

## Degradation in the rumen of treated and untreated soya bean meal proteins

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**Abstract** — In order to estimate the quantity of solubilized nitrogen in the rumen that can contribute to intestinal feed non-ammonia nitrogen, degradation in the rumen was studied both for a control soya bean meal (cSBM) and a treated one (tSBM) to assess simultaneously the kinetics of: 1) protein disappearance from rumen bags and 2) rumen fluid contents in various nitrogen fractions in the rumen fluid: total nitrogen (Nt), ammonia nitrogen (NH<sub>3</sub>-N), non-ammonia nitrogen (NAN), peptides-N and amino acids-N, and true protein (protein-N). Measurements were taken on four sheep fed successively with four diets consisting of hay plus one of the two meals, in the proportion of 40 % and 20 % control meal (cSBM 40 % and cSBM 20 %), 40 % treated meal (tSBM) and a control diet based on hay alone supplemented with starch and urea (HSU). The effective degradability estimated with the nylon bag method was 0.727 and 0.502 for cSBM and tSBM, respectively. Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) showed that the cSBM conglycinins were degraded at a higher rate in the rumen than glycinins. The same proteins were degraded at a slower rate in the tSBM. Most of the nitrogen degraded in the rumen fluid was in NH<sub>3</sub>-N form, and only 30 % as NAN. For cSBM, NAN comprised protein-N (concentration was low 1 and 2 h after feeding), peptides-N and amino acids-N, while for the treated meal the NAN comprised solely peptides-N and amino acids-N. These results showed that for soya bean meal – a feed in which the dietary proteins are strongly degraded at moderate rates – very little of the solubilized proteins from the bags can escape degradation in the rumen. Whatever the meal studied (treated or untreated), the estimation of feed NAN in rumen fluid able to escape degradation in the rumen compared to the degraded proteins (calculated from the degradation in nylon bags) was negligible (about 1 %). (© Elsevier / Inra).

**control soya bean meal / treated soya bean meal / protein degradation / rumen fluid composition / electrophoresis**

**Résumé** — **Dégradation dans le rumen d'un tourteau de soja traité ou non.** Afin de connaître la quantité d'azote non ammoniacal, solubilisée dans le rumen et pouvant contribuer au flux d'azote intes-

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tinal d'origine alimentaire, la dégradation des protéines d'un tourteau de soja témoin (cSBM) et traité (tSBM) a été étudiée. Nous avons estimé en cinétique : 1) la disparition des protéines alimentaires dans le rumen en utilisant la méthode des sachets de nylon 2) la teneur en différentes fractions azotées du liquide du rumen: azote total (Nt), azote ammoniacal ( $\text{NH}_3\text{-N}$ ), azote non ammoniacal (NAN), N peptidique (peptides-N) et l'azote des acides aminés (amino acids-N), et l'azote protéique (protein-N). Les mesures ont été effectuées sur quatre moutons nourris successivement avec quatre régimes à base de foin additionné soit de 40 ou 20 % de tourteau témoin (cSBM 40 % et cSBM 20 %) soit de 40 % de tourteau traité (tSBM) ou 40 % d'un mélange témoin d'amidon et d'urée (HSU). La dégradabilité théorique estimée in situ est respectivement de 0,727 et 0,502, pour cSBM et tSBM. Les électrophorèses sur gel de polyacrylamide indiquent que les conglycinines (7S) du tourteau de soja témoin sont dégradées plus rapidement dans le rumen que les glycinines (11S) ; ces mêmes protéines étant dégradées plus lentement pour le tourteau traité. Dans le liquide du rumen, les teneurs en Nt et  $\text{NH}_3\text{-N}$  sont élevées 1 h et 2 h après le repas et diminuent progressivement (jusqu'à 7 h après le repas). La majorité de l'azote dégradée dans le jus de rumen l'est sous forme de  $\text{NH}_3\text{-N}$ , et 30 % seulement sous forme de NAN. Pour le tourteau non traité, le NAN est constitué de protéines (dont la teneur est peu élevée 1 h et 2 h après le repas) alors que, pour le tourteau traité, le NAN est constitué uniquement de N peptidique et de N acides aminés. Ces résultats indiquent que pour le tourteau de soja, aliment dont les protéines alimentaires sont fortement dégradées mais à des vitesses peu élevées, très peu de protéines solubilisées des sachets peuvent échapper à la dégradation jusqu'au stade de  $\text{NH}_3\text{-N}$  dans le rumen. Quel que soit le tourteau étudié, la quantité de NAN d'origine alimentaire présente dans le jus de rumen et transitant avec la phase liquide est négligeable (environ 1 % de l'azote dégradé estimée à partir des sachets de Nylon). (© Elsevier / Inra)

#### **tourteau de soja témoin / tourteau de soja traité / dégradation des protéines / composition du jus de rumen / électrophorèse**

## **1. INTRODUCTION**

In ruminants, the protein value of a feed is based on an estimate of the protein quantity of dietary and microbial origin absorbed in the small intestine. Dietary nitrogen that escapes degradation in the rumen is thus a significant factor in determining the protein value. Many experiments have demonstrated the beneficial effects of technological processes of feeds, especially heat treatment, in reducing the degradation of their crude protein in the rumen without decreasing digestibility in the small intestine. Moreover, increasing numbers of studies carried out in vitro and in situ with various feeds show that dietary proteins are degraded at different rates according to their structure and location in the plant tissue. Furthermore, soluble proteins can remain intact for several hours (8–12 h) in the incubation medium [4, 9, 14, 26]. Similarly, some peptides from the proteolysis can accumulate temporarily in the liquid phase of the ruminal content and become transit with it.

