PROTEIN DEGRADABILITY OF PEA AND FABA BEAN IN THE RUMEN OF SHEEP

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ABSTRACT

Protein degradability of untreated and heat treated samples of pea, high tannine faba bean variety (HTFB) and low tannine faba bean variety (LTFB) were examined. Heat treatment was carried out for 40 min at 125 °C in a laboratory oven. Protein degradability was determined by means of nylon bag technique using two sheep. Samples were incubated in the rumen for 3, 6, 12, 18, 24 and 36 h. Effective protein degradabilities (EPD) in the rumen were calculated on the basis of degradation characteristics and theoretical outflow rate 0.05 h^{-1}. Concentrations of crude protein in pea, HTFB and LTFB were 218, 295 and 286 g kg^{-1} dry matter respectively. Pea sample was characterised by lower EPD (649 g kg^{-1}) than HTFB (700 g kg^{-1}) and LTFB (687 g kg^{-1}). EPD of heat treated pea (651 g kg^{-1}), HTFB (692 g kg^{-1}) and LTFB (700 g kg^{-1}) were not significantly different from untreated samples ($P>0.1$). The results indicate that neither mild heat treatment nor faba bean variety affect the protein degradability in the rumen.

INTRODUCTION

There is a large gap in the supply of protein to domestic animals in Europe. Only about 30% of protein materials used in EU animal feed sector is derived from EU sources of supply (Brookes, 2001). Among alternative sources of home-grown protein there are pulses. In 2003, 4.772.056 t of peas and 478.451 t of beans were produced in European countries (FAOSTAT data, 2004). Although pulses account for only about 9 % of total protein used in compound feeds (Brookes, 2001) their role in animal nutrition can not be ignored. The main factor which limits the use of peas and beans in ruminant diets is their high degradation in the rumen. Especially when used in the combination with grassland forages it can lead to inefficient nitrogen utilization with adverse economic and environmental effects.
Several studies indicate that ruminal protein degradability of protein rich materials can be reduced by heat treatment (Faldet, 1991; Aguilera et al., 1992; Chapoutot and Sauvant, 1997; Aufrère et al., 2001). Aufrère et al. (2001) observed that heat treatment effectively reduced the protein degradability of lupin while in pea it was even slightly increased. It indicates that the efficiency of heat treatment may be substrate dependent and that the results obtained by one legume species can not be simply generalized to other species. Protein degradability of pulses may also be reduced by exploring the genetic variability between species and between varieties within species. Makkar et al. (1997) observed lower *in vitro* protein degradability in colour-flowering high tannine varieties than in white-flowering low tannine faba bean varieties.

The aim of the present work has been to investigate the possible differences in protein degradability of low and high tannine faba bean variety and to examine if protein degradability of pea and faba bean may be altered by heat treatment.

MATERIAL AND METHODS

Samples of pea variety Sponzor, high tannin faba bean variety (Kornberški, HTFB) and low tannin faba bean variety (Gloria, LTFB) were examined. Legume grains were harvested manually and the samples were dried at 50°C. Dried samples were ground through a 5 mm screen using laboratory hammer mill and divided into two equal parts. One part of each sample was then heat treated. Heat treatment was carried out for 40 min at 125°C in a laboratory oven. Samples intended for chemical analyses and

enzymatic digestibility were ground through a 1 mm screen. The particle size distribution was measured by a sieving test of dry samples according to ISO (1988). Six sieves with apertures 8, 5, 4, 3, 2 and 1 mm were used. The particle size was reported in terms of geometric mean diameter $d_{gw} = \log^{-1}((\sum(W_i \log đ_i))/ \sum W)$, where $đ_i$ = geometric diameter of particles on i^{th} sieve = $(d_i \times d_{i+1})^{1/2}$, d_i is diameter of screen openings of the i^{th} sieve, d_{i+1} = diameter of screen next larger then i^{th} screen and W_i = weight fraction on i^{th} sieve (ADAS, 1970). Crude protein (CP) was determined by the method of Kjeldahl. Enzymatic *in vitro* protein digestibility (HCl-pepsin) was assessed as described by Naumann et al. (1976).

