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Original article

In situ evaluation of the ruminal and intestinal degradability of extruded whole lupin seed nitrogen

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Summary — The effect of whole lupin seeds (*Lupinus albus* cv Lublanc) at 120, 150 and 195 °C on *in situ* nitrogen degradability (Dg.N) was measured by the nylon bag technique using fistulated non-lactating Holstein cows. The N degradation was evaluated in nylon bags suspended in the rumen; heating the seeds at 120, 150 and 195 °C decreased the Dg.N value: 83.9, 72.9 and 53.0 respectively *vs* 95.3% (rumen outflow rate of 0.06/h). To estimate the total N disappearing in the digestive tract, bags were incubated in the rumen for 16 h, then in a pepsin bath for 2 h and then introduced into the duodenum for subsequently recovery in feces. The whole tract degradability of N was always high, approximately 98.3%. The amounts of N which disappeared in the intestine increased from 3.1 (untreated seeds) to 15.1, 26.3 and 44.7% as the temperature rose to 120, 150 and 195 °C respectively. The PDIN and PDIE contents (g/kg of DM) of the raw whole lupin seeds were 224 and 84 respectively; extrusion elevated these values by 10–32% for PDIN and 57–194% for PDIE. The augmentation in the supply of dietary proteins to the postruminal parts as a result of extrusion could rapidly benefit high yielding cows.

lupin seeds / extrusion / cows / *in situ* degradability / rumen / intestine

Résumé — Évaluation *in situ* des dégradabilités ruminale et intestinale des matières azotées des graines de lupin extrudées. *Quatre vaches laitières taries, de race Holstein, munies de canules (rumen et duodénum) ont été utilisées afin de déterminer l'effet de l'extrusion à 120, 150 et 195 °C de la graine de lupin (*Lupinus albus *cv Lublanc) sur la dégradabilité* in situ *des matières azotées (MA) dans le rumen et l'intestin. Après traitement à 120, 150 et 195 °C, la dégradabilité théorique des MA dans le rumen diminue : 83,6, 89,7, 72,3 et 53,0 respectivement contre 95,1% (avec un taux de passage théorique des particules de 0,06/h). Des sacs incubés pendant 16 h dans le rumen ont été introduits dans l'intestin* via *la canule duodénale et récupérés dans les fèces. La disparition des MA dans l'ensemble du tractus digestif n'est pas modifiée par l'extrusion (98,3%). En conséquence, la fraction azotée d'origine alimentaire qui disparaît dans les régions postruminales augmente avec la température d'extrusion, soit : 15,1, 15,7, 26,3 et 44,7% lorsque les graines sont respectivement traitées à 120, 150 et 195 °C; la disparition intestinale n'étant que de 3,1% pour les graines non traitées. Les valeurs de PDI (g/kg MS) estimées pour le lupin cru sont : 224 (PDIN) et 84 (PDIE); l'extrusion augmente ces valeurs,* ie, *10 à 32% (PDIN) et 57 à 194% (PDIE). La cuisson-extrusion protège les MA de la graine de lupin de la digestion bactérienne dans le rumen et accroît l'apport de MA d'origine alimentaire dans l'intestin grêle.*

lupin / extrusion / vaches / dégradabilité* in sacco */ rumen / intestin

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INTRODUCTION

Large quantities of protein are required by dairy cows to maintain high milk production. This can be enhanced by feeding nondegradable proteins or by protecting supplementary crude protein from excessive breakdown. Attempts have been made to increase the quantity of protein reaching the small intestine of ruminants by heat-treatment (Stern *et al,* 1985; Thomas *et al,* 1987; Stutts *et al,* 1988). Low alkaloid and high protein and lipid contents in sweet white lupin seeds (Cerning-Beroard and Filiatre, 1977) indicate a potential use as a protein supplement in ruminal diets. Unfortunately, poor performances of lactating dairy cows fed lupin seeds have been reported (Guillaume *et al,* 1987); these could be partly due in a reduced true protein in the small intestine. Until now, little information has been reported concerning the benefit of heating this proteaginous seed to reduce protein breakdown in the rumen (Emile *et al,* 1988) and to increase small intestine availability of rumen undegraded dietary nitrogen (N).

Consequently, the objective of our study was to determine the effect of extrusion at 120, 150 and 195 °C on whole lupin seeds (WLS) by measuring: 1) N solubility in artificial saliva and 2) degradation of N contents in nylon bags within the rumen and intestine of non-lactating Holstein cows.