A comparison of the degradation in the rumen of two commercial soya bean meals (a control meal and a treated meal) was made. The aim of this study was to estimate the effect of the technological process on the in situ degradation of proteins in the rumen (nylon bag method), by characterising the proteins in the residues of the nylon bags using electrophoresis. A second objective was to determine whether a certain proportion of the solubilized protein in the ruminal fluid would remain long enough as protein, peptide and amino acids to escape ruminal degradation by transiting with the liquids.

## **2. MATERIALS AND METHODS**

### **2.1. Feeds**

The study was carried out using two different commercial soya bean meals: a control soya bean meal (cSBM) and a treated soya bean meal (tSBM) prepared from the same standard seed batch under trituration conditions: temperature

105° to 110 °C, pressure 2 bar, 15-min cycle and formalin at 0.1 %.

Four ruminally cannulated Texel sheep were studied to compare four diets consisting of grass hay and:

- 40 % of control soya bean meal (cSBM 40 %),
- or 40 % of treated soya bean meal (tSBM),
- or 40 % of an isoenergetic and isoprotein mixture (37/3) of starch and urea (HSU),
- or 20 % of control soya bean meal (cSBM 20 %)

## 2.2. Animal and experimental design

Four ruminally cannulated Texel sheep were included in the study, which consisted of four successive experimental periods of animal feeding using successively soya bean meal and hay and urea (HSU). Each period comprised a 2-week adaptation phase and a 2-week period for measurements. The first week of this latter period was for measuring the in situ degradation kinetics for the soya bean meal, and the second for kinetic sampling of the ruminal fluids. The dry matter intake was limited to 40 g·kg<sup>-1</sup> metabolic weight per day in two meals at 0900 and 1700 hours.

As previously reported for rapeseed meals [5], dry matter and nitrogen degradability were measured using the nylon bag procedure as described by Michalet-Doreau et al. [27].

The soya bean meal in the diet was the same as that used in the nylon bags. After removal from the rumen, the bags were rinsed in cold water, then beaten in a stomacher [25] prior to further washing and drying at 60 °C for 48 h. The stomacher permitted the separation of bacteria from the incubated feed samples.

Samples of ruminal fluid were taken from the sheep on 2 consecutive days, before the morning feed (0 h time) and 1, 2, 4 and 7 h after the meal. The ruminal fluid (150 mL) was filtered, then centrifuged at 120 g. The supernatant was centrifuged at +4 °C, 27 000 g for 20 min to remove nutritional particles and bacteria. The protein was then precipitated with sulfosalicylic acid (400 g·L<sup>-1</sup>) and separated after centrifugation (20 000 g for 10 min.).

The rumen was emptied on each sheep before feeding and 1 and 7 h after feeding to measure the volume of the rumen fluid.

## 2.3. Analyses

The granulometry of the soya bean meals was carried out according to Grenet [18].

The total nitrogen (Nt) content of the feeds, bag residues, as well as the soluble nitrogen of the ruminal fluids (before and after precipitation with sulfosalicylic acid) were determined using the Kjeldahl method. The protein values of the ruminal fluid nitrogen were obtained from the difference, and the ammonia nitrogen (NH<sub>3</sub>-N) values were determined from the acid supernatant (after precipitation with sulfosalicylic acid) by the Conway method [11].

Enzymatic degradability of the soya bean meals was measured at 1 h (DE1) [2].

The cell-wall contents (neutral detergent fibre [NDF] and acid detergent fibre [ADF]) were determined for the feeds using the Van Soest [39] and Van Soest and Wine [40] methods, modified by Giger et al. [17] and Dorléans et al. [12], by carrying out a preliminary treatment with an amylase and a protease. The neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were also determined.

## 2.4. Electrophoresis [5]

The feeds, their residues after in situ degradation, and the ruminal fluid were fractionated using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using separating gels of 12.5 % acrylamide, C = 2.67 %.

## 2.5. Calculations and statistical analysis

The in situ dry matter and nitrogen disappearances in hay and soya bean meals were fitted to the model of Ørskov and McDonald [31]. The effective degradability of nitrogen, Deg (N), was calculated as Deg (N) =

$$a + (bc)/(c + kp), \text{ assuming } kp = 0.06 \text{ h}^{-1} [20].$$

The various degradability parameters for the nylon bags were analysed by a variance analysis (SAS GLM procedure [33]) according to the following factorial model:

$$Y = M + A_i + T_j + E_{ij}$$

where  $M$  is the overall average;  $A_i$ , the animal effect;  $T_{265}$ , the soya bean meal effect and  $E_{ij}$ , the residual error.

For the parameters measured in the ruminal fluid, the average values of the two measurement days were analysed following the same factorial model.

### 3. RESULTS

#### 3.1. Chemical analyses

The chemical composition of the feeds is shown in *table I*. The average diameters of the soya bean meal particles were similar: 0.46 and 0.47 mm for cSBM and tSBM, respectively

The NDF and ADF concentrations were similar for cSBM and tSBM. While the NDIN concentrations were greater for tSBM (7.74 % Nt vs. 2.78 % Nt for the control meal), they were not very high, whereas the ADIN concentration was low whether the meal was treated or untreated. One-hour enzymatic degradability (DE1) was halved by the treatment (13 % Nt vs. 24 % Nt).

The rumen volume was 6.4, 8.7 and 8.1 L at 0, 1 and 7 h, respectively.

#### 3.2. In situ study (*table II*)

The treatment did not change significantly the immediately soluble fraction 'a' or the potentially degradable fraction 'b' of either dry matter or nitrogen. However, it

significantly decreased (0.129 to 0.047) fraction 'c' (rate of degradation of the nitrogen degradable fraction) and thus effective degradability.

