Predicting *In Sacco* Rumen Degradation Kinetics of Raw and Dry Roasted Faba Beans (*Vicia faba*) and Lupin Seeds (*Lupinus albus*) by Laboratory Techniques

P. Yu*, A. R. Egan and B. J. Leury
Department of Animal Production, Institute of Land and Food Resources University of Melbourne
Parkville, Victoria 3052, Australia

ABSTRACT : Two laboratory techniques: (1) an *in vitro* method with two procedures for measuring protein degradabilities and (2) an *in vitro* method with three procedures for measuring protein solubility, were investigated to determine which laboratory techniques could most accurately predict the quantity of rumen protein degradation kinetics of legume seeds after dry roasting under various conditions, in terms of (1) rumen protein disappearance (D_j, where j=0, 2, 4, 8, 12, 24 and 48 h incubation), (2) rumen protein effective degradability (EDCP), (3) the parameters describing rumen degradation characteristics (the soluble fraction: S, the potentially degradable fraction: D, undegradable fraction: U, lag time: T0 and the degradation rate: Kd) and (4) rumen bypass protein (BCP), which were determined by the method accepted internationally at present, *in sacco* nylon bag technique using the standardized Dutch method. Feeds evaluated were the raw and dry roasted whole faba (*Vicia faba*) beans (WFB) and whole lupin (*Lupinus albus*) seeds (WLS), each was dry roasted under various conditions (at 110, 130 or 150℃ for 15, 30 or 45 min). *In vitro* protein degradability (D_1_Auf and D_{24}_Auf) were determined using the modified Aufrère method by enzymatic hydrolysis for 1 h and 24 h using a protease extracted from *Streptomyces griseus* in a borate-phosphate buffer. *In vitro* protein solubility (bf_1_S, bf_2_S, bf_3_S) was measured in a borate-phosphate buffer with three different procedures. Results from laboratory techniques (*in vitro*) were correlated and linearly regressed with *in sacco* results. Of the three procedures of *in vitro* protein solubility evaluated, none of them could predict *in sacco* results with good precision. The highest Pearson correlation coefficient (R^2) was less than 0.50. Of two procedures of *in vitro* protein degradability studied, the D_1_Auf values were closely correlated with *in sacco* parameters: Kd, EDCP and %BCP with high R^2 values: 0.82, 0.85 and 0.85, respectively, and closely correlated with *in sacco* D_j at 2, 4, 8 and 12 h rumen incubation with high R^2 values: 0.83, 0.91, 0.93 and 0.83, respectively. The D_{24}_Auf values could not predict *in sacco* results. The highest R^2 value was less then 0.40. These results indicated that *in vitro* protein solubility measured in borate-phosphate failed to identify differences in the rate and extent of protein degradation of legume seeds after dry roasting under various conditions and thus should not be used to predict rumen degradation, particularly for heat processed feedstuffs. But *in vitro* protein degradability using the modified Aufrère method by enzymatic hydrolysis for 1 h or possibly an intermediate time (>1 h and <24 h) is a promising laboratory procedure to detect effectiveness of dry roasting legume seeds on rumen protein degradation characteristics and could be used as a simple laboratory method to predict the rate and extent of protein degradation in the rumen *in sacco* with high accuracy. The equations to predict EDCP, Kd and BCP of dry roasted legume seeds (WLS and WFB) under various conditions are as follow: For both: EDCP (%)=-1.37+1.06*D_1_Auf (R^2=0.85, p<0.01). For both: Kd (%/h)=-21.81+0.49*D_1_Auf (R^2=0.82, p<0.01). For both: %BCP=103.37-1.07*D_1_Auf (R^2=0.85, p<0.01). (***Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 10 : 1377-1387***)

Key Words : *In Sacco* Rumen Degradation, *In Vitro* Degradability, *In Vitro* Protein Solubility, Dry Roasting, Legume Seeds

INTRODUCTION

For high producing dairy cows, much emphasis has been placed on the need to determine exact protein requirements. Several new protein evaluation systems, such as NKJ-NJF (1985), NRC (1988), UK metabolizable protein (AFRC, 1992), DVE/OEB (Tamminga et al., 1994), have been proposed to predict these requirements by relating them to microbial protein production and to feed protein that escapes rumen degradation (BCP). This follows the principle that the rate and extent of rumen degradation of protein are critically important determinants of the composition of the nutrients (protein:energy balance) absorbed by dairy cows.

When feed protein is degraded rapidly in the rumen, it is usually converted in the main to NH_3. This NH_3 is then utilized with varying efficiency by ruminal microbes. A certain concentration of NH_3 (4.5-5.0 mg NH_3-N/100 ml of rumen fluid; Satter and Roffler, 1975; Slyter et al., 1979) is needed to obtain maximum DM and fiber digestion in the rumen. If NH_3 is in excess of microbial needs, NH_3 is absorbed from the rumen in large quantities and N utilization will be reduced (Chalupa, 1974).

It is essential to know the characteristics of protein degradation in the rumen, as both the degraded protein (contributing to microbial protein synthesis) and the undegraded protein (contributing dietary protein

* Corresponding Author: P. Yu. Department of Animal and Poultry Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada S7N 5A8. Tel: +306-966-4132, Fax: +306-966-4151, E-mail: yupe@sask.usask.ca.
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available to varying degree for digestion in the intestine) are utilized to meet the animals protein requirements. The relative efficiency of protein use and the economic benefit thus depend on these processes and our ability to predict it.

The quantity of BCP depends on not only the total protein content of the feed but also its effective degradability in the rumen (EDCP). Techniques for measurement of the actual degradation of feed proteins by rumen microbes are quite complex (Mahadevan et al., 1979). *In vivo* measurements using duodenal cannulation are difficult and are subject to the problems in distinguishing the BCP from microbial protein and intestinal secretions. They are expensive and time consuming. *In sacco* nylon bag techniques were developed for measuring rumen protein degradation (Oskov and McDonald, 1979). This type of technique offers a better way to simulate the rumen environment, and results of *in sacco* degradation are probably more effective in reflecting true degradation than are other methods, provided the method is well standardized. However, again this approach also requires the use of surgically modified animals. This has led to development of laboratory techniques that may, with an acceptable level of accuracy, predict protein degradation in the rumen more simply and rapidly.

Protein solubility, which has been recognized for a long time as a factor in determining nutritional value of protein for ruminants, has been shown to be related to rumen NH_3 release and this offers one possible indication of ruminal degradation of dietary protein. However there are numerous methods to measure protein solubility. Different methods produce different results.