MATERIALS AND METHODS

Animals and diets

Four non-lactating Holstein cows weighing 600 kg, fitted with ruminal and proximal duodenal cannulas, were used. Animals were housed in individual stalls with free access to both feed and water. Basal diet consisted of 70% Italian rye-grass hay and 30% of hammermilled WLS on a dry matter (DM) basis. Animals were given one half of their assigned diet at 9.00 h and one half at 17.00 h daily. The mean daily DM intake was ≈ 10 kg.

Protein sources

The protein sources tested were WLS (*Lupinus albus* cv Lublanc) raw or extruded* at 120 ($EWLS_{120}$), 150 ($EWLS_{150}$) and 195 °C ($EWLS_{195}$) and ground finely enough to pass through a 1-mm screen; EWLS were from the same batch as RWLS. Chemical composition of the WLS used for *in situ* incubations is listed in table I.

In situ *incubation of protein sources*

Nylon bags made from Blutex T_{50} (Tripette et Renaud, Paris, France) and having a mean pore size of 46 μm were used throughout the study. Five-g samples of milled WLS were placed into 7 x 11 cm heat-sealed nylon bags. Sixty-four bags were prepared for each protein source. Twelve bags per feedstuff (RWLS and EWLS) per cow were introduced into the ventral sac of the rumen immediately before the morning feeding and anchored by a nylon cord string to the cap of the ruminal cannula. Two bags were taken out of the rumen after incubation for 2, 4, 8, 16, 24 and 48 h, briefly rinsed under cold tap water to eliminate surface debris and machine-washed (2 x 5 min) to remove rumen fluid; then nylon bags were dried at 60 °C for 48 h in a forced air oven.

The total N disappearing in the digestive tract was obtained by further incubation of 4 bags (6 x 6 cm; 1.5 g) per feedstuff per cow. After initial rumen exposure for 16 h (Michalet-Doreau *et al,* 1987), the bags were removed, rinsed, incubated at 40 °C for a further 2 h with pepsin solution (3 g/l in 0.1 N HCl), rinsed again and inserted into the small intestine through the

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Table I. Dry matter (DM) content, composition of the DM and nitrogen (N) solubility of the raw and extruded whole lupin seeds.

		EWLS		
	RWLS	*120 °C*	*150 °C*	*195 °C*
Dry matter (% feedstuff)	91.8	95.4	95.6	95.8
Composition of DM (%)				
Protein (N x 6.25)	38.8	38.4	38.1	38.3
Ether extract	8.7	8.3	7.9	7.6
Neutral detergent fiber	24.0	20.8	20.8	19.5
Acid detergent fiber	16.1	16.9	18.6	17.6
Organic matter	95.6	94.9	95.6	95.8
N soluble (% total N)	80.5	33.2	26.9	21.8

Whole lupin seeds: raw (RWLS) and extruded (EWLS).

duodenal cannula (Peyraud *et al,* 1988). Bags recovered from the feces were treated similarly to those removed from the rumen. Bags not recovered within 30 h were discarded (de Boer *et al,* 1987).

Biochemical analyses

Dry matter was determined in feedstuffs (105 °C overnight). Evaluations of organic matter and fat in WLS were made according to the methods recommended by the AOAC (1984), neutral and acid detergent fiber contents of WLS were estimated by the procedure described by Van Soest (1963) and Van Soest and Wine (1967). Nitrogen (Kjeldahl) was determined both in feedstuffs and in residuals; from this, protein was determined as N x 6.25. In addition, soluble N in each protein source (RWLS, EWLS) was determined after extraction in artificial saliva (bicarbonate-phosphate buffer; pH = 6.9) according to the procedure of Vérité and Demarquilly (1978).

Calculations

Disappearance of DM and N from the nylon bags at each incubation time was calculated from the respective amounts remaining after incubation in the rumen. The *in situ* degradability kinetics for DM and N were studied using the exponential model proposed by Ørskov and McDonald (1979) using the Marquardt algorithm for non-linear regression analysis (STATITCF, 1985) :

$$Dg\ (t) = a + b\,(1 - e^{-ct}) \qquad (1)$$

where Dg *(t)* = degradability at time *t*; *a* :=the rapidly soluble component; *b* = the less rapidly degradable component, which disappears at the constant fractional rate *c*, per time *t*. In addition, the degradability (Dg) was estimated according to the equation $Dg = a + (bc) / (c + k)$, where *a*, *b* and *c* are in equation (1) and *k* is the estimated rate of solid outflow from the rumen, assumed to be 0.06/h. The amount of digestible DM and N reaching the intestine was evaluated as the difference between whole tract digestion and Dg.DM or Dg.N.

