vicia faba*, the previous results for seeds of the *Vicia* genus are also very scarce [1, 13, 27]. Even for hairy vetch, no data was found. Our results for common vetch, bitter vetch, and monantha vetch do not disagree with those of these authors. The respective ED values obtained by Valdes et al [27] were 78.1, 73.1, and 71.3. On the other hand, the ED value observed for hairy vetch was similar to that expected from the evolution in this forage through the pod-filling of the ED of CP [14]. Most results of *Vicia faba* (Tab. IV) show a high soluble CP fraction and therefore a high CP degradability, these values being higher than those observed in the present work. Nevertheless, except in the work of Yu et al. [29], these samples were ground through a 1 mm screen, and Hosking [15] observed an important reduction of this fraction with the increase of particle size. This effect was also observed in the disappearance data of Faurie and Peyronnet [8]. On the other hand, most data reported in Table IV, except those of Aguilera et al. [1], do not refer to *V. faba* var. minor. Degradation kinetics parameters reported by these authors were similar to those observed in this work, however, the present ED values were lower, as a consequence of the higher k_p value recorded in our work (2.2 vs. $5.04\%\cdot h^{-1}$). The difference of the ED values for both cultivars agreed with the results of Makkar et al. [19], who observed a negative and close correlation between the tannin content and the CP degradability of faba beans measured in vitro.

The present results showed a high CP degradability for legume seeds, except for some samples with high tannin contents. Hydrolysable tannin-protein complexes can be dissociated by different ruminal bacteria which is not possible for proteins complexed with condensed tannins [20]. So, based on the generally low values observed for the CP undegradable fraction, the content of condensed tannins was low in these seeds with the possible exception of field bean 2. As indicated above, the ED of CP was mainly related to the size of the CP soluble fraction, but, conversely, no correlation was observed with the buffer CP solubility. Madsen and Hvelplund [18] observed a linear and quadratic response of ED of CP to the increase of buffer solubility of CP. So, these seeds, with high values of both CP degradability and buffer CP solubility, will be located in the asymptotic zone, which should explain the lack of a relationship observed between both parameters.

In conclusions, the ED of CP of these seeds is high and, consequently, their protein value is mainly related to the microbial protein synthesis promoted by their fermentation in the rumen, which is reduced with increasing contents of ADIN and tannins.

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