#### 3.3. Ruminal fluid composition (*table III*)

Non-ammonia nitrogen (NAN) is the difference between Nt and NH<sub>3</sub>-N. It therefore consists of amino acids, peptides and true protein (protein-N).

**Table II.** In situ degradation parameters for soya bean meals (cSBM: control soya bean meal; tSBM: treated soya bean meal).

DM	cSBM	tSBM
a	0.302 <sup>a</sup>	0.284 <sup>a</sup>
b	0.701 <sup>a</sup>	0.694 <sup>a</sup>
c	0.113 <sup>a</sup>	0.054 <sup>b</sup>
Deg (DM)	0.740 <sup>a</sup>	0.627 <sup>b</sup>
N		
a	0.162 <sup>a</sup>	0.124 <sup>a</sup>
b	0.876 <sup>a</sup>	0.830 <sup>a</sup>
c	0.129 <sup>a</sup>	0.047 <sup>b</sup>
Deg (N)	0.727 <sup>a</sup>	0.502 <sup>b</sup>

DM: dry matter; N: nitrogen; a: immediately soluble fraction (%); b: potentially degraded fraction (%); c: rate of degradation (h<sup>-1</sup>); Deg: degradability (%) = a + (bc)/(c + k). Different subscripts in the same line correspond to a significant difference ( $P < 0.05$ ).

**Table I.** Chemical composition of the control soya bean meal (cSBM), treated soya bean meal (tSBM) and hay used in the rations (Hay).

	cSBM	tSBM	Hay
Nt (% DM)	7.97	7.82	1.67
DE1 (% Nt) <sup>1</sup>	24.30	12.60	
NDF (% DM)	14.24	16.53	60.33
ADF (% DM)	8.66	7.52	34.81
NDIN (% Nt)	2.78	7.74	39.68
ADIN (% Nt)	0.39	0.44	4.93

<sup>1</sup> One-hour enzymatic degradability (DE1) [2].

Nt: total nitrogen; NDF: neutral detergent fibre; ADF: acid detergent fibre; NDIN: neutral detergent insoluble nitrogen; ADIN: acid detergent insoluble nitrogen.

For all the diets, the concentration of Nt ( $\text{mg}\cdot\text{g}^{-1}$ ) in the ruminal fluid was highest 1 h after feeding and diminished gradually until 7 h after feeding. The  $\text{NH}_3\text{-N}$  concentration was highest and reached its maximum later for cSBM (2 h after feeding for cSBM 40 %) than for tSBM or HSU (1 h after feeding). The concentrations diminished gradually, but at 7 h after feeding they

were still significantly different (cSBM 40 % > tSBM > cSBM 20 %  $\approx$  HSU).

The ruminal fluid Nt and  $\text{NH}_3\text{-N}$  concentrations for the ration containing cSBM 20 % were always significantly lower ( $P < 0.05$ ) than those containing cSBM 40 % and tSBM. On average, the peptide-N and amino acids-N (obtained by differences between NAN and protein-N) were low and

**Table III.** Concentrations of total nitrogen (Nt), ammonia nitrogen ( $\text{NH}_3\text{-N}$ ), non-ammonia nitrogen (NAN), peptides-N, amino acids-N and protein-N in the rumen fluid of rations containing 40 % control soya bean meal (cSBM 40 %), 40 % treated soya bean meal (tSBM), hay + starch + urea (HSU) and 20 % control soya bean meal (cSBM 20 %) before feeding (0 h) and at 1, 2, 4 and 7 h after feeding.