Krishnamoorthy et al. (1982) conducted a study to compare different methods for measuring protein solubility and drew a conclusion that using borate-phosphate buffer provided a measure of protein solubility that best simulated solubility in the rumen and provided a method readily and easily duplicated in any laboratory. However there are three variations of the procedures using borate-phosphate buffer to measure protein solubility; the experiment undertaken sought to identify the method most closely predicting parameters describing rumen protein degradation characteristics, particularly for heat treated feedstuffs.

The Aufrère method is another standardizable laboratory technique to determine protein degradability. This enzymatic method was used successfully to evaluate and predict the rumen degradability of various protein sources (Aufrère and Cartailler, 1988; Aufrère et al., 1991; Cone et al., 1996). In the French PDI system, the Aufrère method is used as a laboratory method for N evaluation (Aufrère et al., 1991). There are two variations of the procedure available for the Aufrère method; experiments reported in this study compared these two procedures.

Little information is available concerning the relationship of results obtained from these two laboratory techniques to that from *in sacco* nylon bag technique (CVB, 1996). Also the little information is available on which to judge the effectiveness with which these two laboratory techniques allow prediction of specific parameters of rumen degradation characteristics (Kd, S, D, T0) and EDCP or BCP, particularly for heat processed feedstuffs under various conditions.

The objectives of this study were:

(1) to examine statistical relationships between protein solubilities from three different procedures and *in vitro* protein degradabilities from modified Aufrère method from two different procedures with *in sacco* results, in terms of rumen protein disappearance (D_j), rumen protein degradation characteristics (S, D, Kd, T0, U) and EDCP or BCP;

(2) to evaluate which, if any, of the laboratory techniques could be used as a simple, routine, uniform and easily duplicated laboratory procedure to identify differences in protein degradation after dry roasting and to predict more accurately *in sacco* rumen degradation kinetics and fermentation characteristics of protein.

MATERIALS AND METHODS

Legume seeds

The WLS and WFB were obtained from a commercial feed company (Peter Gibbs Stock Feeds, Australia). Minor contamination in WLS and WFB were soybean and peas, in all cases contributing less than 0.2%. The chemical composition of WLS and WFB are presented in table 1.

Technological treatments

The methodology used to dry roasting WLS and WFB was as described in Yu et al. (1998, 1999).

Chemical analysis

The DM value was determined by drying at 105℃ to constant weight. The ash value was determined by ashing at 550℃ to constant weight according to AOAC (1984). The OM (DM basis) value was calculated as 1000-Ash (g/kg DM). Total N was assayed by Kjeldahl method (AOAC, 1990) and total CP content was obtained as N multiplied by 6.25. CFat was determined by ether extraction (AOAC, 1984).

The methods were applied also to the respective residues left after *in sacco* and *in vitro* processes as

Table 1. Effect of dry roasting on chemical composition of whole lupin seeds and whole faba beans

Temp. (℃)	Raw	110			130			150			
Time (min)	Raw	15	30	45	15	30	45	15	30	45	SEM
WLS											
DM (g/kg)	921.3^{b}	920.9^{b}	930.4^{ab}	933.2^{ab}	928.8^{ab}	927.8^{ab}	931.9^{ab}	932.9^{ab}	935.1^{a}	933.7^{a}	2.2
Ash (g/kg DM)	27.2	28.3	26.2	27.5	29.4	26.8	26.9	28.3	26.1	25.8	1.0
OM (g/kg DM)	972.8	971.7	973.8	972.5	970.6	973.2	973.1	971.7	973.9	974.2	1.0
CP (g/kg DM)	386.5	387.9	380.7	377.3	379.8	385.0	380.1	392.4	385.0	386.0	6.5
CFat (g/kg DM)	53.9^{a}	53.2^{ab}	53.1^{ab}	50.7^{ab}	49.7^{bc}	48.4^{cd}	45.3^{de}	44.1^{e}	43.9^{e}	41.7^{e}	0.7
WFB											
DM (g/kg)	885.9^{h}	895.6^{g}	900.7^{f}	910.2^{e}	919.0^{d}	920.6^{cd}	923.4^{cd}	924.1^{c}	935.3^{b}	941.0^{a}	0.8
Ash (g/kg DM)	34.7	34.2	33.8	33.4	33.7	34.2	34.4	35.2	34.0	34.2	0.5
OM (g/kg DM)	965.3	965.8	966.2	966.6	966.3	965.8	965.6	964.8	966.0	965.8	0.4
CP (g/kg DM)	317.3	317.5	319.9	318.8	323.1	324.2	318.2	322.0	320.4	310.4	7.5
CFat (g/kg DM)	20.4^{a}	19.9^{ab}	18.7^{abc}	17.1^{bcd}	18.2^{abc}	16.3^{cde}	16.0^{cde}	16.2^{cde}	14.6^{de}	14.0^{e}	0.5

Notes: n=3; SEM: standard error of mean; ^{a,b,c,d} Means with different letters in the same row are significantly different ($p<0.05$) (Tukey' Studentized Range Test).

described in the following sections.

In sacco techniques (Dutch standard)

The description of fistulated dairy cows, animal diets and *in sacco* technique procedure were reported in Yu et al. (1998, 1999).

Modified Aufrère method

In vitro protease degradation of protein was measured using a modified Aufrère method: enzymatic hydrolysis for 1 h (D_1_Auf) and 24 h (D_{24}_Auf) using a protease extracted from *Streptomyces griseus* in a borate-phosphate buffer at pH 8.

Triplicate samples, each of approximately 750 mg was weighed into 80 ml centrifuge tubes, followed by the addition of 75 ml of protease from Streptomyces griseus (type XIV, Sigma P-5147, Activity: approximately 4 units per mg solid) containing borate-phosphate buffer solution (10 ml enzyme solution (2000 mg protease of Streptomyces griseus per liter (L) borate-phosphate buffer) +10 ml tetracycline (Sigma T-3258) solution +10 mg nystatine (Sigma N-3503) per L buffer) to each tube. The tubes were stoppered and were placed in a rack in a waterbath at a temperature of 38℃. After 1 or 24 h, the rack of samples was removed from the waterbath and placed in ice water for 10 min after which the residues were filtered and transferred to Kjeldahl tubes for N analysis.