Protein degradabilities were determined using the nylon bag technique as described by Ørskov *et al*. (1980). Two adult sheep fitted with rumen canulae (40 mm diameter) were used. The sheep were given 220 g of concentrate per day and meadow hay *ad libitum*. The concentrate contained 68 % of ground maize, 29 % of soybean meal and 3 % of vitamin-mineral mix. Samples, equivalent to about 4.5 g dry matter, were weighed into nylon bags (pore size: 45–55 µm, Locker Wire Weavers, Warrington, UK) of the internal size 100 mm × 75 mm and incubated in the rumen of sheep for 3, 6, 12, 18, 24 and 36 h. All determinations were done in duplicates. After incubation the bags were rinsed under running tap water and washed in a domestic washing machine for 30 min. In order to remove microbial matter from undigested material a modified frozen–rethawing technique according to Kamel *et al*. (1995) was used. After the first washing run bags were frozen at –20 ºC and then thawed and washed again using the same procedure as described above. After the second washing cycle, bags were dried at 60 ºC. Washing loss (A) was determined by soaking bags with samples for 1 h in hot water (39 ºC) and then washing and drying them using the same procedure as for samples incubated in the rumen.

Protein degradabilities at different incubation times (PD) were expressed as a proportion of protein which disappeared from the bags during the incubation in the rumen. In the samples from the present experiment protein did not disappear from bags at an exponential rate as usual (Ørskov and McDonald, 1979). Therefore, data of protein degradabilities (PD) were fitted to the linear equation $PD = a + b \times t$, where t represented the incubation time and a and b were parameters of equation. Washing loss (A) was included into line fitting as a zero time incubation. From the course of protein degradation it was evident that the final degradation was achieved at incubation times well below 36 h. Therefore data on 36 h incubation were omitted from line fitting.

Equation for the calculation of effective protein degradability was derived in a similar way as described by (Ørskov and McDonald, 1979) for an exponential equation. The first derivative of linear equation was corrected by the factor f ($f = e^{-kt}$), which represents the fraction of protein which remains in the rumen at t hours after feeding and integrated on a section between t = 0 and t_{1000}. Theoretical time at which a complete protein degradation can be expected (t_{1000}) was calculated as $t_{1000} = (1000 \text{ g kg}^{-1} - a)/b$. Effective protein degradability (EPD) which was in the present experiment supposed to be equal to cumulative protein degradability up to time t_{1000} was calculated as $EPD = a + b/k(1-e^{-kt1000})$. The theoretical particle outflow rate ($k = 0.05\ h^{-1}$) was used in the calculations.

RESULTS AND DISCUSSION

Chemical composition

Chemical composition of the feeds is presented in Table 1. In agreement with data from various feed tables (INRA, 1989; Forschunganstalt für viehwirtschaftliche Produktion, 1994; CVB, 1996; DLG, 1997) the concentration of protein was higher in faba bean than in pea. The difference between the two faba bean varieties was small. In vitro protein digestibilities were similar in all feeds and were not affected by the heat treatment (Table 1). Similar data for in vitro digestibility of pea protein (0.958) was reported by Walhain *et al.* (1992).

Table 1. Chemical composition, particle size and *in vitro* protein digestibility of pea and faba bean samples