Time (hours)	cSBM 40 %	tSBM	HSU	cSBM 20 %
<b>Nt (<math>\text{mg}\cdot\text{g}^{-1}</math>)</b>				
0	0.463 <sup>b</sup> $\pm$ 0.032	0.554 <sup>a</sup> $\pm$ 0.052	0.248 <sup>d</sup> $\pm$ 0.034	0.317 <sup>c</sup> $\pm$ 0.030
1	0.697 <sup>a</sup> $\pm$ 0.142	0.662 <sup>a</sup> $\pm$ 0.171	0.591 <sup>a</sup> $\pm$ 0.009	0.451 <sup>b</sup> $\pm$ 0.058
2	0.687 <sup>a</sup> $\pm$ 0.089	0.561 <sup>b</sup> $\pm$ 0.164	0.521 <sup>b</sup> $\pm$ 0.081	0.404 <sup>c</sup> $\pm$ 0.034
4	0.593 <sup>a</sup> $\pm$ 0.086	0.444 <sup>b</sup> $\pm$ 0.104	0.337 <sup>c</sup> $\pm$ 0.060	0.342 <sup>c</sup> $\pm$ 0.038
7	0.472 <sup>a</sup> $\pm$ 0.064	0.411 <sup>a</sup> $\pm$ 0.070	0.253 <sup>b</sup> $\pm$ 0.052	0.289 <sup>b</sup> $\pm$ 0.033
<b><math>\text{NH}_3\text{-N}</math> (<math>\text{mg}\cdot\text{g}^{-1}</math>)</b>				
0	0.354 <sup>b</sup> $\pm$ 0.036	0.432 <sup>a</sup> $\pm$ 0.046	0.141 <sup>d</sup> $\pm$ 0.010	0.224 <sup>c</sup> $\pm$ 0.031
1	0.466 <sup>a</sup> $\pm$ 0.036	0.465 <sup>a</sup> $\pm$ 0.017	0.460 <sup>a</sup> $\pm$ 0.127	0.319 <sup>b</sup> $\pm$ 0.014
2	0.522 <sup>a</sup> $\pm$ 0.037	0.420 <sup>b</sup> $\pm$ 0.083	0.395 <sup>b</sup> $\pm$ 0.086	0.306 <sup>c</sup> $\pm$ 0.016
4	0.489 <sup>a</sup> $\pm$ 0.070	0.325 <sup>b</sup> $\pm$ 0.076	0.213 <sup>c</sup> $\pm$ 0.049	0.244 <sup>c</sup> $\pm$ 0.026
7	0.389 <sup>a</sup> $\pm$ 0.056	0.294 <sup>b</sup> $\pm$ 0.058	0.141 <sup>c</sup> $\pm$ 0.040	0.188 <sup>c</sup> $\pm$ 0.034
<b>NAN (<math>\text{mg}\cdot\text{g}^{-1}</math>)</b>				
0	0.110 <sup>ab</sup> $\pm$ 0.025	0.121 <sup>a</sup> $\pm$ 0.008	0.107 <sup>ab</sup> $\pm$ 0.029	0.093 <sup>b</sup> $\pm$ 0.016
1	0.231 <sup>a</sup> $\pm$ 0.129	0.198 <sup>ab</sup> $\pm$ 0.070	0.131 <sup>b</sup> $\pm$ 0.028	0.132 <sup>b</sup> $\pm$ 0.035
2	0.165 <sup>a</sup> $\pm$ 0.078	0.141 <sup>ab</sup> $\pm$ 0.061	0.126 <sup>ab</sup> $\pm$ 0.023	0.098 <sup>b</sup> $\pm$ 0.016
4	0.104 <sup>bc</sup> $\pm$ 0.025	0.119 <sup>ab</sup> $\pm$ 0.020	0.124 <sup>a</sup> $\pm$ 0.035	0.098 <sup>c</sup> $\pm$ 0.011
7	0.084 <sup>b</sup> $\pm$ 0.018	0.117 <sup>a</sup> $\pm$ 0.012	0.113 <sup>a</sup> $\pm$ 0.038	0.100 <sup>ab</sup> $\pm$ 0.016
<b>Peptides-N + amino acids-N (<math>\text{mg}\cdot\text{g}^{-1}</math>)</b>				
0	0.050 <sup>b</sup> $\pm$ 0.014	0.065 <sup>a</sup> $\pm$ 0.018	0.064 <sup>a</sup> $\pm$ 0.017	0.044 <sup>b</sup> $\pm$ 0.016
1	0.135 <sup>ab</sup> $\pm$ 0.076	0.163 <sup>a</sup> $\pm$ 0.079	0.094 <sup>b</sup> $\pm$ 0.013	0.091 <sup>b</sup> $\pm$ 0.029
2	0.082 <sup>ab</sup> $\pm$ 0.021	0.113 <sup>a</sup> $\pm$ 0.064	0.093 <sup>ab</sup> $\pm$ 0.017	0.062 <sup>b</sup> $\pm$ 0.010
4	0.071 <sup>b</sup> $\pm$ 0.019	0.086 <sup>a</sup> $\pm$ 0.020	0.094 <sup>a</sup> $\pm$ 0.024	0.058 <sup>b</sup> $\pm$ 0.008
7	0.063 <sup>b</sup> $\pm$ 0.015	0.086 <sup>a</sup> $\pm$ 0.018	0.084 <sup>a</sup> $\pm$ 0.009	0.057 <sup>b</sup> $\pm$ 0.012
<b>Protein-N (<math>\text{mg}\cdot\text{g}^{-1}</math>)</b>				
0	0.060 <sup>a</sup> $\pm$ 0.020	0.057 <sup>a</sup> $\pm$ 0.011	0.043 <sup>a</sup> $\pm$ 0.013	0.050 <sup>a</sup> $\pm$ 0.002
1	0.096 <sup>a</sup> $\pm$ 0.056	0.035 <sup>b</sup> $\pm$ 0.016	0.037 <sup>b</sup> $\pm$ 0.015	0.041 <sup>b</sup> $\pm$ 0.006
2	0.084 <sup>a</sup> $\pm$ 0.042	0.028 <sup>b</sup> $\pm$ 0.015	0.033 <sup>b</sup> $\pm$ 0.009	0.036 <sup>ab</sup> $\pm$ 0.010
4	0.032 <sup>a</sup> $\pm$ 0.009	0.035 <sup>a</sup> $\pm$ 0.007	0.030 <sup>a</sup> $\pm$ 0.011	0.040 <sup>a</sup> $\pm$ 0.003
7	0.021 <sup>b</sup> $\pm$ 0.009	0.030 <sup>b</sup> $\pm$ 0.006	0.029 <sup>b</sup> $\pm$ 0.009	0.044 <sup>a</sup> $\pm$ 0.005

Different subscripts in the same line correspond to a significant difference ( $P < 0.05$ ).

did not vary significantly for HSU, whereas a maximum was observed at 1 h after feeding in ruminal fluid containing cSBM 40 % and 20 %. The peptide-N and amino acids-N for the ration containing tSBM were always higher than cSBM 40 % ( $P < 0.05$  at 4 and 7 h) and cSBM 20 % ( $P < 0.05$ ).

For the ration containing cSBM 40 %, a high concentration of protein-N was found in the rumen fluid (14 % Nt at 1 h and 12.2 % Nt at 2 h after feeding), while low concentrations of protein-N were found for the ration containing tSBM (5 % Nt 1 and 2 h after feeding), cSBM 20 % and HSU.