In vitro protease degradability of protein by enzymatic hydrolysis for 1 h and 24 h were calculated as: D_1_Auf=(N-Nr)/N*100%; D_{24}_Auf=(N-Nr)/N*100%, where, N=total N in original sample (g/kg DM); Nr=residue N in original sample (g/kg DM).

Protein solubility

Raw and dry roasted WLS and WFB were analyzed for soluble protein by extraction in a borate-phosphate buffer solvent with three different procedures (Krishnamoorthy et al., 1982; Analytical Procedure of Protein Solubility, Animal Production, Melbourne University).

Protein solubility (bf_S) was calculated as: bf_S (%)=SCP*100%/TCP, where SCP=soluble N*6.25, in g/kg DM; TCP= total N*6.25, in g/kg DM.

Statistical analysis

Statistical analyses were carried out using the statistical package of SAS (1991). Data were subjected to analysis of variance using Proc GLM (SAS, 1991) to examine the main effects of temperature (Temp), time and interaction between temperature and time. The statistical model used was: Yijk=m+Seriesi+Tempj+Timek+Temp*Timejk+eijk, where, Y=dependent variables in terms of feed composition, protein solubility and *in vitro* degradability; i=1, 2; j=1, 2, 3, 4; k=1, 2, 3, 4.

Comparison of means of dry roasting effects on chemical composition, protein solubility and *in vitro* degradability were carried out using Tukey Studentized Range Test (SAS, 1991). The Proc Corr and Proc GLM (SAS, 1991) were used for linear correlation and regression analysis between *in vitro* and *in sacco* results. Pearson correlation coefficients (R^2) and regression equations are presented.

RESULTS

Chemical composition

Chemical composition of DM, ash, CP, CFat of the raw and dry roasted WLS and WFB are presented in table 1.

Dry roasting had significant effects on the DM and CFat contents ($p<0.01$) but no significant effects on

CP and OM ($p>0.05$). There were no interaction effects ($p>0.05$) between temperature and time for any of DM, ash, OM and CP.

In vitro protease degradation of protein (modified Aufrère method)

The effects of dry roasting on *in vitro* protease degradability of protein of WLS and WFB are presented in table 2.

Raw WFB had a higher protein degradability than raw WLS. The degradabilities of protein of raw WFB and raw WLS were 83.9 and 75.4% at 1 h incubation, 90.9 and 85.3% at 24 h incubation, respectively.

At 1 h of incubation, dry roasting did not significantly affect ($p>0.05$) protein degradability at 110℃, had little effect at 130℃ but exhibited a pronounced effect ($p<0.01$) at 150℃. Relative to raw WFB, the magnitude of these reductions averaged 3.6 and 27.7% at 130 and 150℃, respectively; the corresponding values for WLS were 12.1 and 26.0%, respectively.

After 24 h protease incubation, the difference in *in vitro* protein degradability among treatments was not apparent for either WFB or WLS. Furthermore there were no differences between the raw and the roasted treatments.

Protein solubility

The effects of dry roasting on protein solubility of WLS and WFB are presented in table 3. Although all treatments were similar in CP content, the proportion of CP soluble in borate-phosphate buffer solution sharply decreased ($p<0.01$) with increasing roasting temperature and time.

At 110, 130 and 150℃, the average protein solubility was reduced ($p<0.01$) from 72.4 (raw) to 63.8, 52.2, 20.3% in WLS; from 61.2 (raw) to 56.5, 41.2 and 18.7% in WFB, respectively.

In sacco rumen degradation of protein

In sacco rumen protein disappearances (D_j) (at 0, 2, 4, 8, 12, 24 and 48 h) and *in sacco* rumen degradation kinetics and fermentation characteristics (S, D, Kd, T0 etc) of WLS and WFB after dry roasting under various conditions have been reported by Yu et al. (1998, 1999)

Relationships between *in sacco* and *in vitro* results of the raw and roasted WFB

In sacco rumen disappearance and in vitro solubility and degradability of protein in WFB: A linear regression was used to compare *in vitro* protein degradability and protein solubility of each of the procedures with *in sacco* rumen disappearance (D_j) at each of the incubation times (0, 2, 4, 8, 12, 24 and 48 h). The correlation values and prediction equations obtained in these comparisons are presented in table 4. The major results presented in this table are as follow:

(1) Using this approach, all regression equations were highly significant ($p<0.01$) except those relating D_{24}_Auf with D_j at all incubation times which were not significant ($p>0.05$);

(2) The bf_1_S, bf_2_S and bf_3_S values were highly correlated (R^2=0.77-0.85; 0.86-0.94; 0.64-0.80, respectively) with D_j at shorter times of *in sacco* incubation (0, 2, 4 and 8 h), but after longer periods of incubation (12, 24 and 48 h) this correlation gradually became weaker (R^2=0.42-0.65; 0.56-0.76; 0.31-0.47, respectively);

(3) The highest overall correlation with *in sacco* D_j at all incubation times was obtained using procedure one of the Aufrère method (D_1_Auf) (R^2=0.71-0.98) followed by protein solubility (bf_2_S) with procedure two (R^2=0.56-0.94)

(4) The order from high to low correlation between D_1_Auf and *in sacco* D_j were at 8, 4, 2, 12 and 0 h incubation with R^2 values: 0.98, 0.97, 0.89, 0.88 and 0.86, respectively.

Table 2. Effect of dry roasting on *in vitro* protein degradability in whole lupin seeds and whole faba beans

Temp. (℃)	Raw	110			130			150			
Time (Min)	Raw	15	30	45	15	30	45	15	30	45	SEM
In vitro rumen protein degradability of WFB											
D_1_Auf (%)	83.91^{a}	84.24^{a}	85.03^{a}	86.44^{a}	83.85^{ab}	82.16^{ab}	76.77^{bc}	71.92^{c}	63.96^{d}	46.21^{e}	1.36
D_{24}_Auf (%)	90.90	91.22	88.63	92.31	93.18	89.45	94.53	91.96	89.86	91.82	1.27
In vitro rumen protein degradability of WLS											
D_1_Auf (%)	75.39^{a}	74.25^{a}	70.20^{ab}	69.19^{ab}	70.01^{ab}	65.74^{bc}	63.12^{bcd}	60.37^{cd}	56.66^{d}	44.23^{e}	1.48
D_{24}_Auf (%)	85.25	83.64	83.80	84.14	85.22	85.41	84.90	83.67	84.38	85.15	1.03

Notes: D_1_Auf and D_{24}_Auf: *in vitro* protein degradability by enzymatic hydrolysis for 1 h and 24 h using modified Aufrère method; SEM: standard error of mean; n=3; ^{a,b,c,d} Means with different letters in the same row are significantly different ($p<0.05$) (Tukeys' studentized Range (HSD) Test).