	Pea	Faba bean	
		High tannine variety (HTFB)	Low tannine variety (LTFB)
Crude protein	218	295	286
In vitro protein digestibility			
- untreated sample	0.965	0.950	0.968
- heat treated sample	0.966	0.947	0.968
Particle size (d_{gw}, mm)	2.16	2.12	2.06

d_{gw} = particle size expressed as a geometric mean diameter

Protein degradation in the rumen

Degradation characteristics of pea and faba bean protein in the rumen are presented in Tables 2 and 3. Protein disappeared from the bags linearly (Graph 1). The linear disappearance of protein in the present study is not consistent with the findings in previous studies (Michalet-Doreau and Cerneau, 1991; Babnik et al., 1992; Poncet and Remond, 2002) in which the protein of pea and faba bean disappeared from the bags at an exponential rate. Linear degradation curves of protein from oil seed meals or cakes can be initiated by treatment with formaldehyde (Freer and Dowe, 1984), heat (Babnik and Verbič, 2002) or combination of heat, blood and xylose (Mosimanyana and Mowat, 1992). Neither of such treatments was used to alter degradation characteristics of control samples in the present experiment. Effective protein degradabilities of untreated pea, HTFB and LTFB were 649, 696 and 694 g kg^{-1}. In literature, data on protein degradability of pea and faba bean are highly variable. Relative to data from tables, protein degradabilities in the present study were low. Various feed tables (INRA, 1989; Forschunganstalt für viehwirtschaftliche Produktion, 1994; CVB, 1996; DLG, 1997) suggest values from 760 to 900 g kg^{-1} for pea and from 800 to 860 g kg^{-1} for faba bean. One of the reasons for relatively low protein degradabilities in the present study may lie in sample preparation. Relatively large screen aperture (5 mm) was used for grinding of samples which were incubated in the rumen. An increase in grinding fineness causes an increase in *in sacco* protein degradability and legume seeds seem to be particulary sensitive to grinding. Michalet-Doreau and Cerneau (1991) reported that along with a decrease of grinding screen from 6 to 0.8 mm the protein degradability of pea increased by 12.3%. It was found that in concentrates mastication has no effect on the particle size distribution (Michalet-Doreau et al., 1992) and from this point of view the particle size of concentrate feeds incubated in the nylon bags should be similar to the particle size of feeds in the diet. Due to the considerable effect of particle size on protein degradability, data from the present experiment are relevant only for pea and faba bean of similar particle size, i.e. about 2.1 mm geometric mean diameter (Table 1).

Protein degradability of faba bean has not been affected by the variety (Table 3). Condensed tannins are expected to complex protein and protect it from degradation in the rumen. Therefore, relative to low tannine variety, a lower protein degradability was expected in high tannine variety. The results of the current study are not consistent with the results of Makkar et al. (1997) who reported considerably lower protein degradability for tannine containing varieties compared to tannine free varieties. Disagreements might have been due to different methods used to assess protein degradability or to lower variability in tannine content, which was not assessed in the present experiment. Makkar et al. (1997) used an *in vitro* method in which samples were incubated in small amount of buffered rumen fluid. In the present study, small samples were weighed in nylon bags and incubated directly in the rumen of sheep. Due to relatively small samples which were inserted in the rumen, no indirect effect of tannins on rumen microflora can be expected.

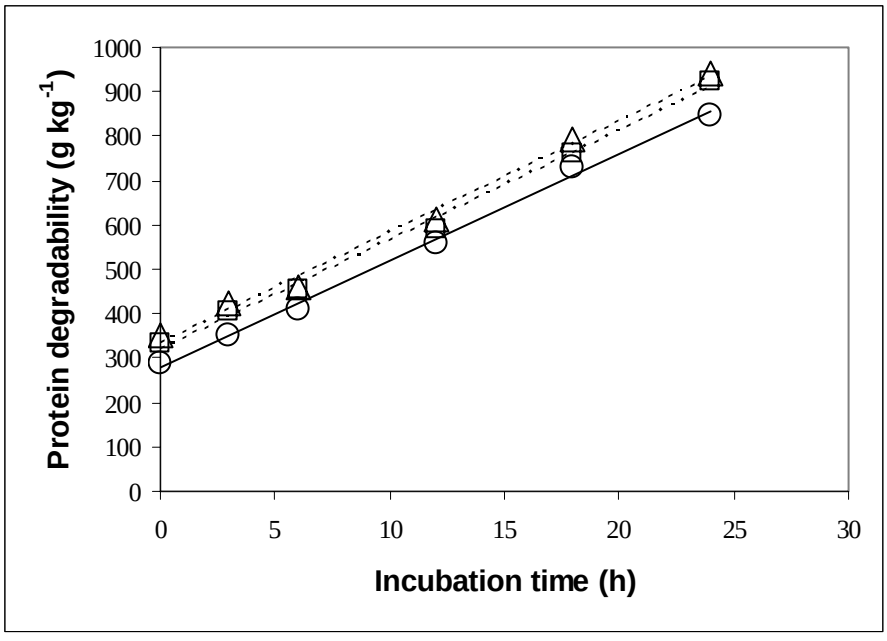
Heat treatment did not affect the protein degradation of pea and faba bean in the rumen (Tables 2 and 3). The results are in disagreement with the results of authors who found that heat treatment effectively reduced protein degradation in pea (Aguilera et al., 1992, Walhain et al, 1992) or faba bean (Cros et al., 1991, Aguilera et al., 1992; Yu et al., 2001). In accordance to the results from the present experiment no