### 3.4. Electrophoresis

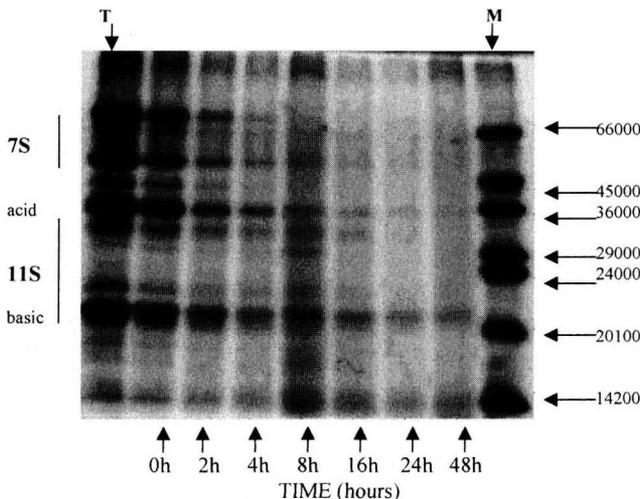
In the feeds, the yield of protein-N (as a percentage of the initial N) obtained by the extraction procedure before electrophoresis was 68 % of Nt and 24 % of Nt for cSBM and tSBM, respectively. Electrophoretic profiles after in situ incubation (figure 1) showed that conglycinins (7S) of cSBM disappeared at 16 h, whereas glycinins (11S) that are more resistant to degradation were observed up to 24 h (acid subunits) and 48 h (basic subunits). However, for tSBM (figure 2), acid and basic glycinins and part of the conglycinins were found at 72 h.

At 0 h (before feeding), for diets of cSBM 40 % and cSBM 20 %, proteins in the ruminal fluid could not be found by electrophoresis, whereas at 1 and 2 h after feeding, proteins were observed (figures 3 and 4). For tSBM, few proteins were observed in the ruminal fluid 1 h after feeding (data not shown).

## 4. DISCUSSION

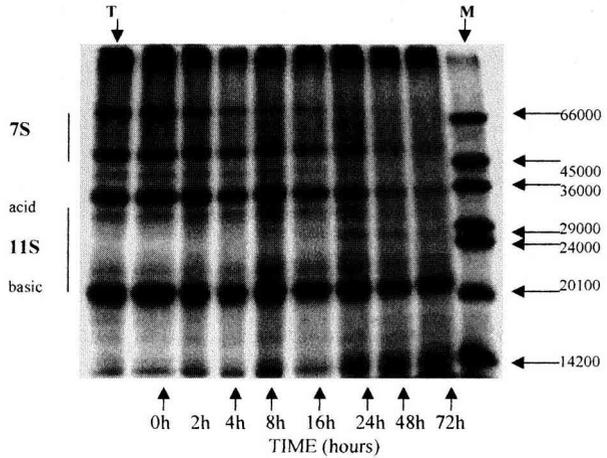
Treatment caused little modification in the ADIN concentration, which is very low (table 1), whether the meal was treated or untreated, and indicate that total availability may not be influenced. Similar results have been found by Faldet et al. [15] and Maiga et al. [24]. In contrast, treatment significantly increased NDIN concentration. The nitrogen content of NDF in feeds is greatly increased by heating, which promotes denaturation of albumins, but not necessarily in ADF, which requires the Maillard reaction to render the protein recoverable in ADF [41].

The Deg (N) obtained for cSBM 0.727 is a little higher than the values obtained by Madsen and Hvelplund [23], Inra [20] and Aufrère et al. [3], but it is close to those of Windschit and Stern [43] and England et al.

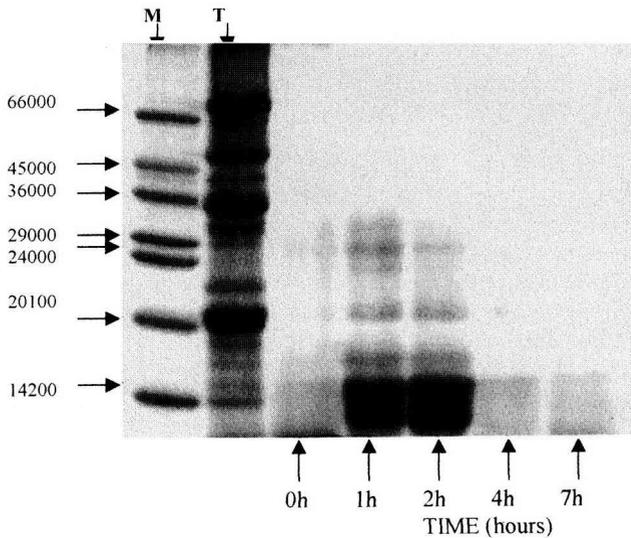


**Figure 1.** Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of control soya bean meal (cSBM) showing residues after in situ incubation for 0, 2, 4, 8, 16, 24 and 48 h in the rumen fluid for sheep receiving a ration of hay and cSBM 40 %. T: control soybean meal; M: molecular mass markers from Sigma.

**Figure 2.** Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of treated soya bean meal (tSBM) showing residues after in situ incubation for 0, 2, 4, 8, 16, 24, 48 and 72 h in the rumen fluid for sheep receiving a ration of hay and tSBM. T: treated soybean meal; M: molecular mass markers from Sigma.