Table 3. Effect of dry roasting on buffer protein solubility (bf_S: % of DM basis) of whole lupin seeds and whole faba beans

Temp. (℃)	Raw	110			130			150			
Time (min)	Raw	15	30	45	15	30	45	15	30	45	SEM
Protein solubility of WLS											
bf_1-S %	73.42^{a}	67.03^{a}	65.76^{a}	64.47^{a}	62.88^{ab}	57.93^{ab}	47.14^{bc}	34.63^{c}	17.74^{d}	10.55^{d}	2.95
bf_2-S %	74.39^{a}	67.69^{ab}	66.16^{b}	63.57^{b}	62.23^{b}	54.39^{c}	47.23^{c}	32.06^{d}	17.10^{e}	7.89^{f}	1.35
bf_3-S %	69.30^{a}	63.24^{ab}	59.34^{b}	56.92^{bc}	57.06^{bc}	50.51^{c}	30.24^{d}	31.78^{d}	18.56^{e}	12.24^{e}	1.33
Mean bf-S %	72.34^{a}	65.98^{ab}	63.75^{b}	61.65^{bc}	60.73^{bc}	54.28^{c}	41.53^{d}	32.82^{e}	17.80^{f}	10.23^{f}	1.77
Protein solubility of WFB											
bf_1-S %	62.71^{a}	59.39^{a}	57.74^{a}	55.17^{ab}	54.29^{ab}	44.59^{bc}	34.43^{c}	34.72^{c}	15.60^{d}	10.37^{d}	2.35
bf_2-S %	62.45^{a}	59.73^{a}	58.05^{ab}	57.69^{ab}	49.73^{abc}	45.15^{bcd}	40.57^{cd}	33.18^{d}	19.70^{e}	3.51^{f}	2.40
bf_3-S %	58.51^{a}	55.85^{a}	54.68^{a}	50.44^{a}	41.98^{ab}	37.56^{ab}	22.17^{bc}	23.02^{bc}	18.19^{bc}	10.13^{c}	4.77
Mean bf-S %	61.22^{a}	58.34^{ab}	56.82^{ab}	54.29^{ab}	48.66^{bc}	42.43^{cd}	32.39^{de}	30.30^{e}	17.53^{f}	8.00^{f}	2.31

Notes: bf_1-S, bf_2-S, bf_3-S: buffer crude protein solubilities by method 1, 2 and 3; SEM: standard error of mean; n=3; ^{a,b,c,d,e,f} Means with different letters in the same row are significantly different ($p<0.05$) (Tukeys' studentized Range HSD Test).

Table 4. Regressions and correlations of *in vitro* protein degradability (D_1_Auf and D_{24}_Auf) and protein solubility (bf_1_S, bf_2_S, bf_3_S) with *in sacco* rumen protein disappearance (D_j, %) at 0, 2, 4, 8, 12, 24 and 48 h incubation and *in sacco* parameters of rumen protein degradation characteristics (S, D, U, Kd, T0, EDCP, %BCP, BCP) in the raw and roasted whole faba beans

In sacco	*In vitro* protein degradability						*In vitro* protein solubility								
	D_1_Auf			D_{24}_Auf			bf_1_S			bf_2_S			bf_3_S		
	a	b	R^2	a	b	R^2	a	b	R^2	a	b	R^2	a	b	R^2
Rumen disappearance of CP															
D0	-9.30	0.70	0.86**	124.03	-0.87	0.04	24.10	0.49	0.81**	24.11	0.49	0.87**	26.62	0.49	0.80**
D2	-3.39	0.68	0.89**	81.17	-0.35	0.01	29.82	0.45	0.77**	29.52	0.46	0.86**	32.98	0.44	0.69**
D4	-15.33	0.98	0.97**	97.61	-0.41	0.003	31.93	0.65	0.85**	31.67	0.66	0.94**	37.20	0.61	0.72**
D8	-19.37	1.26	0.98**	119.82	-0.46	0.003	42.75	0.82	0.81**	42.36	0.83	0.89**	50.28	0.74	0.64**
D12	11.15	1.02	0.88**	112.62	-0.25	0.001	63.18	0.62	0.65**	61.93	0.65	0.76**	69.70	0.54	0.47**
D24	53.41	0.54	0.76**	107.35	-0.13	0.001	81.99	0.31	0.49**	80.82	0.33	0.62**	85.35	0.26	0.34**
D48	88.16	0.13	0.71**	103.84	-0.06	0.004	95.29	0.07	0.42**	94.93	0.08	0.56**	96.05	0.06	0.31**
Rumen degradation characteristics of CP															
S	-9.30	0.70	0.86**	124.03	-0.87	0.04	24.10	0.49	0.81**	24.11	0.49	0.87**	26.62	0.49	0.80**
D	110.01	-0.72	0.85**	-26.64	0.89	0.04	76.05	-0.50	0.81**	76.01	-0.49	0.87**	73.49	-0.50	0.80**
U	-0.71	0.01	0.51**	2.61	-0.03	0.06	-0.14	0.01	0.54**	-0.12	0.52	0.52**	-0.11	0.01	0.57**
Kd	-18.08	0.47	0.96**	47.82	-0.323	0.01	4.06	0.32	0.91**	4.34	0.32	0.94**	6.65	0.30	0.77**
T0	2.75	-0.01	0.28**	6.53	-0.05	0.13	1.99	-0.01	0.09	2.06	-0.01	0.17	1.83	-0.00	0.01
EDCP	25.07	0.76	0.95**	118.87	-0.39	0.01	62.95	0.48	0.76**	62.38	0.49	0.86**	67.28	0.44	0.61**
%BCP	74.93	-0.76	0.95**	-18.87	0.39	0.01	37.05	-0.48	0.76**	37.62	-0.49	0.86**	32.72	-0.44	0.61**
BCP	259.04	-2.61	0.96**	-61.05	1.30	0.01	129.28	-1.67	0.77**	131.09	-1.70	0.87**	114.43	-1.52	0.62**

Notes: D_j= a+b*D_i_Auf (j = 0, 2, 4, 8, 12, 24, 48; i= 1, 24); D_j= a+b*bfi_S (j = 0, 2, 4, 8, 12, 24, 48; i= 1, 2, 3); S, D, U, Kd, T0, EDCP, %BCP, BCP=a+b*D_i_Auf (i= 1, 24); S, D, U, Kd, T0, EDCP, %BCP, BCP=a+b*bf_i_S (i= 1, 2, 3); **: regression equations significant at $p<0.01$ and *: regression equations significant at $p<0.05$.