The PDI (protein digested in the small intestine) contents of the feedstuffs were evaluated using the Dg.N, the undegraded N digestibility in the small intestine calculated as: [(1 – Dg.N) – residual rumen undegraded N] / (1 – Dg.N) and fermentable organic matter data derived from present work and the equations proposed by Vérité *et al* (1987) and Vérité and Reynaud, 1989). The RWLS and EWLS organic matter digestibilities used were 0.91 and 0.88 respectively (unpublished results).

Disappearances of DM and N from bags incubated in the rumen and in the rumen plus intestine were analysed statistically using analysis of variance for feedstuffs or temperature effects within each rumen incubation time. Linear regression and correlation coefficients were used to compare N disappearance from the bags at each of the rumen exposure time with N solubility *in vitro* for all feedstuffs. Results are presented as means with their standard errors. Differences were evaluated statistically using ANOVA, significances being declared at $P < 0.05$.

RESULTS

Nitrogen solubility

Buffer-soluble-N from RWLS and EWLS sources is presented in table I. Solubility (%) of the RWLS-N in artificial saliva was high (80.5). Extrusion at 120, 150 and 195 °C reduced this value by 59, 67 and 73% respectively.

In situ *evaluation of the ruminal degradability*

Loss of DM and N from bags over a 48-h period for each of the WLS studied is given in figure 1. After 2 h of incubation, the disappearance of DM and N from the rumen was significantly higher for RWLS than for EWLS. However, with longer incubations, the effect of processing gradually decreased, so that by 48 h, the loss of DM and N from the treated grains was the same as from the untreated grains. As indicated by data in table II, there were direct linear relationships between the solubility and the disappearance of N. The correlation was influenced by residence time of the bags in the rumen and a significant relationship was only found between

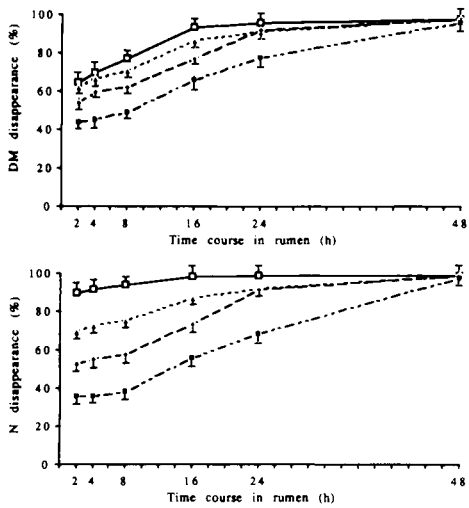

Fig 1. Percentage disappearance of dry matter (DM) and nitrogen (N) of the whole lupin seeds raw (—□—) and extruded at 120 (—▲—), 150 (—◆—) and 195 °C (—■—), from nylon bags as a function of ruminal incubation time. Each vertical bar indicates the standard error of difference between 8 samples.

Table II. Relationships between *in situ* degradability of nitrogen (N) and *in vitro* N solubility of the whole lupin seeds.

Rumen exposure time (h)	*Predicted equations*	*RSE*	*r*
2	$y = 31.62 + 0.74\ x$	10.63	0.91*
4	$y = 33.70 + 0.73\ x$	11.68	0.90*
8	$y = 36.45 + 0.72\ x$	11.70	0.90*
16	$y = 56.36 + 0.54\ x$	10.36	0.87*

y = mean value of *in situ* N degradability (%) and x = N solubility (%). RSD : residual standard deviation; correlation coefficients *(r)* were evaluated from experiments performed with the raw and extruded whole lupin seeds; * significant relationship.

N solubility and N disappearance from bags at 2, 4, 8 and 16 h of rumen incubation.

The parameters defining the equations describing the pattern of DM and N degradation as well as the Dg.DM and Dg.N values are presented in table III. Coefficients of determination *(r^2)* for disappearance of DM and N were significant and the Dg.N values were 82.8 and 95.1% respectively. Processing the WLS at the indicated levels (120, 150, 195 °C) was followed by a decrease in the proportion of the rapidly soluble N fraction *(a)* and both increased the proportion and reduced the breakdown rate *(c)* of the fraction *(b)*. Therefore, extrusion of WLS diminished the Dg.DM value by 3.9% for $EWLS_{120}$, 11.1% for $EWLS_{150}$ and 27.9% for $EWLS_{195}$; the corresponding reductions of the Dg.N value were: 12, 23.7 and 44.3%.