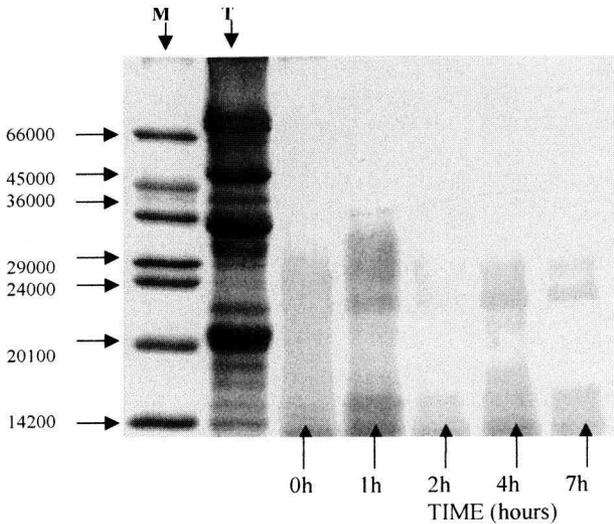
**Figure 3.** Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of control soya bean meal (cSBM) showing proteins in the rumen fluid before the meal (0 h) and 1, 2, 4 and 7 h after feeding for sheep receiving a ration of hay and cSBM 40 %. T: control soybean meal; M: molecular mass markers from Sigma.



[13]. The 'a' value of the immediately soluble fraction of the control meal is low and agrees with the DE1 results and those in the literature, and is due to the high temperature (around 115–120 °C) applied in the technical oil extraction process.

Treatment of the soya bean meal led to lower degradability in the rumen [Deg (N) 0.502], as many authors had already observed [7, 10, 16, 19, 21, 29, 35]. How-

ever, as observed by Stern et al. [36], a significant reduction of the immediately soluble fraction for the treated meal was not found. The difference in degradation between the two meals mainly involved the rate of degradation of the 'b' fraction, probably due to the increased NDIN concentration. Protein that was insoluble in neutral detergent but soluble in acid detergent appeared to have high digestibility, although it usually digested at slower rates than the



**Figure 4.** Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of control soya bean meal (cSBM) showing proteins in the rumen fluid before the meal (0 h) and 1, 2, 4 and 7 h after feeding for sheep receiving a ration of hay and cSBM 20 %. T: control soybean meal; M: molecular mass markers from Sigma.

fractions that were soluble in neutral detergent [41].

Electrophoretic profiles of cSBM showed that soya bean meal conglycinins (7S) were degraded rapidly, whereas glycinins were more resistant (the basic subunits were not degraded, and the acid subunits were only slowly degraded). This agrees with the results of Van der Aar et al. [38], Romagnolo et al. [32], Newbold and Rust [28], Nola et al. [30], Aufrère et al. [4] and Schwingel and Bates [34]. For tSBM, the conglycinins were degraded slower and the glycinins were not degraded. For cSBM, conglycinins were rapidly hydrolysed by proteases in the rumen. The molecular three-dimensional structure was responsible for these properties. Conglycinins are glycosylated and thus had hydrophilic characteristics. These hydrophilic properties caused the molecule to be less compact when solubilized in water [22]. For tSBM, heating facilitates the Maillard reaction between sugar aldehyde groups and the free amino groups of protein, yielding an amino sugar complex. This complex is more resistant than normal protein to enzymatic hydrolysis, and the reversibility of this reaction is dependent upon the temperature and time of heat exposure [37].

The higher Nt,  $\text{NH}_3\text{-N}$  and protein-N contents in ruminal fluid before the first meal (0 h) compared to 7 h afterwards, particularly for the diet tSBM, were due partly to a low degradation rate of this meal, as was observed in the nylon bag technique and the DE1 results, and partly to the smaller volume of the rumen fluid measured at 0 h (6 L) and compared to 8 L after the meal.

In contrast to rapeseed meal with the same degradability [5], and as was also observed by Aharoni et al. [1], most of the soya bean meal proteins were degraded to  $\text{NH}_3$ . Only a low proportion was in protein-N form 1 h and 2 h after feeding for the control meal (cSBM 40 %), and this proportion was even lower for the control meal (cSBM 20 %) and the tSBM meal. These data were confirmed by electrophoresis (figures 3 and 4).

Therefore, little soluble NAN can escape ruminal degradation by leaving the rumen with the liquids. In contrast to these results, Chen et al. [8] demonstrated that for cows receiving rations containing about 20 % normal soya bean meal, the ruminal fluid peptides-N concentration was higher than  $\text{NH}_3\text{-N}$  2 h after feeding, and decreased little when half the distributed soya bean meal was replaced by autoclaved soya bean meal.

As for rapeseed meal [5], in order to estimate the consequences in terms of nutrition it is important to quantify the extent to which dietary NAN solubilized in ruminal fluid can contribute to the intestinal protein flow. From the results for each of the soya bean meals, the proportion of NAN [expressed in crude protein (CP)·kg<sup>-1</sup>·d<sup>-1</sup> of ingested dry matter of soya bean meal] was estimated taking into account the nitrogen fraction from the hay (in the HSU diet). The volume of rumen (8 L) was determined by manually emptying the rumen on each sheep. The fractional passage rate for liquid was estimated at 0.07·h<sup>-1</sup> for a hay intake of 1 kg dry matter·d<sup>-1</sup> [6]. Feed proteins arriving in the intestine (PIA), estimated from the Deg (N) actually measured in the nylon bags, were calculated allowing for a fractional passage rate for solid of 0.06·h<sup>-1</sup> (PIA (g·d<sup>-1</sup> dry matter) = 1.11 × CP(1-Deg (N)) with CP (g·kg<sup>-1</sup> dry matter) [42].