***In sacco* rumen degradation characteristics and *in vitro* solubility and degradability of protein in WFB**

A linear regression and correlation were used to compare *in vitro* protein degradability and protein solubility with each parameter of rumen protein degradation characteristics (S, D, Kd, T0, U, EDCP and BCP). The correlation values and prediction equations obtained in these comparisons are presented in table 4. The major results to be noted in this table are as follow:

(1) Regression equations were significant at $p<0.01$ for all parameters of rumen degradation characteristics except those relating D_{24}_Auf with the various parameters of rumen protein degradation characteristics

which were not significant ($p>0.05$);

(2) In the relationship predicting S and D, bf_2_S was highly correlated (R^2=0.87, 0.87, respectively) as was D_1_Auf (R^2>0.85);

(3) In relationships predicting U and T0, none of *in vitro* results could predict *in sacco* results (R^2<0.60);

(4) Predicting Kd, D_1_Auf was highly correlated (R^2=0.96) as was bf_2_S (R^2=0.94);

(5) For EDCP, %BCP and BCP, D_1_Auf was strongly correlated (R^2=0.95, 0.95 and 0.96, respectively) as was bf_2_S (R^2=0.86, 0.86 and 0.87, respectively);

(6) Highest R^2 obtained for regression equations were those for relationships between D_1_Auf and Kd, EDCP and %BCP (R^2=0.96, 0.95, 0.95, 0.96, respectively).

Relationships between *in sacco* and *in vitro* results of the raw and roasted WLS

In sacco rumen disappearance and in vitro degradability and solubility of protein in WLS: A correlation and regression were used to compare *in vitro* protein degradability and protein solubility with *in sacco* D_j at each incubation time for WLS. The correlation values and prediction equations obtained in these comparisons are presented in table 5. The results obtained for WLS were quite different from those for WFB. The major results to be noted in this table are as follows:

(1) All regression equations were highly significant at $p<0.01$, except those relating D_{24}_Auf with D_j which were not significant ($p>0.05$);

(2) Results from procedure two of Aufrère method; D_{24}_Auf were poorly correlated with *in sacco* D_j (R^2<0.08). Results from procedure one of Aufrère method; D_1_Auf were correlated with *in sacco* D_j with R^2=0.33-0.84);

(3) The highest overall correlation between protein solubility and *in sacco* D_j at all incubation times was obtained by procedure two for protein solubility. The bf_2_S values were highly correlated with D_j at longer incubation times (8, 12, 24, 48 h with R^2 values 0.90, 0.81, 0.88 and 0.87, respectively). However at shorter incubation times (0, 2, 4 h), the correlation was weak (R^2=0.33, 0.53, 0.77, respectively).

***In sacco* rumen degradation characteristics and *in vitro* degradability and solubility of protein in WLS**

The correlation values and prediction equations obtained in those comparisons of *in vitro* protein degradability and protein solubility with *in sacco* parameters of ruminal protein degradation characteristics (S, D, Kd, T0, U, EDCP and BCP) in WLS are presented in table 5. Several major observations on the data in this table are as follows:

(1) Regression equations were significant at $p<0.01$ or $p<0.05$ for all parameters of rumen degradation characteristics except for the relationship between D_{24}_Auf and rumen protein degradation characteristics ($p>0.05$);

(2) Compared with all *in vitro* procedures, the bf_2_S values were the most highly correlated with characteristics of rumen protein degradation *in sacco*, as represented by U (R^2=0.89), EDCP (R^2=0.92) and %BCP (R^2=0.92).

Relationships between *in sacco* and *in vitro* results in WFB and WLS combined

After combining the data for the two feeds, WFB and WLS, combining for a single common linear regression analysis, the correlation between *in sacco* results and *in vitro* results were quite different from either WFB or WLS separately. The correlation and regression analysis results are presented in table 6.

It was found that:

(1) None of the *in vitro* protein solubilities from the three procedures were highly correlated with *in sacco* results, all R^2 values being very low (<0.50);

(2) *In vitro* protein degradability by procedure two (D_{24}_Auf) of the Aufrère method was poorly correlated with *in sacco* results (R^2<0.40);

(3) *In vitro* protein degradability by procedure one (D_1_Auf) of the Aufrère method was highly correlated with *in sacco* parameters of rumen degradation characteristics for WLS and WFB dry roasted at various conditions with the equations to predict EDCP, Kd and %BCP as follows:

For both: EDCP=-1.37+1.06*D_1_Auf
(R^2=0.85, $p<0.01$);
For both: Kd=-21.81+0.49*D_1_Auf
(R^2=0.82, $p<0.01$);
For both: %BCP=103.37-1.07*D_1_Auf
(R^2=0.85, $p<0.01$).

DISCUSSION

Effect of dry roasting on chemical composition

Dry roasting significantly increased the DM content and decreased CFat of WLS and WFB. This could be attributed principally to the water evaporating at the end of the roasting process. These results agreed with the results observed by others (Block et al., 1981; MacGuffey and Schingoethe, 1982; Van Dijk et al., 1983; Asp and Bjoerck, 1984; Cros et al., 1991; Kibelolaud et al., 1993) that heat treatment increased the DM content and decreased the CFat content. The decrease in CFat might be due to fat binding to other components rendering CFat lower in solubility in ether when dry roasted.