In sacco Dg.N values of WLS (raw and extruded) were significantly correlated with the N solubility *(s)*; the regression equation was: Dg.N = 53.9 + 0.54 *s* (*r* = 0.82; *RSD* = 12.6). In addition, correlations were also found between the coefficient *a in situ* and the N solubility *(s) in vitro*; the corresponding equation was: *a* = 12.6 + 0.88 *s* (*r* = 0.92; *RSE* = 12.7).

Evaluation of the intestinal disappearance

Total disappearance of DM and N from bags during transit through the digestive tract in depicted in figure 2. The percentages observed for the whole tract digestion of DM (89.2 – 90.9%) and N (97.5 – 98.7%) were always high. Although processing of WLS significantly depressed Dg.DM and Dg.N values, the whole tract disappearance of these components was not significantly affected. Consequently, the amount of DM which disappeared in the postruminal sections increased from 8.1 (RWLS) to 10.6, 15.6 and 26.8% as the temperature of the extruded grains rose to ≈ 120, 150 and 195 °C respective-

Table III. Coefficients of degradability kinetics, *in sacco* degradability and r^2 values of dry matter and nitrogen of the whole lupin seeds.

		a	*b*	*c*	r^2	*Dg*
Dry matter						
RWLS		43.9 ± 1.1	53.7 ± 2.2	16.0 ± 0.6	0.92	82.8
	120 °C	49.4 ± 1.1	48.1 ± 2.0	10.1 ± 0.5	0.83	79.6
EWLS	150 °C	33.1 ± 2.6	64.3 ± 2.5	10.2 ± 0.5	0.86	73.6
	195 °C	38.4 ± 1.2	57.6 ± 2.0	3.5 ± 0.6	0.90	59.7
Nitrogen						
RWLS		80.7 ± 0.7	18.3 ± 0.7	22.1 ± 0.6	0.96	95.1
	120 °C	56.5 ± 1.7	42.7 ± 2.9	10.4 ± 0.9	0.70	83.6
EWLS	150 °C	27.1 ± 2.5	71.8 ± 2.9	10.2 ± 0.8	0.75	72.3
	195 °C	27.7 ± 2.0	69.9 ± 2.5	8.3 ± 0.5	0.72	53.0

Whole lupin seeds: raw (RWLS) and extruded (EWLS); *a*, *b* and *c* are non linear parameters and Dg the *in sacco* degradability (see p 577); r^2 is the coefficient of determination.

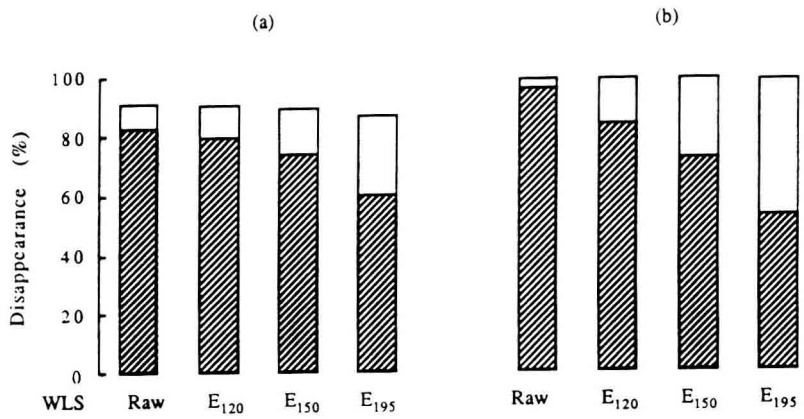

Fig 2. Effect of extrusion on relative proportions of ruminal (▨) and intestinal (□) disappearance of dry matter (a) and nitrogen (b) from whole lupin seeds (WLS), raw and extruded at 120 (E_{120}), 150 (E_{150}) and 195 °C (E_{195}).

ly. The percent intestinal disappearances for N were: 3.1 (RWLS), 15.1 (E_{120}), 26.3 (E_{150}) and 44.7 (E_{195}), the corresponding intestinal digestibilities of the rumen undegraded dietary protein were: 63.2, 91.2, 94.9 and 95.1% (these values are used for estimation of the PDI contents).

The PDIN and PDIE contents (table IV) of the RWLS were 224 and 84 g/kg of DM respectively; heat-treatment increased these values by 10 to 32% for PDIN and by 57 to 194% for PDIE.