Despite the results obtained with hay in the rapeseed meal diet [5], the proportion of NAN in the diet of HSU did not differ from that obtained in the ruminal fluid with the cSBM and tSBM diets and was about 30 % Nt at 1 and 2 h after feeding. Assuming that microbial synthesis and proteolysis were similar for hay in the soya bean meals diets and for HSU, the ratio between the amount of NAN in ruminal fluid able to escape degradation in the rumen and the amount of PIA was insignificant.

The NAN able to escape degradation in the rumen, expressed as a percentage of degraded protein (NAN × 6.25)/(CP × Deg (N)), was 0 to 1 % of the degraded protein, even though it was 5.5 to 7.5 % of the degraded protein for rapeseed meals.

## 5. CONCLUSION

Treated soya bean meal nitrogen was less degraded in the rumen and slower than for the control meal. Protein separation of the bag residues by electrophoresis shows that

the conglycinins and glycinins of the tSBM were degraded slower than that of the cSBM. If the diets were made up of 40 % meal, the nitrogen forms present in the rumen were mainly NH<sub>3</sub>-N. One and 2 h after feeding only 30 % Nt were in NAN form and comprised protein-N, peptides-N, and amino acids-N for the control meal, and essentially peptide-N and amino acids-N for the treated meal.

In contrast to the results obtained for rapeseed meals [5], the increase in Deg (N) caused by a low heating treatment during processing was, in part, not compensated for by ruminal fluid NAN leaving the rumen with the liquid phase.

## REFERENCES

- [1] Aharoni Y., Tagari H., Boston R.C., A new approach to the quantitative estimation of nitrogen metabolic pathways in the rumen, *Br. J. Nutr.* 66 (1991) 407–422.
- [2] Aufrère J., Cartailleur D., Mise au point d'une méthode de laboratoire de prévision de la dégradabilité des protéines alimentaires dans le rumen, *Ann. Zootech.* 40 (1988) 255–270.
- [3] Aufrère J., Graviou D., Demarquilly C., Vérité R., Michalet-Doreau B., Chapoutot P., Predicting in situ degradability of feed proteins in the rumen by two laboratory methods (solubility and enzymatic degradation), *Anim. Feed Sci. Technol.* 33 (1991) 97–116.
- [4] Aufrère J., Graviou D., Michalet-Doreau B., Degradation in the rumen of proteins of 2 legumes: soybean meal and field pea, *Reprod. Nutr. Dev* 34 (1994) 483–490.
- [5] Aufrère J., Graviou D., Demarquilly C., The significance of the degradation products of rapeseed meal proteins in the rumen according to different meal processing techniques, *Ann. Zootech.* 47 (1998) 127–140.
- [6] Baumont R., Jailler M., Dulphy J.P., Dynamic of voluntary intake, feeding behaviour and rumen function in sheep fed three contrasting types of hay, *Ann. Zootech.* 46 (1997) 231–244.
- [7] Broderick G.A., Wallace R.J., Ørskov E.R., Control of rate and extent of protein degradation. In *Physiological aspects of digestion and metabolism in ruminants*, in: Tsuda T., Sasaki Y., Kawashima R. (Eds.), *Proceedings of the 7th International Symposium on Ruminant Physiology*, August 28–Sept 1, 1989, in Japan, 1991, pp. 541–592.