Table 5. Regression and correlation of *in vitro* protein degradability (D_1_Auf and D_{24}_Auf) and protein solubility (bf_1_S, bf_2_S, bf_3_S) with *in sacco* rumen protein disappearance (D_j, %) at 0, 2, 4, 8, 12, 24 and 48 h incubation and *in sacco* parameters of rumen protein degradation characteristics (S, D, U, Kd, T0, EDCP, %BCP, BCP) in the raw and roasted whole lupin seeds

In sacco	*In vitro* protein degradability						*In vitro* protein solubility								
	D_1_Auf			D_{24}_Auf			bf_1_S			bf_2_S			bf_3_S		
	a	b	R^2	a	b	R^2	a	b	R^2	a	b	R^2	a	b	R^2
Rumen disappearance of CP															
D_0	16.77	0.19	0.33**	85.49	-0.66	0.08	25.21	0.08	0.35**	25.38	0.08	0.35**	25.10	0.09	0.38**
D_2	7.77	0.47	0.53**	23.42	0.18	0.00	28.82	0.20	0.55**	29.07	0.19	0.58**	29.31	0.21	0.51**
D_4	-4.70	0.74	0.77**	49.58	-0.07	0.00	29.40	0.28	0.68**	29.58	0.28	0.73**	30.51	0.29	0.59**
D_8	-112.26	5.33	0.84**	65.59	-0.16	0.00	30.07	0.52	0.89**	31.21	0.50	0.90**	32.09	0.53	0.77**
D_{12}	-26.93	1.46	0.75**	285.54	-2.57	0.05	37.22	0.61	0.81**	38.60	0.60	0.81**	40.15	0.62	0.68**
D_{24}	-30.94	1.75	0.80**	117.27	-0.41	0.00	45.81	0.74	0.87**	47.12	0.72	0.88**	47.67	4.59	0.79**
D_{48}	-12.83	1.56	0.81**	187.00	-1.16	0.00	55.04	0.66	0.87**	57.29	0.64	0.87**	58.51	0.67	0.74**
Rumen degradation characteristics of CP															
S	16.77	0.19	0.33**	85.49	-0.66	0.08	25.21	0.08	0.35**	25.38	0.08	0.35**	25.11	0.09	0.38**
D	-15.19	1.18	0.72**	62.99	-0.01	0.00	36.72	0.50	0.78**	37.86	3.22	0.78**	38.79	0.51	0.67**
U	98.41	-1.37	0.82**	-48.49	0.68	0.00	38.07	-0.58	0.89**	36.76	-0.56	0.89**	36.10	-0.60	0.79**
Kd	-6.69	0.23	0.70*	31.03	-0.27	0.02	3.41	0.10	0.75**	3.61	0.09	0.75**	3.95	0.10	0.60**
T0	0.99	-0.01	0.01	13.28	-0.15	0.10	0.652	0.00	0.00	0.58	0.00	0.00	0.52	0.00	0.00
EDCP	-16.83	1.26	0.84**	163.45	-1.16	0.01	38.38	0.53	0.91**	39.56	0.52	0.92**	40.36	0.55	0.79**
%BCP	116.83	-1.26	0.84**	-63.45	1.16	0.01	61.62	-0.53	0.91**	60.44	-0.52	0.92**	59.64	-0.54	0.79**
BCP	500.36	-5.40	0.83**	-249.59	4.71	0.01	263.39	9.42	0.91**	258.62	-2.23	0.91**	254.86	-2.36	0.79**

Notes: Dj = a+b*Di_Auf (j = 0, 2, 4, 8, 12, 24, 48; i= 1, 24); Dj= a+b*bfi_S (j = 0, 2, 4, 8, 12, 24, 48; i= 1, 2, 3); S, D, U, Kd, T0, EDCP, %BCP, BCP=a+b* Di_Auf (i= 1, 24); S, D, U, Kd, T0, EDCP, %BCP, BCP=a+b*bfi_S (i= 1, 2, 3); **: regression equations significant at $p<0.01$ and *: regression equations significant at $p<0.05$.

Effect of dry roasting on protein solubility

Subjecting WLS and WFB to dry roasting had no significant effect on CP content, while it sharply reduced the CP solubility for all heat treatments. Soluble CP in WLS and WFB were high, similar to that reported previously (Benchaar et al., 1991; Kibelolaud et al., 1993). The major proportion of protein in WLS and WFB are globulins, followed by albumins and to a lesser extent by glutelins and prolamines (Varasundharosoth and Barnes, 1985). Albumins and globulins are sensitive to heat treatment, rendering them water insoluble (Van Soest, 1982). Early reports have shown that heat treatment, either dry or wet, substantially decreased the CP solubility (Leonard and Block, 1988; Arieli et al., 1989; Benchaar et al., 1991; Cros et al., 1991; Walhain et al., 1992; Yu, 1995).

The present study showed that using three different procedures to measure protein solubility in borate-phosphate buffer resulted in different results. These variations demonstrate the need for a repeatable and accurate procedure to determine protein solubility representative of protein degradation (rate and extent) in the rumen.

Effect of dry roasting on *in vitro* protease degradation of protein

Aufrère et al. (1991), Cros et al. (1991) and Yu et al. (1996) reported that degradability of CP of raw WFB was 82.7, 89.2 and 90.0%, respectively. In the present study, the result (83.9%) for protein degradability at 1 h incubation was similar to that of Aufrère et al. (1991) but lower than those reported by Cros et al. (1991) and Yu et al. (1996). In comparison, the result (90.9%) for protein degradability value at 24 h incubation of raw WFB was also quite close to that of Cros et al. (1991) and Yu et al. (1996). In the raw WLS, the protein degradabilities at 1 h (75.4%) and 24 h (85.3%) incubation were lower than the results of 94% and 93% observed by Aufrère et al. (1991) and Kibelolaud et al. (1993), respectively. These different results may be due to the different enzymes used, sample variety, sample preparation procedures, incubation time etc.

At 1 h incubation, dry roasting of WFB and WLS did not significantly affect protein degradability at 110℃ and had little effect at 130℃ but exhibited a very pronounced effect at 150℃. Compared with the raw, dry roasting at 150℃/45 min dramatically

Table 6. Regression and correlation of *in vitro* protein degradability (D_1_Auf and D_{24}_Auf) and protein solubility (bf_1_S, bf_2_S, bf_3_S) with *in sacco* rumen protein disappearance (D_j, %) at 0, 2, 4, 8, 12, 24 and 48 h incubation and *in sacco* parameters of rumen protein degradation characteristics (S, D, U, Kd, T0, EDCP, %BCP, BCP) in whole faba beans and whole lupin seeds together