effect of heat treatment on protein degradability of pea was observed also by Aufrère et al. (2001). Disagreements may be due to variation in treatment conditions as well as to properties of treated material. It demonstrates that exact conditions of heat treatment should be applied for an efficient reduction of protein degradation in the rumen.

Table 2. The effect of heat treatment on degradation characteristics of pea protein in the rumen

	Untreated	Heat treated	SEM	Significance
Protein degradability in the rumen (PD, g kg^{-1})				
3 h	351	349	4	NS
6 h	408	421	14	NS
12 h	560	551	23	NS
18 h	731	696	26	NS
24 h	847	861	40	NS
36 h	990	991	2	NS
Coefficients of degradation lines and effective protein degradabilities (EPD) in the rumen				
A (g kg^{-1})	289	306	/	/
a (g kg^{-1})	279	286	15	NS
b (h^{-1})	24.0	23.3	1.8	NS
t_{1000} (h)	30.8	30.8	1.7	NS
EPD (g kg^{-1})	649	651	5	NS

LEGEND: A - protein fraction which was washed out from the nylon bags without incubating them in the rumen; a, b - coefficients from the equation PD = a + b×t, t representing incubation time in the rumen and coefficient b degradation rate of protein; t_{1000} - time at which estimated PD = 1000 g kg^{-1}; EPD - effective protein degradability



Graph 1. Degradation of pea (O), high tannine faba bean (Δ) and low tannine faba bean (□) protein in the rumen of sheep.

Table 3. The effect of variety and heat treatment on degradation characteristics of faba bean protein in the rumen

	Variety*		Heat treatment			Significance	
	High tannine variety	Low tannine variety	Untreated	Heat treated	SEM	Variety	Treatment
Protein degradability in the rumen (PD, g kg^{-1})							
3 h	409	403	413	399	6	NS	NS
6 h	433	469	459	443	19	NS	NS
12 h	622	598	560	621	21	NS	NS
18 h	783	783	777	789	17	NS	NS
24 h	941	944	932	953	12	NS	NS
36 h	984	984	983	985	4	NS	NS
Coefficients of degradation lines and effective protein degradabilities (EPD) in the rumen							
A (g kg^{-1})	351	325	342	335	/	/	/
a (g kg^{-1})	325	318	328	314	6	NS	NS
b (h^{-1})	25.3	25.7	24.7	26.2	0.3	NS	< 0.05
t_{1000} (h)	27.0	26.9	27.5	26.3	0.5	NS	NS
EPD (g kg^{-1})	696	694	694	696	5	NS	NS

* Values in this table are least square means calculated on the basis of untreated and heat treated samples and are therefore slightly different from values for untreated samples presented in the text

LEGEND: abbreviations are defined in Table 2

CONCLUSIONS

Faba bean is characterized by a higher *in sacco* ruminal protein degradability than pea. There were no differences in protein degradation between high and low tannine faba bean variety. Ruminal protein degradation of pea and faba bean was not affected by a mild heat treatment.

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