Table IV. Evaluation of the PDI (protein digested in the small intestine) values (g/kg of DM) of the raw and extruded whole lupin seeds.

Lupin seeds		*PDIA*	*PDIN*	*PDIE*
Raw		13	224	84
Extruded	120 °C	64	246	134
	150 °C	111	264	173
	195 °C	190	295	247

The PDI contents of whole lupin seeds were calculated using the equation proposed by Vérité *et al* (1987) and Vérité and Peyraud (1989). PDIA : PDI supplied by rumen undegraded dietary protein; PDIN : PDIA plus PDI supplied by microbial protein from rumen-degraded protein; PDIE : PDIA plus PDI supplied by microbial protein from rumen fermented organic matter.

DISCUSSION

Though some specific characteristics of proteins, such as structure and presence of disulfide bonds influence their degradability in the rumen (Mahadevan *et al,* 1980), for practical purposes this degradability is often related to protein solubility in mineral buffers (Vérité and Demarquilly, 1978). Unheated WLS proteins, composed primarily of globulins: 87.2% and albumins: 12.8% (Duranti and Cerletti, 1979) were highly soluble both *in vitro* (80.5%) and probably *in situ* (parameter *a* = 80.7%).

This is in agreement with the results of Wohlt *et al* (1973) and Blethen *et al* (1990), who examined incidence of protein type *eg*, albumins, globulins, prolamins and glutelins, on N solubility of various feedstuffs. The high N degradability in RWLS was particularly noticeable, this being associated with high initial solubility. The significant correlation between *in vitro* N solubility, coefficient *a* and Dg.N values shows that it is possible to predict the degradability of N from the *in vitro* results; similarly, Aufrère *et al* (1988) , as well as Chapoutot *et al* (1990) have reported a high correlation between the N degradability *in situ* of feedstuffs and that obtained with the same *in vitro* technique reported in this paper. Our estimates of Dg.N (95.1%) are similar to previous reports for this oilseed, milled through a 0.8–1.0 mm screen (Freer and Dove, 1984; Emile *et al,* 1988; Demarquilly *et al,* 1989) whereas Valentine and Bartsch (1988) found a lower Dg.N value (81.5%). Subjecting WLS to extrusion at 120, 150 and 195 °C reduced the solubility of proteins, thus lowering their susceptibility to ruminal degradation. This finding is in agreement with those of others on the effects of heat-treatment on both N solubility and N degradability of various grains: soybeans, horsebeans, cottonseeds and canola (Mir *et al,* 1984; Michalet-Doreau *et al,* 1985; Deacon *et al,* 1988; Arieli *et al,* 1989; Cros *et al,* 1991).

Total tract N disappearance was extremely high for RWLS (98.2%) as well as for EWLS (98.3%). Consequently, extrusion at 120, 150 and 195 °C appeared to have potential for decreasing ruminal degradation without reducing intestinal digestibility of WLS proteins. It is interesting to note that the apparent intestinal digestibility values of RWLS (0.63) and EWLS (0.94) agree well with mean values of true digestibility in the small intestine of undegraded dietary protein given in the INRA tables (Demarquilly *et al,* 1989) for various raw oilseeds: rapeseeds, horsebeans, linseeds, RWLS (0.60) or extruded: soybeans (0.85). The ruminaly bypass proteins may be least available in the intestine because much of the readily digested proteins are degraded by microorganisms, leaving only more refractory portions. These findings concur with those of Arieli *et al* (1989), who showed that extrusion (140 – 160 °C) of whole cottonseeds tended to elevate the apparent intestinal digestibility of N (0.76 *vs* 0.64); meanwhile, processing cottonseeds at 180 °C diminished the intestinal digestibility (0.22 *vs* 0.64). In the French protein system, the protein value of feeds and the animal requirements are both expressed in terms of true protein truly digestible in the small intestine. The results regarding PDI contents obtained for RWLS are in accordance with those of Andrieu *et al* (1989) for this untreated oilseed. All heat-treatments were followed by an increase in the PDI values; furthermore, the estimated PDI concentrations for EWLS are comparable to those reported for the extruded soybeans in the INRA tables (Andrieu *et al,* 1989).

We conclude that WLS proteins were effectively protected from ruminal breakdown by extrusion at 120, 150 and 195 °C without adverse effect on protein total degradability. The increase in the supply of dietary protein to the postruminal sections as a result of extrusion could rapidly benefit high yielding cows.

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