In sacco	*In vitro* protein degradability						*In vitro* protein solubility								
	D_1_Auf			D_{24}_Auf			bf_1_S			bf_2_S			bf_3_S		
	a	b	R^2	a	b	R^2	a	b	R^2	a	b	R^2	a	b	R^2
Rumen disappearance of CP															
D_0	-14.38	0.72	0.71**	-109.55	1.67	0.36**	29.27	0.17	0.10*	28.62	0.18	0.13*	29.83	0.18	0.10*
D_2	-4.48	0.68	0.83**	-61.09	-1.19	0.24**	32.61	0.24	0.27**	32.04	0.26	0.32**	34.04	0.24	0.24**
D_4	-20.23	1.01	0.91**	-110.37	1.84	0.29**	35.64	0.35	0.27**	34.75	0.37	0.33**	38.26	0.33	0.22**
D_8	-31.76	1.39	0.93**	-150.22	2.47	0.28**	42.72	0.52	0.34**	41.97	0.54	0.39**	47.16	0.48	0.26**
D_{12}	-14.46	1.31	0.83**	-129.30	2.37	0.26**	55.83	0.50	0.31**	54.84	0.52	0.36**	60.86	0.44	0.21**
D_{24}	21.57	0.95	0.65**	-34.72	1.41	0.14*	66.52	0.48	0.44**	65.91	0.50	0.50**	70.33	0.45	0.34**
D_{48}	48.23	0.64	0.45**	-1.05	1.07	0.12*	76.97	0.36	0.37**	77.08	0.36	0.39**	80.26	0.32	0.27**
Rumen degradation characteristics of CP															
S	-14.38	0.72	0.71**	109.55	1.67	0.36**	29.27	0.17	0.10*	28.62	0.18	0.13*	29.82	0.18	0.10*
D	74.59	-0.23	0.06	118.16	-0.68	0.05	52.91	0.12	0.04	53.72	0.10	0.03	54.80	0.08	0.02
U	39.79	-0.49	0.36**	91.39	-0.99	0.14*	17.82	-0.28	0.31**	17.66	-0.28	0.32**	15.38	-0.26	0.23**
Kd	-21.81	0.49	0.82**	-80.79	1.07	0.38**	6.59	0.14	0.17**	6.34	0.15	0.20**	7.79	0.13	0.13*
T0	0.14	0.01	0.06	-9.37	0.12	0.38**	1.49	-0.01	0.03	1.51	-0.01	0.04	1.44	-0.01	0.02
EDCP	-1.37	1.06	0.85**	-105.39	2.04	0.30**	55.32	0.41	0.32**	54.70	0.43	0.37**	58.89	0.38	0.24**
%BCP	101.37	-1.07	0.85**	205.39	-2.04	0.30**	44.68	-0.41	0.32**	45.30	-0.43	0.37**	41.11	-0.38	0.24**
BCP	420.19	-4.45	0.77**	999.49	-10.19	0.39**	174.98	-1.54	0.24**	177.91	-1.62	0.28**	160.54	-1.39	0.17**

Notes: D_j=a+b*D_i_Auf (j=0, 2, 4, 8, 12, 24, 48; i=1, 24); D_j=a+b*bfi_S (j=0, 2, 4, 8, 12, 24, 48; i=1, 2, 3); S, D, U, Kd, T0, EDCP, %BCP, BCP=a+b *D_i_Auf (i=1, 24); S, D, U, Kd, T0, EDCP, %BCP, BCP=a+b*bfi_S (i= 1, 2, 3); **: regression equations significant at $p<0.01$ and *: regression equations significant at $p<0.05$.

decreased *in vitro* protein degradability from 83.9 to 46.2% (WFB) and 75.4 to 44.2% (WLS). Aufrère et al. (1991) observed a similar trend in that extrusion reduced *in vitro* protein degradability from 80 to 48% in WLS and 75 to 35% in WFB with increasing temperatures.

After incubation for 24 h, the proportions of protein degraded for both WLS and WFB were increased but no differences were found among dry roasting treatments, averaging 91.3% for WFB and 84.5% for WLS. These results were similar to those observed by Yu (1995) that no differences in protein disappearance at 24 h incubation were found among the treatments for pressure toasted whole horse bean cv. Alfred. Aufrère et al. (1991) reported that *in vitro* protein degradability of WLS at 24 h incubation was not significantly altered by extrusion: 88 (raw) vs. 89% (extruded), but *in vitro* protein degradability of WFB was significantly reduced by extrusion from 84 (raw) to 74% (extruded).

In general, *in vitro* protein degradability after 1 h incubation was decreased with increasing temperature and time; whereas with longer incubation, differences among the treatments of dry roasting were not apparent.

Relationship between *in sacco* results and protein solubility

Protein solubility in the rumen is of considerable nutritional significance as a factor influencing protein degradation in the rumen (Krishnamoorthy et al., 1982). There are two laboratory systems to measure protein solubility: a) the detergent system and b) the solvent system. Van Soest (1989) concluded that the detergent system could not accurately predict quantitatively the rumen degradation of protein. In comparing different solvents, Krishnamoorthy et al. (1982) concluded that borate phosphate buffer was the preferred solvent for measuring protein solubility to predict rumen degradation. However Krishnamoorthy et al. (1982) did not relate any estimate obtained from protein solubility studies directly to rumen degradation in terms of the parameters describing rumen degradation kinetics and/or fermentation characteristics of protein.

In the present study, the linear correlation and regression between protein solubility in borate-phosphate buffer and *in sacco* rumen protein degradation were evaluated. The results obtained were not promising in that protein solubility in borate-phosphate buffer could not predict with high R^2 values

the *in sacco* rumen disappearance at any incubation time, nor any parameters describing rumen degradation characteristics of protein (S, D, U, Kd, EDCP and BCP). The reasons presumably were that dry roasting reduced buffer protein solubility sharply with increasing temperature and time, whereas dry roasting did not dramatically reduce *in sacco* protein degradation until roasting temperature rose to 150℃. Therefore the linear correlation between buffer protein solubility and *in sacco* results was very poor.

We also tested curvilinear correlations between buffer protein solubility and *in sacco* results, using the best curve fit analysis (SAS, 1991), but these were also poor. For example, curvilinear correlation between buffer protein solubility (average) and *in sacco* rate and extent of rumen protein degradation (Kd, EDCP) by polynomial (y=a+bx+cx2), logarithmic (y=a+b* log(x)) and exponential (y=a+b*10(cx)), (R^2 values) were 0.41, 0.26, 0.28 vs. 0.17 (simple linear) for Kd and 0.52, 0.43, 0.36 vs. 0.32 (simple linear) for EDCP, respectively (detailed data not shown).

Other authors have also found that protein solubility does not permit prediction of rumen protein degradability of concentrate feeds (pelleted) with good precision (De Boever et al., 1984; Madsen and Hvelpund, 1985).

The methods using the buffer protein solubility of feeds thus provide only a very broad guide to the protein degradation of feeds in the rumen. They do not permit direct estimations of rate and extent of degradation of the insoluble protein (D fraction) that is susceptible to microbial degradation in the rumen. From results in Yu et al. (1998, 1999), it is clear that *in sacco* methods can detect not only soluble fraction (S) but also the insoluble but potential degradable fraction (D) and that the proportion of these as well as the rate of degradation (Kd) are each differently affected by dry roasting at various conditions.