- [8] Chen G., Sniffen C.J., Russell J.B., Concentration and estimated flow of peptides from the rumen of dairy cattle: effects of protein quantity, protein solubility, and feeding frequency, *J. Dairy Sci.* 70 (1987) 983–992.
- [9] Cherney D.J.R., Volenec J.J., Cherney J.H., Protein solubility and degradation in vitro as influenced by buffer and maturity of alfalfa, *Anim. Feed Sci. Technol.* 37 (1992) 9–20.
- [10] Coenen D.J., Trenkle A., Comparisons of expeller-processed and solvent-extracted soybean meal as protein supplements for cattle, *J. Anim. Sci.* 67 (1989) 565–573.
- [11] Conway E.J., *Microdiffusion Analysis and Volumetric Error*, Crosby Lockwood, London, 1957.
- [12] Dorleans M., Mandran N., Sauvant D., Study of the use of a protease with the van Soest procedure, *Anim. Feed Sci. Technol.* 61 (1996) 129–136.
- [13] England M.L., Broderick G.A., Shaver R.D., Combs D.K., Comparison of in situ and in vitro techniques for measuring ruminal degradation of animal by-product proteins, *J. Dairy Sci.* 80 (1997) 2925–2931.
- [14] Fahmy W.G., Aufrère J., Graviou D., Demarquilly C., El-Shazly K., Comparison between the mechanism of protein degradation of two cereals by enzymatic and in situ methods, using gel electrophoresis, *Anim. Feed Sci. Technol.* 35 (1991) 115–130.
- [15] Faldet M.A., Loss V.L., Broderick G.A., Satter L.D., Chemical, in vitro and in situ evaluation of heat-treated soybean proteins, *J. Dairy Sci.* 74 (1991) 2548–2554.
- [16] Faldet M., Satter L., Broderick G., Determining optimal heat treatment of soybeans by measuring available lysine chemically and biologically with rats to maximize protein utilization by ruminants, *J. Nutr.* 122 (1992) 151–160.
- [17] Giger S., Thivend P., Sauvant D., Dorléans M., Journaix P., Étude de l'influence préalable de différentes enzymes amylolytiques sur la teneur en résidu NDF d'aliments du bétail, *Ann. Zootech.* 36 (1987) 39–48.
- [18] Grenet E., Taille et structure des particules végétales au niveau du feuillet et des fèces chez les bovins, *Ann. Biol. Anim. Bioch. Biophys.* 10 (1970) 643–657.
- [19] Hussein H.S., Cameron M.R., Fahey G.C., Merchen N.R., Clark J.H., Influence of altering ruminal degradation of soybean meal protein on in situ ruminal fiber disappearance of forages and fibrous byproducts, *J. Anim. Sci.* 73 (1995) 2428–2437.
- [20] Inra, Recommended allowances and feed tables, in: Jarrige R. (Ed.), *Ruminant Nutrition*, JL Eurotext, London and Paris, 1989.
- [21] Lykos T., Varga G.A., Effects of processing method on degradation characteristics of protein and carbohydrate sources in situ, *J. Dairy Sci.* 78 (1995) 1789–1801.
- [22] Lynch G.L., Van der Aar P.J., Berger L.L., Fahey C.C., Merchen N.R., Proteolysis of alcohol-treated soybean meal proteins by *bacteroides ruminicola*; *Bacteroides amylophilus*, pepsin, trypsin, and in the rumen of steers, *J. Dairy Sci.* 71 (1988) 2416–2427.
- [23] Madsen J., Hvelplund T., Protein degradation in the rumen. A comparison between in vivo, nylon bag, in vitro and buffer measurements, *Acta Agric. Scand. Suppl.* 25 (1985) 103–124.
- [24] Maiga H.A., Schingoethe D.J., Henson J.D., Ruminal degradation, amino acid composition, and intestinal digestibility of the residual components of five protein supplements, *J. Dairy Sci.* 79 (1996) 1647–1653.
- [25] Merry R.J., McAllan A.B., A comparison of the chemical composition of mixed bacteria harvested from the liquid and solid fractions of rumen digesta, *Br. J. Nutr.* 50 (1983) 701–709.
- [26] Messman M.A., Weiss W.P., Use of electrophoresis to quantify ruminal degradability of protein from concentrate feeds, *Anim. Feed Sci. Technol.* 49 (1994) 25–35.
- [27] Michalet-Doreau B., Vérité R., Chapoutot P., Méthodologie de mesure de la dégradabilité in sacco de l'azote des aliments dans le rumen, *Bull. Tech. CRZV Theix Inra* 69 (1987) 5–7.
- [28] Newbold J.R., Rust S.R., Rumen proteolysis of constituent proteins of soybean meal, in: *Proceedings of the 100<sup>th</sup> Meeting of the British Society of Animal Production*, 1997.
- [29] Nishimuta J.F., Ely D.G., Boling J.A., Ruminal bypass of dietary soybean protein treated with heat, formalin and tannic acid, *J. Anim. Sci.* 39 (1974) 952–957.
- [30] Nola R., Greppi G.F., Enne G., Nordio C., Fractional protein degradation of seed meals in bovine rumen, *Proc. Soc. Nutr. Physiol.* 3 (1994) 47.
- [31] Ørskov E.R., McDonald I., The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage, *J. Agric. Sci. Camb.* 92 (1979) 499–503.
- [32] Romagnolo D., Polan C.E., Barbeau W.E., Degradability of soybean meal protein fractions as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, *J. Dairy Sci.* 73 (1990) 2379–2385.
- [33] SAS, Statistical Analysis Systems Institute Inc., SAS/Stat Software, General Linear Model, SAS Institute, Cary, NC, USA, 1985.
- [34] Schwingel W.R., Bates D.B., Use of sodium dodecyl sulfate polyacrylamide gel electrophoresis to measure degradation of soluble soybean proteins by *Prevotella ruminicola* GA33 or mixed ruminal microbes in vitro, *J. Anim. Sci.* 74 (1996) 475–482.

- [35] Sherrod L.B., Tillman A.D., Effects of varying the processing temperatures upon the nutritive values for sheep of solvent-extracted soybean meal and cottonseed meal, *J. Anim. Sci.* 21 (1962) 901.
- [36] Stern M.D., Santos K.A., Satter L.D., Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle heat-treated whole soybeans, *J Dairy Sci.* 68 (1985) 45–56.
- [37] Stern M.D., Varga G.A., Clark J.H., Firkins J.L., Huber J.T., Palmquist D.L., Evaluation of chemical and physical properties of feeds that affect protein metabolism in the rumen. Symposium: Metabolic relationships in supply of nutrients for milk protein synthesis, *J. Dairy Sci.* 77 (1994) 2762–2786.
- [38] Van der Aar P.J., Berger L.L., Wujek K.M., Mastenbroek I., Fahey J.R., Relationship between electrophoretic band patterns and in vitro ammonia release of soluble soybean meal protein, *J. Dairy Sci.* 66 (1983) 1272–1276.
- [39] Van Soest P.J., Use of detergent in the analysis of fibrous feed. I. A rapid method for the determination of fiber and lignin, *J. Assoc. Agric. Chem.* 46 (1963) 829–835.
- [40] Van Soest P.J., Wine R.H., Use of detergents in the analysis of fibrous feeds, IV. Determination of plant cell-wall constituents, *J. Assoc. Off. Agric. Chem.* 50 (1967) 50–55.
- [41] Van Soest P.J., Nitrogen metabolism, in: *Nutritional Ecology of the Ruminant*, 2<sup>nd</sup> edn., Cornell University Press, New York, 1994, pp. 290–311.
- [42] Vérité R., Michalet-Doreau B., Chapoutot P., Peyraud J.L., Poncet C., Révision du système des Protéines Digestibles dans l'intestin (PDI), *Bull. Tech. CRZV Theix Inra* 70 (1987) 19–34.
- [43] Windschitl P.M., Stern M.D., Evaluation of calcium lignosulfonate-treated soybean meal as a source of rumen protected protein for dairy cattle, *J. Dairy Sci.* 71 (1988) 3310–3322.