These results suggest that buffer protein solubility can not be used as a means to determine optimal processing conditions or to rank or predict the effect of heat treatment on rumen degradation characteristics of protein for the heat processed legume seeds.

The present study of protein solubility in borate-phosphate buffer with three different procedures has further showed that even using the same buffer, the different procedures produced different results. These variations demonstrate that the need for a repeatable and accurate method of determining protein solubility representative of protein degradation in the rumen has not been met.

The addition of proteolytic enzymes to the buffer solutions may make it possible to take into account the degradation of insoluble proteins, which would improve the prediction of protein degradation in the rumen compared with solubility in a buffer only.

Relationship between *in sacco* results and *in vitro* protein degradability

Rumen degradation characteristics of protein: The D_1_Auf values were highly linearly correlated with EDCP, Kd and %BCP (R^2>0.80) measurements (*in sacco*). Using the best curve fit analysis (SAS, 1991), the correlation between D_1_Auf and EDCP was not improved. For curvilinear correlations between D1_Auf and EDCP by polynomial (order=2), logarithmic and exponential, the R^2 values were 0.85, 0.84, 0.81 vs. 0.85 (simple linear), respectively (detailed data not shown).

The D_{24}_Auf values were poorly correlated with the various parameters of rumen degradation characteristics (S, D, U, Kd, EDCP and BCP). Curvilinear correlations between D_{24}_Auf values and *in sacco* results were also very poor; for curvilinear correlations between D_{24}_Auf and the rate and extent of rumen degradation (Kd, EDCP) by polynomial, logarithmic and exponential, R^2 values were 0.46, 0.42, 0.33 vs. 0.38 (simple linear) for Kd and 0.35, 0.34, 0.28 vs. 0.30 (simple linear) for EDCP, respectively.

These results indicate that by using Aufrère method with enzymatic hydrolysis at 1 h or an intermediate time 1 h and <24 h using a protease extracted from *Streptomyces griseus* in a borate-phosphate buffer at pH 8, it may be possible to predict *in sacco* the rate and extent of protein degradation in the rumen with high accuracy, particularly for processed feedstuffs under various conditions.

Rumen disappearance of protein

In the present study, *in vitro* rumen protein degradability (D_1_Auf) at 1 h incubation was highly correlated with *in sacco* rumen protein disappearance at shorter incubation times 2-12 h (with the high R^2 values ranging from 0.83 to 0.93). However, the correlation between *in vitro* protein degradability and the amount of protein disappearing in the rumen *in sacco* was influenced greatly by length of incubation; variability increased markedly with increasing incubation time. After longer incubation, the strength of the correlation gradually declined. At 4-8 h incubation, the highest correlation coefficients were observed (R^2=0.91-0.93). Shorter periods (0-2 h, R^2=0.71-0.83) and longer periods of incubation (12-24 h, R^2=0.83-0.65; 48 h, R^2=0.45) showed less stability in the relationship. These results are favorably comparable to those reported by Grummer and Clark (1982) that 4-8 h incubation had the higher correlation coefficients (0.94, 0.89) than at 0-2 h incubation (0.87, 0.86).

The early phase of the *in sacco* degradation curve is very much subject to the condition of preparation and rumen exposure of the material in the bags, and

is one of the principal factors addressed in attempts to standardize the method.

This may also be related to substrate factors such as NH_3 concentration. Grummer and Clark (1982) observed that concentration of NH_3-N in the rumen of cows peaked at 1 to 2 h post-feeding and then declined to their lowest level at 8 h after feeding and then increased again from 8 h after feeding. High NH_3 concentrations in the rumen decreased the rate of protein degradation; this was attributed to decreased bacterial protease activity. From 4-8 h after feeding, the low NH_3 concentrations may have resulted in a stimulation of proteolytic enzymes and increased the rate of N disappearance from nylon bags.

Crawford et al. (1978) and Proos-Floyd et al. (1985) reported that the correlation between protein solubility (autoclaved ruminal fluid) and *in sacco* rumen disappearance became weaker after longer incubation, but tentatively attributed this to either reduced susceptibility of the remaining protein to enzymatic attack or to feedback inhibition of enzyme action by end products. In methodological terms it may also be a function of measurement error, because as incubation time increased, a smaller sample size was available for breakdown. It is for this reason that great care was taken in this experiment to model the likely residue weights in the *in sacco* time sequence and set sufficient starting sample weights to reduce the impact of this factor.

In the present study, dry roasting did not significantly affect the *in vitro* enzymic degradability of protein at 24 h of incubation. The D_{24}_Auf values were thus similar among all the treatments of dry roasting WFB and WLS, while *in sacco* results showed that dry roasting did affect rumen degradation characteristics (Kd, S, D, U etc) substantially. Incubation for 24 h was sufficiently long for the protease extracted from *Streptomyces griseus* to degrade almost all the protein in feed samples. These results indicate that the effectiveness of dry roasting under various conditions on legume seeds could be detected by *in sacco* technique, but not by Aufrère method with enzymic digestion incubated for 24 h.

CONCLUSIONS

Protein solubility in borate-phosphate buffer fails to identify differences in the rate and extent of protein degradation of legume seeds after dry roasting under various conditions. The consequences are that the method equally may not be applied effectively to compound feeds, particularly pelleted material. Using buffer protein solubility as a means to determine optimal processing conditions for processed feedstuffs for ruminant may be misleading.

In vitro protein degradability using a modified Aufrère method with enzymatic hydrolysis for 24 h by a protease extracted from *Streptomyces griseus* in a borate-phosphate buffer does not detect the effectiveness of dry roasting on *in sacco* rumen protein degradation. The protease hydrolysis removes almost all protein in 24 h incubation.

The most promising method was based on *in vitro* protein degradability using a modified Aufrère method with enzymatic hydrolysis for 1 h. Correlations obtained had high R^2 values of *in vitro* methods evaluated to predict rumen degradation and predicted better than any other, in terms of the rate and extent of rumen degradation of protein of dry roasted legume seeds. However, there may be an intermediate time >1 h and <24 h that may provide a better correlation. The modified Aufrère method appears useful as a simple laboratory method for protein evaluation of feedstuffs heat processed such as dry roasted legume seeds and may serve as a useful guide in ranking materials.

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